

# Institutes of the Royal Netherlands Academy of Arts and Sciences

---

## Progress Report 1982

North-Holland Publishing Company  
Amsterdam/Oxford/New York, 1983

Verhandelingen der Koninklijke Nederlandse  
Akademie van Wetenschappen,  
Afdeling Natuurkunde, Tweede Reeks, deel 81



---

## Contents

Netherlands Institute for Brain Research, Amsterdam

Hubrecht Laboratory, International Embryological  
Institute, Utrecht

Centraalbureau voor Schimmelcultures, Baarn/Delft

Institute for Ecological Research,  
Heteren/Oostvoorne

Limnological Institute, Nieuwersluis/Oosterzee

Delta Institute for Hydrobiological Research, Yerseke



# **Netherlands Institute for Brain Research**

---

## **Progress Report 1982**

**Edited by N.E. van de Poll, D.F. Swaab and W. Chen-Pelt**

Nederlands Instituut  
voor Hersenonderzoek,  
IJdijk 28, 1095 KJ Amsterdam,  
The Netherlands

## CONTENTS

Research teams and participants	- 3
Historical background	- 5
The organization of research	- 6
I. Development and plasticity of the cerebral cortex	- 8
II. Interaction of the nervous system and behavior during maturation	- 14
III. Brain-endocrine interactions during maturation and adaptation	- 18
IV. Development and plasticity of behavior	- 26
V. Mathematical and computational aspects of neurobiology	- 34
Mechanics workshop	- 38
Electronics workshop	- 39
General technical service	- 40
Guest workers and work-visits abroad	- 40
Publications	- 41
Abstracts (incl. posters)	- 49
Papers read (seminars, etc.)	- 54
Teaching	- 56
Miscellaneous	- 60
Seminars given at the Institute	- 62

## RESEARCH TEAMS AND PARTICIPANTS

Director: Prof.Dr. D.F. Swaab  
Manager: H. Sijtsma

### I. Development and plasticity of the cerebral cortex

Dr. H.B.M. Uylings (head)  
Drs. C.G. van Eden (PhD student)  
Drs. M. Hofman (PhD student)  
Drs. E.M.D. Hoorneman (PhD student; from 1-9-1982)  
Drs. W. van Norde (temp.)  
Dr. R.W.H. Verwer  
M. Dozy  
P. Evers  
C. de Raay

### II. Interaction of the nervous system and behavior during maturation

Dr. M.A. Corner (head)(NIBR and University of Amsterdam)  
Dr. R.E. Baker  
Drs. H.L.M.G. Bour (PhD student; until 1-11-1982)  
Dr. A.M.M.C. Habets  
Drs. F. van Huizen (PhD student)  
Dr. M. Mirmiran  
Dr. H.J. Romijn (coordinator electron microscope service)  
M.T. Mud  
H.F. Pronker  
P. Wolters

### III. Brain-endocrine interactions during maturation and adaptation

Prof.Dr. D.F. Swaab (head)(NIBR and University of Amsterdam)  
Dr. G.J. Boer (coordinator radio-isotope laboratory)  
Dr. R.M. Buijs  
Drs. E. Fliers (PhD student FUNGO)  
Drs. J. Kruisbrink (temp.)  
Dr. F.W. van Leeuwen  
Dr. P. Pévet (University of Amsterdam)  
Dr. Chr.W. Pool (coordinator animal care facilities)  
Drs. P.J. van der Sluis (PhD student)  
Drs. G.J. de Vries (PhD student)  
B. Fisser  
E. de Graaf (FUNGO/ University of Amsterdam)  
J.J. van Heerikhuize  
A.A. Sluiter

### IV. Development and plasticity of behavior

Dr. N.E. van de Poll (head)  
Dr. J.P.C. de Bruin  
Drs. F.H. de Jonge (PhD student, Psychonomie)

Dr. H.G. van Oyen (until 1-9-1982)  
 Drs. J. Scholtens (alternative military service;  
 PhD student Psychonomie, from 1-4-1982)  
 Drs. J. Slopsema (alternative military service;  
 until 21-8-1982)  
 Dr. H.H. Swanson  
 S.M. de Jong-van Zanten  
 E.M. Verbraak  
 S.M. van der Zwan (until 1-10-1982)

#### V. Mathematical and computational aspects of neurobiology

Dr. H.L. Walg (head; until 1-4-1982)  
 Dr. J. van Pelt (head; from 1-4-1982)  
 Drs. A.J. Noest (from 1-7-1982)  
 M. Timmerman

##### Mechanical workshop

A. Kamstra (head)  
 M. Westdorp

##### Electronics workshop

J. Overdijk (head)  
 R. Nooy

##### General technical service

J.C. de Jong (head)  
 L. Tibbertsma

##### Animal care facilities

R. Hofer  
 N. de Vries (until 1-6-1982)  
 E. Tanger (from 1-8-1982)

##### Drawing department

H. Stoffels

##### Photography department

A.T. Potjer (head)  
 T.C. Sypkens-Potjer

##### Secretariat

W. Chen-Pelt  
 P.J. van Nieuwkoop  
 J. Sels  
 A. van der Velden

##### Administration

H. de Vijlder  
 N. van der Poel

##### Library

C. Winkler

##### Household service

M.A. Scheermeijer-Beuker (head)  
 H.H. Barbe-Scheermeijer  
 C. de Haas-Joele  
 J.W. Pals-Cappon  
 M. de Vos-Harthoorn

##### Canteen

C. de Groot

## HISTORICAL BACKGROUND

At a meeting of the International Association of Academics, held in Paris in 1901, the anatomist Wilhelm His proposed that research into the nervous system should be placed on an international footing. In 1904 this resulted in the formation of the International Academic Committee for Brain Research, which pointed out that 'the time is not far distant when the study of the millions of brain cells will have to be divided amongst researchers in the way that astronomers have been obliged to divide the millions of stars into various groups'.

The committee set itself the task of 'organizing a network of institutions throughout the civilized world, dedicated to the study of the structure and functions of the central organ....'. The first country to respond to this ambition was the Netherlands: on the basis of a report drawn up by Profs. C. Winkler and L. Bolk, the Royal Netherlands Academy of Arts and Sciences (KNAW) applied to the government for permission to found an institute for brain research. On June 8th, 1909, the 'Netherlands Central Institute for Brain Research' was opened in a wing of the then newly erected Department of Anatomy and Embryology of the University of Amsterdam.

The first director was Prof. C.U. Ariens Kappers, who gained international fame with his work in the comparative anatomy of the nervous system. Much of the material used in his research, including a considerable number of human and animal brains, is still at the institute. He regarded the study of the development of the brain as being essential to the understanding of the normal and pathological structure and function of the nervous system. That his contention is still valid is evidenced by the central place this approach occupies in the research program of the institute today.

Prof. C.U. Ariens Kappers was succeeded in 1946 by Prof. B. Brouwer, whose principal field of study was pathological anatomy. A member of his staff, Prof. J. Drooglever-Fortuyn, introduced electrophysiology to the institute's field of research.

After the death of Prof. Brouwer in 1949, the institute was expanded and reorganized to allow for a multi-disciplinary approach to brain research. In 1952, Prof. S.T. Bok (one of the pioneers of quantitative morphological analysis of the brain, especially the cerebral cortex) was appointed director. After his retirement in 1962, he was succeeded by Prof. J. Ariens Kappers, whose special field of study was the circumventricular organs. Under his direction, research into the structure and function of the pineal gland became an important part of the institute's work.

On the first of October, 1978, Dr. D.F. Swaab, acting director since November 1975, was appointed to be the new director. In December 1979 the office of 'extraordinary professor' of neurobiology at the University of Amsterdam was conferred upon him. The construction of new quarters for the institute within the complex of the Academic Medical Center of the University of Amsterdam is now nearing completion.

The institute's supervisory committee consists of Profs. J. Joosse (chairman), A.H.M. Lohman, H. van Crevel, F.H. Lopes da Silva, J.L. Slangen and H.K.A. Visser and convenes frequently with Prof. D.F. Swaab, H. Sijtsma and Dr. D. van der Mei (KNAW). Dr. R. Balász (MRC Developmental Neurobiology Unit, London, England) advises the committee in biochemical matters.

## THE ORGANIZATION OF RESEARCH

As of the first of January, 1977, the central research theme 'Maturation and Adaptation of the Nervous System' is being investigated by five multi-disciplinary research teams, which in the coming period will be working along the following lines:

### I. Development and plasticity of the cerebral cortex

Neurotransmitters, hormones and environmental conditions are essential for normal development of the cortex. This team centres its research on the question how and to what extent these factors influence the development and plasticity of the cortex. Attention is mainly directed to the effects they have on the prefrontal cortex of rat as well as man. In rats, because of the experimental opportunities such study offers; in man because such research is adherent to the neuropathological study of the prefrontal cortex area in case of abnormalities in its development. The research also encompasses the study of the structural differences in the cortex between the sexes, and between the right and left hemisphere of the brain. Quantitative and qualitative light and electron microscopic techniques constitute the core of this research, whereby autoradiographic techniques and, in collaboration with members of research team III, immunocytochemical procedures are employed. The aspects of physiology and behavior are being investigated in coordination with members of research teams II and IV, while the chemical aspects are being investigated together with members of research team III.

### II. Interaction of the nervous system and behavior during development

During the period of synapse formation in the central nervous system, rhythmical patterns of spontaneous bioelectrical activity are both widespread and pronounced. These lead, among other things, to the strikingly vigorous movements shown by foetal and neuronal animals during 'active' (or REM) sleep. The degree to which these neuronal discharges contribute to the emergence of ordered networks (synaptic organization) is still very poorly understood. Research team II is approaching this problem in vivo by studying - in collaboration with research teams I (neuroanatomical effects), III (biochemical effects) and IV (behavioral effects) - the consequences for brain development of chronically suppressing active sleep in infant rats. In addition, a tissue culture 'model' system allows for a more controlled manipulation of neuronal activity during development, with subsequent assays for structural and functional abnormalities, than would be possible in the intact animal. In collaboration with research team V, endeavors are also being made to develop a computer model of cortical network, in order to test hypotheses about the reciprocal relationship between structural and functional maturation. Techniques used by the research team include electrophysiology, electron microscopy and culturing in vitro, while quantitative morphological, immunocytochemical, behavioral and computer techniques are contributed by research teams I, III, IV and V, respectively.

The two electron microscopes that are available at the institute are supervised by this research team.

### **III. Brain-endocrine interactions during maturation and adaptation**

Brain cells produce certain substances, such as neuropeptides, which exert an influence upon the nervous system itself as well as on the pituitary gland and, by their hormonal actions, on other organs as well. An appropriate maturation process and a well adjusted functioning of the nervous system is also dependent on hormones produced elsewhere in the body.

The interaction between nervous and endocrine systems is being studied during the early growth and the maturation stage of the nervous system, in relation both to parturition and to the individual's adaptation to its environment. In this framework the question is also studied how the pineal gland determines the most favorable season for birth.

The emphasis in the research of this group lies on the function of neuropeptides during the above mentioned processes. Research team I is studying the morphological consequences of the effects produced by peptides on brain development. The effects of gonadal hormones on peptidergic fiber growth, and their selectivity as regards extrahypothalamic connections in culture, are being studied in collaboration with research teams III and II, as are the electrophysiological properties of peptidergic synapses. The functional implications of the extrahypothalamic pathways and their sexual dimorphism are studied in collaboration with research team IV.

The research team is engaged in the application of immunocytochemical methods, applicable to both light and electron microscopic work, along with biochemical and physiological techniques, radio-immunoassays and clinical observations.

### **IV. Development and plasticity of behavior**

Under the influence of sex hormones during critical phases of development, changes take place in the central nervous system which may markedly affect the behavior of an individual in adult life. In the rat this sensitive period occurs around the time of birth. The brain shows growth spurts at different stages in various parts of the brain, and the periods during which sex hormones influence the central nervous system may be related to this phenomenon. Between this sensitive period, when hormones are active, and adulthood lies a long period of development during which environmental (including social) factors may interact with the organizational effects of the hormones. Sexual dimorphism of brain and behavior forms an important part of the research conducted in collaboration with other research teams, such as morphological aspects (team I), relations sleep patterns (team II) and biochemical and immunological aspects (team III).

The influence of sex hormones on several aspects of adult behavior such as sex, sexual behavior and aggression, and some forms of learning are being investigated. Special emphasis will be placed on the possible identification and localization of associated functional differences in the nervous system, using lesion and implantation techniques. Since the consequences of organizational effects of sex hormones sometimes only appear under certain conditions, the relationship between hormones and specific behaviors in adult animals is also considered.

### **V. Mathematical and computational aspects of neurobiology**

This group has a twofold task. First, it is concerned with all automation and data processing activities and with the management of the institute's

computersystems including the powerful DIGITAL VAX 11/780 system. Secondly, it studies the growth and adaptation phenomena from a theoretical/mathematical point of view. The main attention has been paid to the analysis of structural (topological) properties of dendritic trees in cooperation with research team I. This way of analysis enables the study of growth processes and the quantification of rules along with the growth proceeds. As such it offers the tools for studying the implication of experimental conditions on dendritic growth. Other fields of interests concern the bioelectric activity in neural networks. By means of computer simulation programs the activity of neurons in a distributed model is studied in relation to variables like network connectivity and synaps efficiency. This study is performed in cooperation with research team II where the bioelectric activity is measured in dissociated neural tissue cultures.

## I. DEVELOPMENT AND PLASTICITY OF THE CEREBRAL CORTEX

Neurotransmitters, hormones and environmental conditions are essential for the normal development of the cortex. The research of our team is orientated towards the normal development of the cortex and the extent to which the above mentioned factors are of influence on the cortical development and plasticity. The conditions involved in our studies include undernutrition, monoamine deficiency, neuropeptide deficiency (together with research team III) and subcutaneous clonidine injections (affecting REM sleep) (together with research team II). Emphasis is put on the study of (a) normal development, (b) impairment in this development and (c) the potential for recovery. In this project, the presence of structural sexual dimorphism and lateralization of the cortex are also being studied. The cytoarchitecture of neurons, dendrites, axons and synapses is studied both qualitatively and quantitatively (together with research team V) using light and electron microscopical, immunocytochemical (with research team III) and histochemical techniques. This project is partly represented in the FUNGO workgroup, 'Development and Aging of the Brain and Behavior' (nr. 13-51-38).

### Theme 1 - Effects of nutritional rehabilitation and environmental enrichment on cerebral and cerebellar cortex of previously undernourished rats

The experimental conditions investigated (in collaboration with Dr. P. McConnell, Dept. Human Anatomy, Univ. of Oxford, U.K.) were the following: three groups of rats were undernourished from birth through 30 days of age; one group was then sacrificed, while the other two were given nutritional rehabilitation ad libitum up to 150 days post partum. One of the last two groups was housed under standard laboratory conditions, the other in an environment, which was 'enriched' both non-socially and socially. For each of the three groups there was a control group which received adequate nutrition throughout life.

This year the dendritic measurements of all the groups studied were completed. The metrical, topological orientation and density analyses of the branching structures of about 1,200 cells are in progress. The occipital cortex showed a significant reduction in thickness and cross-sectional area of about 10% and 18%, respectively for the day-30 underfed group. A striking finding is that the dendritic branching structure of the multipolar non-pyramidal neurons in the occipital cortex at day 30 is not affected by undernutrition, while in contrast the

dendrites of the pyramidal cells in the superficial layers are seriously affected. Apparently, types of neurons within the same part of the cortex are differentially affected. In contrast to the cerebellar neuron types, the neuron generation of both cell types has been completed before the start of postnatal undernutrition. Thus a difference in neuron generation of both cerebral neuron types could not be a plausible factor, as it was for the cerebellar neurons. The total basal dendrite length per cell and the total number of segments per cell (indicative of frequency of branching) were significantly smaller by day 30 in the undernourished group (18% and 15%, respectively). The number of basal dendrites per neuron and the length of terminal segments were also significantly smaller (7% and 11% respectively), in contrast to the length of the intermediate segments. The length of these intermediate segments did not significantly deviate. No difference was found in the orientation of the dendritic field of pyramidal cells between the control and the underfed groups. The density of the dendritic network, however, was significantly different, especially in a laminar zone just below the soma.

During the first analyses of the data it appeared that our tracking system for 3-dimensional measurements of dendrites was not sufficiently safeguarded against all errors made by the operator. The measuring computer program has now been changed (by research team V), so that it warns the operator whenever these rare errors occur. Hippocampus tissues from our earlier mentioned undernutrition studies are being studied to determine whether or not parts of the hippocampus were affected by undernutrition. This study is especially interesting, since the granule cells in the fascia dentata proliferate postnatally. The volume of the fascia dentata, the CA4, CA3, CA1, the subiculum and the hippocampus as a whole will be determined in Nissl-stained sections with the digitizer MOP Kontron/AM02. The measurements were nearly completed in 1982.

The electron microscopical study of quantitative alterations occurring in the synaptogenesis in the rat cerebellum after neonatal undernourishment was continued. The rat cerebellum is an important area to be studied, since its development is mainly postnatal. The measurements were directed towards the synaptic contacts between parallel fibers and Purkinje cell dendritic spines. Those contacts form the majority of synaptic structures in the cerebellar molecular layer. Both the number of synapses and the number of spines per unit volume of tissue (i.e., numerical density) have been estimated for each individual animal of the 30-day control and underfed group. No significant difference in numerical density of the spines could be established between these two groups of animals. It cannot be precluded that the wide inter-animal variation masked an effect. Preliminary results suggest that void spines (i.e., spines without synaptic contacts) occur in 30-day control rats, whereas undernourished rats seem to have spines with more than one synapse. For the numerical density analyses two approaches were used. The first is a new method, which does not require that the structures under analysis have a disk-like or sphere-like shape. A second approach is the procedure of modified 'unfolding method of Saltykov'. This procedure determines the numerical density under the assumption that the structures of interest have a disk-like or a spherical shape. Both procedures correct for the cutting effect which is related both to the size of the structure examined and the thickness of the section. Computer programs have been developed for both procedures on the VAX computer.

## **Theme 2 - The development of the prefrontal cortex**

Our research theme examines the structural development of the prefrontal cortex (pfc) and the influence of dopamine on the development.

The pfc is nearly the only meso/neocortical area that receives dopaminergic innervation. Parallel to our studies, the involvement of the pfc areas in specific types of behavior is analyzed in research team IV. The development of cytoarchitectonically different subareas in the prefrontal cortex (pfc) was examined in Wistar rats. The distinguishable subareas are, for the medial pfc, the prelimbic area, the anterior cingulate area and the precentral medial area, and for the orbital pfc the dorsal and ventral anterior insular areas. From the literature, it is known that projections from the medial dorsal nucleus of the thalamus terminate in a specific pattern within these cytoarchitectonically defined boundaries. They also coincide with borders visible in the distribution of heavy metals and the AChE activity. Under standard laboratory conditions, a total of 108 rats were bred; 6 male and 6 female rats for 9 different stages viz., day 1,6,10,14,18,24,30,60 and 90 post partum (dpp). From a study of the Nissl-stained sections, the following conclusions were drawn: (1) the cytoarchitectonical criteria used to delineate pfc subareas in the adult brain could be applied also to developing rats from 18 dpp; (2) at 6 dpp, the first subdivision in different subareas could be made; (3) a heterogeneous cortical plate was observed at 1 dpp, which may correspond to the future, heterogeneous cytoarchitecture of the pfc characterizing its subareas. Using serial Nissl sections, the developmental increase in size of the different pfc subareas was determined with a volumetric analysis using the MOP AMO2 and computer programs developed in collaboration with research team V. The first analysis showed that growth curves could be established sufficiently accurate to conclude that the orbital pfc subareas reach their maximal value at least 6 days after those of the medial pfc subareas, viz. at days 30 and 24, respectively. In addition, for both the orbital and the medial pfc subareas a so-called overshoot in their size was found at days 30 and 24, respectively. Moreover, those different subareas of the pfc, which receive the afferent input from a same area of the medial dorsal thalamic nucleus (MD), followed the same growth curve. Thus, the shape of the growth curve of the prelimbic area (a medial pfc subarea) is similar to those of the insular cortices (orbital pfc subareas), but reaches its maximum value 6 days earlier. A further analysis has been started to examine the possible presence of lateralization and sexual dimorphism in volume size of the pfc subareas during the development and the adult stages.

In 1982 a start was made with examining the influence of the dopaminergic innervation upon the development of the pfc. Since dopaminergic fibers are present in the pfc a few days before birth, dopamine lesions have to be made prenatally. The intention is to compare the pfc development in a control group and in a dopamine-lesioned group. A pilot study is being undertaken to explore the techniques of chemical dopamine lesions, injected intraventricularly at the age of 17 embryonic days. The effectiveness of these chemical lesions is controlled with high pressure liquid chromatography, determining the content of dopamine, noradrenaline and dopamine metabolites in collaboration with the Department of Pharmacology, Univer. of Leyden).

### Theme 3 - The structural development of the visual cortex in the rat

This study is being conducted in collaboration with Dr. J.G. Parnavelas (Univ. of Texas, Dallas, USA) and is directed towards describing the development of different neuronal types in different layers (viz. pyramidal and non-pyramidal neurons in layers II/III and V, and IV). In the course of our study three hypotheses are investigated: that (1) neurons located in ontogenetically older layers mature earlier; (2)

projection, pyramidal neurons mature earlier than local circuit non-pyramidal neurons and (3) larger cell types mature before smaller ones; irrespective of their generation time and their final laminar location. We analyzed the soma size and dendritic branching pattern of layer II and layer V small and large pyramidal cell types and non-pyramidal layer IV neurons. Given the questions, the following developmental stages have been selected, day 6,10,14,18,30 and 90 pp. This year the dendritic measurements of the last group, i.e., day 30, were completed. Preliminary results indicate that we could falsify each of these hypotheses. For example, (a) the small pyramidal neurons in layer V mature earlier than the large pyramidal neurons; (b) the non-pyramidal neurons in layer IV mature later than the small pyramidal neurons in layer V, but definitely not later than the pyramidal cells of layer II. Further analysis of our data on this aspect is in progress. With a new analysis method it appeared that frequencies of topological structures of the basal dendritic trees of layers II/III pyramidal cells and those of the dendrites of multipolar non-pyramidal cells differ significantly from an at random segmental growth model and do not deviate significantly - but deviate systematically - from the terminal growth model at all developmental stages analyzed.

The orientation and the density analysis of the development of the basal dendritic network in collaboration with Prof. A. Ruiz-Marcos (Cajal Institute, Madrid) showed the growth zones within the dendritic network. First around the soma and at later stages in a half-spherical shell, directed towards the white matter. When day-30 neurons have been analyzed, a complete description can be given.

A new method for the topological analysis of trees has been developed in collaboration with research team V, for the analysis of a small sample sizes. This is of special importance for the first postnatal developmental stages, since in these stages many small trees (nr. of terminal segments smaller than 4) occur. Trees with more than 4 terminal segments can be used for comparison with growth models.

#### **Theme 4 - Effects of clonidine on cortical growth and its responsiveness to postweaning rearing in an enriched environment**

This project is carried out in collaboration with research team II. Clonidine, an alpha 2 receptor agonist, induces REM sleep deprivation in rats. Our study showed that after daily subcutaneous clonidine injections between days 8 and 21 post partum the growth response of the cerebral cortex to an enriched environment from day 30 pp until 75 pp was absent. Standard laboratory conditions served as a control. In addition, the cortical weight of both the enriched and the standard groups of preweaning clonidine-treated rats was significantly smaller than the standard control group at 75 days of age. Furthermore, a further study revealed that if clonidine injections were given immediately after a daily 2.5-hr exposure to an enriched environment from 28 until 65 days post partum, the growth response of the cerebral cortex was also absent, as compared to control-standard and clonidine-standard groups. These results indicate that clonidine (active sleep) interferes with the development of the cerebral cortex and its plastic response capacities on enriched environmental stimulation (for further reports see research team II).

## Theme 5 - The influence of vasopressin deficiency on the rat brain

In previous experiments we have found that in midsagittal sections of the cerebellum, the size of the cerebellar cortex and white matter was significantly smaller in homozygous-diabetes insipidus (HOM-DI), vasopressin-deficient rats as compared with those areas in the heterozygous-diabetes (HET-DI), vasopressin-containing, rats in other experiments. It was shown (research team III), that the DNA content of the cerebella in HOM-DI rats was about 15% lower than that in HET-DI rats. This study could, however, not distinguish what cell type was deficient. Therefore, the number of Purkinje cells and granule cells were analyzed. Counts were performed in lobe VI, in midsagittal Nissl-stained sections of 4  $\mu$ m. The analysis of days 12, 24 and 180 post partum HOM-DI and HET-DI rats showed no significant differences in the number of Purkinje cells, whereas the number of granule cells determined at 24 dpp, was significantly reduced. At 24 dpp, the granule cell generation has nearly been completed. These findings indicate, therefore, a differential effect of vasopressin deficiency (directly or indirectly) on cerebellar neuron generation. It may also indicate that the cerebellar neuron generation is affected after embryonic day 16, the day before which Purkinje cells have proliferated. During the analysis it appeared to be necessary to develop a new method for granule cell counting, since the used methods were insufficient. The method was the following: a weighed systematical sampling indicated ten count-frames within the granular layer of lobe VI. Within these count frames the area/diameter of granular nucleus profiles were measured with the MOP AMO2 digitizer from microphotographs. A modified Saltykov procedure derived from these observed diameters of granular cell nuclei, the histogram of the 'real' diameters, and herewith the number of granule cells per volume zone within lobe VI could be determined.

## Theme 6 - Encephalization in mammals

Mathematical relations between energy metabolism, brain size and longevity in adult mammals were determined, based upon data from the literature. The analysis was carried out by linear regression in which the reduced major axis was used as method of curve fitting. It was found that the ratio of brain weight to basal metabolic rate in mammals is directly proportional to the degree of encephalization, i.e., to the evolutionary level of brain development, as established by measuring the deviation from the interspecific brain-body weight equation for mammals. It appears, furthermore, that the oxygen consumption of the brain, cerebral cortex and cortical neurons in normal adult mammalian species can be estimated by using gross brain weight as the principal parameter. According to these findings it turns out that encephalization in mammals can be completely formulated in terms of energetics. Furthermore, it was demonstrated that the energy metabolism of the cerebral cortex relative to that of the whole brain increases with the degree of encephalization. By using the encephalization coefficient and a body weight factor correlated with the standard metabolic rate of an animal as main variables in an allometric equation we were able to predict the maximum potential life span of a great number of mammalian species, including man.

Based upon these relationships derived from extant mammals further research was done on the progressive enlargement of the brain and that of the cerebral cortex in fossil hominids. It was shown that the evolution of the hominid brain was correlated with that of an increase in life span

and that both occurred in bursts, interspersed with long periods of little or no evolutionary change. The line along which these parameters (i.e., brain size and life span) evolved support the hypothesis that species do not change gradually as put forward in the Neo-Darwinian theory, but in relatively 'short' episodic 'bursts'. Finally the brain-body weight relationship in neonatal placental mammals has been formulated, revealing relationships between birth weight, somatic oxygen consumption and degree of encephalization, similar to those found for adults. According to this model, the degree of encephalization in the newborn (the extent of enlargement of the brain with respect to body size) as well as in the adult mammal is a function of two major components: (a) a body size-related component connected with the energy metabolism of the animal, which in the fetus is determined by the maternal energy supply; (b) a non-somatic evolutionary component correlated with the length and rate of rapid brain growth. A biometric analysis, furthermore, revealed that the maternal energy supply to the fetal mass at term accounts for about 14% of the standard maternal energy supply to the fetal mass is the principal determining factor, besides litter size and neonatal brain size, in setting limits to length of gestation.

## Methodology and instrumentation

### Topologies

Topological properties of neuronal trees are important parameters in the analysis of the growth (see also theme 3). New methods are in development - in collaboration with research team V to analyze these properties. Topological procedures were developed for binary branching structures, therefore trifurcations and cut segments might obviate these procedures. Pyramidal basal dendrites occasionally contain trifurcations, while in other neuronal types such as Purkinje cells trifurcations occur more frequently. Although trifurcations reduce the discriminative topological information, we showed that our methods for testing terminal or segmental growth are still applicable, without an assumption regarding the division of one trifurcation into two bifurcations. This makes our method for testing terminal or segmental growth most attractive as compared with the other in the literature existing methods. In addition, we have derived analytical formulas to determine the expected number of subtrees of a certain size in a population of neuronal trees grown according to both terminal and segmental growth models. Together with some other fundamental relations for tree structures, these formulas form the basis of a theoretical concept on topological analysis, which incorporates the vertex analysis as well. Vertex analysis is a topological method derived by Berry, analyzing the different bifurcation and terminal tip points of a tree. We are currently investigating the relation between the vertex analysis and our method. Furthermore, a study was started on the combination of topological and metrical tree analyses (see also the report of research team V).

### Semi-automated dendrite tracking system

This system has been connected on line with the Interdata computer and passed the first tests. On-line connection with the DEC VAX computer is in progress (see the reports of the mechanics and electronics workshops). In addition, as mentioned in theme 1, the acquisition computer program for this system has been improved and rewritten in Fortran for the VAX computer by research team V.

## II. INTERACTION OF THE NERVOUS SYSTEM AND BEHAVIOR DURING MATURATION

During the period of synapse formation in the central nervous system, rhythmical patterns of spontaneous bioelectrical activity are widespread and pronounced. The degree to which these neuronal discharges contribute to the emergence of ordered networks (synaptic organization) is still poorly understood. This research team is approaching this problem in vivo by studying the contribution of active (i.e., REM) sleep to brain development, in collaboration with research teams I (neuro-anatomical effects), III (biochemical effects) and IV (behavioral effects). In addition, an in vitro 'model' system allows for a more controlled manipulation of neuronal activity during development, with subsequent assays for structural and functional abnormalities. A collaboration with research team V is attempting to develop a computer model of neuronal networks in tissue culture, in order to test hypotheses about the reciprocal relationship between cortical structure and function. Techniques used by the team include electrophysiology, electron microscopy and culturing in vitro, while quantitative morphological, immunocytochemical, behavioral and computer techniques are contributed by research teams I, III, IV and V, respectively. This research team is represented (unsubsidized) in the FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior' (nrs. 1351-17, -35 and -36).

### Theme 1 - Neuronal basis of sleep/wake rhythms and their developmental significance

The analysis of AS(active sleep)-deprivation effects in developing rats was rounded off in the form of a doctoral dissertation. Several of the experiments implicated the cerebral cortex as being especially vulnerable to interventions which diminish REM sleep during development. It therefore became desirable to learn more about the 'sensitive period' during which the requirement for AS is at its greatest. This period presumably occurs after the time when cortical neurons begin to discharge 'spontaneously' during sleep. Studies involving electrophysiological recordings at the cellular level were therefore continued during this past year.

Using glass micropipettes, single unit recordings were made under chloral hydrate anaesthesia from layers IV and V of the occipital cortex in developing rats. In confirmation of our previous results using microwires implanted in free-moving animals, we were unable to detect any spontaneously active neurons prior to postnatal day 10. The earliest extracellular action potentials were long in duration (5-10 msec), fired for the most part singly, and were not activated by sensory stimulation. From day 11-12, cortical units discharged in bursts of 3 to 5 action potentials, which were closely associated with bursts of EEG slow waves ('trace alternant' pattern). The units have extracellular spike durations of 2 msec or less, and respond to sensory stimuli (auditory, visual and tactile) by briefly increasing their discharge rate. Systemic injections of sodium glutamate caused a simultaneous increase in both spontaneous unit firing and EEG slow-wave activity, which mimicks the pattern normally seen in the cortex two or three days later. In adult cerebral cortex too, the neurons tend to fire in bursts of 3-5 spikes and to increase their firing rate briefly following a sensory stimulus. Iontophoretic application of glutamic acid excites the neurons in most cases, whereas inhibitory responses to GABA are sometimes seen. The earliest appearance of neuronal responses to these types of local chemical stimulation is being investigated.

The data analysis from our study of neuronal discharges within the pontine reticular formation (PRF) during different sleep/wake stages in developing rats was completed this year. The increase in spontaneous firing during active sleep (AS) with respect to the quiet sleep (QS) level, which was reported last year to occur abruptly between 14 and 15 days after birth, was found by single unit analysis to entail an absolute reduction in QS firing levels, as well as the expected increase during AS. In addition, whereas up to 14 days most neurons appear to be activated to an equal degree during AS and active wakefulness (AW), a functional differentiation is clearly present in older animals. Thus PRF cells either fire more intensely during AS than during AW, or vice versa. This activity pattern is not readily reconcilable with the notion of an 'executive' role for the PRF neurons in generating the state of AS. In order to test this point experimentally, extensive unilateral PRF lesions were made in 17-day-old rats which had earlier been implanted with recording microwires on the intact side. Despite an abundant production of normal AS epochs in the experimental animals, no compensatory increase in firing of the residual PRF neurons was observed; indeed, the post-operative firing levels during such epochs were actually lower than prior to lesioning.

A pilot study has been carried out this year to investigate possible age-related changes in sleep patterning in rats. In agreement with human studies on sleep and aging, we were able to demonstrate the following changes in 24-month-old male Wistar rats: (i) a decline in AS (absolute amount as well as percentage of total sleep time), and (ii) an increased proportion of wakefulness (W) at the expense of sleep. These changes occurred mostly during the light phase of the circadian rhythm. Studies were also carried out, in collaboration with research team IV, on sexually dimorphic aspects of sleep patterns in adult rats.

## **Theme 2 - Tissue culture models of developing structure and function in the cerebral cortex**

In order to understand the basis for the development of spontaneous bioelectric activity within neural networks, it is necessary to take an inventory in a quantitative fashion of the various cellular elements. Immunocytochemical (ICC) staining of dissociated fetal rat cerebral cortex cultures has therefore been started in collaboration with research team III, with special emphasis upon the appearance and maturation of putative inhibitory neurons. The optimal antibody concentration for glutamic acid decarboxylase (GAD), a marker for local synthesis of the inhibitory neurotransmitter gamma-amino-butyric acid (GABA), was determined. Antigen loading by means of colchicine pretreatment proved unsuccessful, in that toxic phenomena (such as deterioration of neurites) overrode the improvement in stainability. On the basis of five developmental series, cultured for 4 to 33 days in a serum-containing nutrient medium, the following tentative conclusions were drawn. (1) Numerous GAD-positive neuronal cell bodies appear as early as the 1st week in vitro, with a characteristic eccentric cytoplasmic accumulation of immunoreactive (IR) deposits; both bipolar and multipolar IR neurons can be observed, while IR varicosities appear along some of the neurites. (2) After an additional week in vitro, IR neurites have become widely distributed, and appear to innervate cell bodies as well as neurites. (3) An extensive IR network (which often is regionally distributed) has formed by the end of the third week in culture, revealing numerous innervated cells. However, IR-containing cell bodies are seldom any longer seen at this age. GAD-ICC staining is now in progress also for

cultures grown in a chemically defined medium (vide infra). Preliminary ICC-screening for substances other than GABA has given positive results for glial fibrillary acid protein (an astrocyte marker), carbonic anhydrase (an oligodendrocyte marker), vasoactive intestinal peptide, and somatostatin.

Experiments were carried out this year in order to improve the serum-free, chemically defined medium (CDM) used for reproducibly culturing fetal rat cerebral cortex tissue in vitro. These studies involved dose-response relationships for several unsaturated fatty acids (linoleic, linolenic, arachidonic and docosahexanoic), for vitamins A and E, and for choline, biotin, L-carnitine, D(+)galactose, glutathione and ethanolamine. The results led to an improved CDM in which reaggregated fetal rat cerebral cortex cells can be kept in good condition for as long as 3-4 weeks. This survival time equals our earlier findings, as well as the reports of other investigators using serum-supplemented medium. The application of microscopic and electrophysiological techniques to the same preparation places extraordinary demands on the culturing procedures, including optimal control over substrate adhesiveness, glial proliferation and neuronal plating density. At an inoculation density of 0.5 mm<sup>3</sup> of tissue per culture dish, individual cells were not overgrown with glia as was the case with higher densities, while clusters of reaggregated cells were still able to be formed. In order to overcome the glial suppressant properties of CDM, the cultures were exposed to a prolonged start in horse-serum, followed by a gradual transition to 100% CDM. Under these conditions a confluent glial 'carpet' forms, without overgrowing the neuron, within 4-7 days in vitro. Spontaneous bioelectric activity could be recorded in 40 out of 53 cultures grown in CDM (aged 5-33 days): in the 14-24-day group almost all (i.e., 27/29) cultures were active, whereas only ca. 50% of the older and of the younger ones showed any spontaneous discharges.

The role of spontaneous bioelectric activity in synapse development in dissociated fetal rat occipital cortex tissue, cultured in CDM, was studied by reversibly blocking action potential discharges by means of tetrodotoxin (TTX). Pilot experiments revealed that  $5 \times 10^{-8}$ M TTX is the threshold concentration for acute suppression of bioelectric activity, whereas  $10^{-7}$ M is required for chronic suppression during several days. Control as well as experimental cultures were electrophysiologically sampled throughout the first three weeks in vitro. The controls were electrically active from day 14 onwards, whereas the experimental cultures all proved indeed to be functionally silent. After each recording session the culture was processed for electron microscopy, using either ethanolic phosphotungstic acid or osmium tetroxide staining. After sectioning, quantitative stereological methods were used to determine: (1) the number of presynaptic dense projections, (2) the number of synapses per volume of neural tissue, and (3) the length of the postsynaptic densities. Analysis revealed that the morphological maturation of presynaptic dense projections was retarded in the TTX-treated cultures. In addition, the synapses in the electrically silent cultures showed both a lower density (25% reduction in number per unit area) and lengthened postsynaptic densities (12% longer contact zones) after three weeks in vitro. These effects can be accounted for on the basis of either of the following two mechanisms being operative to a greater extent in bioelectrically active than in silent cultures: (i) the active zones (i.e., presynaptic dense projections plus postsynaptic density) of large synapses eventually split up into smaller ones; and/or (ii) an increased number of small-sized synapses are formed via enhanced terminal branching of neurites. We are now using serial ultrathin and semi-thin (0.5  $\mu$ m) sectioning in order to answer this question.

In order to pinpoint the exact relationships between the explosive synapse formation during the second and third week in vitro and the appearance and development of spontaneous bioelectric activity, the time course of action potential discharges has been studied in three HS-grown culture series of dissociated fetal rat cortex. The observed pattern confirms the earlier qualitative findings: isolated spikes appeared at the end of the first week; highly stereotyped burst patterns were present during the second week; and, at the end of the third week, fluctuations in the order of a few minutes were often seen in the frequency of action potential discharges. One of these series - out of which 76 single units were selected - has been investigated using a computer program for spike train analysis. This program first selects stationary samples which contain 500 intervals. The following sets of parameters are then computed: (i) parameters concerning the interval distribution, (ii) parameters which describe the temporally or serially ordered correlations within the spike train, and (iii) parameters which specify the burst characteristics. The interdependency of these parameters has been determined using factor analysis. Important independent parameters were: (1) the deviation from a Poisson process, as revealed by the Anderson-Darling test for departure from a flat spectrum of counts (i.e., time dependencies); (2) the deviation from a renewal process (dependencies in interval sequence), as revealed either by the Anderson-Darling test for departure from a flat spectrum of intervals (first-order correlations) or by the Markov value (higher-order correlations) and (3) the deviation from an exponential interval distribution, as revealed by the Anderson-Darling test. In total, 17 parameters were computed for the 76 units, which were then plotted as a function of age in vitro. Each parameter showed a large variation at all ages, so that only a few developmental trends could unequivocally be detected. Considering all parameters together in a cluster analysis, it became possible to discriminate between a period before and a period after 12 days in vitro. Further analysis of each unit, using the coefficient of variation for the mean firing levels over successive 1 min epochs, revealed an increase in this parameter after 20 days (thus confirming our qualitative impression that oscillations in the order of several minutes were present in these older preparations). A parallel can be drawn with the development of the rat cortex in vivo, where at postnatal day 12 a rapid maturation of EEG and neuronal firing patterns takes place.

### **Theme 3 - Developmental mechanisms underlying specificity in the formation of neuronal interconnections**

Further experiments have been carried out on the role played by functional activity in the development of selective synaptic connections under in vitro conditions. An attempt was made to circumvent the deleterious effects of xylocaine on serum-grown neural tissues by using a more selective bioelectric blocking agent. Organotypic explants of fetal mouse spinal cord with attached dorsal root ganglion (DRG) were therefore cultured in the presence of tetrodotoxin (TTX), both in serum-supplemented (HSM) and serum-free (CDM) media (the use of CDM-grown cultures in these experiments was prompted by previous observations showing enhanced preservation of cord cytoarchitecture, coupled with abundant evoked polysynaptic activity that increased with age in vitro). In both HSM and CDM, sensory afferents from the DRG formed fiber bundles which penetrated the cord explant. In HSM control cultures these fiber entrances were predominantly dorsal (in 20 out of 33 explants), as had

been reported in a previous study. HSM-TTX, CDM and CDM-TTX grown cultures, on the other hand, showed no such preference, the sensory afferents entering the cord from the dorsal and ventral sides in equal numbers. When recorded in control medium, less spontaneous activity was present in HSM-TTX cultures after 4 weeks in vitro than in the CDM, CDM-TTX and HSM-control cultures. There was no significant difference between CDM-control and CDM-TTX cultures with respect to the number of spontaneously active points.

Fixed latency (i.e., monosynaptic) DRG-evoked responses showed no overall numerical differences in any of the culture groups examined. The distribution of such points, however, was affected by treatment with TTX in the cultures grown in HSM, a highly significant dorsal preference was observed in the control, but not in the experimental series. This was true whether the DRG fibers mostly entered the cord explant dorsally or ventrally. Polysynaptic evoked activity showed an increase with age in the number of active points in both the CDM-TTX and the CDM-control cultures. Nevertheless, there was a significant difference (in favor of the control group) in the number of polysynaptically evoked points recorded at 4 weeks in vitro. In contrast, HSM-control and HSM-TTX cultures showed no difference in the number of evoked polysynaptic points that could be recorded. In these recent experiments no group (not even the HSM-controls, in contrast to earlier series) showed a differential distribution of polysynaptically active points at any age. We conclude that suppression of spontaneous bioelectric activity can affect both the specificity of DRG monosynaptic connections and the degree of spread of evoked responses within the spinal cord, but that the physico-chemical conditions under which the neuronal network develops plays a large part in the expression of such effects.

A previous study showed that, upon the addition of the glycosaminoglycan chondroitin sulphate (CS) to CDM, a significant restoration of the dorsal spinal cord innervation preference by the DRG afferent fibers can be achieved. A similar restoration has now been observed when the sugar galactose-1-phosphate is added to the CDM. Since both the CS and galactose compounds are highly negatively charged membrane surface molecules, it became of interest to determine whether or not it was the charged nature of the molecules that was responsible for the return of preferential dorsal innervation and fiber entry. Experiments have therefore been initiated using both glucose-1-phosphate and a variety of galactose containing sugars and gangliosides. Preliminary results indicate that the negatively charged sugar, glucose, not enhance dorsal cord preference by DRG fibers, whereas D(+) galactose, galactosamine and N-acetylgalactosamine all result in significant dorsal preferences. A mixture of purified bovine gangliosides also restores dorsal selectivity on the part of DRG afferents. Thus, it appears that specific types of molecules (presumably located within the nerve cell membrane) may be required for the expression of selective dorsal innervation tendencies by sensory nerve fibers within the spinal cord.

### III. BRAIN-ENDOCRINE INTERACTIONS DURING MATURATION AND ADAPTATION

In this project the production and secretion of peptides by nerve cells, and their actions on the brain, are being studied. Emphasis is laid on (1) vasopressin and oxytocin cells, i.e., on their sites of production, transport, release, reception and interaction with other neurotransmitter systems in the rat and human brain, (2) the possible involvement of these neuropeptides in brain development and labor, and

(3) their changes during aging. Furthermore, new methods are being developed to allow the study of specificity in immunocytochemistry and the distribution of receptor sites; special attention is paid by to the pineal gland in relation to adaptation to environmental changes. The main disciplines include immunological techniques, electrophysiology, light and electron microscopy and biochemistry, while clinical material is obtained in collaboration with various university clinics in the Netherlands and the United Kingdom. Parts of this project are represented in the FUNGO project nrs. 13-35-07, 13-35-33 and 13-51-30 (the last two with financial support).

#### **Theme 1 - The sites of production, transport, release and reception of vasopressin and oxytocin**

Various hypothalamic peptides, such as vasopressin (VP) and oxytocin (OXT) influence central processes. These peptides were known to be synthesized in the paraventricular (PVN) and supraoptic nucleus (SON), while the suprachiasmatic nucleus (SCN) produces VP only. In the past year 4 additional sites of VP synthesis have been discovered in the colchicine-pretreated rat. After such treatment VP- and neurophysin-immunoreactive cell bodies were found in the bed nucleus of the stria terminalis (BST), dorsomedial hypothalamus (DMH), medial amygdaloid nucleus (AME) and the locus coeruleus (LC)(anti-rat neurophysin was obtained from Dr. A.G. Robinson, Univ. of Pittsburgh, USA). Especially in the BST the number of parvocellular cell bodies was prominent (at least 300 VP neurons unilaterally). These cells are predominantly multipolar, though sometimes bipolar, and have a width and length of approximately 9 and 16  $\mu\text{m}$ , respectively. The cells in the DMH and AME are also parvocellular, but their number is smaller than in the BST (10-15 and 20-25  $\mu\text{m}$ , respectively). In the LC approximately 80 medium-sized (width 18-40  $\mu\text{m}$ , length 27  $\mu\text{m}$ ) multipolar cells are present. In the VP-deficient homozygous (HOM) Brattleboro rat no staining of these cells was seen. No additional OXT cell bodies were found after colchicine treatment.

The VP-immunoreactive material in the BST, LC and lateral septum was studied by means of gel isoelectric focusing of acid-ethanol extracts and VP RIA of the gel fractions. In all areas only a single peak at the pI of VP was found.

By means of immunocytochemical techniques, using purified antisera, it has been demonstrated that in addition to the 'classical' projections from the PVN and SON towards the neural lobe, many areas of the brain are densely innervated by VP and OXT fibers. Lesioning of the PVN did not result in a decreased innervation of the lateral septum, while a disappearance of practically all oxytocinergic innervation of the brain was found. This study revealed that probably only the VP fibers projecting to the medulla and the spinal cord are derived from the PVN. In search of the origin of the VP innervation of the lateral septum, tracers were injected, resulting in the finding of retrogradely labelled cells in many sites of the brain, among which the BST, but not in the PVN, SON or SCN. Unilateral lesions of the BST indeed caused a dramatic decrease in VP fibers in the lateral septum, the diagonal band of Broca, anterior amygdaloid area, lateral habenular nucleus, periventricular grey and locus coeruleus. After such lesions no changes were found in the innervation patterns of VP fibers in the hippocampus, the interpeduncular nucleus, dorsal raphe, substantia nigra and entorhinal cortex.

Immunoelectron microscopy has revealed that VP and OXT fibers terminate synaptically in different regions of the brain. So far these

peptide-containing synapses do not seem to be different, neither in their morphology nor in their mode of action, from the classical aminergic and amino acid transmitter-containing synapses. Consequently, they provide a morphological substrate for the influence of these peptides upon central processes. In the nucleus of the solitary tract a comparison has been made regarding the ultrastructural localization of VP, OXT, substance P (SP) and enkephalin (Enk). For the demonstration of VP and OXT the use of Triton X-100 in the incubation fluids was a necessity, whereas SP and Enk were readily demonstrable without it. Morphologically, no difference was found between VP and OXT terminals, whereas for Enk and SP endings the preservation of the ultrastructural details was improved without the use of Triton. In order to allow the ultrastructural localization of VP and OXT in the human brain, a series of experiments was performed in rats. Preliminary results indicate that the fixation of brain tissue should take place within a postmortem period of 8 h, since after this time lapse most of the ultrastructural details required to visualize synaptic contacts are largely lost. In spite of the paucity of human brain material, indications are at hand that also in the human nucleus tractus solitarius (NTS) VP and OXT fibers terminate synaptically.

By means of *in vitro* incubation of rat brain slices of e.g., the NTS and septum, it was demonstrated that VP and OXT could be released following a depolarizing stimulus. This calcium-dependent, potassium- or veratridine-stimulated release was only found in those regions where VP and/or OXT fibers electromicroscopically show synaptic specializations. Thus, in addition to the presence of peptidergic synapses another major criterion has been fulfilled for considering these peptides to be neurotransmitters in the CNS.

A start was made with visualizing the binding sites for VP in the rat brain and kidney, using (1) autoradiography and (2) immunocytochemistry. For the first procedure unfixed brains were frozen, and then sectioned with a cryostat. The sections were found to bind tritiated VP and are now processed for autoradiography. Using the immunocytochemical procedure, staining of binding sites was accomplished in the Brattleboro rat kidney. Binding sites were to be localized following preincubation of fixed kidney vibratome and paraffin sections with VP, mainly in connecting ducts of the kidney. Current work is directed towards improvement of the staining and controlling for the specificity of the immunocytochemical staining. The procedure, in addition, is modified in order to allow the visualization of binding sites for VP in the brain of Brattleboro and Wistar rats.

In the past years morphological evidence has been obtained that glial cells (pituicytes) in the neural lobe mediate the inhibition by of VP and OXT release by opioid peptides. In collaboration with Dr. J.A. Bidlack (Univ. of Rochester, Rochester, N.Y., USA) a study was started to localize immunoelectronmicroscopically opioid receptor proteins in the rat neural lobe. For this study poly- and monoclonal antibodies directed against the protein(s) are used. Immunoreactivity was found in vibratome semithin Epon sections of the neurointermediate lobe. At present, immunoelectronmicroscopy is performed.

The electrophysiological postsynaptic properties of vasopressin, especially in the septum and the periventricular nucleus, are being studied in collaboration with research team II. A recording system was set up, which allows the determination of unit activity, firing rate and (averaged) evoked potentials, which in addition, offers the possibility of iontophoresis of drugs. This will make experiments possible in which the effect of stimulation of VP afferents and of iontophoretic application of VP on the electrophysiological properties of target cells are measured. In search of the functional significance of the VP

innervation of the lateral septum, the effect of an injection of VP via a permanent canule into the lateral septum on water balance, was studied in water-loaded rats. Such a central injection of a minute amount of VP (100 pg) resulted in a significantly reduced urine production. Since this does not seem to be the result of an increased peripheral release of VP, studies will be undertaken to elicit the mechanism of action.

## Theme 2 - Neuropeptides in development and labor

VP and OXT were found by RIA to be present in early fetal life. Hypothalamic extracts, studied by isoelectric focusing in polyacrylamide gels containing dimethylformamide, combined with RIA, revealed PI characteristics of these peptides similar to those found in the adult brain. Extrahypothalamic areas are currently investigated.

From the 12th postnatal day onwards, a marked and persistent sex difference developed with respect to the density of the immunocytochemically stained vasopressinergic fiber network in the rat lateral septum and lateral habenular nucleus. Males castrated on the first postnatal day showed, when examined on the 26th day, a fiber density which was as low as in intact females. In male rats castrated on postnatal day 7 a fiber density developed which was intermediate to that of male and female rats, while castration on the 14th postnatal day had no effect upon the development of the male fiber density. Testosterone administration to either neonatally castrated males or female rat induced a development of the VP fiber network, indistinguishable from that seen in normal male rats. This effect of testosterone took place irrespective of the moment testosterone was given, i.e., in the first, second or third postnatal week of life. Castration of adult males caused the fiber density in the lateral septum to decrease after a period of three weeks.

The brain growth deficiency of the VP-deficient homozygous (HOM) Brattleboro rat, which in earlier experiments could not be prevented by postnatal supplementation of VP, could partially be restored by VP therapy of the pregnant mother. Daily pitressin-tannate injections or continuous administration throughout pregnancy of 8-lysine-VP or VP from a subcutaneously implanted Accurel device resulted in a larger brain weight of the HOM pups as measured at 1 month postnatally. Other parameters investigated so far, like cerebellum and medulla oblongata weights and their DNA content, known to be affected seriously in the HOM, increased towards heterozygous (HET) control levels. Optimal conditions for dosage rate and site of VP delivery and the sensitive period are currently investigated. In support of these results, which show that the impairments have a prenatal origin, is the now completed investigation of brain parameters shortly after birth. The growth retardation of body and brain of HOM pups already exists at the first postnatal day when compared to HET pups also born from a HOM mother. Cerebral cortex and medulla oblongata weights were the most severely affected regions, while the cell content of the cerebellum - but not its weight - was reduced. This indicates that for the later most seriously affected structure of the adult HOM brain, the prenatal events are crucial.

In cooperation with Dr. D. Gash (Univ. of Rochester, Rochester, N.Y., USA) studies were continued on the transplantation of VP neurons in the brains of HOM Brattleboro neonates, the rationale being to develop a technique to counteract the effects of their genetic deficiency. Donor tissue from Long-Evans fetuses containing the PVN and SON was implanted by means of a manually manipulated canule, after the tissue had been minced and concentrated. Using the bottom of the IIIrd ventricle of 5-day-old HOM neonates as implantation site, VP neurons of fetuses aged

between 13 and 17 days survived much better in the recipient brain than those of older fetuses or from newborn Long-Evans rats. Evaluation was performed 1 month after grafting, using immunocytochemistry for VP as marker. However, even with survival and fiber outgrowth of the grafted VP neurons the diabetes insipidus of the recipients appeared not be alleviated. For this reason an attempt was made to graft at a site where the VP neurons are normally located. However, unilateral implantation of fetal SON tissue at the site of the SON of the Brattleboro neonates, resulted in grafts which were hardly visible microscopically one month later, and, if present, VP neurons could not be identified immunocytochemically. Also a kainic acid treatment of the SON on the day prior to implantation (in order to lesion the existing cells) yielded no better results. Other approaches like, e.g., cell suspension implantation and electrical lesioning of the implantation site are currently planned.

The Accurel technique as a continuous peptide delivery system has proved to be very potent. Accurel polypropylene tubing (a microporous hollow fiber of small dimensions, supplied by ENKA Research Laboratories, Obernburg, W-Germany) lumen-filled with VP, LVP, OXT, alpha-MSH or enkephalin, heat-closed at both ends and enfilmed with a layer of collodion, releases the peptides in a constant, reproducible and continuous fashion upon immersion in physiological media. Implantation of VP/Accurel/collodion devices in VP-deficient Brattleboro rats showed long-term and dose-dependent alleviation of diabetes insipidus for 2 up to 10 weeks. VP excretion by the urine showed a constancy of release up to the week that diuresis started to increase again.

In vitro tests confirmed the assumption that the driving force of the constant delivery of vasopressin from the Accurel is the adsorption equilibrium of the peptide at the large internal polypropylene surface, whereas the application of collodion encapsulating is necessary to prevent the entry of surfactants that interfere with this adsorption. Diffusion of vasopressin takes place as long as the concentration within the water phase of the Accurel (its void volume) and the outside the preparation are not equalized. If immersed in a higher concentration of VP than internally determined by the adsorption equilibrium, the device starts to absorb VP. In a superfusion system, which is probably more comparable with the in vivo situation, a constant release can be obtained. The applicability of the Accurel/collodion delivery module in the fetus and as a brain probe gave promising results in pilot experiments, and will be continued.

OXT/Accurel devices, subcutaneously implanted into day-16 pregnant Wistar rats, advanced birth as expected, but appeared to protract the course of birth in the first birth intervals. Moreover, pups born from OXT-treated mothers were lower in weight and showed a reduced diuresis at 1 month of age. The reduced diuresis was also induced in pups that had been reared by postnatally OXT-treated mothers. The outcomes show that OXT therapy in the perinatal period, as for instance used for advancing birth, might have hitherto unknown permanent effects on the offspring.

Fetal OXT and VP are supposed to be involved in (a) the production of amniotic fluid, (b) the process of labour, and (c) fetal development. In order to enable the monitoring of disturbances of these processes in the rat by OXT and VP assays in amniotic fluid samples, we first had to establish what the fetal brain contributed to the levels of these peptides (in collaboration with H.P. Oosterbaan, 's Hertogenbosch). Removal of the fetal brain in fetuses of pregnant Wistar rats and data from 16 human anencephalics showed that the bulk of OXT in maternal plasma and amniotic fluid was not derived from the fetal brain. In addition, umbilical cord blood OXT was measurable within the normal range in 3 out of 5 anencephalics without the normal concomitant high VP

levels. The question whether these OXT levels are derived from the maternal brain or from alternative sources is now under investigation. Studies in heterozygous female Brattleboro rats mated with HOM males showed the absence of VP in HOM amniotic fluid, so that VP in the amniotic fluid must be derived from the fetus. The lack of VP in the homozygous fetuses did not influence the amount of amniotic fluid as compared with their heterozygous littermates. The increased production of amniotic fluid observed after removal of the fetal rat brain and the hydramnios found in most human anencephalics consequently does not seem to be caused by a lack of fetal VP. The data obtained so far show, therefore, that the fetal brain is not an important source for amniotic fluid OXT in contrast to its importance for the VP levels. Umbilical cord OXT might be derived - at least partly - from the mother, while umbilical cord VP seems to originate from the fetus.

### Theme 3 - Vasopressin and oxytocin in the aging human and rat brain

Aging is generally considered to be concomitant with degenerative changes, e.g., weight and cell loss in the central nervous system. A study of changes occurring with increasing age in the peptidergic neurons producing VP and OXT has several advantages as compared with 'conventional' neurons. Using immunocytochemistry, the VP and OXT producing cells can be identified during aging, while the synthetic activity can be measured by determining quantitatively the distribution of thiamino-pyrophosphatase (TPP-ase) an enzyme specific for the Golgi apparatus. Moreover, the consequences of aging can be followed in the peripheral circulation and urine by assaying the hormones. In the vasopressinergic neurons of the human SON and PVN, changes were observed related to aging, i.e., decreased immunocytochemical reactivity after the 8th decade of life. These cellular changes are currently being described in quantitative terms. A sex difference was found in the rostro-caudal extension of vasopressinergic neurons of the SCN, average values of females being 1.5 times those of males. No clearcut effect of age was observed on immunocytochemical reactivity or morphology. With respect to the investigation of vasopressinergic and oxytocinergic pathways in the human brain, the septum verum, the hippocampus and the amygdala have been studied so far. The density of the VP and OXT innervation of the septum showed a marked individual variation. In addition it was found that the medial septal nucleus was more densely innervated than the lateral septal nucleus, which is in contrast with the high fiber density in the rat lateral septum. No differences related to age or sex have been observed so far. The density of VP and OXT fibers in the septum and especially in the hippocampus and the amygdala, was found to be less than in the rat brain. The locus coeruleus, however, also contains dense networks of VP and OXT fibers in the human brain.

In the male Wistar rat, the activity of the hypothalamo-neurohypophyseal system was studied in animals from 3 to 32 months of age. Plasma levels of VP were increased in the 32-month-old animals. Plasma levels of OXT in 32-month-old animals did not differ from the levels found in the youngest group, but were higher than in 11-month-old animals. Hormone synthetic activity in the SON, as measured by the distribution of TPP-ase, was similar in the 3- and 32-month-old animals. In the PVN, however, an increase was found in the 32-month-old animals. Urine production decreased during aging. In other words, instead of a loss of HNS function, as has been suggested in the literature, an increased neurosecretory activity was observed in aged rats.

#### **Theme 4 - Methodological developments concerning specificity in immunocytochemistry**

Specificity of the immunocytochemical localization of a compound requires knowledge of the binding characteristics of the first antiserum. The specificity can only be investigated by (1) separating all compounds present in the tissue, and (2) determining the affinity of an antiserum for each of these compounds. With the development of the press-blotting technique, peptides can be fixed after having been separated by gel isoelectric focusing and subsequently stained by means of an immunocytochemical procedure. In this way a complete affinity spectrum of antisera for tissue peptides can be obtained. This technique complements the procedures for high molecular weight proteinaceous compounds by SDS-gel electrophoresis. Acid-ethanol neurohypophyseal extracts (MW < 10,000) showed the presence of a large number of anti-VP and anti-OXT immunoreactive bands, using the PAP procedure. After adsorption of the VP-antiserum with synthetic VP, only 3 bands remained, which were also identifiable with non-immune serum. The other bands are apparently caused by cross-reaction of the antibody and will be further characterized.

Another aspect in the procedure for defining specificity in immunocytochemistry is the quantification of the reaction towards a defined tissue antigen in a model system. Our defined antigen substrate sphere (DASS) method has now proved its predictive value for tissue staining.

#### **Theme 5 - The role of the pineal in the adaptation to environmental changes**

Most mammals including man live in environments with strong seasonal changes. The daily photoperiod and the annual cycle in environmental temperature are the most striking examples in temperate regions and so is the annual cycle in rainfall in tropical regions. Animals need to adapt themselves to these variables by e.g., hibernation, moult of skin, fur and plumage, migration etc. The essence of seasonal reproductive cycles is that animals are barred from delivery of young during seasons that would not allow an optimal survival of the offspring and thus of the species. The pineal is known to be a part of the brain that is strongly implicated in such adaptive processes, either via the production and release of peptides and/or proteins, or via indoleamines.

By combining different techniques we are trying to identify the pineal peptides and to establish how their synthesis and release are regulated (in collaboration with Dr. I. Ebels and Dr. H.P.J.M. Noteborn, Univ. of Utrecht, and Dr. A. Reinharz, Univ. of Geneva, Switzerland). At present we are trying to purify and isolate a peptide which has a terminal sequence of Pro-Arg-GlyNH<sub>2</sub>, like VP and AVT. This compound is thought to be responsible for the AVT-biological activity detected by various bioassays in the mammalian pineal gland. An in vitro culture system has been developed to study synthesis and release of pineal peptides. All identified 5-methoxyindoles (melatonin, 5-hydroxytryptamine, 5-methoxytryptophol, 5-methoxytryptophan etc.), which are synthesized in the pineal, appeared to strongly influence the production of protein/peptidergic factors by the pineal. This demonstrates that one of the target organs for melatonin and other 5-methoxyindoles is the pineal itself.

Melatonin has been - and still is - regarded as the pineal hormone 'par excellence'. Last year, however, we managed to demonstrate that a different 5-methoxyindole, i.e., 5-methoxytryptamine (5-MT) also has a

strong effect on sexual activity. Depending on the mode and time of administration, and the photoperiodic conditions, 5-MT presented an effect that could be qualified either as anti- or as pro-gonadal. In addition, the indoleamine metabolism in the retina and the Harderian gland was found to be similar to that occurring in the pineal. Moreover, in these organs - like in the pineal - their synthesis was dependent on environmental parameters.

Combining all these observations, we have suggested that all 5-methoxyindoles are implicated in a system enabling the pineal and some other parts of the brain to perceive, differentiate and integrate information from the environment. In response, the pineal would synthesize and release proteic/peptidic hormone(s) which have an effect on the function of the reproductive system.

#### Miscellaneous

A better understanding of the morphology and topography of astrocytes in the normal human brain is a prerequisite for the study of the development and differentiation of brain tumors. In collaboration with Prof. F.C. Stam and Drs. W. Kamphorst (Free Univ. of Amsterdam) a study was initiated to use antibodies raised against glial fibrillary acid protein (GFAP), a marker for astrocytes. The morphology and distribution of GFAP-containing glial cells was studied immunocytochemically using the PAP technique in brains from patients of 15 to 91 years of age. Fetal and neonatal brains are currently under investigation. A morphological continuum was found, ranging from GFAP-negative glial cells, via slightly GFAP-positive gracile cells, to strongly positive large cells with the classical appearance of fibrous astrocytes. In Bergmann cells, only the radial fibers were stained (except in those brains where occasional GFAP-positive cell bodies were found). In the subpial layers, sclerotic GFAP-positive cells were present; bipolar glial cells were seen in the taeniae of the choroid plexus, while a few positive cells, sometimes with positive processes, were found in the ependyma (tanycytes). GFAP-positive neurons and their axons were sometimes found to stain in the visual cortex, thalamus and pallidum; the basket fibers around cerebellar Purkinje cells were also stained on occasion. In the 91-year-old brain, a clearcut increase in the number of GFAP-positive glial cells was found in almost all areas, whereas in the younger age groups no age-dependent changes were observed. The thickness of the GFAP-positive subpial and subependymal glial layers did not change with age. However, the number of positive glial cells in the molecular layer of the cerebrum was distinctly larger in the older age group. In the cortices of the older brains a GFAP-positive glial corona was found around senile plaques, and only in these cortices were stained glial cells observed with the classical aspect of protoplasmatic astrocytes. In the final stage of neurofibrillar degenerations, positive glial fibers seem to grow between the remains of parallel neurofibrils.

As in previous years, the radioimmunoassays for VP, OXT, AVT and alpha-MSH have been applied to a large number of investigations by members of our group itself, as well as to collaborative studies with investigators from other laboratories.

With Dr. C. Grimmelikhuisen (Max Planck Institut, Heidelberg, GFR) assays of VP and OXT were performed on Hydra extracts. The RIA data allow to conclude that the immunocytochemistry on hydra shows reaction of compounds that different from the genuine VP and OXT.

After contacts with Dr. I.W. Henderson (Univ. of Sheffield, UK) about the occurrence of hydronephrosis in the Brattleboro rat strain, this

condition was routinely investigated in our breeding series, but only occasionally was hydronephrosis observed in 1-month-old rats.

In collaboration with Dr. C. Heyting (Anthropogenetics, Univ. of Amsterdam), Dr. W. van Raamsdonk (Dept. Zoology, Univ. of Amsterdam), and Dr. F.J.M. Raemakers (Dept. Histopathology, Univ. of Nijmegen) the work on the ICC localization at 10 nm filaments components and other cytoskeletal proteins as possible markers for changes in cell activity was continued. The optimal tissue processing and immuno-incubation conditions were determined. Much effort has been put into the determination of the specificity of the ICC reactions. With different antibodies against the 70 KD neurofilament protein, GFAP, vimentin, keratin, myosin and actin, an inventory was made of the ICC reactivities in young, adult and aged rat brains.

In collaboration with Dr. R.W.H. Verwer (research team I) a start was made with the EM immunocytochemical localization of cytoskeletal proteins.

Within the framework of obtaining neuronal cell markers in collaboration with Dr. C.H. Heyting an antibody against a carbonic anhydrase II was raised and characterized.

In collaboration with Drs. F.T. Russchen, H. Groenewegen and P. Voorn (Free Univ. Amsterdam) a study concerning the distribution of various neuropeptides in the nucleus accumbens and amygdala of the cat was performed. Immunoreactivity for a number of peptides (e.g., Substance P, somatostatin and enkephalin) was shown to be present in cell bodies and fibers.

#### IV. DEVELOPMENT AND PLASTICITY OF BEHAVIOR

Research in this project is concerned with the study of the development of social behavior (aggression and sexual responses), and of emotional learning aspects of behavior. These modes, which in adulthood exhibit clearcut sex differences, are being studied in relation to the lasting consequences of gonadal hormones and environmental factors, acting on the brain during pre- and early postnatal development.

The work continues along three lines: (1) Activating effects of gonadal hormones upon sexual behavior and aggression. In this theme behavioral specificity of male and female gonadal steroids upon the male and female's sexual and aggressive behavior is investigated. Special attention is paid to the way these hormones are involved in the motivational aspects of sexuality and to how these activated sexual and aggressive tendencies interact within and between individuals. (2) Sex differences in learning and emotionality. It has been well established now that both sexes differ in behavioral adaptation to changes in the environment. These differences are studied in learning situations and in tests in which behavioral changes due to social experiences occur. (3) An increasing amount of data now relate the sex differences in behavior to sexual dimorphism in structural and functional aspects of the brain. In our project special attention is paid to the functions of the prefrontal cortex and the preoptic-anterior hypothalamic area with respect to the sex differences in behavior.

An increasing interest in the study of behavior in other research teams at the institute has stimulated several joint projects (e.g., effects of underfeeding and enriched environment on behavioral development with research team I, and behavioral effects of REM sleep deprivation with research team II). Parts of this project are represented in the FUNGO workgroups, 'Development and Aging of the Nervous System and Behavior'

(nr. 13-51-14) and 'Brain and Behavior' (nr. 13-31-55), the BION workgroup, 'Ethology', and the Psychonomy workgroup, 'Comparative and Physiological Psychology'. Behavioral research on sexual motivation in rats is supported by ZWO-Psychonomy (nr. 15-25-09). A 3-year project was started on aggressive behavior, also subsidized by ZWO-Psychonomy (nr. 15-25-14). Together with P.F. Brain (Univ. of Swansea) a grant from the European Training Programme for Brain and Behavior was obtained to study the role of aromatization in androgenic stimulation of aggression in rats and mice.

## Theme 1 - Activating effects of gonadal steroids upon sexual and aggressive behavior

### Sexual behavior

Sexual behavior and aggression are clearly activated by gonadal steroid hormones in the adult male and female rat. Sexual dimorphism in these behaviors not only results from the long lasting changes in the organization of the neural substrate by hormonal influences during development, but also should be seen as a result of a complex interaction of hormonal factors environmental stimuli and the brain in the adult. In this theme the behavioral effects of male and female gonadal hormones upon sexual and aggressive behavior. Special attention is paid to the possible role hormones play in the integration of sexual and aggressive behavior. Such an approach made a more detailed behavioral analysis necessary with special emphasis upon the motivational aspects of behavior.

The use of partners with a high stimulus quality observational parameters which are restricted to copulatory behavior provides only limited insight into the motivational aspects of sexuality. Therefore special attention was focused upon proceptive behaviors - acts that enhance sexual activity in the partner - thus indicating the animal's urge to engage in sexual behavior. All research so far has concentrated on females. Proceptive behavior of females can be observed in the interaction with males. However, procedural and environmental factors obscure quantitative measurements, as for instance the level of the male's activity or the size of the testcage which influence the possibility for sexual initiation. Various procedures were screened to optimize the test situation. Males and females were tested in cages of different sizes or in a situation where the male was strapped into a harness, thus restricting its range, and giving the females maximum freedom of movement and choice of contact. Especially the latter procedure accentuated the females proceptive behavior when stimulated by hormones. Different dosages of progesterone given to estradiol pretreated females - a treatment which activated the females' receptivity - resulted in such high levels of proceptivity that no dose effects could be detected by scoring this behavior. However, it was found that the behavior of the male was differentially affected.

As in most mammals female rats not only show feminine sexual responses, but also show mounting behavior, which is a prominent element of the males' sexual repertory. This has been considered as a sign of increased feminine sexual motivation and proceptivity. On the other hand, mounting in the female has been interpreted as 'homosexual' behavior - possibly resulting from perinatal hormonal stimulation. A possible approach is to investigate whether an individual female prefers to mount a specific (female) partner and whether mounting is positively or negatively correlated with lordosis. Attempts to establish hormonal effects on

mounting in females were hampered by individual variability. Research is now focused on developing experimental procedures which will yield more consistent results.

Not only typical female, but also male gonadal steroids may affect female sexuality. It has been suggested that progesterone and testosterone influence proceptive behavior and sexual motivation. Sexual receptivity and proceptivity were measured in sexual interaction tests after treatment with estradiol benzoate (EB), progesterone (P), testosterone propionate (TP) and dihydrotestosterone propionate (DHTP) in various combinations. While EB alone activated both receptive and proceptive behaviors, TP elicited these behaviors only in supraphysiological doses (500 µg/rat). DHTP (a non-aromatizable androgen) inhibited the estrogen-activated sexual responses in a dosage-dependent manner. P in the dosage used (100 µg/rat) facilitated EB-, TP- and EB/DHTP-activated feminine responses, although TP/P were ineffective in activating receptivity and proceptivity. Sexual motivation in females was measured in the 'semi-open field' test, where the experimental animal has the choice of sitting near one or another partner (who may have received different hormone treatments). This test measures attractivity and sociosexual orientation. In the 'Y-maze', the female can elect to receive a sexual reward by copulation with an active male. These tests are now being used to evaluate the behavioral specificity of gonadal hormone effects in females and to compare effects with observations during a sexual interaction. When given the choice in a Y-maze, estrogen-treated females with a moderate score of receptivity did not prefer a male to a female and did not allow the males to have sexual contact. In contrast, estrogen/P-treated females preferred sexually active males and readily engaged in sexual behavior. In the semi-open field (where sexual gratification is not possible) estrogen-treatment did not elicit preference for a male as opposed to an ovariectomized female. The question whether hormonal influences on sexual preferences of females confounded by other variables such as the living conditions and sexual experience, is currently being investigated.

#### Aggressive behavior

Both sexual behavior and aggression may be activated by gonadal hormones. The interaction of these behaviors and the role male and female hormones play in this respect is important for the understanding of gonadal influences upon social behavior.

In contrast to the many studies on aggressive responses in mice, the rat has not been used very frequently for this kind of research. Wistar rats are notorious for their lack of aggressiveness under laboratory conditions. Only a restricted pattern of aggressive defensive behavior is influenced by testosterone. In contrast Tryon Maze Dull (S3) rats consistently show aggression. Three aspects of 'intermale' aggression were systematically compared in S3 males and females: the activating effects of testosterone, the relevance of the hormonal state of the opponent and the topography of the activated patterns of behavior. With a large dose TP (500 µg) increased aggression in males and females equally. TP-treatment of the opponent was an additional factor which significantly increased aggression in both sexes. Even when aggressive interactions were submitted to a more detailed statistical treatment with a discriminant analysis of the aggression parameters, no differences between males and females could be detected. TP in a lower dose (100 µg/animal) again activated equal levels of aggression in males and females. This is an important finding, because it argues against a sex difference in sensitivity to testosterone due to perinatal organizing

effects. Mounting and lordosis occurred frequently in the females during these tests. The measurement of estrogenic stimulation of aggressive responses is relevant for the understanding of androgenic stimulation of this behavior. According to the 'aromatization-hypothesis' conversion of androgens to estrogens in the brain is essential for behavioral stimulation. In males, EB was as effective as TP in activating fights. In contrast, the females showed very low levels of aggression, especially when injected with EB and tested against EB-treated females. Thus males and females showed equal aggressive responses with TP-treatment, but differed after estrogen treatment. This was also true for the aggression provoking qualities of the opponent. TP-treated females were better aggression-provoking stimuli than estrogen-treated opponents of the same sex. Males did not respond to EB-treatment with lordosis. Estrogen-treated females, on the other hand, in addition to being aggressive, also frequently showed lordosis, particularly when tested against TP-treated (female) opponents. Thus TP-treated females not only provoked aggression, but also elicited feminine sexual responses in EB-treated female animals. This led us to the question whether a possible relationship between sexual and aggressive behavior would provide an explanation for the low levels of aggression in EB-treated females as compared to the males. Correlation coefficients calculated on the basis of the individual scores of aggression and lordosis or proceptive responses, however, did not reveal any such relationship. Apparently, these behavioral systems function relatively independently.

In a further series of experiments the effects of TP and EB were compared with those of DHTP, either in combination with estrogen, or alone. Adding DHTP to EB-injections was found to inhibit feminine sexual responses and to enhance masculine sexual behavior. The methods employed in this experiment were basically the same as in previous studies. Groups of gonadectomized male and female rats were treated daily with TP (100  $\mu\text{g}/\text{animal}$ ), EB (2  $\mu\text{g}/\text{animal}$ ), EB/DHTP (1000  $\mu\text{g}/\text{animal}$ ) or DHTP for 14 days. Paired encounters between animals of the same sex were studied in confrontations with a TP-treated opponent (100  $\mu\text{g}/\text{animal}$ ). As in all earlier tests for aggression, many sexual responses were seen. Mounts most frequently occurred in the females, which probably reflects the better stimulus properties of the female opponent. Mounting occurred in all groups of females, but was remarkably high in the EB/DHTP group. Significant levels of lordosis were observed in the EB-treated females, whereas DHTP, when added to EB, completely suppressed feminine sexual responses. Low but consistent levels of lordosis occurred in the TP-treated females. Aggression in males and females was not very intensely activated by these low doses, although substantial levels of fighting were seen in all groups, the lowest in the oil-treated animals. In the males the highest levels of aggression were seen in the EB/DHTP and DHTP groups. Further experiments now in progress reveal that when in females a higher dose of EB (10  $\mu\text{g}$ ) is combined with DHTP, aggression is significantly higher than in the EB group. Feminine sexual responses then are frequent in the EB group, and virtually absent in the EB/DHTP group. The results obtained so far stress the close relationship between sexual tendencies and aggression and the behavioral specificity of male and female gonadal hormones in this respect.

## Theme 2 - Aversively motivated learning and emotionality

Analysis of the influences of gonadal hormones upon reactions to novel and aversive stimuli reveal that sex differences in open-field behavior (ambulation, rearing and defecation) can be changed by neonatal hormonal

manipulation. In contrast, the presence or absence of testosterone in adulthood hardly affected open-field behavior, but was a critical variable in the manifestation of sex differences in passive avoidance, effects of testosterone depending upon the sex of the animal. An interesting parallel was found in the sex differences in behavioral changes as a consequence of agonistic experiences. As with passive avoidance, sex differences were only present in intact or testosterone treated males, but not in females (see below).

Since being defeated in a fight is a strongly aversive experience, we decided to investigate whether males and females respond differently. A paradigm was developed for the induction and study of effects of winning or losing upon subsequent behavior in rats. When tested against S3 rats, WEzob rats always are quickly defeated, whereas when tested against Wistars the S3 rats are more aggressive and usually becomes dominant. After three days of confrontations either with an S3 or with a Wistar, WEzob losers were tested against the WEzob winners on the fourth day and retested after a period of 14 days. The behavior of losers in these encounters is characterized by a significant inhibition in aggression and social initiative along with an increase of freezing. Winners, when confronted with losers, showed high levels of aggression and more autogrooming. Their location in the test cage was clearly affected by the agonistic experiences, the winner being more often localized on the opponent's side of the cage, i.e., the side of the test cage to which the loser has been adapted, prior to testing. Finally losers showed a significant decrease in body weight after 4 days of testing. The behavioral effects were still present 14 days later. These tests were done with males.

Further experiments were aimed at investigating whether there is a sex difference in the modification of agonistic responses by experience, and if so, whether testosterone is involved in the mediation of this response. Gonadectomized male and female WEzob rats were treated either with TP or with OIL and subjected to the winner/loser paradigm. Males and females reacted in a totally different way to victory or defeat, and the presence of testosterone was a critical factor for this sex difference to become manifest. Males and females already reacted differently to TP-treatment during the induction of winning or losing. TP-treated males (but not females) which became losers showed a marked inhibition of approach during these tests. In the confrontation of WEzob winners and losers this sex difference and its dependence upon testosterone became even clearer. A permanent winner/loser effect upon aggression, in which losers showed reduced levels of aggression, was only found in the TP-treated males. This effect was still present when similar tests were conducted 14 days later. This sex- and hormone-dependent effect was expressed very clearly in the amount of time spent on the opponent's side of the cage: only TP-treated male losers showed a strong and permanent tendency to remain on their own side of the test cage, in contrast to male winners. The more drastic consequences of defeat in the TP-treated males was confirmed by marked effects upon body weight: a significant weight loss occurred in TP-treated male losers as opposed to winners. Apparently intact males and gonadectomized males treated with testosterone react similarly to winning and losing. Aggression and defeat are known to affect endogenously produced levels of gonadal androgens but the behavioral significance of this phenomenon is a matter of dispute. The present results indicate that a relatively high and constant level of testosterone does not prevent the inhibition of aggression and the occurrence of submissive behaviors in losing animals. This interpretation is in agreement with earlier results on the effects of androgens on submission in mice.

This line of research was continued on the basis of a research grant from Psychonomie (ZWO). Recently developmental brain anomalies were found in the CPB-WEzob strain of rats. There was a relatively frequent occurrence of severe somatic deficiencies, i.e., enlarged ventricles, one or both optic nerves missing. Therefore, a comparative study was undertaken on aggressive behavior of several strains to select rats with levels of aggression similar to those shown by WEzob rats. These strains were supplied by TNO. WKY-BN, WAG, SD, LEW and SHR male rats were tested against Wistar and against S3 males in order to establish whether one of these strains showed intermediate levels of aggression. The Long Evans strain was selected. Subsequent experiments partly replicated data of earlier work with the WEzob. In addition, effects upon other behavior tests were studied, i.e., open field, passive avoidance and tests for sexual behavior in an encounter with a receptive female.

### **Theme 3 - Neural substrates underlying aggressive, sexual and maternal behavior**

#### **The prefrontal cortex**

In previous experiments behavioral consequences of thermal PFC lesions were studied, which demonstrated that damage to the orbital subarea of the PFC resulted in an increase in aggressive and locomotor behavior. Subsequently, the question was considered if these behavioral functions of the orbital PFC were due to its innervation by dopaminergic fibres. Microinjections of the neurotoxin 6-hydroxydopamine (6-OHDA) into the orbital PFC resulted in a 68% depletion of DA (dopamine), but failed to affect locomotor activity. However, subtle changes in aggression were witnessed. Animals treated with 6-OHDA showed significantly less lateral threat behavior, and, whenever bouts of vehement aggression occurred, showed signs of being dominated by the control animals. Since we cannot exclude the possibility that more drastic behavioral changes might have been found, if the DA depletion had been more complete, experiments are considered to administer 6-OHDA to the cells of origin of the mesocortical dopaminergic system, since it is unlikely that a better DA depletion (without causing greater non-specific damage) can be achieved by local injections of 6-OHDA directly into the orbital PFC (collaboration with Dr. J. v.d. Gugten, Univ. of Utrecht).

The results of previous experiments suggest that the orbital PFC exerts an inhibitive modulation on other areas of the brain involved in the regulation of aggressive behavior. The lateral hypothalamus seems to be the most likely candidate. To investigate this possibility brain stimulation techniques were used. Stimulation electrodes were implanted in the lateral hypothalamus (LH, bilateral), while a moveable electrode was implanted in the orbital PFC (OF, unilateral). Whenever stimulation of one or both LH electrodes elicited aggressive behavior, the OF was stimulated simultaneously. Preliminary results indeed suggest that OF stimulation increased the threshold for LH stimulation elicited aggression. The moveable electrode used for OF stimulation has a range of 3 mm and traversed the cortical layers of the OF in steps of 300  $\mu$ m. This feature enables one to determine which cortical layers exert such an inhibitive role. Although histological evaluation of the electrode tracks is still in progress, behavioral results suggest that this inhibitive influence is limited to one or perhaps two layers of the orbitofrontal cortex. These experiments are performed in cooperation with Dr. A.M. v.d. Poel (Univ. of Leyden).

Studies on the functional development of the PFC were hampered by

problems (enlarged brain ventricles, poorly developed or absent optic nerves) in rats of the WEzob strain, the strain used so far in these studies. As a consequence of the strain-comparison mentioned in the previous section the Long-Evans Hooded rat strain was chosen for further studies on the functional development of the PFC.

#### Perinatal androgens and sexual dimorphism of the brain

The establishment of sexual dimorphism by the action of androgens on the developing brain was investigated using graded doses of testosterone (newborn female rats received 0, 2.5, 5 or 10  $\mu$ g TP). A range of somatic and behavioral observations was then performed. Androgenized females with irregular cycles showed receptive but not proceptive behavior, (i.e., soliciting behavior towards a male partner). These females mounted frequently, but this form of sexual behavior occurred predominantly in those females that also showed lordosis upon being mounted by a male. No differences could be established after treatment with EB and P. The expected sex difference in ambulation in the open-field test, females ambulating more than males, were still apparent after the relatively low doses of neonatal TP used in this experiment. The responses of these androgenized Wistar females to more aggressive S3 females were evaluated to ascertain if sexually dimorphic responses to defeat are programmed by perinatal endocrine influences. There was no difference in aggression or in submissive behavior between experimental females and controls.

A pilot study was started to investigate the behavioral concomitants of TP-treatment during the second postnatal week in females. This treatment was found to result in a marked and persistent difference with respect to the density of immunocytochemically stained vasopressinergic fibers in the rat lateral septum in experiments in research group III. Females treated with TP on days 8 and 11 showed a disturbance of the ovarian cycle, i.e., they were in constant estrus, but the age of vaginal opening was normal. Lordosis quotients were relatively low - even after substantial doses of estrogen and progesterone, but there was no difference between these females and the OIL-treated controls. Attractivity of the experimental females, however, turned out to be extremely low, as was shown by the very low levels of sexual activity of the stimulus males used for behavioral testing.

#### Maternal behavior

Maternal behavior is a category of social behavior spontaneously shown by post-parturient female rats. In many mammalian species, the father and sometimes other colony members of both sexes may also participate in the care of infants. Strange males and females, on the other hand, usually ignore or attack pups. The origin of sex differences in parental behavior and the importance of the hormonal milieu for its initiation has not been clearly established. It has been suggested that unfamiliar pups present an aversive stimulus which must be overcome before parental behavior can be elicited. Since males and females respond differently to aversive stimuli, a sexually dimorphic response to infants might be expected. Sex differences, environmental and hormonal influences on parental behavior are therefore being investigated.

The first study was carried out in collaboration with research team III. In our strain of Wistar rats, 4 out of 8 virgin females became maternal within 8 days of constant contact with pups. An attempt was made to accelerate the onset of maternal behavior by mimicking the hormonal conditions characteristic of pregnancy and parturition. We were

particularly interested in oxytocin, which is secreted in large amounts from the posterior pituitary at the time of delivery in order to induce uterine contractions and later milk let-down. Research team III has shown by immunohistochemical methods that oxytocinergic fibers innervate various brain areas. The possibility that this hormone might play a role in the 'spontaneous' induction of maternal behavior, without prior sensitization to pups, was investigated. Virgin females were ovariectomized and primed with estrogen for 10 days. A polyethylene cannula was placed in the lateral ventricle of the brain and fixed to the skull with dental cement. Injection of oxytocin (400 ng) into the cerebral ventricles was not effective in inducing maternal behavior in estrogen-primed virgins within 2 hours of exposure to pups. Although estrogen was necessary for eventual sensitization to occur, oxytocin did not influence the number of females which became maternal within 8 days of pup exposure. The interaction of hormones and experience in the elicitation of maternal behavior will be further examined.

The experiment on maternal behavior required leaving 3-10 day-old pups for 24 hours with a potential foster mother. It was observed that when the pups were ignored and their body temperature dropped to room temperature, their weight gain on being returned to the lactating mother was less than that of pups deprived of food for the same length of time but given warmth and care by a foster mother who had become maternal. This phenomenon was more systematically investigated. It was found that pups which were allowed to cool lost less weight during the period of separation but also regained less weight on being returned to their mother than underfed pups kept warm by the foster mother, so that the total growth rate was drastically curtailed. Developmental indices such as nipple growth in females, hair growth and eye opening were also delayed. After being replaced with their mother continuously after age 16 days, these pups resumed normal growth, and sexual maturation occurred at the same age as in controls (although at a lower weight). A concomitant finding was that Active Sleep (REM) was abolished during the periods of cooling, so that pups were effectively deprived of Active Sleep for at least 50% of the time. In collaboration with research team II we are investigating whether this results in later behavioral changes similar to those observed after early Active Sleep deprivation during the suckling period produced by pharmacological or mechanical means.

Together with research team II, the behavioral consequences of chronic deprivation of active sleep, by means of pharmacological agents injected during postnatal development were further investigated.

A study was undertaken on sex differences in diurnal sleep pattern in rats. Suppression of active sleep by estrogen was found in gonadectomized males but not in females. Sleep-wakefulness cycles were then recorded from intact and gonadectomized male and female Wistar rats with EEG electrodes placed on the occipital cortex and EMG electrodes in the neck muscle. During EEG recording animals were placed in a sound- and temperature-controlled box with a reversed 12-12 light dark cycle. Wakefulness, quiet and active sleep were automatically registered in minutes per hour for a 12-hour light-dark regimen. Intact males, when compared with castrated males did not show significant differences in the characteristics of sleep stages and wakefulness, although there was a tendency for castrated males (like ovariectomized females) to have more quiet sleep during the dark phase. A single estrogen injection had no effect on the subsequent sleep-wakefulness rhythms of castrated males and ovariectomized females. Thus, in males the results of preliminary experiments could not be confirmed. Sleep registrations in five intact females with regular estrus cycles showed significantly less quiet sleep in the dark and light periods on the day of (pro)estrus than during the

metestrus phase. During diestrus quiet sleep levels were comparable with those of gonadectomized females and these values were between those of estrus and metestrus. No differences in active sleep pattern were found in the different stages of the estrus cycle. On the basis of questions raised in the Department of Clinical Psychology of the 'Onze Lieve Vrouwe Gasthuis' regarding possible central effects of a beta-sympaticomimetic agent, Ritrodin hydrochloride (Prepar) a pilot study was started on influences of this compound in male and female rats. Based on longitudinal studies in women receiving this treatment to inhibit premature labor, it was established that emotional lability and anxiety are psychological side-effects. Experiments with rats were initiated to investigate influences of this drug on open-field behavior and passive avoidance learning in a first attempt to explore influences upon emotionality. The dosage used in these experiments was chosen on the basis of acute effects upon heart rate - ECG recordings being monitored in anaesthetized animals in research team II. Preliminary results indicate that open-field activity was indeed affected by treatment with this agent, even in a dosage with minor peripheral effects, although further experiments will have to elucidate whether an increase in anxiety should in fact be regarded as a cause of these behavioral changes.

Together with Dr. K.Boer (Wilhelmina Gasthuis) and Dr. Cohen (Clinical Psychology, Utrecht) a research project was started to investigate whether enhanced endogenous levels of testosterone, which may occur in women with a polycystic ovarian syndrome, can be related to changes in emotionality and aggressiveness. For this project questionnaires were selected to assess whether psychosexual and behavioral characteristics are related to elevated plasma levels of testosterone. Additionally, a questionnaire for measuring motivational aspects of sexuality was designed and the Buss-Durkee inventory for the measurement of hostility was translated. Interviewing patients will start after a selection has been made of adequate subgroups and controls. The results will be compared with data from transsexuals (female to male) receiving longterm hormonal treatment.

## V. MATHEMATICAL AND COMPUTATIONAL ASPECTS OF NEUROBIOLOGY

This Group V is concerned with (1) all automation activities, including the management of the institute's data-processing equipment and procedures and (2) theoretical/mathematical aspects of growth and adaptation of the nervous system. This year, the institute's main computer system, a VAX 11/780 from DIGITAL (in use since April 1981) has proved its reliability by reaching a high percentage up-time. The system now plays a central role in the experimental practice and text-processing activities. With the addition of 1 Mbyte of main memory this year, the system now comprises a CPU, 1.5 Mbyte main memory, 67 Mbyte disk storage, tape drive, plotting line printer, high-quality printer, 5 video terminals and a Laboratory Peripheral Accelerator sub-system with one A/D-conversion and 3 digital I/O interfaces. The second system, a Perkin-Elmer (Interdata) model 70 computer, operating independently from the VAX system, was used mainly for running programs which service real-time on-line experiments and perform file management and basic statistics. The third and oldest system, an IBM 1130 computer, has passed its last active days and now awaits dismantling.

Within the scope of activities directed towards an optimal performance of the VAX/VMS operating system, several routines were written to improve the system house-keeping. For instance, SYEOUT keeps track of all system

and device runtime errors and produces histograms for further diagnosis; TAPETST is a general routine for checking data in arbitrary formats written on tape and offers a useful facility to search locations of corrupted data on tape files.

A new standard for contiguous multi-file data structures was developed and an extensive set of file handling and management routines (FILMANGR) is now being implemented. Apart from several runtime routines for accessing data in the file, extending the file with new data etc., the FILMANGR-package offers the research worker several user-friendly routines for editing already stored data and making his data files up to date and error free. In connection with the FILMANGR package, a set of routines has been written for interactively creating data files via a terminal (TERMINPT). The new file structure consists of variable length data records and supports mixed data types.

The installation of the well-documented SPSS package (Statistical Package for the Social Sciences) has considerably improved the accessibility of programs for statistical analysis of experimental data and SPSS is now frequently used by many research workers. Furthermore, due to the SPSS facilities, the number of statistical programs still running on the Perkin-Elmer computer that has to be converted to the VAX is substantially reduced. Some programs - not provided for by the SPSS package- have been converted, for instance, analysis of variance (ANOVA) for repeated measures on more than one factor. The plot facilities of the LPAO printer/plotter are used by the PLOTXY program which also offers options for polynomial fitting the experimental data.

To improve the accessibility of the I/O interfaces of the LPA-subsystem (digital I/O, A/D and D/A), a connection has been made between these interfaces and a distribution panel mounted in a 19 inch rack that stands separately from the VAX system. Of one digital I/O interface all I/O and control lines are further extended to a user panel in this rack and accessible via BNC connectors. Circuits for over-voltage protection of input signals and short-circuit protection of output lines form part of this extension. Research workers can now easily connect their signal-producing equipment to the VAX system.

### Project-related activities

In collaboration with research team I

#### a) Digitizer programs

The set of programs acquiring and processing data from the MOP-AM02 digitizer (still connected to the Perkin-Elmer computer, has been extended with MOPSERIAL. With this program measurements on identified structures in series of parallel slides are performed and the volumes of the structures from the area measurements and the between-slide distances calculated. In this way, volumes have been determined of prefrontal cortex areas. Results can be written on magnetic tape in SPSS format for further statistical analysis.

#### b) Topological analysis of branching patterns

The program for the manipulation and evaluation of treestructures (TREEHAND) has been extended considerably. Apart from the already developed classification routines (MICTOP), it comprises simulation procedures for treecutting (dedicated and random) as well as routines to determine subtree and segment probabilities dependent on order and to calculate symmetry coefficients. This subject will be described in more detail under the entry RESEARCH.

c) Semi automatic dendrite measurement system

This year the mechanics and electronics departments have finished their work on the construction of the second microscope for dendrite measurements. For further details concerning the principles of operation of stage positioning and feedback procedures, we refer to the concerning parts of this and previous progress reports. The newly built interfaces (Electronics department) for data transfer between microscope and computer and for microscope steering, have been tested by means of modified assembler routines on the Perkin-Elmer computer. The hardware is now ready for connection to the VAX system while the corresponding data acquisition software, rewritten for LPA-usage during this year, is ready for testing.

In collaboration with research team II

Acquisition of spike trains

A program has been written for the acquisition of spike trains via the LPA-subsystem. The spike-producing equipment is directly connected to the digital input interface while the spikes, represented as unit pulses are interpreted by the LPA system as interrupts and are registered in buffers as time-stamped events from the actual digital input channel. Filled buffers are written via direct memory access (DMA) into main memory. The data are then available for further processing such as extensive time series analysis.

In collaboration with research team III

a) Radioimmunoassays

The transfer of data from the liquid scintillation counter (LSC) has been changed. Up to now the data were punched directly on papertape and printed on a teletype. After the conversion of the programs for analysis to the VAX, this medium was no longer appropriate, owing to the lack of a papertape reader on the VAX. Now the data from the LSC are stored on floppy disks of the Comm-Stor communication/data storage system which supports its own filemanagement and directories. The data in these files are transported via a serial (RS 232) communication line to the VAX for further analysis.

b) Semi automatic image processing system (with group I)

The important role of picture analysis in the institute's experimental practice (e.m.- and l.m.-photographs, autoradiographs etc.) and the extensive range of possibilities for image processing offered by microcomputer based image processing systems, has raised the need to introduce these techniques at the institute. Although several image processing systems are commercially available, the assembly of an instrument from board-level components appeared much cheaper and offers the possibility to develop a system, optimally fitted to the needs of the research workers. For instance, the system is intended to be used for cytospectrophotometric measurements on immunocytochemically (ICC) stained sections, absorption measurements of ICC stained fibres, the characterization of cortical areas in Nissl coloured slides, the quantification of grain densities in autoradiographs and stereological analysis.

The system as finally proposed and ordered consists of a video part (a 512x512 pixel (8 bit) color video monitor with light pen, a TV camera front end and video memory) and a microcomputer part (a Kontron Psi-82 microcomputer with winchester and floppy disk drive) and a high data rate communication line with the VAX.

In collaboration with research team IV

#### Operant conditioning system

Much attention has been paid to the development of a new concept of an operant conditioning system. Owing to the aged hardware that controlled the four operant conditioning cages ('Skinner Boxes') up to now, the terminated factory service possibilities and the limitation to operate not more than four cages, the Grason-Statdler system is now ready for replacement. The new system has to meet the requirements of operating up to 16 cages, controlling several schedules per cage and improving the flexibility in designing conditioning experiments. The system hardware finally proposed consists of a set of cages, designed and built by NIH's mechanics workshop, each controlled by a stand-alone operating micro-processor. Each of these slave processors is connected to a common master microprocessor in a star network. The conditioning programs will be developed on the master and are down-loaded to the slaves for running the experiments. Experimental data are stored in slave memory and are transferred to the master when the experiment is finished. Permanent data storage and extensive data analysis is planned on the VAX-system via a communication line between the master and the VAX. A market evaluation resulted in the selection of the Kontron Psi-82 microcomputer and additional single board microprocessors which were delivered around the turn of the year.

#### Research

##### Topological analysis of branching patterns

The theoretical/methodological work concerning the analysis of structural (topological) properties of branching patterns has been continued in collaboration with research team I. An important question concerned the development of a classification procedure with the property that all observed branching patterns, irrespective of their degree (number of terminal segments), could be administrated in one frequency distribution. Up to now, only frequency distributions of trees of equal degree could be constructed. A small sample of observed trees, containing trees of different degrees, then gives rise to nearly empty distributions, which are unsuitable for hypothesis testing. The goal to classify all observed trees in one distribution could be reached by the development of dichotomous classification rules. These rules imply that the subtree pairs (subtrees emerging from a bifurcation point) of all bifurcation points in all observed trees are assigned to one of the two classes of a two-class distribution. The assignment depends on the degrees of both subtrees and on criteria determined by the growth hypothesis. In this way the trees are unravelled in their constituting parts. This approach makes sense because the way in which a tree divide its terminal segments over its two subtrees is statistically highly dependent of the way the tree has been grown. The expected probabilities of the two classes must be calculated on basis of the growth model and the actual number of assignments to both classes.

Other questions which are still in elaboration concern the occurrence of multifurcations (branching points from which more than two subtrees emerge), and the effect of random cutting in trees on the frequency distributions of the corresponding ambilateral (3-D topological) types. The last question is important because many times samples of observed branching patterns contain trees which are not complete and have cut branches, e.g. caused by sectioning or incomplete staining. Mostly these trees are ignored in the analysis or only a part of the structure is

processed. However, in view of the fact that (1) many 'cut' structures are found in experimental data obtained from slides, (2) reconstruction of trees in a series of slides is very time consuming and (3) a lot of effort had to be spent in gathering the experimental data (e.g., measuring dendritic branching patterns), it is important to study the implications of cut branches for the analysis.

## MECHANICS WORKSHOP

An enumeration of the major projects constructed by this department is outlined below. Electronic equipment for these projects was developed by the institute's electronics workshop.

### For research team I

A dip-device for dipping slides in photographic emulsion has been constructed in such a way that the thickness of photographic emulsion on top of histological sections can easily be determined. In addition, two temperature controlled perspex cool-cages have been constructed, cooled by water pumped along spirals.

The new microscope system developed for measuring the arborization of neuronal dendritic and/or axonal fields has been completed this year. For a short description of its specific characteristics we refer to previous progress reports. This year the system has been tested and, after minor corrections, the mechanical construction work rounded off.

### For research team II

Extensive revisions were carried out on several 'Schwarzer' polygraph machines, involving the pen and the paper transport system as well as cleaning and repainting of the casing of the instrument (see also the report of the electronics workshop).

### For research team III

Two microtomes were outfitted with stainless steel knife-holders constructed in such a way that a variety of disposable knives can be mounted for special purpose cutting.

0.5 mm long, steel cannulae were made in order to be placed in the rat lateral septum by means of a stereotact. Thus, it was possible to inject vasopressin into this specific brain area in unanaesthetized animals. Plastic incubation boxes were constructed enabling standardization of antiserum incubations of tissue sections of various sizes.

Furthermore, a brain tissue grafting device has been developed. After uptake of small volumes of fetal brain tissue in a cannula, it allows the easy placing of that tissue in the brain of most rat neonates after a fixed manual penetration of the skull, followed by an upwards movement of the cannula whereby its mandril keeps its position. The device has been designed with an adjustable depth of penetration, volume of the graft, and upwards movement.

In addition to the routine repair and maintenance work, there was construction of such accessories as chassis and front panels for apparatus being constructed by the electronics workshop, production of storage trays of synthetic fabrics for storing small tubes, and modifications on a wide variety of equipment (microscopes, centrifuges, gamma counters, micromanipulators, microscissors, micropipettes, microtomes, microdrives and photographic equipment). For the photography and drawing department an illumination installation was constructed in order to make the photo-reproduction of large sized material possible.

## ELECTRONICS WORKSHOP

A description of some of the larger projects developed by this department is outlined below.

### For research team I

The work on the construction of a new microscope based semi-automatic dendrite measuring system has been rounded off. The final activities comprised the design of the definitive electronic circuits, the building of all printed circuit boards and the installation of the interface into the console of the system. The subsequent tests have established the proper functioning of all system electronics and mechanical hardware. In these tests the system was still connected to the Interdata computer.

### For research team II

Work on the revision of the Schwarzer polygraphs for Group II was continued, and the first machine was made fully operational on the basis of test runs in the laboratory. This project entails (1) the replacement of all vacuum tubes by solid-state components in five machines, and (2) the improvement of the basic design so as to give more stable registrations and to facilitate the simultaneous monitoring of experimental data which are stored on analog tape for subsequent analysis.

### For research team III

A start has been made with the design of a new microprocessor controlled operant-conditioning system for the study of learning in rats. The electronics of this system include a coupling between slave microprocessors (controlling the experiment) and their cages via serial to parallel conversion interfaces and the logic and drivers for the control of all functions in the cage such as lamps, retractable levers, feeder, audio generator and shock generator. The project has passed its designing phase, and printed circuit boards are now being constructed. The program, running in the slave processor, controls the conditioning experiment through a serial RS3 communication line. After the parallel conversion, each bit of the 16 bit word controls a single function of the conditioning cage.

### For research team V

An interface rack for the accommodation of all home-built interfaces between the experimental set-ups and the VAX computer system has been installed. All digital I/O, A/D and D/A interfaces controlled by the LPA subsystem are now easily accessible via this rack by means of a permanent parallel connection of all data and control lines. Of one digital I/O interface these lines (bit for bit) are further connected to a distribution panel with BNC connectors for user access. Circuits for over-voltage protection of input signals and short-circuit protection for output signals form part of this distribution panel. Additionally, to be independent of the TTL logic voltage levels, conversion circuits allow the user to present normal positive-active signals to the BNC connectors. This rack will, among other things accommodate the interfaces for the semi-automatic microscope systems. Trouble-shooting and repair of the terminal interface of the Interdata computer system was carried out.

## GENERAL TECHNICAL SERVICE

As in previous years, this department has devoted most of its time to assisting in the design and manufacture of new laboratory plant- and equipment unattainable as yet at laboratory supply stores (e.g., aggression cages) and/or in the adaptation of existing laboratory equipment to meet the researcher's requirements.

While awaiting the transfer from our temporary buildings to our future permanent quarters (which are nearing completion) the scope of this department's duties has also included the repair and maintenance of the buildings, the electricity, heating, gas and water supplies (including water drainage and sewage), and the fixed installations, such as air conditioning, refrigeration and cooling units, the auxiliary generator, the integrated alarm system, various compressors, the toxic fumes disposal unit, the isotope laboratory, and the radioactive material storage cupboard. This part of their duties has become increasingly extensive and time-consuming, as a result of the necessity for finding short-term inexpensive solutions to breakdowns, leakages, and a multitude of other exigencies arising from failing foundations. In addition to the administration of alcohol supplies, this department supervizes the gas cylinders required by various research teams at the institute laboratories.

## GUEST WORKERS AND WORK-VISITS ABROAD

Baker, R.E. at Dr. L. Urban, Dept. of Veterian Physiology, Iowa State Univ., Ames, Iowa (USA), December 23-24

Boer, G.J. at Dr. D.M. Gash, Dept. of Anatomy, Medical Center, Univ. of Rochester, NY (USA), February 14-26.

Buijs, R.M. at Prof. M. Le Moal, Dept. of Neurobiol. de Compart., Univ. of Bordeaux II, December 6-8.

De Vries, G.J. at Dr. R.G. Hill, Dept. of Pharmacology, Medical School, Univ. of Bristol (UK), Januari 18 - February 10.

Gash, D.M. (guest worker from the Univ. of Rochester at Rochester, USA) for the continuation of cooperative work on the transplantation of fetal vasopressin neurons in the brain of Brattleboro neonates, September 19-27 (research team III).

Haldar-Misra, C. (guest worker from the Varanase Univ., India) for the ultrastructural study of the pineal gland of mammals in organ culture, September 1980 - October 1982 (research team III).

Ravid, R. (guest worker from the Hebrew Univ., Jerusalem, Israel) for immunocytochemical localization of vasopressin receptors, November 1, 1982 - December 31, 1983 (research team III).

Renier, J. (guest worker from the Limburgs Universitair Centrum, Diepenbeek, Belgium) to learn the technique of serum-free culturing of nerve tissue, September 9-10 (research team II).

Oosterbaan, H.P. (gynecologist in training, Univ. of Utrecht) for research on oxytocin and vasopressin in relation to development and labor in rat and human (research team III).

Pévet, P. at the Institute de Biochimie Cellulaire et Neurochimie, Bordeaux (France), November 15-18; at the Institut de Neurophysiologie et Psychophysiologie, Marseille (France), November 22-24; at the Institut de Biologie Animale, Talence (France), December 6-8; and at the Institute of Evolution, Univ. of Haifa (Israel), October 1-7.

Swaab, D.F. at Dr. H. Belmaker, the Sarah Herzog Psychiatric Hospital, Jerusalem (Israel), January 30; at Prof. M.A. Klingberg, Univ. of Tel-Aviv, Tel-Aviv (Israel), January 31-February 1; at Prof. W. Meier-Ruge et al., Sandoz AG medizinische Grundlagenforschung, Basel (Switzerland), March 22; at Prof. Ph. Richard et al., Inst. de Physiol., Strasbourg (France), May 4; at the Weizmann Institute of Science, Rehovot (Israel), June 23.

Swanson, H.H. at Prof. B. Mess, Dept. of Anatomy, Univ. Medical School, Pecs (Hungary), March 26-28; at the Ethical Committee of the Internat. Soc. for Research on Aggression, Mexico City (Mexico), August 13-16; and at Dr. A. Cuaron, Dept. of Endocrinology, National Soc. Services Hospital, Mexico City (Mexico), August 18-20.

Vallet, P (guest worker from the Univ. of Geneve, Dept. of Physiol.) to learn retrograde tracing in combination with immunocytochemistry, January-December (research team III).

#### PUBLICATIONS

Baker, R.E., A.M.M.C. Habets, E. Brenner and M.A. Corner - Influence of growth medium, age in vitro and spontaneous bioelectric activity on the distribution of sensory ganglion evoked activity in spinal cord explants. *Develop. Brain Res.* 5, 329-341.

Baker, R.E., K. Matesz and L. Urban - Peripheral reinnervation patterns and dorsal root ganglion cell topography in skin grafted *Rana pipiens* frogs. *Brain Res. Bull.* 7, 635-638.

Bakhuis, W.L. and H.L. Bour - Organization of sleep and wakefulness in the perinatal chick (*Gallus domesticus*). In: *Brain and Behavior in the Fowl*. T. Ookawa (Ed.), Japan Sci. Soc. Press, Tokyo, in press.

Balemans, M.G.M., P. Pévet, S. van Benthem, C. Haldar-Misra, I. Smith and M. Hendriks - Day/night rhythmicity in the methylating capacities for different 5-hydroxyindoles in the pineal, the retina and the Harderian gland of the golden hamster (*Mesocricetus auratus*) during the annual seasons. *J. Neural Transm.* 56, 53-72 (1983).

Boer, G.J., K. Boer and D.F. Swaab - On the reproductive and developmental differences within the Brattleboro strain. *Ann. N.Y. Acad. Sci.* 394, 37-47.

Boer, G.J., and Patel, A.J. - Disorders of cell acquisition in the brain of rats deficient in vasopressin (Brattleboro mutant). *Neurochem. Int.*, in press

Boer, G.J. and D.F. Swaab - Longterm effects on brain and behavior of early treatments with neuropeptides. In: *Application of Behavioral*

Pharmacology in Toxicology. G. Zbinden, V. Cuomo, G. Racagni and B. Weiss (Eds.), Raven Press, New York, pp.251-263 (1983).

Boer, G.J., H.B.M. Uylings, A.J. Patel, K. Boer and R. Kragten - The regional impairment of brain development in the Brattleboro diabetes insipidus rat; some vasopressin supplementation studies. Ann. N.Y. Acad. Sci.394, 703-719.

Boer, G.J., C.M.F. Van Rheenen-Verberg and H.B.M. Uylings - Impaired brain development of the diabetes insipidus Brattleboro rat. Develop. Brain Res.3, 557-575.

Boer, K. and D.F. Swaab - Oxytocine en de baring. In: Voortgang en Visie. 25 jaar Verloskunde en Gynaecologie. Gedenkboek Prof.Dr. G.J. Kloosterman. P.E. Treffers et al. (Eds.), Bohn, Scheltema en Holkema, Utrecht, pp.216-226 (1983).

Boorsma, D.M., A.C. Cuello and F.W. van Leeuwen - Direct immunocytochemistry with a horseradish peroxidase conjugated monoclonal antibody against substance P. J. Histochem. Cytochem.30, 1211-1216

Bowden, N., N.E. van de Poll, H.G. van Oyen, P.F. Brain and H.H. Swanson - Gonadal steroids and aggressive behaviour in male and female rats. Aggres. Behav.8, pp.182-184.

Buijs, R.M. - The ultrastructural localization of amines, amino acids and peptides in the brain. In: Chemical Transmission in the Brain. R.M. Buijs et al. (Eds.), Progress in Brain Research, Vol.55, Elsevier Biomedical Press, Amsterdam, pp.167-183.

Buijs, R.M. - Tissue treatment in immunocytochemistry. In: Immunocytochemistry and its Application in Brain Research. F.W. Van Leeuwen et al. (Eds.), Handbook 2nd EMBO Practical Course, Amsterdam, pp.49-55.

Buijs, R.M. - Vasopressinergic and oxytocinergic pathways, synapses and central release. In: Neuroendocrinology of Vasopressin, Corticotiberin and Opiomelanocortins. A.J. Baertschi and J.J. Dreifuss (Eds.), Academic Press, London, pp.51-60.

Buijs, R.M. - Vasopressin and oxytocin, chemical messengers in the brain. In: Neurotransmitter Interaction and Compartmentation. H.F. Bradford (Ed.), Plenum Press, London, 675-685.

Buijs, R.M., P. Pévet and D.F. Swaab - Chemical transmission in the brain (Eds.), Progress in Brain Research, Vol.55, Elsevier Biomedical Press, Amsterdam.

Buijs, R.M. and J.J. Van Heerikhuizen - Vasopressin and oxytocin release in the brain; a synaptic event. Brain Res.252, 71-76.

Buijs, R.M., De Vries, G.J., Van Leeuwen, F.W., and Swaab, D.F., Vasopressin and oxytocin: Distribution and putative functions in the brain. In: The Neurohypophyseal Structure, Function and Control. Progress in Brain Research, Vol. 60, B.A. Cross, G. Leng (Eds.), in press.

- Corner, M.A. and H.J. Romijn - Spontaneous, evoked and epileptiform electrical activity and their cyto-morphological basis in the cerebral hemispheres of the chick embryo. In: Brain and Behavior in the Fowl. T. Ookawa (Ed.), Japan. Sci. Soc. Press, Tokyo, pp.297-305 (1983).
- De Bruin, J.P.C. - Neural correlates of motivated behaviour in fish. In: Advances in Vertebrate Neuroethology. J.-P. Ewert et al. (Eds.), Plenum Press, London, in press.
- De Bruin, J.P.C. - Prefrontal cortex lesions and social behaviour. In: Functional Recovery from Brain Damage. M.W. van Hof and G.Mohn (Eds.), Elsevier Biomedical Press, Amsterdam, pp.239-258.
- De Bruin, J.P.C. - Samenhang tussen hersen- en gedragsontwikkeling. Vakblad voor Biologen 62, 166-168.
- De Jonge, F.H. and B.J. Meyerson - Attractivity of male and female rats after early endocrine manipulation. Horm. Behav.16, 1-12.
- De Vries, G.J. and W. Best - Gonadal steroids and the development of a sex difference in the vasopressinergic innervation of the brain. In: Integrative Neurohumoral Mechanisms. Physiological and Clinical Aspects. Endroczi (Ed.), Elsevier Biomedical Press, Amsterdam, in press.
- De Vries, G.J., W. Best and A.D. Sluiter - The influence of androgens on the development of a sex difference in the vasopressinergic innervation of the rat lateral septum. Develop. Brain Res., in press.
- De Vries, G.J. and R.M. Buijs - The origin of the vasopressinergic and oxytocinergic innervation of the rat brain; with special reference to the lateral septum. Brain Res., in press.
- Fliers, E. - Aging brain and ergot alkaloids. Sandorama 1, 34-36.
- Gash, D.M., G.J. Boer, M.F.D. Notter and J.R. Sladek Jr. - Transplanted vasopressin neurons and central nervous system effects of vasopressin. In: The Neurohypophysis. B.A. Cross and G. Leng (Eds.), Progress in Brain Research, Elsevier Biomedical Press, Amsterdam, in press.
- Goodfellow, C.F., M.G.R., Hull, D.F. Swaab, J. Dogterom and R.M. Buijs - Oxytocin deficiency at delivery with epidural analgesia. Brit. J. Obstet. Gynaecol., in press.
- Grimmelikhuijzen, C.J.K., K. Dierickx and G.J. Boer - An ancestral oxytocin/vasopressin-like peptide is present in the nervous system of hydra. Neurosci.7, 3191-3199.
- Haldar-Misra, C. and P. Pévet - The influence of noradrenaline on the process of protein/peptide secretion in the mammalian pineal organ: comparative in vitro studies. Cell Tiss. Res.224, 33-44.
- Haldar-Misra, C. and P. Pévet - The influence of different 5-methoxyindoles on the process of protein/peptide secretion characterized by the formation of granular vesicles in the mouse pineal gland: An in vitro study. Cell Tiss. Res. 230, 113-126 (1983).
- Haldar-Misra, C. and P. Pévet - The influence of melatonin on the process of protein/peptide secretion in the pineal gland of the rat and hamster: an in vitro study. Cell Tiss. Res., in press.

Hofman, M.A. - A two-component theory of encephalization in mammals. *J. theor. Biol.*99, 571-584.

Hofman, M.A. - Encephalization in mammals in relation to the size of the cerebral cortex. *Brain Behav. Evol.*20, 84-96.

Holder, F.C., M.O. Schroeder, M. Pollatz, J.M. Guérne, B. Vivien-Roels, P. Pévet, R.M. Buijs, and J. Dogterom - A specific and sensitive bioassay for arginine-vasotocin: description, validation and some applications in lower and higher vertebrates. *Gener. comp. Endocr.*47, 483-491.

Hoorneman, E.M.D. and R.M. Buijs - Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning. *Brain Res.*243, 235-241.

Kamstra, A.W. and H.B.M. Uylings - Teflon-disk for processing loose, serial celloidin sections. *Stain Technol.*, in press.

Karasek, M. and P. Pévet - Ultrastructural aspect of the secretory processes in mammalian pineal gland (in Polish). *Potspy Biologic Komorki (Post Biol. Kom)*, in press.

Koch, A.L., R.W.H. Verwer and N. Nanninga - Incorporation of diaminopimelic acid into the old poles of *Escherichia coli*. *J. Gen. Microbiol.*, in press.

Kovacs, G.L., R.M. Buijs, B. Bohus and Tj.B. Van Wimersma Greidanus - Microinjection of arginine(8)-vasopressin antiserum into the dorsal hippocampus attenuates passive avoidance behavior in rats. *Physiol. Behav.*28, 45-48.

Mirmiran, M. - Experimental studies on the significance of active (c.q. REM) sleep for maturation of brain and behavior in the rat. Doctoral Dissertation, Univ. of Amsterdam, Dept. of Medicine, 118 pp.

Mirmiran, M. - "Oneiric" behavior during active sleep induced by bilateral lesions of the pontine tegmentum in juvenile rats. In: *Sleep-1982*. W.P. Koella (Ed.), Karger, Basel, pp.236-239.

Mirmiran, M. and M.A. Corner - Neuronal discharge patterns in the occipital cortex of developing rats during active and quiet sleep. *Develop. Brain Res.*3, 37-48.

Mirmiran, M. and H.B.M. Uylings - The environmental enrichment effect upon cortical growth is neutralized by concomitant pharmacological suppression active sleep in female rats. *Brain Res.*261, 331-334.

Mirmiran, M., J. Scholtens, N.E. Van de Poll, H.G. Van Oyen and M. Corner - Developmental effect of chronic REM sleep deprivation in the postnatal rat. In: *Sleep-1982*. W.P. Koella (Ed.), Karger, Basel, pp.386-389.

Mirmiran, M., J. Scholtens, N.E. Van de Poll, H.B.M. Uylings, J. Van der Gugten and G.J. Boer - Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. *Develop. Brain Res.*7, 277-286, (1983).

Mirmiran, M., H.B.M. Uylings and M.A. Corner - Chronic REM-sleep deprivation prior to weaning in male rats counteracts the effectiveness

of subsequent environmental enrichment on cortical growth. *Develop. Brain Res.*7, 102-105 (1983).

Mirmiran, M., H. Van den Dungen and H.B.M. Uylings - Sleep patterns during rearing under different environmental conditions in juvenile rats. *Brain Res.*233, 387-398.

Noteborn, H.P.J.M., I. Ebels, P. Pévet, A.C. Reinharz, C. Neacsu and C.A. Salemink - Comparison of some peptidergic and proteic ovine pineal fractions with a bovine B5 pineal fraction. *J. Neural Transm.*55, 27-44.

Pévet, P. - Anatomy of the mammalian pineal gland. In: *The Pineal Gland*. R. Reikn (Ed.), Elsevier Biomedical Press, New York, pp.1-75 (1983).

Pévet, P. - Is 5-methoxytryptamine a pineal hormone? *Psychoneuroendocrinology*, in press.

Pévet, P. - Peptides in the pineal gland of vertebrates. Ultrastructural, histochemical, immunocytochemical and radioimmunological aspects. In: *The Pineal Organ: Photobiology-Biochronometry-Endocrinology*. A. Oksche and P. Pévet (Eds.), Elsevier Biomedical Press, Amsterdam, pp.211-236.

Pévet, P. - Pineal peptides in the fetus and in young and adult mammals. In: *Proc. of the Symp. on the Developmental Neurobiology of the Melatonin Rhythm generating System*. D.C. Klein (Ed.) S. Karger A.G., Basel, pp.157-181.

Pévet, P. - The different classes of proteic and peptidergic substances present in the pineal gland. In: *The Pineal Gland and its endocrine Role*. J. Axelrod et al. (Eds.), Plenum Press, London, in press.

Pévet, P. - The 5-methoxyindoles different from melatonin: their effects on the sexual axis. In: *The Pineal Gland and its Endocrine Role*. J. Axelrod et al. (Eds.), Plenum Press, London, in press.

Pévet, P. and C. Haldar-Misra - Effect of orally administered melatonin in reproductive function in the golden hamster. *Experientia* 38, 1493-1494.

Pévet, P. and C. Haldar-Misra - Effect of 5-methoxytryptamine on testicular atrophy induced by experimental or natural short photoperiod in the golden hamster (*Mesocricetus auratus*). *J. Neural Transm.*55, 64-84.

Pévet, P. and C. Haldar-Misra - Morning injections of large doses of melatonin, but not of 5-methoxytryptamine administered late in the afternoon. *J. Neural Transm.*55, 85-93.

Pévet, P. and C. Haldar-Misra - Daily 5-methoxytryptamine injections inhibit short-day-induced testicular atrophy in golden hamsters. *J. Neural Transm.*55, 95-99.

Pool, Chr.W., R.M. Buijs, D.F. Swaab, G.J. Boer and F.W. Van Leeuwen - On the way to a specific immunocytochemical localization. In: *Neuroimmunocytochemistry*. C. Cuellar et al. (Eds.), IBRO Handbook Series 'Methods in Neurosciences', John Wiley and Sons, Ltd., New York/London, in press.

Pool, Chr.W., R.M. Buijs, D.F. Swaab, G.J. Boer and F.W. Van Leeuwen - Specificity in immunocytochemistry. In: Immunocytochemistry and its Application in Brain Research. F.W. Van Leeuwen et al. (Eds.), Handbook 2nd EMBO Practical Course, Amsterdam, pp.93-128.

Pool, Chr.W., Diegenbach, P.C. and Sluiter, A.A. - Antibody quantification in immunocytochemistry. In: Immunocytochemistry and its Application in Brain Research. F.W. Van Leeuwen et al. (Eds.), Handbook 2nd EMBO Practical Course, Amsterdam, pp.145-157.

Romijn, H.J. - Columnar organization, local circuitry and synaptology of the mammalian cerebral cortex. Acta Morphol. Neerl.-Scand.20, 237-249.

Romijn, H.J., A.M.M.C. Habets, M.T. Mud and P.S. Wolters - Nerve outgrowth, synaptogenesis and bioelectric activity in fetal rat cerebral cortex tissue cultured in serum-free, chemically defined medium. Develop. Brain Res.2, 583-589.

Romijn, H.J., F. van Huizen, P.S. Wolters and A.M.M.C. Habets - Further attempts to obtain a serum-free medium for long-term cerebral cortex cultures. Biology of the Cell 45, 34.

Salt, T.E., G.J. De Vries, R.E. Rodriguez, P.M.B. Cahusac, R. Morris and R.G. Hill - Evaluation of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-substance P as an antagonist of substance P responses in the rat central nervous system. Neurosci. Lett.30, 291-295.

Sedlacek, J. and M.A. Corner - Monoaminergic regulation of spontaneous motility in the chick embryo. In: Behavior in the Fowl. T. Ookawa (Ed.), Japan Sci. Soc. Press, Tokyo, pp.61-70 (1983).

Slopesma, J.S., J. Van der Gugten and J.P.C. De Bruin - Regional concentrations of noradrenaline and dopamine in the frontal cortex of the rat: dopaminergic innervation of the prefrontal subareas and lateralization of prefrontal dopamine. Brain Res.250, 197-200.

Swaab, D.F. - Comments on the validity of immunocytochemical method. In: Cytochemical Methods in Neuroanatomy. V. Chan-Palay and S.L. Palay (Eds.), Alan R. Liss, Inc., New York, pp.423-440.

Swaab, D.F. - Hersenontwikkeling van de foetus in gevaar. Proc. Symp. 'Het zich ontwikkelende kind in gevaar', Hoorn, in press.

Swaab, D.F. - Neuropeptides: Their distribution and function in brain. In: Chemical Transmission in the Brain. R.M. Buijs et al. (Eds.). Progress in Brain Research, Vol.55, Elsevier Biomedical Press, Amsterdam, pp.97-122.

Swaab, D.F. and G.J. Boer - Neuropeptides and brain development. Current perils and future potentialities. Develop. Physiol., in press.

Swaab, D.F. and M. Mirmiran - Possible mechanisms underlying the teratogenic effects of medicines on the developing brain. In: Proc. 13th CINP Congress, J. Yanai (Ed.), in press.

Swaab, D.F. and M. Mirmiran - The influence of chemicals and environment on brain development: 'Behavioral Teratology'. Proc. World Congress UNAPEI on 'Prevention of Physical and Mental Congenital Defects', M. Marois (Ed.), in press.

- Swaab, D.F., G.J. Boer, K. Boer, H.P. Oosterbaan and P.R. Oosting - Neurohypophysial and intermediate lobe peptides in intrauterine growth and labour. In: Proc. IBRO Symp. on 'Vasopressin, Corticoliberin and ACTH-related Peptides', A.J. Baertschi and J.J. Dreifuss (Eds.), pp.343-352.
- Swaab, D.F., M.A. Corner and W. Chen-Pelt - Progress Report 1981, of the NIBR, Proceedings of the Netherlands Academy of Arts and Science 79(2).
- Swanson, H.H. - Parental behaviour and population control. In: Parental behaviour in Rodents. R.W. Elwood (Ed.), John Wiley, Chichester, 1983, pp.259-291 (1983).
- Swanson, H.H. - The use of animals in behavioural research. Council of Europe 'Forum' 3, VIII-XI.
- Swanson, H.H., N.E. van de Poll and J. van Pelt - Influence of the estrous cycle on heterosexual aggression in two strains of rats (S3 and WEzob). *Horm. Behav.* 16, 395-403.
- Swanson, H.H., P. McConnell, H.B.M. Uylings, H.G. Van Oyen and N.E. Van de Poll - Interaction between pre-weaning undernutrition and post-weaning enrichment on behavior and brain development in male and female rats. *Behav. Process.* 8, 1-20 (1983).
- Swanson, H.H., N.E. Van de Poll and J. Scholtens - Correlation of effects of neonatal testosterone on vaginal cyclicity, sexual, aggressive and other sexually dimorphic behaviour patterns in rats. Proc. 'Integrative Neurohumoral Mechanisms', in press.
- Swanson, H.H., N.E. Van de Poll and J. Van Pelt - Heterosexual aggression during the oestrus cycle in two strains of rats (S3 and WEzob). *Aggressive Behaviour* 8, pp.185-187.
- Swanson, H.H., N.E. Van de Poll and J. Van Pelt - Influence of the oestrus cycle on heterosexual aggression in two strains of rats (S3 and WEzob). *Horm. Behav.* 16, 395-403 (1983).
- Swanson, H.H. and N.E. van de Poll - Effects of isolation and enrichment following early handling on sexual maturation and behavior in male and female rats. *J. Reprod. Fert.*, in press.
- Szekely, G., Matesz, K., R.E. Baker and M. Antal - The termination of cutaneous nerves in the dorsal horn of the spinal cord in normal and skin-rotated frogs. *Exp. Brain Res.* 45, 19-28.
- Tielen, A.M., F.W. Van Leeuwen and F.H. Lopes da Silva - The localization of leucine-enkephalin immunoreactivity within the guinea pig hippocampus. *Exp. Brain Res.* 48, 288-295.
- Uylings, H.B.M. and H.K.P. Feirabend - Klassieke kleurmethode voor licht-microscopisch onderzoek van het zenuwstelsel. In: *Technics Manual of Netherlands Neuroanatomists Verhaart Meetings*, in press.
- Uylings, H.B.M. and A.H.M. Lohman - De ontwikkeling van het cerebrum. *Vakbl. Biol.* 63 (No.4), 64-68 (1983).

- Uylings, H.B.M., R.W.H. Verwer, J. Van Pelt and J.G. Parnavelas - Topological analysis of dendritic growth at various stages of development. *J. Microsc. (Oxford)*, in press.
- Van den Dungen, H.M., R.M. Buijs, Chr.W. Pool and M. Terlou - The distribution of vasotocin and oxytocin in the brain of the rainbow trout. *J. Comp. Neurol.* 212, 146-157.
- Van de Poll, N.E., N.J. Bowden, H.G. Van Oyen, F.H. De Jonge and H.H. Swanson - Gonadal hormonal influences upon aggressive behavior in male and female rats. In: *Psychopharmacology of Sexual Disorders*. M. Segal (Ed.), John Libbey and Company Ltd., London, in press.
- Van de Poll, N.E., F.H. De Jonge, H.G. Van Oyen and J. Van Pelt - Aggressive behaviour in rats.: effects of winning or losing on subsequent aggressive interaction. *Behav. Process.* 7, 143-155.
- Van de Poll, N.E., J. Smeets, H.G. Van Oyen and S.M. van der Zwan - Behavioral consequences of agonistic experience in rats: sex differences and the effects of testosterone. *J. Comp. Physiol. Psych.* 96 (No.6), 893-903.
- Van de Poll, N.E., S.M. Van der Zwan and H.G. Van Oyen - Sexual behavior in female rats born in all-female litters. *Behav. Brain Res.* 4, 103-109.
- Van de Poll, N.E., H.G. Van Oyen, J. Smeets and F.H. De Jonge - Effects of agonistic experiences in rats. *Aggress. Behav.* 8, pp.149-151.
- Van Leeuwen, F.W. and G.J. de Vries - Enkephalin-glial interaction and its consequences for vasopressin and oxytocin release from the neural lobe. In: *the Neurohypophysis*. B.A. Cross and G. Leng (Eds.), *Progress in Brain Research*, Vol.60, Elsevier Biomedical Press, Amsterdam, in press.
- Van Leeuwen, F.W. - Enkephalin immunoreactivity in fibers terminating in a synaptoid fashion in pituicytes in the rat neural lobe. In: *Regulatory Peptides, from Molecular Biology to Function*. E. Costa and M. Trabucchi (Eds.), Raven Press, *Advances in Biochemical Pharmacology* 33, 203-208.
- Van Leeuwen, F.W. - Enkephalin in the rat neural lobe. Immunocytochemical evidence for its presence within synaptic elements on pituicytes. In: *Chemical Transmission in the Brain*. R.M. Buijs et al. (Eds.), *Progress in Brain Research*, Vol.55, Elsevier Biomedical Press, Amsterdam, pp.253-264.
- Van Leeuwen, F.W. - Immunoelectron microscopy in neuroscience. In: *Immunocytochemistry and its Application in Brain Research*. F.W. van Leeuwen et al. (Eds.), *Handbook 2nd EMBO Practical Course*, pp.167-174.
- Van Leeuwen, F.W. - Specific immunocytochemical localization of neuropeptides: a utopian goal? In: *Techniques in Immunocytochemistry* Vol.1. G.R. Bullock and P. Petrusz (Eds.), Academic Press, New York, pp.283-299.
- Van Leeuwen, F.W. and A.R. Caffé - Immunoreactive vasopressin cell bodies in the rat bed nucleus of the stria terminalis. *Cell Tiss. Res.* 228, 525-534 (1983).

Van Leeuwen, F.W., Chr.W. Pool and A.A. Sluiter - Enkephalin immunoreactivity in presynaptic elements on glial cells in the rat neural lobe. *Neurosci.8*, 229-241 (1983).

Van Leeuwen, F.W., D.F. Swaab, R.M. Buijs and J. Sels - Immunocytochemistry and its application in brain research. *Handbook 2nd EMBO Practical Course (Eds.)*, pp.1-386.

Van Oyen, H.G., S.M. Van der Zwan, N.E. Van de Poll and H.G. Walg - Punishment of food rewarded lever holding in male and female rats. *Physiol. Behav.*26, 1037-1040.

Van Pelt, J. and R.W.H. Verwer - New classification methods of branching patterns. *Acta Stereol.*, in press.

Van Pelt, J. and R.W.H. Verwer - The exact probabilities of branching patterns under terminal and segmental growth hypotheses. *Bull. Math. Biol.*, in press.

Vaughan, M.K., B.A. Richardson, L.Y. Johnson, R.J. Reiter, P. Pévet and C. Neacșu - Effects of bovine pineal peptidic fraction (E5) on plasma pituitary levels of Lh, FSH and prolactin. *Experientia* 38, 871-872.

Verwer, R.W.H. and D.M.G. De Groot - The effect of shape assumptions on the estimation of the numerical density of synapses from thin sections. In: *Chemical Transmission in the Brain*. R.M. Buijs et al. (Eds.), *Progress in Brain Research*, Vol.55, Elsevier Biomedical Press, Amsterdam, pp.195-203

#### ABSTRACTS (incl. posters)

Baker, R.E. - The development of connections between fetal mouse dorsal root ganglia and spinal cord explants grown in the presence of chondroitin sulfate. *Proc. EMBO Workshop, 'Synaptic Connectivity in Development and Regeneration'*, Eilat (Israel).

Baker, R.E. - The development of sensory afferent connections in organotypic spinal cord explants grown in serum-free medium. *EMBO Workshop, 'Molecular Mechanisms of Brain Development'*, Strasbourg (France).

Balemans M.G.M., P. Pévet, J. van Benthem and C. Haldar-Misra - Seasonal rhythmicity in the capacity of hydroxyindole-O-methyltransferase for the synthesis of different 5-methoxyindoles in the pineal, the retina and the Harderian gland of the hamster. *J. Endocrinol.*94, 37P.

Best, W. and G.J. de Vries - Influence of testosterone on the development of a sex difference in the vasopressinergic innervation of the rat brain. *Proc. 23rd Dutch Feder. Meeting, Amsterdam*, 24.

Boer, G.J. and J. Kruisbrink - Long-term in vitro release of vasopressin and oxytocin with a newly developed Accurel technique. *Proc. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK)*, C12.

Buijs, R.M. - Vasopressinergic and oxytocinergic pathways, their synaptic terminals and release. Proc. IBRO Satellite Symp. on Vasopressin, Corticoliberin and ACTH-related peptides, Geneva (Switzerland), A4.

Buijs, R.M. - The distribution and release of neurohypophyseal peptides in the brain. Proc. 3rd Annual Meeting contact group Psychoneuroendocrinology, Liege (Belgium), p.2.

Buijs, R.M. - The distribution and release of vasopressin and oxytocin in the CNS. Proc. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK), p.6.

Corner, M.A., R.E. Baker, A. Habets and E. Brenner - Development of sensory ganglion evoked responses in spinal cord explants cultured in media of different chemical composition. Neurosci. Lett., Suppl.10, S122.

De Jonge, F.H. and N.E. van de Poll - Sexual motivation of the female rat. Low Countries Meeting (Psychonomy), DÜsseldorf (Germany).

De Jonge, F.H. and N.E. van de Poll - Sexual motivation and proceptive behavior in the rat. 4th ESCAB Conf., Bielefeld (Germany).

De Vries, G.J. and W. Best - Influence of gonadal steroids on a sex difference in the vasopressinergic innervation of the lateral septum of the rat. Neuroscience, Suppl.7, S54.

De Vries, G.J. and W. Best - Influence of gonadal steroids on the development of a sex difference in the vasopressinergic innervation of the brain. Proc. Integrative Neurohumoral Mechanisms Congress, Budapest (Hungary).

De Vries, G.J. and Buijs, R.M. - In search of the origin of the vasopressinergic innervation of the lateral septum. Proc. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK), A17.

De Vries, G.J., W. Best and A.A. Sluiter - Action of androgens on the development of a sex difference in the vasopressinergic innervation of the brain. Proc. Internat. Symp., 'Sexual differentiation, basic and clinical aspects', Carmel (USA).

Fliers, E., D.F. Swaab and C.W. Pool - The human and rat hypothalamus-neurohypophyseal system (HNS) during aging. Proc. 23rd Dutch Feder. Meeting, Amsterdam, 127.

Gash, D.M., G.J. Boer, M.F.D. Notter and J.R. Sladek Jr. - Transplanted vasopressin neurons: effects on the central nervous system. Proc. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK), p.8.

Habets, A. - Quantitative aspects of developing spontaneous bioelectric activity in dissociated cerebral cortex cultures. Proc. NATO Adv. Study Inst., 'Organizing Principles for Neural Development', Povoá do Varzim (Portugal).

Haldar-Misra, C. and P. Pévet - The influence of 5-methoxyindoles on the process of protein/peptide secretion in the mouse pineal gland. An ultrastructural in vitro study. J. Endocrinol.94, 27P.

- Haldar-Misra, C. and P. Pévet - Effect of melatonin on pineal peptide/protein synthesis. An ultrastructural study in the mouse pineal in vitro. *Gen. Comp. Endocrinol.*46, 356.
- Heyting, C.H., C.W. Pool and W. van Raamsdonk - Localization of neurofilament protein and glial fibrillary acidic protein in the developing rat brain. *Proc. 3rd Internat. Meeting of the Internat. Soc. for Develop. Neuroscience, Patras (Greece)*, p.117.
- Hoorneman, E.M.D, and R.M. Buijs - Vasopressinerge zenuwvezelbanen in rattehersenen na laesie van de nucleus suprachiasmaticus. *Ned. T. Geneesk.*126 (nr.30), 1392.
- Kragten, R. and G.J. Boer - Early postnatal vasopressin administration in the Brattleboro mutant rat and its effect upon brain growth. *Proc. 23rd Dutch Feder. Meeting, Amsterdam*, 245.
- Kragten, R. and G.J. Boer - Perinatal vasopressin administration and its effect on brain development in the diabetes insipidus Brattleboro rat. *J. Endocrinol.*94, Suppl., 29P.
- Kruisbrink, J. and G.J. Boer - Controlled release of vasopressin from Accurel and its application in the Brattleboro rat. *J. Endocrinol.*94, Suppl., 27P.
- Noteborn, H.P.J.M., I. Ebels, P. Péradioimmunological aspects of some peptidic and proteic ovine pineal fractions. *J. Endocrinol.*94, Suppl., 27P.
- Pévet, P. - Physiological effects of pineal indoleamines different from melatonin. *NATO Advanced Study Institute 'The Pineal Gland and its Endocrine Role'*, Erice (Sicily, Italy).
- Pévet, P. - Peptidergic and proteic substances in the vertebrate pineal. Ultrastructural, histochemical, radioimmunological and physiological aspects. *NATO Advanced Study Institute 'The Pineal Gland and its Endocrine Role'* Erice (Sicily, Italy).
- Pévet, P. - Is 5-methoxytryptamine a pineal hormone? *Neuroendocrine Lett.*4, 140.
- Pévet, P. - La 5-methoxytryptamine. Une hormone de la glande pineale? *Annales d'Endocrinologie* 43, 113.
- Pévet, P., and Haldar-Misra, C. - 5-Methoxytryptamine: antigonadal and progonadal effects in male golden hamster. *J. Endocrinol.*94, Suppl., 94P.
- Pévet, P. and C. Haldar-Misra - Anti- and progonadatropie effecten van 5-methoxytryptamine bij de mannelijke goudhamster. *Ned. T. Geneesk.*126, 222.
- Pévet, P., C. Haldar-Misra and T. Ocal - Effect of 5-methoxytryptophan and 5-methoxytryptamine on the reproductive system of the male golden hamster. *Gen. Comp. Endocrinol.*46, 357.
- Van Oyen, H.G., N.E. van de Poll, S.M. van der Zwan and S. de Jong - Passive and active avoidance behavior in male and female rats. *22nd Dutch Feder. Meeting, Utrecht. Ned. T. Geneesk.*, in press.

- Pool, Chr.W., C.H. Heyting and W. van Raamsdonk - Cytoskeleton and myofibrillar proteins in neurons in the rat brain. Proc. 23rd Dutch Feder. Meeting, Amsterdam, 342.
- Romijn, H.J., Van Huizen, F., Wolters, P.S., and Habets, A.M.M.C. - Further attempts to obtain a serum-free medium for long-term cerebral cortex cultures. *Biology of the Cell* 45, p.34.
- Romijn, H.J., F. van Huizen, P.S. Wolters and A.M.M.C Habets - An improved serum-free chemically defined medium for long-term cortex cultures. First Europ. Conf. on Serum-free Cell Culture, Heidelberg (Germany).
- Russchen, F.T., Groenewegen, H.J., and Van Leeuwen, F.W. - Differential origins of 'limbic' and 'striatal-like' efferent connections of the nucleus accumbens in the cat. *Neurosci. Lett.*, Suppl.10, S425-426.
- Swaab, D.F. - Vasopressin and oxytocin in the developing, adult and aging brain. 13th CINP Congress, Symp. 'Peptides in the Brain', Jerusalem (Israel).
- Swaab, D.F. - Vasopressin and oxytocin neurons in various stages in life. In: Manual/Abstractbook on 2nd EMBO Practical Course 'Immunocytochemistry and its application in brain research', Amsterdam, pp.287-293.
- Swaab, D.F. - Introduction in Manual/Abstractbook on 2nd EMBO Practical Course 'Immunocytochemistry and its application in brain research', Amsterdam, pp.29-31.
- Swaab, D.F. - Peptidische verbindingen in het centrale zenuwstelsel. Boerhaave Cursus, Leiden, in press.
- Swaab, D.F. and E. Fliers - Aging of the brain. Proc. 23rd Dutch Feder. Meeting, Amsterdam, 408.
- Swaab, D.F., G.J. Boer, H.P. Oosterbaan and P. Oosting - The fetal brain-pituitary-adrenal axis and labour. Proc. IBRO Satellite Symp. on Vasopressin, Corticoliberin and ACTH-related Peptides, Geneva (Switzerland), A30.
- Swaab, D.F., F.W. van Leeuwen and R.M. Buijs - Preface in Manual 2nd EMBO Practical Course 'Immunocytochemistry and its applications in brain research', Amsterdam, pp.9-10.
- Swanson, H.H. - Interactions of early undernutrition, environmental enrichment and maternal care on growth and behavior of rats. 2nd Europ. Winter Conf. on Brain Res., Chamonix (France).
- Swanson, H.H. and J. Scholtens - Physiological and behavioural consequences of treating newborn female rats with low doses of testosterone. 5th Biennial Meeting, Internat. Soc. for Research on Aggression, Mexico City (Mexico).
- Swanson, H.H. and N.E. van de Poll - Correlation of effects of neonatal testosterone on vaginal cyclicity, sexual, aggressive and other sexually dimorphic behaviour patterns in rats. Proc. Integrative Neurohumoral Mechanisms, Budapest (Hungary).

Swanson, H.H., N.E. van de Poll and J. Scholtens - Effects of low doses of testosterone at birth on oestrus cycles, sexual and aggressive behaviour in rats. Low Countries Meeting on Comparative and Physiological Psychology, Düsseldorf (W-Germany).

Swanson, H.H., N.E. van de Poll and J. Scholtens - Low doses of neonatal androgen: effects on reproductive function and aggressive behavior in female rats. Autumn Conf. BION workgroup, 'Ethology', Texel.

Uylings, H.B.M. - Morfometrie van 3-D neuromorfologische structuren in lichtmikroskopische coupes. Methodendag of the Dutch Neuroanatomists Verhaert Meeting, Leiden.

Uylings, H.B.M., P. McConnell and H.L. Walg - Differential effects of undernutrition and its subsequent rehabilitation on non-pyramidal and pyramidal neurons in occipital cortex. Neurosci, Suppl.7, S216-217.

Uylings, H.B.M., H.L. Walg, H. Overdijk, A.W. Kamstra, R.W.H. Verwer and J. van Pelt - The analysis of 3-D neuronal branching patterns. Neurosci. Lett., Suppl.10, S30-31.

Van Benthem, J., I. Ebels, P. Pévet, C. Haldar-Misra and M.G.M. Balemans - Seasonal rhythmicity in the capacity of the pineal gland of the golden hamster to synthesize different 5-methoxyindoles and the influence of reduced neopterin on this capacity. J. Endocrinol.94, 36P.

Van de Poll, N.E. - Gonadal Hormones: effects upon sexual and aggressive behavior in male and female rats. Low Countries Meeting (Psychonomy), Düsseldorf (Germany).

Van de Poll, N.E. - Sexverschillen in agressief gedrag van de rat. Autumn Meeting Ethology (BION), Texel.

Van de Poll, N.E., N.E. Bowden, H.G. van Oyen and P.F. Brain - Effects of gonadal hormones on the male and female rats' aggressive and sexual behavior. 4th ESCAB Conf., Bielefeld (Germany).

Van der Sluis, P.J., G.J. Boer and C.W. Pool - Fixation and immunocytochemical detection of small peptides after isoelectric focusing. Proc. 23rd Dutch Feder. Meeting, Amsterdam, 395.

Van Huizen, F., H.J. Romijn, A.M.M.C. Habets and P.S. Wolters - Age and function related changes of synaptic ultrastructure in rat cerebral cortex cultures. Proc. NATO Adv. Study Inst. 'Organizing principles for neural development', Povoia (Portugal).

Van Huizen, F., H.J. Romijn, P.S. Wolters and A.M.M.C. Habets - Development of synaptic contacts in fetal rat cerebral cortex cultures in relation to developing bio-electric activity. Neurosci. Lett., Suppl.10, S499.

Van Leeuwen, F.W. - Enkephalin immunoreactivity in presynaptic elements on glial cells in the rat neural lobe. Proc. 3rd. Internat. Conf. on the Neurohypophysis, Babraham (UK), p.16.

Van Leeuwen, F.W. and A.R. Caffé - New loci in the rat brain containing immunoreactive vasopressin. Proc. 3rd Internat. Conf. on the Neurohypophysis, Babraham (UK), A16.

Van Leeuwen, F.W. and A.R. Caffé - A new locus in the rat brain containing immunoreactive vasopressin: the nucleus occultus. *Neurosci. Lett.*, Suppl.10, S499-500.

Van Norde, W. and H.B.M. Uylings - Underdevelopment of cerebellar lobes in the diabetes insipidus Brattleboro rat. *Acta Morphol. Neerl.-Scand.*, in press.

Van Pelt, J. and R.W.H. Verwer - A new method for the analysis of branching patterns. First Europ. Symp. of the Internat. Soc. for Stereology, Sheffield (UK), July 5-7.

Verwer, R.W.H. and D.M.G. de Groot - Effekt van vorm-aanname op de schatting van de numerieke dichtheid van synapsen. Methodendag of the Dutch Neuroanatomists Verhaert Meeting, Leiden.

Verwer, R.W.H., De Raay, C., Uylings, H.B.M., The effect of undernourishment and subsequent nutritional and environmental rehabilitation on spines and synapses in the rat cerebellum. *Neurosci. Lett.*, Suppl.7, S220.

Verwer, R.W.H., Uylings, H.B.M., Van Pelt, J., and Parnavelas, J.G. - Topological analysis of dendritic branching patterns at various stages of cerebral development. Twelfth Internat. Symp. on Stereology, Sheffield (UK).

Vivien-Roels, B. and P. Pévet - La glande pineale est-elle capable d'integrer parallelement des variations de lumiere et de temperature: l'exemple des reptiles. *Annales d'Endocrinologie* 43, 112.

#### PAPERS READ (seminars, etc.)

See also 'abstracts' and 'teaching' sections

Baker, R.E. - Influence of growth medium, age in vitro and spontaneous bioelectric activity on the distribution of sensory ganglion evoked activity in spinal cord explants. Lab. de Biologie Moleculaire, Collège de France, Paris (France), April; The development of connections between fetal mouse dorsal root ganglia and spinal cord explants grown in the presence of chondroitin sulfate. EMBO Workshop on 'Synaptic Connectivity in Development and Regeneration', Eilat (Israel), November.

Boer, G.J. - Accurel polypropylene and its use for controlled release of hormones. AKZO Research Centre, Arnhem, February; Longterm effects on brain and behavior of early treatments with neuropeptides. Internat. Workshop, 'Application of Behavioral Pharmacology in Toxicology', Capri (Italy), April; Vasopressin and the developing brain. Dept. of Anatomy, Univ. of Rochester (USA), February.

De Bruin, J.P.C. - Samenhang tussen hersen- en gedragsontwikkeling. Nederlandse Dierkundige Vereniging. Symp. 'Hersenswerk', Amsterdam, January.

Buijs, R.M. - Vasopressinergic and oxytocinergic pathways, synapses and central release. IBRO satellite Symp., 'Vasopressin, corticoliberin and ACTH-related pathways', Geneva (Switzerland), March; Tissue treatment in

immunocytochemistry; 2nd EMBO practical course, 'Immunocytochemistry and its Application in Brain Research', Amsterdam, May; The distribution and release of vasopressin and oxytocin in the CNS. 3rd Int. Conf. on the Neurohypophysis, Babraham (UK), September; The distribution and release of neurohypophysial peptides in the brain; 3rd Annual Meeting of the Contact Group, 'Psychoneuroendocrinology', Liege (Belgium), September; Vasopressin and oxytocin: their role in neurotransmission Univ. of Bordeaux II, Lab. de Neurobiol. des Compart., Bordeaux (France), December.

De Jonge, F.H. - Sexuele motivatie bij de rat. Psychonomy workgroup, 'Comparative and Physiological Psychology, Utrecht, October.

De Vries, G.H. - A sex difference in the vasopressinergic innervation of the brain. Dept. of Pharmacol., Medical School of Bristol, (UK), February; Influence of gonadal steroids on the development of a sex difference in the vasopressinergic innervation of the brain. Congr., 'Integrative Neurohumoral Mechanism: Physiological and Clinical Aspects', Budapest (Hungary), March.

Fliers, E. - Some functional and morphological aspects of dementia; a review. Ver. Gerontol. Inst. Amsterdam, Amsterdam, October.

Habets, A.M.M.C. - Kwantitatieve aspecten van spontane bioelektrische activiteit tijdens ontwikkeling cerebrale cortex weefsel in vivo. Kring Elektrofysiol. Ver. voor Biofysica, MFI-TNO, Utrecht, November.

Mirmiran, M. - 'Oneiric' behavior during active sleep in rat. Dept. of Comp. Physiol. Psychol., Univ. of Nijmegen, September; Behavioral teragenicity of medicines used during pregnancy, Dept. of Obstet. and Gynaecol., Univ. of Nijmegen, November.

Pévet, P. - The different classes of proteic and peptidic substances present in the pineal gland. 12th Int. School of Pharmacol., NATO Advanced Study Inst., Erice-Trapini (Italy), June; Physiological effects of pineal indoleamines different from melatonin. 12th Int. School of Pharmacol., NATO Advanced Study Inst., June; Is 5-methoxytryptamine a pineal hormone? XIII Int. Congr. of the Internat. Soc. of Psychoneuroendocrinol., Tubingen (FRG), July; La glande pineale de mammiferes. Une glande neuroendocrine synchronisatrice. Inst. de Biochimie Cellulaire et Neurochimie, Bordeaux (France), November; La glande pineale des mammiferes. Une glande endocrine synchronisatrice: une etude immunocytochimique, radioimmunologique et ultrastructurale. Inst. de Neurophysiologie et Psychophysiologie, Marseille (France), November; La glande pinéale de mammiferes. Inst. de Biologie Animale, Talence (France), December.

Swaab, D.F. -The influence of medicines on the fetal brain development, Univ. Hospital of Groningen, Dept. of Developm. Neurol. and Dept. of Obstet. and Gynec., March; Vasopressin and oxytocin during development and aging. Univ. Hospital of Utrecht, Dept. of Psych., March; The fetal brain-pituitary-adrenal axis and labor. IBRO Satellite Symp., 'Vasopressin, corticoliberin and ACTH-related peptides', Geneva (Switzerland), March; Life span changes in vasopressinergic and oxytocinergic neurons in the rat and human brain. Univ. Louis Pasteur, Inst. de Physiol., Strasbourg (France), May; Vasopressin and oxytocin in the developing adult and aging brain. 13th CINP Congr., Jerusalem (Israel), June; Medicines and brain development of the child. 13th CINP

Congr., Jerusalem (Israel), June; 'The influence of chemicals and environment on brain development: behavioral teratology. World Conf. by the UNAPEI 'Prevention of Physical and Mental Congenital Defects', Institut de la Vie, Strasbourg (France), October; Influence of foetal and neonatal environment on physical, psychological and intellectual development. Introduction Report Workshop no.11 at the UNAPEI World Conf.; Amniotic fluid pollution. Symp., 'Verloskunde 200', Obstet. Clinic, Rotterdam, November.

Van de Poll, N.E. - Biosocial aspects of behavior. Psychobiology, Tilburg, April; Criminality and aggression: neurobiological and animal behavioral backgrounds, Stichting Studiecentrum Rechtspleging, Utrecht, November; Sexdifferences in aggression in rats. Autumn Conference Ethology (BION), Texel, December.

Van Leeuwen, F.W. - New loci in the rat brain containing immunoreactive vasopressin. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK), September; Enkephalin immunoreactivity in presynaptic elements on glial cells in the rat neural lobe. 3rd Internat. Conf. on the Neurohypophysis, Cambridge (UK), September; The distribution of vasopressin immunoreactive cellbodies in the rat brain. Netherlands Neuroanatomists Verhaert Meetings, Amsterdam, October; The nucleus occultus, a new vasopressin immunoreactive cell group within the rat brain. FUNGO Workshop, 'Regulation of Hypophyseal Functions', Utrecht, October.

Van Eden, C.G. - Verdeling van zware metalen, AChE en 5HT in de prefrontale cortex van de rat. Netherlands Neuroanatomists Verhaert Meetings, Amsterdam, April.

Verwer, R.W.H. - Stereology: pre and contra of the method of Cruz-Orive. Dept. Morphology, Interuniversitair Oogheekundig Instituut, Amsterdam, October.

## TEACHING

### a. Students

Best, W. (pharmacy student, Univ. of Amsterdam): 'The influence of castration and testosterone supplementation on the development of a sex difference in the vasopressinergic innervation of the brain' (research team III).

Boelé-In 't Veld, C.E.I. (psychologist): literature study on 'The anatomy and function of the amygdala complex in the rat and possible sexual dimorphism in structure and function' (research team IV).

Bolwerk, E. (biology student, Free Univ., Amsterdam): 'Induction of maternal behavior with oxytocin' (research team IV).

Brenner, E. (biology student, Univ. of Utrecht): 'Behavioral consequences of early REM-sleep deprivation in rats' (research team II).

Burger, J. (chemistry student, Univ. of Amsterdam): 'Activational effects of testosterone-propionate on sexual motivation and copulatory behavior' (research team IV).

- Caffé, A.R. (biology student, Univ. of Utrecht): 'Immunocytochemical localization of enkephalinergic cell bodies in the hypothalamus of the rat' (research team III).
- Fiolet, M. (linguistics student, Univ. of Amsterdam): 'Capita Selecta in the neurosciences related with patho-linguistics. team I/III).
- Guldenaar, S.E.F. (biology student, Univ. of Utrecht): 'Exohypothalamic vasopressinergic and oxytocinergic innervation of the human brain' (research team III).
- Heinsbroek, R. (biology student, Univ. of Amsterdam): 'Dexamethason and the reaction on novel aversive stimulation in females' (research team IV). Helle, N. (linguistics student, Univ. of Amsterdam): 'Capita Selecta in neurosciences related with patho-linguistics' (research team I).
- Kayser, B.E.J. (medical student, Univ. of Amsterdam): 'Paraventricular neurones in the rat hypothalamic slice: lucifer yellow injection and immunocytochemical identification' (research team III).
- Kerkhoven, J. (biology student, Univ. of Amsterdam): 'Immunoelectron microscopy of vasopressin and oxytocin in the human CNS' (research team III).
- Kragten, R. (biology student, Univ. of Amsterdam): 'Effect of vasopressin suppletion on the developing diabetes insipidus Brattleboro rat' (research team III).
- Lieshout, N. (psychology student, Univ. of Utrecht): 'The involvement of the medial preoptic anterior hypothalamic continuum in sexual motivation' (research team IV).
- Partiman, T. (medical student, Univ. of Utrecht): 'Physiological studies on the involvement of pontine reticular neurons in the generation of active (REM) sleep in infant rats' (research team II); 'Development of vasopressinergic and oxytocinergic neurons in the human brain' (research team III).
- Schlüter, N. (biology student, Free Univ., Amsterdam): 'Fetal hypothalamic transplantations in the third ventricle of Brattleboro rats during early postnatal development' (research team III).
- Snellen, F. (medical student, Univ. of Amsterdam): 'Influence of beta-sympatico mimetical upon measures of anxiety in male and female rats' (research teams II and IV).
- Suttorp, O. (medical student, Univ. of Utrecht): 'Sex differences in sleep patterns in rats' (research team II and IV).
- Van den Hoof, P. (biology student, Univ. of Amsterdam): 'Electrophysiological and morphological consequences of chronically suppressing the GABA-ergic neuronal activity in cerebral cortex cultures' (research team II).
- Van der Horst, S. (student Agriculture School of Alkmaar): practical course in the Institute's animal care department.

Van der Togt, Chr. (medical student, Univ. of Amsterdam): 'Theoretical aspects of development of bioelectric activity in neuronal tissue cultures' (research team V).

Van der Wal, N. (biology student, Free Univ., Amsterdam): 'Vasopressinergic and oxytocinergic innervation of the human hippocampus' (research team III).

Van der Wal, J.K. (chemistry student, Univ. of Amsterdam): 'The effect of immersion fixation on the preservation of the ultrastructure during immunoelectron microscopy' (research team III).

Van Dongen, A.M.J. (biology student, Univ. of Utrecht): 'Electrofysiological aspects of neuronal maturation in tissue cultures of fetal rat cerebral cortex' (research team II).

Van Gool, W.A. (medical student, Univ. of Amsterdam): 'Sleep patterns in aged rats' (research team II).

Voorn, P. (biology student, Free Univ., Amsterdam): 'Vasopressinergic and oxytocinergic synapses in the medulla oblongata' (research team III).

#### b. Lectures and theses

Baker, R.E. - Lectures on 'Ontogenetic development' and 'Development of the vertebrate nervous system', City College of Chicago, Soesterberg, April 7.

Boer, G.J. - 'Neuropeptides and brain development', Univ. of Rochester, NY, Med. Graduate School, Rochester, NY (USA), February 24.

Corner, M.A. - Integrated lectures on 'Physiology of higher nervous processes', Univ. of Amsterdam, Dept. of Clin. Psychol., October; committee member for the PhD thesis of M. Mirmiran, Univ. of Amsterdam, June 17.

De Bruin, J.P.C. - Lecture on 'Ethology and Neurobiology', Univ. of Amsterdam, Dept. of Biology, March 9 and November 9.

Buijs, R.M. - 'Tissue treatment in immunocytochemistry', 2nd EMBO practical course, 'Immunocytochemistry and its applications in brain research', Amsterdam, May 28.

Swaab, D.F. - Interview with videoprogram 'Brain Research' for biology students, Free Univ., Amsterdam, January; lecture 'Hersenveroudering dementie en therapie', Biological Psychiatry, Amsterdam, January 20; lecture 'Brain growth and development in the growth cycle' Univ. of Amsterdam, Med. School, January 21; lectures 'Neuroendocrine regulation of the menstrual cycle: Activating effects of sexhormones. Organizing effects of sexhormones.' Univ. of Amsterdam, Med. School, January 26; lecture 'Microscopy of the cerebellum', Univ. of Amsterdam, Med. School, February 4; lectures 'The limbic system' and 'Hypothalamus and limbic system', Univ. of Amsterdam, Medical School, February 11; lecture 'Microscopy of the cortex cerebri', Univ. of Amsterdam, February 24; lectures 'Aging and demention', Univ. of Amsterdam, Medical School, March 15; 'Vasopressin and oxytocin neurons in various stages of life. 2nd EMBO

practical course 'Immunocytochemistry and its application in brain research', Amsterdam, May 24-28; post-academic course 'Ontwikkeling van het neuron', Univ. of Amsterdam, Dept. of Psych., September 8; 'Peptidergic connections in the central nervous system', Boerhaave course for post-graduate medical teaching, Leyden, November 11-12; committee member of PhD thesis of H.J.L.A. Ruis, Univ. of Nijmegen, February 26; committee member for PhD thesis of H.W.M. Steinbusch, Univ. of Nijmegen, April 1; committee member for PhD thesis of G. Schmitt-Ehret, Univ. Louis Pasteur, Strasbourg (France), May 4; co-referent for PhD M. Mirmiran, Univ. of Amsterdam, June 17.

Swanson, H.H. - Lectures on 'The sociobiology of the gerbil', course in animal behavior, Univ. of Amsterdam, Dept. of Biology, March 17 and November 9.

Romijn, H.J. - Lecture on 'Netwerkinformatie en synapsvorming van foetaal hersenschorsweefsel in vitro', Univ. of Utrecht, Dept. of Psychophysiology, November 27; 'Hersenen en bewustzijn', Student Soc. PANDA, Amsterdam, May 12.

Uylings, H.B.M. - (together with D.F. Swaab) Lecture on 'Microscopic neurohistology of the central nervous system' and practical introduction for the student-assistents, Univ. of Amsterdam, Med. School, September 13.

Van de Poll, N.E. - Integrated lectures on 'Gonadal hormones and behavior: Comparative aspects of social behavior. Gonadal hormones and behavior. Gonadal hormones, brain and behavior. Gonadal hormones developmental aspects in man', Univ. of Utrecht, Dept. of Physiol. Psychol., May. Van Leeuwen, F.W. - 'Immunoelectron microscopy in neuroscience', 2nd EMBO practical course, 'Immunocytochemistry and its applications in brain research' Amsterdam, May 28. practical course, 'Immunocytochemistry and its application in brain research', Neth. Inst. for Brain Research and Univ. of Amsterdam, Amsterdam, May 24-28.

### **c. Second EMBO Practical Course: Immunocytochemistry and its application in Brain Research**

This practical course was held in the Netherlands Institute for Brain Research, May 24-28, 1982. The organizing committee (F.W. van Leeuwen, D.F. Swaab, R.M. Buijs and J. Sels) compiled at the request of EMBO (Dr. J. Tooze) a program dealing with the rapidly developing field of immunocytochemistry and has dealt with the selection of 18 postgraduates (see below) out of 110 applicants. A teaching staff of 19 international scientists gave introductory lectures, practiced immunocytochemistry at the laboratory tables and presented demonstrations partially by means of posters. The lecturers were: G.J. Boer (NIBR, Amsterdam, The Netherlands), D.M. Boorsma (Free Univ., Amsterdam, The Netherlands), R.M. Buijs (NIBR, Amsterdam, The Netherlands), A.C. Cuello (Univ. of Oxford, England), J. de Mey (Janssen Pharmaceutica Research Lab., Beerse, Belgium), P. Diegenbach (Univ. of Amsterdam, The Netherlands), L.F. Eng (Stanford Univ., USA), L.-I. Larsson (Univ. of Aarhus, Denmark), C.W. Pool (NIBR, Amsterdam, The Netherlands), J. Schipper (Free Univ., Amsterdam, The Netherlands), J.W. Slot (Univ. of Utrecht, The Netherlands), L.A. Sternberger (Univ. of Rochester, NY, USA), D.F. Swaab (NIBR, Amsterdam, The Netherlands), D. van der Kooy (Univ. of Toronto,

Ontario, Canada), P. van der Sluis (NIBR, Amsterdam, The Netherlands), F.W. van Leeuwen (NIBR, Amsterdam, The Netherlands), W. van Raamsdonk (Univ. of Amsterdam, The Netherlands), J.K. Wamsley (Univ. of Utah, Salt Lake City, USA), and B. Zipser (Cold Spring Harbor Lab., NY, USA).

The following applicants were selected: H. Bodenmuller (Max-Planck-Institut Heidelberg, FRG), A. Calas (Univ. of Bordeaux, Talence, France), U. di Porzio (Lab. of Molecular Embryology, Naples, Italy), M. Dornay (The Weizman Inst. of Science, Rehovot, Israel), M. Dubois-Dauphin (Univ. of Geneva, Switzerland), F. Eckenstein (Max-Planck-Institut fur Biochemistry, Martinsried, FRG), J. Geysen (Univ. of Louvain, Belgium), M.R. Hanley (Imperial College, London, England), E.M.D. Hoorneman (Free Univ., Amsterdam, The Netherlands) of A.N. Karamanlidis (Univ., Thessaloniki, Greece), R. Morris (Univ., Bristol, England), Y. Olsson (Uppsala Univ., Sweden), R. Ravid (The Hebrew Univ., Jerusalem, Israel), D. Riche (Lab. of Physiology, CNRS, Gif-sur-Yvette, France), P.G. Vallet (Univ. of Geneva, Suisse), A.N. van den Pol (Univ. of Oxford, England), R.G. Williams (Univ. of Liverpool, England) and L. Zaborsky (Sемmelweis Univ., Budapest, Hungary).

In addition, several aspects of the immunocytochemical techniques (e.g., pretreatment of the tissue, raising monoclonal antibodies, choice of staining procedure, method- and serum specificity, quantification of the immune reaction, double staining, light- and electronmicroscopical immunocytochemistry, receptor localization, combination of immunocytochemistry with tracing of neuronal pathways) were thoroughly discussed in various informal sessions with the students. Members of research team III have been incorporated in the actual organization of the practical sessions (especially the technicians) and discussions. The practical part of the course was interrupted by a public mini-symposium on 'Recent Immunocytochemical Findings in Neurobiology', held in the building of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, on May 27. A course manual (386 pp.) has been prepared containing not only abstracts on the several introductory lectures and demonstrations, but also full descriptions of the immunocytochemical procedures performed during the course. The course manual has been offered for sale to applicants who could unfortunately not participate.

#### MISCELLANEOUS

Baker, R.E. - project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'.

Boer, G.J. - supervisor of the radioisotope laboratory of the NIBR; chairman of organizing committee of VIIIth Dutch-British Endocrine Meeting, Noordwijkerhout, August 25-27; member of advisory board of the Dutch Society of Endocrinology; referee for Molec. Cell. Endocr. (1x); Acta Endocr. (1x) and Cell Tiss. Res. (1x).

Corner, M.A. - project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'; member of organizing committee for the 12th Internat. Summer School of Brain Res., Amsterdam, August 1983; member of program committee for the 4th Ann. Meeting of the Internat. Soc. for Develop. Neurosci., Salt Lake City (USA), July 1983; regional secretary of the Internat. Soc. for Develop. Neurosci.; member of

advisory boards of Develop. Brain Rev., Develop. Neurosci., and Neurosci. Biobehav. Rev.; referee for Brain Res. (1x); research advisor for Dept. of Clin. Psychol., Univ. of Amsterdam; consultant for Elsevier Biomedical Press 'Neurosci. Alerting Service'.

Buijs, R.M. - referee for Acta Endocrinol. (2x) and J. Neurosci. Meth. (1x).

De Bruin, J.P.C. - project leader in BION workgroup, 'Ethology',; and in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'; secretary of BION workgroup, 'Ethology'; coordinator in BION project group 'Behavioral Mechanisms'; organizer of the Autumn Conf. of the BION workgroup, 'Ethology', Texel, December 16-17; referee for Adv. in Vertebrate Neuroethol. (3x), Behav. Proc. (3x) and Behav. Brain Res. (1x).

Habets, A.M.M.C. - project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'.

Pévet, P. - project leader in FUNGO workgroup, 'Regulation of the Hypophyse Functions', secretary-treasurer of the European Pineal Study Group; editor of the ESPG-newsletter; referee for J. Endocrinol. (1x), J. Neural Transm. (3x), Psychoneuroendocrinol. (2x), Cell Tiss. Res. (2x), Reprod. Nutrition Develop. (1x); reviewer in grant project for National Sci. Fndt. (USA).

Romijn, H.J. - organizer of Round table, 'Nerve tissue in (serum-free) tissue culture' for the tissue culture workgroup of the Ned. Ver. v. Celbiol., Utrecht, November 16; photographic contribution to Canad. J. Appl. Sport Sci.; technical assistance with the setting up of a procedure for serum-free culturing of nerve tissue at Depts. Veter. Bioch. and Veter. Virol., Univ. of Utrecht; book reviews in Dagblad 'Trouw' on 'Hersenen en onderzoek' (F. Kappetijn, Dienst Wetenschapsvoorlichting bij de KNAW, Amsterdam, 1981), 'Het omega effect. Verklaring van de geheimzinnige wisselwerking tussen hersenen en geest' (G.J. Taylor 'The Natural History of Mind', Elsevier, Amsterdam, 1980), 'Onthouden en vergeten' (V.J. Nicholson, Van Gorkum en Comp., Assen, 1980).

Swaab, D.F. - project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'; secretary of the Dutch Comm. of the Internat. Brain Res. Organiz. (IBRO); reg. secretary of the Internat. Soc. for Develop. Neurosci. (ISDN); member of program committee for the 3rd Internat. Meeting of the ISDN; secretary-treasurer of the Van den Houten Fund for the NIBR; member of the Internat. Sci. Comm. of the Europ. Sci. Fndt's Training Program in Brain and Behavior Research (ETP-BBR); committee member for Child Psychiatry (Univ. of Amsterdam), of Teratology of Chemical Compounds (Health Council) and Gerontology (FUNGO and ZWO); member of committee '2e fase' training in Neurobiology (Dept. of Biology) and Neurosci. (Med. School, Univ. of Amsterdam); chairman of Minisyp., 'Neuropeptides', VIIIth Dutch-British Endocrine Meeting, Noordwijkerhout, August 25; member of advisory board of Acta Endocrinol. and J. Neural Transm.; member of editorial board of J. Develop. Physiol., J. Neurosci. Meth., J. Molec. Cell Endocrinol., and Peptides; referee for J. Endocrinol. (2x), Europ. J. Pharmacol. (2x), Europ. J. Obst. Gynaecol., and Reprod. Biol. (2x); verklaring van trefwoord 'Neurosecretie', Winkler Prins Encyclopedie, Deel 16, Elsevier, Amsterdam, p.316.

Swanson, H.H. - organizer of Round table, 'Interactions of early undernutrition, environmental enrichment and maternal care on growth and behavior of rats', 2nd Europ. Winter Conf. on Brain Res., Chamonix (France); chairman of session of XIIIth Internat. Congr. of Internat. Soc. of Psychoneuroendocrinol., Tubingen (Germany); member of advisory committee for project of Psychonomy; member of committee of Europ. Brain and Behav. Soc.; member of advisory council of Internat. Soc. of Psychoneuroendocrinology; member of ethics committee of Internat. Soc. for Res. on Aggression; external advisor on project grant in Psychobiology for National Science Fndt., Washington DC (USA); referee for Behav. Brain Res. (1x), Acta Endocrinol. (1x), and Animal Behav. (1x); book reviews in American Scientist on 'Environmental factors in mammal reproduction' (D. Gilmore and B. Cook, Univ. Park Press, Baltimore, 1981), and in Molecular and Cell Biology (p.409) on 'Neuroendocrine Regulation and Altered Behaviour', (P.D. Hrdina and R.L. Singhal, Eds., Croom Helm Ltd., London, 1981).

Uylings, H.B.M. - project leader in FUNGO workgroup, 'Development and Aging of Nervous System and Behavior'; executive member of organizing committee for the bimonthly Netherlands Neuroanatomists Verhaart Meetings; organizer together with Drs. E. Marani, G. Vrensen and Prof. A.H.M. Lohman of Neuroanatomical Methods-day, 'Reconstruction and Research of 3-dimensional neuroanatomical structures'; external referee for tenure position at Dept. of Cell Biol. Univ. of Texas, (USA); external referee for grant application to the Niels Stensen Fndt.; referee for Eur. J. Pharmacol. (1x), J. Microsc. (1x), J. Neurosci. Meth. (1x) and J. Devel. Physiol. (1x).

Van de Poll, N.E. - project leader in FUNGO workgroup, 'Brain and Behavior', and Psychonomy workgroup, 'Comparative and Physiological Psychology'; member of board of Dobberke Fndt. for Comparative Psychology; coordinator of Psychonomy workgroup, 'Comparative and Physiological Psychology'; Twinning Grant ETP together with P.F. Brain (Univ. of Swansea, UK), 'Is aromatization involved in androgen stimulation of aggression in rats?'; member of the Council of the International Society for Research on Aggression (ISRA); member of organizing committee of 2nd Conf. of the ISRA, Zeist, 1983.

Van Leeuwen, F.W. - chairman of workgroup, 'Immunoelectron Microscopy' of the Netherlands Soc. for Cell Biol.; reviewer of research grant program NATO-OTAN; referee for J. Neurosci. Meth. (2x), Regulatory Peptides (1x), and Acta Endocrinol. (1x).

Van Pelt, J. - referee for J. Neurosci. Meth. (1x).

#### SEMINARS GIVEN AT THE INSTITUTE (organization Dr. R.W.H. Verwer)

February 5 - Dr. C. Grimmelikhuyzen (Abt. Biophysik, Max Planck Institut fur Medizinische Forschung, Heidelberg, Germany): Neuropeptides and hydra.

March 3 - Prof. H.G.J.M. Kuypers (Afd. Anatomie, Erasmus Univ., Rotterdam): Nieuwe methoden voor het vervolgen van zenuwverbindingen in de hersenen.

- March 15 - Prof. J.S. Rosenblatt (Inst. of Animal Behavior, Rutgers Univ., Newark N.J., USA): Maternal behavior in the rat.
- March 23 - Dr. R.W. Oppenheim (Neuroembryology Laboratory, Division of Mental Health, State of North Carolina, USA): Adaptations and regression during development of the nervous system.
- March 24 - Dr. W.T. Greenough (Dept. of Physiology, Univ. of Illinois, USA): Development and aging in neurobiology.
- March 29 - Dr. P.D. Coleman (Dept. of Anatomy, Univ. of Rochester, NY, USA): Dendritic branching in the aging nervous system.
- May 7 - Prof. P. Cohen (Groupe de Neurochimie, Univ. Pierre et Marie Curie, Paris, France): Plurifunctional forms of neurophysin in the hypothalamo-neurohypophyseal tract: the coenophorin hypothesis.
- April 27 - Dr. A. Reinharz (Div. d'Endocrinologie, Hopital cantonal de Geneve, Switzerland): Neurophysins and neuropeptides in the mammalian pineal gland.
- June 18 - Prof. M. Jouvet (Dept. of Experimental Medicine, Univ. Claude Bernard, Lyon, France): Some recent developments in the experimental study of sleep mechanisms and functions.
- August 24 - Prof. B.T. Pickering (Dept. of Anatomy, Med. School, Univ. of Bristol, UK): Precursors of neurohypophysial hormones: some answers and some more questions.
- September 1 - Dr. W.S.T. Griffin (Health Science Center, Dept. of Cell Biology, Univ. of Texas, Dallas, USA): Regulation of nucleotide and protein synthesis in developing cerebellum.
- September 22 - Dr. D. Gash (Dept. of Anatomy, Med. School, Univ. of Rochester, NY, USA): Functional CNS transplants in mammals; four successful model systems.
- September 24 - Dr. C. Straznicky (Dept. of Human Morphology, The Flinders Univ. of South Australia, South Australia): Factors controlling the formation of orderly connections in the frog visual system.
- October 29 - Dr. F. van Haaren (Psychologisch Inst., Technische Hogeschool, Tilburg): Interakties tussen operanten en Pavloviaanse konditioneringsprocedures.



# **Hubrecht Laboratory International Embryological Institute**

---

## **Progress Report 1982**

**Edited by J. Faber**

Hubrecht Laboratorium,  
Uppsalalaan 8,  
3584 CT Utrecht,  
The Netherlands

## CONTENTS

Management and staff 4

History and objectives of the institute 5

Introduction 6

I. Research Group: Embryogenesis in Amphibians 7

A. SYMMETRIZATION OF THE EGG 8

1. *The role of gravity in the establishment of the dorso-ventral axis – a microgravity analysis* 8

2. *Analysis of the cytoskeleton and its role in symmetrization of the fertilized egg* 9

B. CLEAVAGE, MESODERM FORMATION AND GASTRULATION 10

1. *The cleavage pattern and the formation of the mesoderm and the blastopore* 10

C. STRUCTURE AND FUNCTION OF THE PLASMA MEMBRANE AND CORTEX 11

1. *Regional differences in dynamic plasma membrane properties of the egg* 11

2. *Freeze-fracture electron microscopy of membrane changes in progesterone-induced maturing oocytes and in eggs* 12

3. *Calcium detection in organelles near the animal hemisphere egg cortex by X-ray microprobe analysis* 13

II. Research Group: Organogenesis in Mammals 14

III. Research Group: Morphogenesis in Cellular Slime Moulds 14

D. MORPHOGENESIS AND PATTERN FORMATION 16

1. *Differentiation and the cell cycle* 16

2. *Monoclonal antibodies* 20

3. *Computer simulation of cell sorting* 20

IV. Research Group: Membrane Regulation 21

E. MEMBRANE REGULATION IN GROWTH AND DEVELOPMENT 21

1. *Growth factor-membrane interaction* 23

2. *Differentiation and development* 29

3. *Other projects* 35

V. Other Research Projects 37

1. *Early reptilian development with special reference to the origin of the primordial germ cells* 37

2. *Plasma membrane dynamics in early molluscan embryos* 38

3. *Designing in vitro test systems for pre-screening of teratogens* 38

4. *Membrane fluidity and the action of adrenocorticotrophic hormone (ACTH) on the brain synaptic plasma membrane* 40
5. *Ageing and lipid fluidity of brain synaptic plasma membranes: effect of ACTH* 40
6. *Lateral diffusion of plasma membrane components during the cell cycle* 41
7. *Research carried out in the amphibian facility* 41

VI. *Miscellaneous* 42

VII. *Papers published and accepted for publication in 1982* 42

## Management and staff

### *Director*

vacancy as from November 1979

### *Acting Director*

J. Faber, Ph.D.

### *Laboratory Manager*

Elizabeth A. Berends

### *Supervisor of Animal Care*

Romee Verhoeff-de Fremery, M.Sc.

### *Experimental Morphology; Curator of the Central Embryological Collection*

Elze C. Boterenbrood, Ph.D.

### *Experimental Morphology; Cinematography*

K. Hara, Ph.D.  
(until April 1982)

### *Histo- and Cytochemistry*

Geertje A. Ubbels, Ph.D.

### *Ultrastructural Research*

J.G. Bluemink, Ph.D.

### *Tissue and Organ Culture*

Kirstie A. Lawson, Ph.D.  
(on leave in U.S.A. as from  
August 1982)

### *Developmental Physiology*

A.J. Durston, Ph.D.

### *Biophysics*

S.W. de Laat, Ph.D.

### *Biochemistry*

P.T. van der Saag, Ph.D.

## RESEARCH ASSOCIATES AND GUESTS

### *Prof. of Exper. Embryol., University of Utrecht*

P.D. Nieuwkoop, Ph.D.

### *Honorary research associate*

W.P. Luckett, Ph.D. (San Juan,  
Puerto Rico, U.S.A.)

### *Temporary research associates*

J. Boonstra, Ph.D. (until May 15)

T.G. Brom, M.Sc.

L.H.K. Defize, M.Sc. (from July 1)

W.J.A.G. Dictus, B.Sc.

M.H.F. van den Hoef, M.Sc.

W.H. Moolenaar, Ph.D.

Christine L. Mummery, Ph.D.

S.A. Nelemans, M.Sc. (from July 1)

C.J. Weijer, M.Sc. (until March 1)

E.J.J. van Zoelen, Ph.D.

### *Guest workers*

Johanna E. Speksnijder, M.Sc. (Utrecht)

Sue-A. McDonald, B.Sc. (Ridge, MD,  
U.S.A.)

### *Visiting scientists*

Brigitte Albers, M.Sc. (Köln, B.R.D.)

P. Cardellini, Ph.D. (Padua, Italy)

Rosine Chandebois, Ph.D., Prof.

(Marseille, France)

Chiu-Nan Lai, Ph.D. (Houston, TX,  
U.S.A.)

R.J. Hill, Ph.D. (Copenhagen, Denmark)

M. Hershkowitz, Ph.D. (Rehovot, Israel)

Jolanta Karasiewicz, Ph.D. (Warsaw,  
Poland)

J. Mácha, Ph.D. (Prague,  
Czechoslovakia)

Valeria Matranga, Ph.D. (Palermo, Italy)

R.W. Merriam, Ph.D. (Stony Brook, NY,  
U.S.A.)

F. Milan, B.Sc. (Padua, Italy)

N. Romani (Innsbruck, Austria)

*Graduate students (University of Utrecht)*

R. Aerts	H.-J. Kuiper
M. de Boer	W. de Lau
M. Heideveld	M. Mulder
J. Janssen	

OTHER STAFF (partial)

*Technicians (semi-scientific staff) Library*

Christina E. van den Brink	Oeke E.H. Kruythof
Winnie W. Cator	Nora Pulle-Starke
Alie Feijen	Anne-Miek Vernooij-Wiss

E.C.A. Freund

W.J. Hage

Lilian Joosen

C.H. Koster

W.A.M. van Maurik

P. Meyer

Jenny Narraway

Theodora M.J. van Oostwaard

L.G.J. Tertoolen

Johanna P.M. Timmermans

F.J.M. Vervoordeldonk

F.A.M. van de Wiel

*Photography and Art*

L. Boom

Carmen L. Kroon-Lobo

*Head of Domestic Service*

G. van Garderen

*Technical and Electronics Workshop*

J.H. Beeker

L.J. de Kam

H.L. Krielen

W. Leurink

J.L. van Lun

C. Mook

H.R. Reitsma

*Secretariat*

Engelina C. Ekelaar

Eveline J.G.M. Hak

Dorothy J.S. Parsons

*Administration*

A. van den Breul

B.H.H. de Deugd

History and objectives of the institute

The Hubrecht Laboratory was founded in 1916 in memory of the Utrecht zoologist and embryologist Prof. A.A.W. Hubrecht. It is a semi-governmental institution operating under the supervision of the Royal Netherlands Academy of Arts and Sciences. The total personnel numbers about 60.

The Laboratory has a statutory commitment to the International Society of Developmental Biologists to carry into effect certain of its aims.

The objective of the Laboratory is to function as an *international research and service centre for developmental biology*. To ensure a multi-disciplinary approach to the many problems of development seven disciplines are being practised, each applying a variety of experimental procedures (see p. 4).

The Laboratory houses the Central Embryological Library (collection of

reprints covering large parts of developmental biology) and the Central Embryological Collection (microscope slides and material preserved in alcohol).

Individual guest workers are welcome at the Laboratory. Partial financial support is available in special cases only.

## Introduction

The Hubrecht Laboratory is an institute for fundamental research in developmental biology. Its scientific objective is a multidisciplinary approach to the problems of development. The central theme of research is the origin of the multicellular organism, and more particularly *the origin of patterns of cell differentiation* within the organism. This theme can be viewed from three major levels of biological organization: the organismal, the cellular and the molecular level. In defining the major problems to be studied the Laboratory as a whole is always keeping these three levels in mind because they are deemed equally essential in the approach to the main theme of research.

Obviously not all problems can be studied in a single developing system because the requirements made of the system vary with the problem. The present research programme concentrates on a limited number of systems carefully chosen for their suitability. Problems primarily derived from the organismal and cellular levels are being studied in amphibian eggs and embryos, in mammalian organ rudiments, and in cellular slime moulds (which can be regarded as simple model systems for animal embryos). Problems derived primarily from the cellular and molecular levels are being studied with the aid of certain mammalian cell lines cultured *in vitro*; these are used as model systems for membrane regulation of the cell cycle and of the initiation of cell differentiation.

The methods used are to some extent determined by the level from which a problem derives, but all problems are studied by subcellular, cellular and supracellular approaches, in varying proportions. The ultimate aim is to achieve a maximally integrated modern approach to the central theme. In the coming years this will no doubt require a concerted effort to expand the expertise available at the Laboratory, particularly in the areas of cellular, subcellular and molecular biology. Already now there is at these levels of analysis a connecting thread running through much of the work done at the Laboratory: *the cell membrane and the cell surface associated with it*.

In 1982 the research was still carried out in four *research groups*. Their current work is described in sections I - IV, which together represent the main research programme of the Laboratory. Projects carried out outside the research groups proper are described in section V; they often have a distinct relation to the work of the research groups.

Wherever possible the material in this Report is arranged according to the levels of biological organization from which the problems derive, each time starting with the organismal level.

While this report was being written a new research programme was drawn up, which will go into effect in 1983. The research will then be carried out in three Sections: Pattern Formation (head Dr. A. J. Durston; encompassing the Research Groups I - III in this report); Membrane Regulation (head Dr. S. W. de Laat; encompassing Research Group IV); and a new Section of Gene Regulation (which is to start in 1984 and will,

among other things, use methods of genetic manipulation and recombinant-DNA techniques). In the new research programme the emphasis will be on *early amphibian and mammalian embryogenesis*; the work on cellular slime moulds and mammalian organogenesis will gradually be phased out. An English version of the programme is available on request.

## 1. Research Group: Embryogenesis in Amphibians

*Members:* J.G. Bluemink, E.C. Boterenbrood, G.A. Ubbels, R. Verhoeff-de Fremery; T.G. Brom (res.assoc.), W.J.A.G. Dictus (res.assoc.), M.H.F. van den Hoef (res.assoc.); E.C.A. Freund, W.J. Hage, C.H. Koster, J.M. Narraway, F.J.M. Vervoordeldonk.

*Guest workers:* P.D. Nieuwkoop, B. Albers (Cologne, B.R.D.), P. Cardellini (Padova, Italy), J. Mácha (Prague, Czechoslovakia), A. Merionato (Padova, Italy), R. Merriam (Stone Brook, N.Y., U.S.A.), J.E. Spek-snijder (Utrecht, Netherlands).

*Collaboration:* K.E. Dixon (Bedford Park, S.A., Australia), J.C. Gerhart (Berkeley, CA, U.S.A.), M.W. Kirschner (San Francisco, CA, U.S.A.), J.-C. Beetschen (Toulouse, France), B. Picheral (Rennes, France), K. Rzehak (Kraków, Poland), J. Paleček (Prague, Czechoslovakia), E.E. Baulieu (Paris, France), N.H. Verdonk (Utrecht, Netherlands), K. Hara (Aichi, Japan).

The aim of this research group is to understand the principles underlying the generation of an individual organism as exemplified by the amphibian embryo. Apart from practical reasons, the amphibian embryo has been taken as the object of study because these embryos have a regulative type of development, they are self-supporting entities, and their development is considered to be representative for most other vertebrates except mammals. We take the viewpoint that in order to arrive at a generative theory of morphogenesis it will be necessary to study development at all levels (i.e., from the molecular to the organismal level), but always taking the organism as the entity of biological organization. At present our research is focussed on *mechanisms underlying cell diversification* during cleavage and in the blastula, gastrula and neurula stages. The following aspects are being studied more in particular, because of their potential role in early morphogenesis: 1. the structure and function of the cell surface and the cell membrane; 2. the structure of the cytoskeleton and its function in the redistribution of cytoplasm and in morphogenetic movements (also in relation to gravity); 3. the regulation of the cell cycle; 4. the role of nucleo-cytoplasmic interactions; 5. the structure and function of cell contacts.

Although the organism is taken as the point of reference of our research, problems of morphogenesis and pattern formation are increasingly formulated in terms of cell properties and cell capabilities. At the subcellular level there are many problems that are amenable to molecular approaches, e.g. membrane-mediated functions and cytoskeleton-mediated activities. It is on the cellular/subcellular level that amphibian embryology should be centered today.

## A. SYMMETRIZATION OF THE EGG

### 1. *The role of gravity in the establishment of the dorso-ventral axis - a microgravity analysis (Xenopus laevis)*

Generally speaking in anurans the animal-vegetal axis foreshadows the antero-posterior axis of the embryo. Upon fertilization the animal cap contracts around the sperm entry point (SEP) and later this side becomes the ventral side of the embryo. As in other anuran species in about 70% of cases the blastopore appears opposite the SEP<sup>1</sup>. Centrifugation experiments have shown that gravitationally induced rearrangements of the yolk predictably determine the dorso-ventral axis of the embryo, regardless of the original position of the SEP or the grey crescent<sup>2</sup>. This suggests a role of gravity in the determination of dorso-ventral polarity, an assumption which can only be proved definitively by fertilizing and growing eggs under microgravity conditions. An experimental proposal submitted to the European Space Agency in 1980 passed several selection procedures and was accepted as part of the payload in Biorack, which is planned to fly in the Space Shuttle scheduled for June 25th, 1985 (the "D-1 flight").

Two questions will be analysed: 1. Can fertilization take place under microgravity conditions? and 2. Is cap contraction around the SEP the sole determining factor for the establishment of the dorso-ventral axis under such conditions? When fertilization is indeed successful, the blastopore is expected to appear within the 160-200° meridians from the SEP (0°).

The work for the preparation of this flight reported last year was continued. It is obvious that the biological material should be of outstanding quality, to bridge the period of maximally 24 hr expected to elapse between the delivery of the material by the experimenters and the start of the experiment in Biorack. Therefore animals from the 1981 stock at the Hubrecht Laboratory are being selected and the relevant parameters are stored in a computer. Unfertilized eggs can be stored at 11°C in full-strength MMR (modified amphibian Ringer's<sup>1</sup>) for 18 hr before fertilization without harmful effects on development. Abnormal rotation of orientation is a characteristic feature in eggs kept too long before artificial fertilization (see previous report, sect. I.A.1.i). At 11°C this only occurs after about 18 hr of storage. Since the eggs are expected to be under microgravity conditions within a 6-12 hr period after having been stripped from the female, any spontaneous rearrangement of yolk is not expected before fertilization is effected. Eggs centrifuged at 3 g for 30 min. were fertilizable and developed normally, also after 12 hr of storage at 11°C. Therefore excess-g during ascent will not interfere with the experimental set-up.

The experimental material will consist of six groups of eggs or embryos fixed in 0.5% buffered glutaraldehyde: one group immediately before fertilization, one between fertilization and first cleavage, two around the time of gastrulation, and two control groups kept in a 1-g centrifuge (one between fertilization and first cleavage, and one around the time of gastrulation).

Since the Biorack experiment can only be successful when it is performed as early as possible during the flight and the time of payload specialists will be limited during the first day of the mission, we de-

cided to develop a fully automatic experiment container. This is being developed in close cooperation with a Dutch company (B.V. Ontwikkelingsmaatschappij CCM, Nuenen).

The housing for eggs, testes and various fluids will consist of a block of perspex containing six cylindrical compartments, equipped with springloaded pistons for sequential operation. Gas exchange will be possible between the egg compartment and the surrounding air, which is essential for the development of gastrulae. The experimental cycle will be controlled by a microprocessor with electronic timers, which initiate wire heaters for the release of the pistons. Non-return valves prevent uncontrolled mixing of fluids. Each cylinder block will be mounted inside a standard Biorack container Type 1 with inside measures of 83.5 x 40.4 x 20.4 mm.

During ascent six containers will be kept in a passive thermal conditioning unit (11°C) on the mid-flight deck. As soon as the Space Shuttle is under microgravity conditions Space Lab, and subsequently Biorack will be activated, the six experiment containers (one for each experimental group) will be installed in the 22°C incubator by the payload specialists, and at the appropriate times the microprocessors will individually switch on each experiment container. After completion of each experimental cycle the samples will stay in the fixation fluid during the rest of the flight. The payload specialists will return the experiment containers from the 22°C incubator to the mid-flight deck, where they will stay under ambient temperature until 6 hr after landing. The material will then be returned to the Hubrecht Laboratory. Various samples will be processed for *in toto* observation (localization of the blastopore in relation to the SEP) and histological analysis. The former will be performed by immunolabelling and scanning electron microscopy. For this purpose anti-sperm serum has been raised and an appropriate method is being elaborated.

1. Kirschner, M.W. *et al.* (1980) - Symp. Soc.Dev.Biol. 38, 187-215.
2. Gerhart, J. *et al.* (1981) - Nature 292, 511-516.

## 2. Analysis of the cytoskeleton and its role in symmetrization of the fertilized egg (*Xenopus laevis*)

Earlier results (see sect.VII, publ.34) suggest that the action of the spermaster and additional cytoskeletal and cytomuscular structures is essential for the formation of distinct cytoplasmic localizations, which may cause selective gene activation (see report for 1980, sect.I.D.2). Therefore an analysis of the structure and function of the cytoskeleton and cytomusculature in the fertilized egg is being made with the aid of cytochemical methods and application of various drugs.

Although tubulin is a very conservative protein, various authors have found that antisera raised against tubulin from different sources do not always react with all other tubulins. Therefore, as a first step in our analysis, and in close cooperation with the Institute of Experimental Zoology in Prague, we developed a method for the isolation of tubulin from *Xenopus* eggs and a procedure for tubulin staining in paraffin sections of early *Xenopus* embryos by specific FITC-fluorescence (see publ.18). Because aspecific fluorescence of the yolk granules inter-

feres with the analysis of fine-fibrillar systems, and because permanent slides are preferable for such analysis, we modified Sternberger's PAP method in such a way that various tissue antigens can be demonstrated in sections of Bouin d'Hollande-fixed eggs and in sodium borohydrate-pretreated sections of glutaraldehyde-fixed eggs. A paper on this method is in preparation.

Preliminary observations showed an animal-vegetal gradient of tubulin, whereas actin was mainly concentrated in the cortical region. Both substances were found in the "dorsal cytoplasm" and in the spermaster region, which may well be related with the action of the spermaster in early cytoplasmic shifts.

This staining method is now being used for a much more detailed analysis of eggs at different stages between fertilization and first cleavage. Several fibrillar systems can be distinguished with various antisera; they differ in extension as well as in nature.

## B. CLEAVAGE, MESODERM FORMATION AND GASTRULATION

### 1. *The cleavage pattern and the formation of the mesoderm and the blastopore (Xenopus laevis)*

Cleavage waves have been observed in time-lapse cinematographs of the animal cap of 15 embryos of *Xenopus laevis* during the period preceding gastrulation (see previous report, sect.I.B.1). These waves have now been studied in more detail. They are the result of the sequential starting of blastomere divisions. When the duration of cycles begins to lengthen the sequence of divisions changes in successive cycles, so that finally in all embryos the cleavage waves take a similar direction, i.e. they travel from ventral left to dorsal right. Moreover, together with the lengthening of cycles the propagation of the waves slows down in successive cycles. This is due to cycle lengthening in the animal cap being more pronounced dorsally and on the right than ventrally and on the left, as could be confirmed by detailed determination of the durations of the cycles in 12 different sectors of the animal caps of all 15 embryos.

The constant relation between the direction of the waves and the embryonic axes, achieved shortly before the beginning of gastrulation, raises the possibility that the waves are related to the formation of the mesoderm in the equatorial zone of the blastula, where the embryonic axes are primarily established. In a preliminary investigation of the cleavage pattern in the dorsal equatorial zone the most pronounced and earliest cycle lengthening was found in the region of the future blastopore. This suggests that the lengthening of cycles may be generated by the dorsal endodermal mass, so that a close relationship may exist between cycle lengthening and mesoderm induction.

In order to investigate more accurately the temporal and spatial mitotic activities in the equatorial zone, a time-lapse camera assembly was constructed with which one egg can be filmed simultaneously from the animal, the dorsal and the ventral side. It is expected that study of such films will provide insight into the timing and extension of inductive activities propagating in the equatorial zone, as well as into the differential cellular activities leading to the mesodermal pattern and the formation of the blastopore.

## C. STRUCTURE AND FUNCTION OF THE PLASMA MEMBRANE AND CORTEX

The central question in developmental biology is how the single egg develops into a system of many different cell types organized in a specific pattern. There is considerable evidence that the plasma membrane is a primary site for the control of growth, division, communication and differentiation of cells. It is still unclear whether the egg plasma membrane has a role in the control of egg polarity, axis formation, and tissue patterning. It is against this background that we are interested to learn more about the structure and function of the *Xenopus* egg membrane during early development.

### 1. Regional differences in dynamic plasma membrane properties of the egg (*Xenopus laevis*)

In the *Xenopus* egg animal/vegetal polarity is expressed at the surface level as a difference in the sensitivity to agents like progesterone and beta-mercaptoethanol and in the ability to fuse with sperm. Earlier freeze-fracture studies by us have shown that at the ultrastructural level the plasma membrane organization of the animal half differs from that in the vegetal half in that there are more  $\leq 7$  nm intramembranous particles (IMPs) and more IMP-free domains in the former. In 1981 we have reported about regional differences in the dynamic membrane properties of fertilized *Xenopus* eggs, comparing animal vs. vegetal, and preexisting vs. newly-formed plasma membrane (see previous report, sect.I.D.1). This year we have elaborated these observations using unfertilized eggs, albino eggs and an alternative lipid probe. The results can be summarized as follows.

In unfertilized as well as fertilized eggs the animal half has a lower lipid mobility than the vegetal half and there is a sharp transition in the equatorial zone. Using fluorescence photobleaching recovery (FPR) and the fluorescent lipid analogue 5-(N-hexadecanoyl)amino fluorescein (HEDAF) the mean diffusion coefficient in unfertilized eggs is  $D = 1.5 \pm 0.3 \times 10^{-8}$  cm<sup>2</sup>/s for the animal and  $D = 7.6 \pm 1.3 \times 10^{-8}$  cm<sup>2</sup>/s for the vegetal half. The respective mobile fractions (MF) are  $0.49 \pm 0.04$  and  $0.44 \pm 0.03$ . In fertilized eggs the mean diffusion coefficient is  $D < 0.01 \times 10^{-8}$  cm<sup>2</sup>/s for the animal half and  $D = 2.8 \pm 0.4 \times 10^{-8}$  cm<sup>2</sup>/s for the vegetal half. The mobile fractions are  $< 0.05$  and  $0.66 \pm 0.05$ , respectively. Lateral diffusion measurements with the lipid analogue 5-(N-tetradecanoyl) amino fluorescein (TEDAF) show similar results. As a consequence of fertilization a rapid fusion of cortical granules takes place and a large amount of new membrane material is incorporated into the egg plasma membrane. The structural membrane organization involved, as shown by freeze-fracture studies (see publ.23), probably also reflects the onset of lipid immobilization in the animal plasma membrane. We think that lipid immobilization functions in the block to polyspermy but have no idea how it is brought about.

The data provide evidence for an animal/vegetal polarity in dynamic plasma membrane properties and suggest that intermediate values for D and MF may exist in the equatorial region. In the unpigmented zone of the equatorial region of the fertilized egg D is higher than at the vegetal pole ( $D = 4.4 \pm 0.5 \times 10^{-8}$  cm<sup>2</sup>/s), but MF is identical. However, in the pigmented zone both D and MF are comparable to those

in the animal half. This implies that there is a steep transition in lipid mobility across the equator, rather than a gradient.

The regional difference in lipid mobility thus correlates well with polarity in surface pigmentation. To find out whether there is a causal relationship, FPR measurements were made on eggs of albino *Xenopus* that contain no premelanosomes<sup>1</sup>. It was found that the D and MF values of the animal and vegetal halves are comparable to those found in pigmented eggs, showing that there is no causal relation with pigment distribution. The sharp transition of D across the equator is probably related to the segregation of ecto- and endoderm later in development. The significance for embryonic axis formation will be tested by egg rotation experiments. Eggs that have been rotated through 180° may give rise to normal embryos under certain conditions. We expect that the animal/vegetal difference in plasma membrane lipid fluidity will be reversed in such eggs.

1. Bluemink, J.G. and O.A. Hopperskaya (1975) - Wilhelm Roux's Arch. Devel. Biol. 177, 75-79.

2. *Freeze-fracture electron microscopy of membrane changes in progesterone-induced maturing oocytes and in eggs (Xenopus laevis)*

When progesterone interacts with the plasma membrane of the full-grown *Xenopus* oocyte (stage VI) a sequence of events is triggered leading to the reinitiation of meiosis and to the formation of the fertilizable egg. Major changes have been found post-hormone-treatment (PHT) in the enzymic, transport, electrical and contractile properties of the surface membrane. Experimental evidence exists that immediate membrane changes are related to signal transduction, whereas late membrane alterations, which lead to competence for cortical granule extrusion and for the polyspermy block, are dependent on protein synthesis during maturation. So far we know relatively much about the changes in functional properties of the plasma membrane but nothing about the ultrastructural aspects. We have analysed the membrane organization at five different stages by freeze-fracture electron microscopy, using membranes taken from the animal half of defolliculated oocytes and eggs. The following stages were chosen: (i) full-grown, unripe oocytes; (ii) 5 min progesterone-stimulated oocytes; (iii) oocytes at germinal vesicle breakdown (GVBD) after 5 hr of progesterone treatment; (iv) fertilizable eggs stripped during oviposition; and (v) zygotes 30 min post-fertilization.

Our data show that noticeable changes take place in the plasma membrane during maturation and fertilization. A distinction could be made between a rapid PHT response taking several minutes and a slow response taking several hours. The earliest effect observed (5 min PHT) is an overall reduction in IMP density from  $996 \pm 43$  to  $821 \pm 21$  IMPs/ $\mu\text{m}^2$ . After 5 hr exposure to progesterone (5 hr PHT) the plasma membrane has changed, showing a mosaic of IMP-poor domains and areas occupied by IMP-dense membrane. The IMP-poor domains correspond to the positions of cortical granules. The densities of the IMP-poor and IMP-dense regions computed separately were  $975 \pm 36$  and  $1327 \pm 34$  IMPs/ $\mu\text{m}^2$ , respectively. After pooling the data the overall IMP density appeared to have increased by 13% since defolliculation, i.e. from  $996 \pm 43$  to  $1133 \pm 47$  IMPs/ $\mu\text{m}^2$ . The main increase in IMP density was not in the domains

above cortical granules but in "unshielded" areas. In fertilizable eggs the mosaic of IMP-poor and IMP-dense regions was still obvious. The IMP-poor domains showed no change in IMP density since the preceding stage. Computation of the overall density, taking IMP-poor and IMP-dense regions together, showed a 35% increase since GVBD, i.e. from  $996 \pm 43$  to  $1482 \pm 66$  IMPs/ $\mu\text{m}^2$ . In zygotes 30 min after fertilization the IMP density had increased from  $996 \pm 43$  to  $1647 \pm 19$  IMPs/ $\mu\text{m}^2$ , i.e. by 65% since the stage of defolliculation. Comparing the patterns of IMP density we arrived at the conclusion that the IMP density increases by 48% during maturation, the relatively larger increase (35%) occurring after GVBD and another 17% after fertilization. The increase in IMP density, which is slow, steady and selective (i.e., the IMP-poor domains are not involved) is interpreted as evidence that new proteins are incorporated into the membrane. The plasma membrane "shielded" by cortical granules does not show such an increase. We presume that the IMP-poor domains are regions specialized for fusion with the membrane of cortical granules.

Although there is a steady increase in IMP density during maturation there is also a transient fast decrease after 5 min exposure to progesterone. Following the view that progesterone interacts with the plasma membrane, this decrease can be explained as a hormonal effect on the membrane lipids, as a result of which proteins are expelled from the membrane or become invisible by a change in conformation. The question whether progesterone acts by affecting membrane viscosity needs to be investigated with the FPR technique. The results have been submitted for publication (see publ.23).

### 3. Calcium detection in organelles near the animal hemisphere egg cortex by X-ray microprobe analysis (*Xenopus laevis*)

Surface contraction of non-muscular animal cells is basic to processes of cytokinesis, endo- and exocytosis-mediated transport, cell shape changes during cell migration, and morphogenetic movements. The mechanical forces are thought to be generated by contraction of actin filaments closely associated with the surface membrane. Firm evidence exists that in the amphibian egg contraction is triggered by  $\text{Ca}^{++}$ , presumably by translocation of compartmentalized  $\text{Ca}^{++}$  in the egg cortex.  $\text{Ca}^{++}$  translocation by  $\text{Ca}^{++}$ -sequestering membranous reticulum in the cortex is a possible mechanism. In this study we were interested in visualizing  $\text{Ca}^{++}$  in cellular compartments, which presumably is translocated when massive contraction is provoked. To this end unfertilized eggs in a relaxed and in a contracted state were prepared for the demonstration of  $\text{Ca}^{++}$  deposits at the ultrastructural level using  $\text{Ca}^{++}$  precipitants and X-ray microprobe analysis.

Inside the egg, in mitochondria and in vesicles of endoplasmic reticulum, granules were seen containing  $\text{Ca}^{++}$ . These deposits were reduced by exposing sectioned material or egg fragments to EGTA. Noticeable amounts of  $\text{Ca}^{++}$  were revealed by X-ray microprobe analysis of sectioned yolk and pigment granules, although no  $\text{Ca}^{++}$  deposits were seen in these organelles. The finding of  $\text{Ca}^{++}$  deposits in mitochondria and membranous elements of the endoplasmic reticulum is in line with findings in many other types of cell. Comparing specimens taken from eggs in the relaxed and in the contracted state, no evidence for  $\text{Ca}^{++}$

relocation could be found. We assume that the methods used were not sensitive enough to demonstrate the movement of  $Ca^{++}$  during induced contractions.

## II. Research Group: Organogenesis in Mammals

*Members:* K.A. Lawson; W.W. Cator (temporary)

K.A.L. devoted the first half of the year to the final analysis of material to be published, and to making several publications ready for the press.

In August she left for a leave of absence of nine months in the U.S.A., where she worked in the laboratory of Prof. R. Pedersen, Dept. of Radiobiology and Environmental Health, Univ. of California, San Francisco, CA. Here she learned methods of postimplantation mouse embryo culture, and started work on cell lineage in the mouse embryo, using techniques of cell marking by injecting markers into single cells. The results will be reported next year.

The work on epithelial-mesenchymal interactions in organogenesis will be discontinued.

## III. Research Group: Morphogenesis in Cellular Slime Moulds

*Members:* A.J. Durston, C.J. Weijer (res.assoc.); A. Timmermans, F.v.d. Wiel.

*Guest workers:* S.A. McDonald (Princeton, NJ, U.S.A.).

*Graduate students:* W.de Lau, M. Heideveld, R. Aerts, M.de Boer.

*Collaboration:* H. Jongkind (Rotterdam), A. Lindenmayer (Utrecht).

Embryonic development involves the transition from a genetically defined undifferentiated structure (usually a fertilized egg) to a recognizable adult, containing quantitatively defined ratios of differentiated cell types in quantitatively defined patterns and shapes. This transition is often homeostatically regulated. Removing part of a developing embryo typically initiates a recovery process, whereby a normally proportioned embryo is reformed.

The developmental process occurs via a tree of decisions by individual cells. One frequent type of decision is an apparent binary switch, whereby an undifferentiated cell differentiates either to cell type A or to cell type B. Like the whole developmental process, such binary switches are presumably quantitatively regulated as regards number and position of the differentiated cell types, and they can also be homeostatically regulated. They are an exceedingly important part of the developmental process that has hardly been investigated in any experimental system. Understanding them is of fundamental importance for understanding development in general; disturbances of development via environmental teratogens; and the homeostatic defects evident in cancer.

During the last few years, we have been working on morphogenesis and cell differentiation in the cellular slime mould *Dictyostelium discoideum* (Dd). This is a simple multicellular micro-organism (genome only 8 x larger than that of *E. coli*), whose differentiation sequence

consists of only one binary switch and associated temporal changes. The undifferentiated Dd cells (amoebae) are induced to differentiate by starvation. They then aggregate chemotactically after an 8-hour lag and each aggregate develops into an approximately cylindrical multicellular slug, consisting of a spatial pattern of two cell types (anterior prestalk cells and posterior prespore cells). The pattern of differentiation in the slug is quantitatively defined and is also homeostatically regulated, since if a slug is sectioned to separate the prestalk and prespore zones, each zone regenerates a whole slug. The prestalk and prespore cells later differentiate irreversibly into stalk and spore cells in a fruiting body. We chose to work with this organism because of its simplicity, and also because of practical advantages. It is possible to obtain very large numbers of Dd cells (ca.  $10^{11}$ ). The organism is haploid, and has good conventional and molecular genetics and many developmental mutants. There is also a large literature containing much background information relevant to Dd pattern formation.

Our work over the last few years has concentrated on formation of the prestalk/prespore pattern in Dd and has led us to the conclusion that this occurs via two separable processes: a cell type homeostasis mechanism, which produces and maintains the correct ratio of prestalk and prespore cell types, but does not arrange these in a spatial pattern, and a cell sorting mechanism, which arranges prestalk and prespore cells in an axial pattern, via chemotaxis of prestalk cells to 3', 5', cyclic AMP (cAMP)<sup>1-4</sup>. Our work on these two aspects over the past years has involved making time-lapse films<sup>1-3</sup>, electrophysiological investigations<sup>5</sup>, density gradient separation of the cell types<sup>6</sup> (publ.27) and investigation of their properties, via a variety of methods (see also <sup>4</sup>). We found that the Dd cell types differ in cAMP-modulated cell adhesiveness<sup>7</sup> and cAMP oscillation frequency<sup>8</sup>. These differences may account for their different behaviour leading to cell sorting and also for other features of morphogenesis. We also examined the properties of cell type homeostasis, which we were able to obtain *in vitro*, and which appears to be modulated by feedback inhibition, one inhibitor being cAMP<sup>9</sup>. This year, we have concentrated on the origin of the Dd cell types and the relation of differentiation to the cell cycle. We also started a programme to seek cell type-specific monoclonal antibodies and started to look into the possibility that Dd can be used as a pre-screening system for teratogenic agents (see sect.V.3).

1. Durston, A.J., F. Vork and C. Weinberger (1978) - In: Biochemical and Biophysical Information Transfer in Recognition (Eds. J.G. Vassileva-Popova and E.V. Jensen), Plenum, New York, 693-708.
2. Matsukuma, S. and A.J. Durston (1979) - J. Embryol. exper. Morphol. 50, 243-251.
3. Durston, A.J. and F. Vork (1979) - J. Cell Sci. 36, 261-279.
4. Durston, A.J. and C. Weijer (1980) - Vakblad voor Biologen 16, 320-327.
5. Weijer, C., A.J. Durston and S.W. de Laat - submitted.
6. Weijer, C., S. McDonald and A.J. Durston - submitted.
7. Weijer, C., W. de Lau and A.J. Durston - submitted.

8. Weijer, C., S. McDonald and A.J. Durston - submitted.
9. Weijer, C. and A.J. Durston - submitted.

## D. MORPHOGENESIS AND PATTERN FORMATION IN *DICTYOSTELIUM DISCOIDEUM*

### 1. Differentiation and the cell cycle

As reported previously (see reports for 1980 and 1981) we developed a density-gradient centrifugation method to separate the Dd cell types, and exploited this to characterize their properties. One of our findings was that cell subpopulations of different densities can be separated very early in Dd development (in fact, even at  $T_0$ , i.e. before development begins), and that different early subpopulations are later destined to become the prestalk and prespore cell types respectively. If very early low-density cells are labelled with a fluorescent marker (TRITC) and followed through development, they are later found in the prestalk zone of the slug. Early high-density cells are later found in the prespore zone of the slug.

Density-gradient centrifugation is known to separate phases of the cell cycle in various cell types (e.g. yeast<sup>1</sup>) and it occurred to us that the differently destined subpopulations separable from  $T_0$  Dd cells (which appear identical to each other, but are known to be actively dividing) might consist of different cell cycle phases, and also that the cell types might differ in cell cycle behaviour throughout development. This conclusion is already indicated by the facts that prestalk and prespore cells in the slug stage differ in the incidence of mitosis<sup>2</sup> and of nuclear <sup>3</sup>H-thymidine incorporation<sup>3</sup>. The investigations reported below were initiated to investigate the relationship between differentiation and the cell cycle in Dd.

1. Hartwell, L.E. (1970) - *J. Bacteriol.* 104, 263.
2. Bonner, J.T. and E.B. Frascella (1952) - *J. Exp. Zool.* 121, 561-572.
3. Durston, A.J. and F. Vork (1978) - *Exp. Cell Res.* 115, 454-457.

*i. Cell cycle properties of the density classes.* We wished to determine the occurrence of particular cell cycle phases in density classes from various stages in development. We therefore measured their DNA content (per cell) (usually using Hoechst 33258<sup>1</sup>, but findings were also checked in particular cases with DAPI<sup>2</sup>, DABA<sup>3</sup> and the Burton method<sup>4</sup>).<sup>☆</sup> We also measured <sup>3</sup>H-thymidine incorporation and growth characteristics, for density classes separated at the beginning of development ( $T_0$ ). For growth characteristics, cells from each density class were re-inoculated into growth medium and cell number, DNA content and <sup>3</sup>H-thymidine incorporation were followed with time. We reasoned that if gradient

<sup>☆</sup> All of these methods were DNA specific, and stoichiometric with our material over an appropriate range, and, as far as checked, all showed similar relationships as regards DNA content, between particular density classes and developmental stages. The methods all showed somewhat different absolute values, no doubt due to their differing chemical specificities.

fractions contain purified cell cycle phases, they should grow synchronously, their synchrony characteristics being determined by the cell cycle phase (e.g. G<sub>1</sub> cells should make DNA after a lag and then double after another lag, etc.). This growth analysis is applicable because unseparated T<sub>0</sub> cells, centrifuged similarly as the density classes (controls), grow exponentially without appreciable lag.

The results of this analysis were complex enough to defy a simple interpretation, and will not be described in full. Some important points follow. The DNA content (Hoechst) of Dd cells decreases during development. It is about 2-3 μg/10<sup>6</sup> cells for vegetative cells; about 2 μg/10<sup>6</sup> cells for aggregation-stage cells and about 1-1.5 μg/10<sup>6</sup> cells for slug cells. This confirms previous published results. The DNA content of cells and its decrease during development also varied from experiment to experiment (as much as a factor 2 variation at the vegetative stage). This variation is partly due to a varying degree of multinuclearity (typically about 10% in vegetative cells, but sometimes as much as 50%; usually no multinuclearity (=0%) by the aggregation stage). We suspect that Dd cells also have a variable mitochondrial DNA content.

The DNA contents of density fractions from each stage also showed variations, but the same was not generally true of their multinuclearity. Differences in multinuclearity contribute little to differences in DNA content between different density fractions. In early stages (T<sub>0</sub> and aggregation) there was typically a low-density fraction (containing cells which are destined to be prestalk and not constituting more than about 5% of the total population) which had low DNA content (ca. 1 μg DNA/10<sup>6</sup> cells). The next lowest density fractions typically had high DNA content (ca. 2-3 μg/10<sup>6</sup> cells) and the densest fractions (which have a tendency to become prespore) had intermediate values. In the slug stage the lowest density fractions (prestalk-enriched) have an intermediate DNA content (about 1.5 μg/10<sup>6</sup> cells), and the highest density fraction (prespore-enriched) has fairly low DNA content (about 1-1.5 μg/10<sup>6</sup> cells).

<sup>3</sup>H-thymidine incorporation was examined only for the T<sub>0</sub> density fractions; at this stage incorporation, though variable, tends to peak in the middle fractions (in cells with high to intermediate average DNA content). <sup>3</sup>H-thymidine pulse labelling at T<sub>0</sub> was also used to follow the fate of "S-phase" cells, by this criterion, during later development, and this showed, interestingly, that T<sub>0</sub> incorporating cells disappear specifically from the prespore fraction in the slug stage.

The growth experiments showed that all T<sub>0</sub> gradient fractions were synchronous, but the data were difficult to interpret because most fractions showed multiple synchrony steps. The only simple result was that for the least dense fraction. This makes DNA synchronously in one step, after a 6-hr delay, and then never divides again within the 10 hr period over which the cells were followed. This result, together with the low DNA content for this fraction, may mean that T<sub>0</sub> low-density cells are in G<sub>1</sub>-phase of the cell cycle and also that they have an extended cell cycle (since normal Dd cells would divide within 8 hrs). This interpretation is open to question, because we do not know, for example, whether the observed DNA synthesis step is due to nuclear or to mitochondrial DNA synthesis.

To summarise and interpret, our findings were complicated by three factors: the (variable) presence of multinucleate cells (accounted for); the (possibly variable) availability of mitochondrial DNA (not accounted

for); and the fact that most density fractions are obviously mixtures of different cell cycle phases (not accounted for). It was clear to us that we needed another approach to the problem, and we therefore undertook flow-fluorimetric measurements of DNA content in individual cells and nuclei from particular developmental stages and density fractions (see ii below). The only two tentative conclusions that could be made so far were that low-density  $T_0$  cells, which are prestalk destined, are possibly in  $G_1$ -phase of the cell cycle and that  $^3H$ -thymidine-incorporating (possibly S-phase)  $T_0$  cells are also prestalk destined. Both findings suggest that cells early in the  $T_0$  cell cycle later become prestalk. This conclusion was confirmed in detail by a separate study (see iii below).

1. Lebarca, C. and K. Paigen (1980) - *Analytical Biochem.* 102, 344-352.
2. Brunk, C., K. Jones and T.W. James (1979) - *Analytical Biochem.* 92, 497-500.
3. Erwin, B.G., C. Stoscheck and J.R. Florini (1981) - *Analytical Biochem.* 110, 291-294.
4. Burton, K. (1956) - *Biochemistry* 62, 315-323.

ii. *Flow-fluorimetric measurements.* For reasons given under i above we decided to make a flow-fluorimetric analysis of the DNA content of individual cells and individual isolated nuclei from Dd developmental stages and density fractions. This was started in 1982, in collaboration with Prof. H. Jongkind, Department of Cell Biology, Erasmus University, Rotterdam. For these measurements we used Hoechst 33258 (as above) and also mithramycin<sup>1,2</sup>. Both were DNA-specific for our cells, and also stoichiometric with increasing material within a particular range, and both gave similar results in flow fluorimetry. Propidium iodide<sup>3</sup> was also tried, but proved unsatisfactory since it gave a high cytoplasmic background after various treatments to remove RNA. In our initial runs we measured the DNA content of ethanol-fixed Dd cells of various stages and densities, using Hoechst.

These measurements showed the surprising result that populations of all developmental stages and densities each showed only one rather broad peak of fluorescence intensity for mononucleate cells. All vegetative populations and early developmental populations showing a sufficient degree of multinuclearity also showed a minor peak (up to about 10% of the area of the major peak) of binucleate cells at exactly double the fluorescence intensity of the major peak. The relative positions (fluorescence intensities) of the major peaks from various developmental stages and density fractions mirrored the relationships between bulk DNA measurements from the same stages and fractions. These findings suggested to us: firstly, that the vegetative Dd cell cycle is unlikely to contain both a substantial  $G_1$  and a substantial  $G_2$  phase in the way suggested by previous investigators<sup>4,5</sup>, but that it consists principally of one cell cycle phase (and various observations suggest that this is  $G_2$ ); secondly, that there is probably considerable variation in the amount of mitochondrial DNA in any particular type of cell (since all peaks seen were broad but also rather symmetrical, and also since different populations of a given type - e.g. unseparated vegetative cells - had somewhat variable peak values); thirdly, that cells of different stages and

densities differ either in their mitochondrial DNA content or in their majority cell cycle phase.

To look into these aspects further, we decided to isolate and measure fluorescence of nuclei from various Dd cell types and stages. The results will be reported next year.

1. Hill, B. and S. Whatley (1975) - FEBS Letters 56, 20-23.
2. Cissman, H. and R. Tobey (1974) - Science 184, 1297-1298.
3. Fried, J., A. Perez and B. Clarkson (1976) - J. Cell Biol. 71, 172-181.
4. Katz, E. and L. Bourgignon (1974) - Devl. Biol. 36, 82-87.
5. Zada-Hames, I. and J. Ashworth (1977) - In: Development and Differentiation in the Cellular Slime Moulds. (Eds P. Cappucinelli and J.M. Ashworth). Developments in Cell Biology Vol.1. Elsevier/North Holland.

iii. *Labelling of synchronous cells.* The approaches above (i and ii) were made to investigate the cell cycle phases of density fractions obtained at time zero ( $T_0$ ) and at various developmental stages. As a different approach to the relationship between differentiation and the cell cycle we decided to make synchronously dividing cells, to label particular cell cycle phases (with TRITC: see previous report, sect. III.F.1.i), and to follow the fate of labelled cell cycle phases during later development (prespore destined or prestalk destined?) after mixing with unlabelled asynchronous cells<sup>1</sup>.

In order to perform this experiment we needed to develop a non-disturbing cell synchronization method. There are two existing synchronization methods for Dd cells, but both are physiologically disturbing, since they involve the use of mutant cells and temperature shocks<sup>2</sup> and of stationary (growth-arrested) cells<sup>3</sup>, respectively. Neither is suitable for the experiment outlined above. We therefore developed a new method (a modification of mitotic shake-off<sup>4</sup>), and this proved satisfactory for our purpose.

We used this method to show that, from the beginning until the middle of the vegetative Dd cell cycle, there is a gradually increasing tendency for cells to differentiate later as prestalk cells, until a maximum effect of 90% labelled cells becoming prestalk is reached. After this point the tendency is abruptly lost. According to <sup>3</sup>H-thymidine incorporation measurements and cell counts made during our experiments, the early, prestalk-generating part of the cell cycle corresponds to G<sub>1</sub> (1 hr), S-phase (4 hr) and the first part of G<sub>2</sub> (1 hr). We note that our density separation experiments had already predicted that G<sub>1</sub> and thymidine-incorporating cells are prestalk destined (see i above). Cells late in the cycle (later G<sub>2</sub> and mitosis) are not prestalk destined.

1. McDonald, S. and A.J. Durston (1983) - J. Cell Sci., in press.
2. Katz, E. and L. Bourgignon (1974) - Devl. Biol. 36, 82-87.
3. Soll, D., J. Yarger and M. Mirick (1976) - J. Cell Sci. 20, 513-523.
4. Terasima, T. and L. Tolmarch (1961) - Nature 190, 1210-1211.

## 2. Monoclonal antibodies

Investigation of Dd differentiation requires the availability of markers specific for the prestalk and prespore cell types. There are a number of good markers (enzymes, polyclonal and monoclonal antibodies, and cDNA probes) for the prespore cell type, but few satisfactory markers for prestalk cells. There is now (1983) a published report of several cDNA probes specific for prestalk cells, but there is still a dearth of good markers for this cell type. We thus decided, in 1982, to try to isolate monoclonal antibodies against prestalk cells. To this end we started the hybridoma technique in this Laboratory (and later set up a hybridoma facility in collaboration with the Membrane Regulation research group). Initially we injected mice with whole, purified prestalk cells, isolated directly from slugs by microsurgery, to ensure minimum destruction of cell-type specific antigens. It was anticipated that the enzymatically mediated dissociation necessary for efficient density-gradient purification of the cell types from slugs would strip the cells of (possibly cell type-specific) surface components. Dd-positive clones were screened out and checked for cell type specificity, using an ELISA against homogenates of each cell type (prepared from the same cells on the inoculation antigen). Three fusions of the above type yielded 50 Dd-specific clones, none of which was markedly cell-type specific. Because this approach appeared to have a low yield of cell-type specific clones, and because a similar approach is now being used by another Dd group, we decided to switch to injecting purified Dd antigens which are known to have cell-type specificity. This approach will be pursued in 1983.

## 3. Computer simulation of cell sorting

Our previous work (see reports for 1980 and 1981) provided evidence that Dd prestalk and prespore cells sort out during development via chemotaxis to cAMP<sup>1,2</sup>. We also showed that these cell types differ in adhesiveness (prespore cells being apparently more adhesive than prestalk cells; adhesion being stimulated by cAMP; and prespore adhesion being more stimutable by cAMP than prestalk adhesion)<sup>3</sup>. We had formed the opinion that Dd cell sorting might be directed by chemotaxis, but might be dependent on an adhesiveness difference between the cell types which preferentially impedes prespore cell movement. On the other hand, other authors have postulated, and obtained evidence suggesting to them that Dd cells sort out due to adhesive differences alone<sup>4,5,6</sup> or due to chemotactic differences alone<sup>7</sup>.

In order to provide a vehicle for testing these various ideas and to determine their capabilities to account for known features of Dd cell sorting and morphogenesis, we decided to make computer simulations of Dd cell sorting. A Fortran programme was constructed in collaboration with Prof. A. Lindenmayer, Dept. of Theoretical Biology, University of Utrecht, in which cells were represented (similarly as done previously by Goel<sup>8</sup>) as regularly shaped units in a two-dimensional array, and movement of the units was governed by appropriate algorithms representing chemotaxis, relay and cell-cell adhesion. Work in 1982 was concentrated on constructing an appropriate programme and starting to use this to examine the situation where there are adhesion differences only (and cells neither relay nor chemotact), to see whether this can generate cell

sorting phenomena as seen in Dd. This work will be continued in 1983 and the results will be reported next year.

1. Matsukuma, S. and A.J. Durston (1979) - *J. Embryol.exper.Morphol.* 50, 243-251.
2. Durston, A.J. and F. Vork (1979) - *J. Cell Sci.* 36, 261-279.
3. C. Weijer, W. De Lau and A. Durston, submitted.
4. Feinberg, A., W. Springer and S. Baronides (1979) - *Proc.Natl.Acad. Scis.* 76, 3977-3981.
5. Lam, T.Y. *et al.* (1981) - *Differentiation* 20, 22-28.
6. Tasaka, M. and I. Takeuchi (1979) - *J. Embryol.exper.Morphol.* 49, 89-102.
7. Inouye, K. and I. Takeuchi (1982) - *Exp.Cell Res.* 138, 311-318.
8. Goel, N. (1971) - *J. Theoret.Biol.* 33, 171-188.

#### IV. Research Group: Membrane Regulation

*Members:* S.W. de Laat, P.T. van der Saag, L.H.K. Defize (Ph.D. student), J. Boonstra (res.assoc.), M.H.F. van den Hoef (res.assoc.), W.H. Moolenaar (res.assoc.), C.L. Mummery (res.assoc.), S.A. Nelemans (res.assoc.), E.J.J. van Zoelen (res.assoc.); C.E. van den Brink, A. Feijen, L. Joosen, W.A.M. van Maurik, P. Meyer, Th.M.J. van Oostwaard, L.G.J. Tertoolen.

*Guest workers:* M. Hershkowitz (Israel), R. Hill (Denmark), J. Karasiewicz (Poland).

*Graduate students:* J. Janssen, H.-J. Kuiper.

*Collaboration:* J.J.M. van den Bercken (Utrecht, Netherlands), J.A.M. van den Biggelaar, A.W.C. Dorresteyn (Utrecht, Netherlands), J. Boonstra, R. van Wijk (Utrecht, Netherlands), S.K. Brahma (Utrecht, Netherlands), W.H. Gispen, H. Zwiers (Utrecht, Netherlands), K.W.A. Wirtz (Utrecht, Netherlands), M. Gadenne (Brussels, Belgium), R. Hill (Copenhagen, Denmark), J. Schlessinger, M. Shinitzky (Rehovot, Israel), G.J. Todaro, D.P. Twardzik (Frederick, MD, U.S.A.), S. Varon (San Diego, CA, U.S.A.).

*Financial support:* Research presented in this section is supported in part by the Netherlands Cancer Foundation (Koningin Wilhelmina Fonds) and Shell International Research Corporation.

#### E. MEMBRANE REGULATION IN GROWTH AND DEVELOPMENT

In this research group the molecular regulation of mammalian cell proliferation and diversification is being studied at present, with emphasis on the role of the plasma membrane. The development of a multicellular organism results from the temporally and spatially controlled proliferation and diversification of its cells. The relationship between cell proliferation and cell diversification is a crucial one for understanding the

control of development. Scheduled cell proliferation in itself can function as a driving force for morphogenesis. Equally important is the notion that during their individual life history, or cell cycle, cells go through a programme of phenotypic modifications which restricts their capacity to respond adequately to appropriate external stimuli - in terms of altered growth behaviour or gene expression - to certain phases in the cell cycle. Apparently the mechanism for the regulation of cell proliferation and cell diversification are interlinked. Terminal differentiation provides the most obvious example of such a link: cells reaching their ultimate differentiated fate usually become arrested in their growth. But also during the early phases of embryonic development changes in cell cycle properties could play a fundamental role in the process of cell diversification. Moreover, it is important to realize that specific interference with the normal differentiation pathways of cells in development could be one of the fundamental causes of carcinogenesis.

Simplifying the problem, there are two sides to the understanding of the molecular control of cell differentiation. On the one hand one needs to resolve the mechanisms by which the expression of particular genes is specified, while on the other hand detailed knowledge is required as to the nature of the appropriate signalling molecules and the molecular routes by which such signals exert their effect at the gene level. Ideally, one would like to see both aspects going hand in hand in developmental biology. For practical reasons, however, we have so far restricted ourselves to the signalling side of the problem. Here the plasma membrane is a major control site, and its regulatory functions are evident from the following considerations:

- the plasma membrane forms the barrier between the cell and its environment, and all extracellular influences (e.g., hormones; growth, differentiation and transformation factors) on intracellular processes are mediated and modified by the properties of the plasma membrane;
- cell-cell interactions modulating cellular growth, differentiation and transformation characteristics are also under the direct control of the properties of the cell surface;
- the plasma membrane is directly involved in the molecular interactions by which processes like growth, differentiation and transformation are regulated through selective membrane permeability to ions and nutrients and through membrane-bound enzymes that control the intracellular level of critical constituents.

In this context our research has been directed mainly at the analysis of 1. the molecular mechanisms by which defined growth and differentiation factors, such as Epidermal Growth Factor (EGF) and Nerve Growth Factor (NGF), are involved in the control of cell proliferation and differentiation through their binding to specific plasma membrane-bound receptor molecules; and 2. the mechanism by which cells can modulate their competence to respond to such factors during the cell cycle or differentiation by alterations of appropriate plasma membrane properties.

To this end we are studying the properties of relevant receptor molecules and the nature and relevance of the early responses of the cell evoked by ligand-receptor interaction. In addition, the causal relationships are being studied between the composition, ultrastructure and physico-chemical properties of the plasma membrane on the one hand, and on the other hand the expression of receptor sites, the functioning

of membrane-bound enzymes and transport systems responsible for the production of molecules, and the transport of ions and molecules involved in the control of growth and differentiation.

The following methods are employed in these studies: a. *biochemical* methods for the analysis of 1. plasma membrane composition (lipids and proteins); 2. regulation of membrane-bound enzymes involved in membrane ion transport ( $(\text{Na}^+, \text{K}^+)\text{-ATPase}$ ); 3. membrane transport of ions and nutrients (tracer fluxes); 4. properties of specific membrane receptors; and 5. isolation and properties of Transforming Growth Factors (TGFs); b. *biophysical* methods for the analysis of 1. mobility properties of specific membrane molecules (fluorescence recovery after laser photobleaching); 2. hydrodynamic properties of membrane lipids (fluorescence polarimetry); and 3. electrical membrane properties and ion transport (electrophysiology); c. (*ultra*)*structural* methods: 1. freeze-fracture electron microscopy for structural analysis of the membrane; 2. scanning electron microscopy for the analysis of cell surface architecture; and 3. time-lapse cinematography for the analysis of cell cycle kinetics; d. *immunological* methods: 1. preparation of monospecific antisera by conventional techniques; and 2. preparation and characterization of monoclonal antibodies to specific membrane receptors.

The nature of the questions to be resolved and of the processes to be studied requires the availability of cell systems in which growth and differentiation can be initiated or modulated in synchronous, large populations of cells. Embryonal tumour cell lines provide such experimental systems. Embryonal tumour cells can originate in different periods of embryogenesis and organogenesis.

### 1. Growth factor-membrane interaction

In this section experimental approaches will be reported which deal with questions related to the molecular mechanisms by which specific growth factors (EGF, NGF, TGFs) act upon different transformed and untransformed target cells (mouse neuroblastoma (Neuro-2A, N1E-115) cells, rat pheochromocytoma (PC12) cells, human epidermoid carcinoma (A431) cells, mouse embryonal carcinoma (F9, PCC4, PCC13) cells, mouse fibroblast (3T3) cells, normal rat kidney (NRK) cells, and human foreskin fibroblasts). In addition, the isolation, partial purification and characterization of a Transforming Growth Factor from Neuro-2A-conditioned medium will be reported.

#### 1a. Early ionic events upon growth factor action

Addition of growth factors such as EGF to quiescent cells induces a rapid influx of  $\text{Na}^+$  resulting in a stimulation of the  $\text{Na}^+, \text{K}^+$  pump. Investigations in our Laboratory have revealed that this growth factor-dependent  $\text{Na}^+$  pathway represents an electrically silent  $\text{Na}^+, \text{H}^+$  exchange system which can be blocked by the diuretic drug amiloride<sup>1,2</sup> (see also previous report, sect. IV, 1a, and publ. 14). This raised the possibility that a change in cytoplasmic pH ( $\text{pH}_i$ ) might be involved in the early action of growth factors.

In collaboration with Dr. R. Y. Tsien (University of California, Berkeley, U.S.A.) it has been possible to assess the ionic basis of  $\text{pH}_i$  homeostasis in normal human foreskin fibroblasts by monitoring changes in  $\text{pH}_i$  during growth stimulation with newly developed fluorescent  $\text{pH}_i$  probes<sup>3</sup>. Our data indicate that  $\text{pH}_i$  in fibroblasts is tightly regulated by an amiloride-

sensitive  $\text{Na}^+$ ,  $\text{H}^+$  exchange system, which rapidly restores  $\text{pH}_i$  to its resting level ( $\sim 7.05$  at  $30^\circ\text{C}$ ) after a prior acid load. This  $\text{pH}_i$  recovery is accompanied by  $\text{Na}^+$  uptake and net efflux of  $\text{H}^+$ ; these processes do not occur in  $\text{Na}^+$ -free medium and are reversibly inhibited by  $0.5 \text{ mM}$  amiloride. Activation of the  $\text{Na}^+$ ,  $\text{H}^+$  exchanger by EGF or serum factors leads to a rise in  $\text{pH}_i$  of about  $0.2 \text{ pH}$  unit within  $20 \text{ min}$ . This  $\text{pH}$  shift is markedly potentiated by insulin. Thus,  $\text{Na}^+$ ,  $\text{H}^+$  exchange does not only play a fundamental role in  $\text{pH}_i$  homeostasis but may also function as a transmembrane signal transducer in the action of growth factors (Moolenaar *et al.*, submitted for publication). Experiments designed to assess the physiological effect of a growth stimulant-induced rise in  $\text{pH}_i$  are currently in progress.

Human epidermoid carcinoma (A431) cells possess an unusually high number of EGF receptors ( $2-3 \times 10^6$  receptors/cell). This cell type is therefore commonly used as a model system for the study of the EGF receptor<sup>4</sup>. However, EGF fails to stimulate DNA synthesis in A431 cells. Using the fluorimetric technique for measuring  $\text{pH}_i$  we have identified a normally functioning  $\text{Na}^+$ ,  $\text{H}^+$  exchange system in A431 cells. Interestingly, EGF fails to stimulate  $\text{Na}^+$ ,  $\text{H}^+$  exchange and  $\text{Na}^+$ ,  $\text{K}^+$  pump activity in this system. Thus, there seems to be a correlation between EGF-induced  $\text{Na}^+$ ,  $\text{H}^+$  exchange activity and the initiation of DNA synthesis. In concordance with this hypothesis are preliminary findings obtained with Rous sarcoma virus (RSV)-transformed rat embryo fibroblasts (Rat-1 cells). Serum growth factors are unable to stimulate amiloride-sensitive  $\text{Na}^+$  influx and  $\text{Na}^+$ ,  $\text{K}^+$  pump activity in these cells. In view of the functional similarities between the RSV-oncogene product (protein kinase  $\text{pp60}^{\text{src}}$ ) and EGF receptors<sup>5</sup>, this observation supports the concept that oncogene products can usurp the molecular circuits designed for and utilized by normal polypeptide growth factors. Work is in progress to test this hypothesis further.

1. Moolenaar, W.H., C.L. Mummery, P.T. van der Saag and S.W. de Laat (1981) - *Cell* 23, 789-798.
2. Moolenaar, W.H., J. Boonstra, P.T. van der Saag and S.W. de Laat (1981) - *J. Biol. Chem.* 256, 12883-12887.
3. Rink, T.J., R.Y. Tsien and T. Pozzar (1982) - *J. Cell Biol.* 95, 189-196.
4. Cohen, S., H.T. Haigler, G. Carpenter, L. King and J.A. McKanna (1979) - *Cold Spring Harbor Conf. Cell Prolif.* 6, 131-142.
5. Erikson, R.L., A.F. Purchio, E. Erikson, M.S. Collett and J.S. Brugge (1980) - *J. Cell Biol.* 87, 319-325.

*1b. Monoclonal antibodies to cell surface antigens: immunological approach to growth factor receptors*

In 1975 Köhler and Milstein<sup>1</sup> demonstrated that individual clones of normal antibody-secreting cells (antigen-activated B-lymphocytes) could be immortalized by fusion with plasmacytoma (myeloma) cells, the transformed counterparts of B-lymphocytes. The hybrid cells obtained are called hybridoma's and have characteristics of both parent cells: they have the capacity to propagate indefinitely under routine cell culture

conditions and to produce the antibody the B-lymphocyte was programmed for. Since these hybridoma's can be grown under clonal conditions, the production of an antibody with single specificity is possible. The hybrid cells can be stored in liquid nitrogen and recultured indefinitely, provided variant cells that no longer produce antibody are eliminated when necessary by recloning. Antibody may be obtained from the hybridoma's as culture medium or as ascitic fluid. Culture medium usually contains the antibody together with fetal calf serum. Ascitic fluid is produced when hybridoma's are grown in the peritoneal cavity of mice and contains a hundredfold higher concentration of antibody than culture medium (for recent reviews see<sup>2-4</sup>). Monoclonal antibodies have some revolutionary practical advantages over conventional antisera, but they also have limitations. All the molecules of a given monoclonal antibody are identical in amino acid sequence and hence in recognition properties, so that the antibody can have exceptional specificity. A monoclonal antibody is insignificantly contaminated by other irrelevant antibodies, and it will therefore give a low background in assays and staining reactions. A monoclonal antibody can be reproduced and distributed indefinitely and its properties will always be the same. A classical antiserum contains antibodies to a number of antigenic determinants on its target antigen, whereas a monoclonal antibody will bind to one determinant only. An antiserum therefore provides quite a precise identification of its target antigen: an unknown molecule that can compete for all its antibody molecules, as in a classical radio-immunoassay, will almost certainly be identical to the known target antigen. In contrast, a monoclonal antibody is unable to distinguish between a group of different molecules that all bear the antigenic determinant it recognizes, or even between determinants that have sufficient structural similarity to bind the antibody. Also, monoclonal antibodies will not usually precipitate their target antigen because they can only cross-link antigen into dimers rather than form a lattice. The major advantage of the monoclonal antibody technique over the possibilities of conventional antibodies is the fact that it is unnecessary to purify the antigen in the former, provided adequately selective screening assays are available. This advantage is also obvious in the first project the monoclonal antibody technique was employed for in our current research. It was decided to prepare monoclonal antibodies against the EGF receptor because 1. the EGF receptor is an important cell surface protein molecule involved in growth control and can serve as a model for other growth factor/hormone receptor molecules in the plasma membrane (for a review see publ.29); 2. important molecular properties of the EGF receptor are already known. It consists of a single transmembrane polypeptide chain (Mol. weight 170 Kd) containing an intrinsic tyrosine kinase on the cytoplasm-oriented side, which phosphorylates tyrosine residues on itself and on other target proteins upon EGF binding (publ.29); 3. EGF plays an important role in embryonic development of mammals (publ.29). Therefore the study of EGF receptor expression during development can further elucidate this role.

To obtain monoclonal antibodies against EGF receptor, plasma membrane vesicles were prepared from human epidermoid carcinoma (A431) cells carrying an exceptionally high number of EGF receptors ( $2-3 \times 10^6/\text{cell}$ )<sup>3</sup>. Mice were subcutaneously injected with 0.1 mg protein of a membrane vesicle preparation in Freund's complete adjuvant. After two weeks the

same dose was injected intra-peritoneally without Freund's, while after another two weeks the final booster injection was given similarly. Four days later the mice were sacrificed and the isolated spleen cells were fused to myeloma cells, the non-secreting cell line SP2/0 of Balb/c origin. Hybridoma's were distributed over 96-well tissue culture plates under hybridoma-selecting conditions. The first screening of culture supernatants was an ELISA (enzyme-linked-immuno-sorbent-assay) on a fixed monolayer of A431 cells, using peroxidase-antimouse serum as second antibody. Forty hybridoma cultures that reacted very strongly in this test were grown at a larger scale, subsequently stored in liquid nitrogen, and the corresponding culture media were used for more specific screening tests: 1. an ELISA on protein blots of A431 membrane proteins separated by SDS polyacrylamide gel electrophoresis (so-called Western blotting procedure). Ten of the forty culture supernatants reacted positively with a 170 Kd protein on these blots; 2. cell rounding test: EGF causes rapid morphological changes (rounding up) of A431 cells in the absence of  $Ca^{2+}$ . Antibodies against the EGF receptor can be expected to induce similar morphological changes. Twenty-two supernatants reacted positively in this test; 3. an ELISA on EGF-treated A431 cells vs. non-treated cells. Binding of EGF to its receptor results in receptor internalization and thus in a lower number of receptors in the plasma membrane (so-called "down-regulation"). Therefore supernatants the binding of which was reduced in EGF-treated cells were scored positively in this test: seventeen supernatants displayed such reduced binding; 4. EGF receptors can be solubilized by non-ionic detergents (Triton X-100) while retaining their EGF binding capacity<sup>6</sup>. Solubilized receptors were incubated with hybridoma supernatant, after which the possibly formed antigen-antibody complexes were immunoprecipitated with rabbit anti-mouse antibodies coupled to protein A-Sepharose beads. The beads were centrifuged off and the resulting supernatant was tested for remaining EGF binding capacity. So far, ten supernatants were found to precipitate between 50 and 100% of the EGF binding activity originally present.

These results permit the conclusion that monoclonal antibodies against EGF receptor will be available soon, and that the production of monoclonal antibodies against other specific membrane molecules can be expected in this Laboratory in the coming years.

1. Köhler, G. and C. Milstein (1976) - *Eur.J.Immunol.* 6, 511-519.
2. Goding, J.W. (1980) - *J.Immunol.Meth.* 39, 285-308.
3. Eisenbarth, G.S. (1981) - *Anal.Biochem.* 111, 1-16.
4. Edwards, P.A.W. (1981) - *Biochem.J.* 200, 1-10.
5. Cohen, S., H. Ushiro, C. Stoscheck and M. Chinkers (1982) - *J.Biol.Chem.* 257, 1523-1531.
6. Cohen, S., G. Carpenter and L. King Jr. (1980) - *J.Biol.Chem.* 255, 4834-4842.

*1c. Transforming growth factors (TGFs): isolation and characterization from neuroblastoma cell-conditioned medium*

When compared to their normal counterparts a variety of transformed

cells have lost the ability to bind certain specific polypeptide growth factors, such as epidermal growth factor (EGF), nerve growth factor (NGF) or insulin-like growth factors (IGFs). Todaro and co-workers<sup>1,2</sup> have shown that this inability to bind externally provided ligands is due to the fact that these cells have (re-)acquired the ability themselves to synthesize and release specific growth factors. In particular, certain tumour cells which have no EGF membrane receptors measurable by means of the <sup>125</sup>I-EGF binding assay produce potent growth factors (TGFs) that are in some unknown way related to EGF.

TGFs are mitogenic peptide hormones with the additional ability of conferring a transformed phenotype on untransformed cells and inducing them to form growing colonies in soft agar, a process which is reversible upon removal of TGF. Best characterized is a group of TGFs with molecular weights ranging from 6 to 30 Kd, which compete with EGF for binding to its receptor but are antigenically unrelated to it. These so-called TGF $\alpha$  have been detected both in cell extracts and in conditioned culture medium of a variety of virally, chemically and spontaneously transformed cells, and recently also in the urine of patients with disseminated tumours. There are indications that TGF $\alpha$  production by tumour cells results from an inappropriate re-expression of an embryonic gene upon cell transformation. TGF $\alpha$  has been detected in extracts of mouse and rat embryos, and in the urine of pregnant women. However, the possible role of TGFs during embryonic development is still obscure.

Recently Anzano and co-workers<sup>3</sup> have shown that the ability of TGF $\alpha$  to induce a transformed phenotype in non-transformed cells requires the additional presence of a second type of growth factor, now called TGF $\beta$ . This polypeptide (12 Kd) does not compete with EGF for receptor binding and has been detected in a variety of neoplastic and non-neoplastic tissues. It has no mitogenic or soft agar-growth-inducing activity by itself, but acquires the latter activity in the presence of either TGF $\alpha$  or EGF. It is therefore disputable whether this factor should be called a growth factor. Some authors therefore prefer to designate TGF $\beta$  as a growth factor-modulator protein.

Besides these two types of TGF, in a variety of transformed cells, embryocarcinoma cells and embryonic tissues mitogenic TGFs have been identified which do not compete with EGF for receptor binding nor are potentiated by EGF. The exact characteristics of this group of TGFs are still unclear since the cellular receptors involved have not yet been identified.

We have reported earlier (Progress Report 1980, sect.IV.I.14) that Neuro-2A neuroblastoma cells can be cultured permanently in a chemically defined serum-free medium consisting of Dulbecco's modified Eagle's and Ham's F12 medium (1:1) supplemented solely with sodium selenite (30 nM) and transferrin (0.01 mg/ml). The growth rate in this medium is comparable to that observed in serum-containing medium. The capacity of these cells to grow without added exogenous growth factors distinguishes Neuro-2A cells from almost all other normal and transformed cell lines, which have an absolute requirement for growth factors in their culture medium. This observation prompted us to investigate whether Neuro-2A cells are capable of producing polypeptide growth factors. This investigation was carried out in collaboration with Dr. G.J. Todaro and Dr. D.P. Twardzik (National Institute of Health, Frederick, MD, U.S.A.) and for this purpose E.J.J. van Zoelen stayed in their Laboratory for four months.

Neuro-2A cells were grown in the medium described above and the conditioned medium was collected, filtered to remove cellular debris, and lyophilized. This material was then extracted with 1 M acetic acid, dialysed against 0.2 M acetic acid, and again lyophilized. This extract was then brought on a Bio-Gel P-100 column in 1 M acetic acid to achieve separation of proteins according to molecular weight. Figure 1 shows the elution profile of this column as obtained from four litres of conditioned medium. Most of the protein (Fig. 1A) has a molecular weight above 29 Kd (calibrated with marker protein of known molecular weight), mainly due to the presence of transferrin in the medium. The mitogenic activity, however, as tested on quiescent 3T3 cells, shows its major peak between 15 and 20 Kd. This activity does not compete with EGF for binding to the

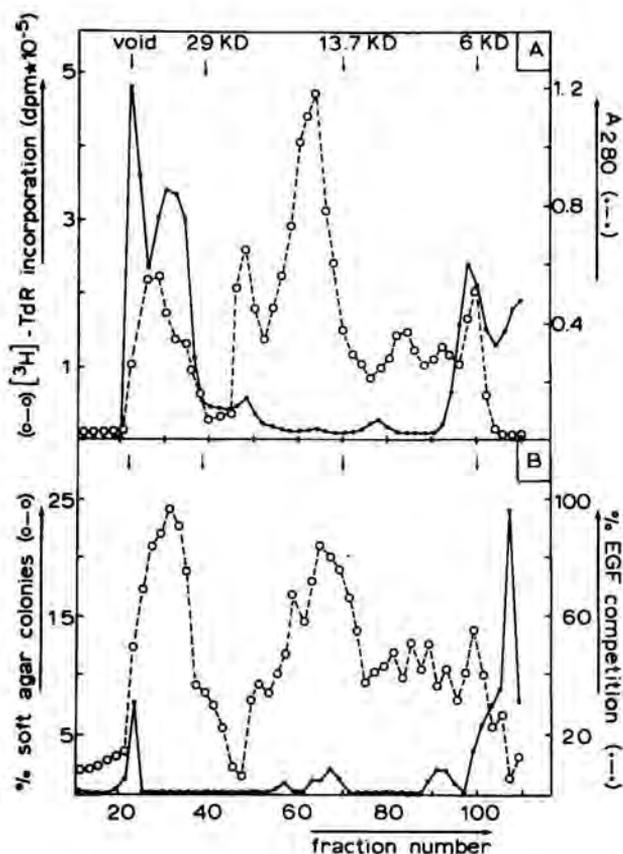


Fig. 1. Elution profile of Neuro-2A-conditioned medium on a Bio-Gel P-100 column (100 x 2.6 cm). 4 l. conditioned serum-free medium was extracted with 1 M HAc and eluted from the column in the same buffer; 3.5 ml fractions were collected. Aliquots were assayed for protein content (A<sub>280</sub>; A) and mitogenic activity towards quiescent 3T3 cells (0.1 ml; A), and for competition with <sup>125</sup>I-EGF for binding to fixed A431 cells (0.8 ml; B) and induction of soft agar growth of normal rat kidney cells (0.5 ml; B).

EGF receptor (Fig.1B), emphasizing that this factor is not identical to TGF $\alpha$ . However, the mitogenic activity correlates very well with the ability to induce growth of untransformed cells in soft agar (Fig.1B), demonstrating that this neuroblastoma cell-derived growth factor belongs to the class of transforming growth factors. We therefore call it ND-TGF. Stability tests have shown that ND-TGF is heat- and acid-stable but sensitive to proteolytic enzymes and sulphhydryl compounds.

These observations suggest that Neuro-2A cells can grow in the absence of externally supplemented growth factors because they produce growth factors themselves. It remains to be established, however, whether Neuro-2A cells can be mitogenically stimulated with the ND-TGF preparation (autocrine growth stimulation). In order to achieve further purification the preparation will be subjected to HPLC (high pressure liquid chromatography). The purified preparation will be tested for its ability to induce soft agar growth in the absence of TGF $\beta$ , and will be used to identify the receptor molecule involved in the mitogenic action of ND-TGF, and to prepare antibodies against it.

1. Todaro, G.J. *et al.* (1981) - J.Supramolec.Struct.Cell.Biochem. 15, 287-303.
2. Todaro, G.J. *et al.* (1982) - In: Tumor cell heterogeneity, origin and implications. Bristol-Myers Cancer Symposia (eds. A.H.Owens *et al.*) Vol.4, Academic Press, New York, pp.205-224.
3. Anzano, M.A. *et al.* (1982) - Cancer Res. 42, 4776-4778.

## 2. Differentiation and development

In this section experiments will be reported that mainly concern the *in vitro* differentiation of a number of embryonal tumour cell systems currently employed in this Laboratory: mouse neuroblastoma (N1E-115), rat pheochromocytoma (PC12), and mouse embryonal carcinoma (F9, PC13) cell. Important areas of study are the regulation of receptor expression, cholesterol and lipid synthesis, growth and differentiation in defined serum-free culture media, and the regulatory role of the cell cycle. The establishment of pluripotential cell lines derived directly from early mouse embryos is in progress. Mouse oocytes and embryos are being studied with ultrastructural and biophysical methods. (The morphological differentiation of neuroblastoma cells is being studied as a possible *in vitro* test system for teratogenic agents - see Section V.3).

### 2a. EGF receptor expression and lipid metabolism in N1E-115 neuroblastoma cells

As described in the previous report (sect.IV.H.3a and IV.H.3b) a substantial decrease in the number of EGF receptor sites occurs during morphological differentiation of neuroblastoma (N1E-115) cells upon serum deprivation. It is probable that the acquisition of competence for differentiation is related to this observed loss of EGF receptor expression. Moreover, important changes in the lipid composition of isolated plasma membranes have been found, which could greatly affect the dynamic behaviour of lipids and proteins in the plasma membrane. Cholesterol/phospholipid ratio and sphingomyelin content both increased significantly

upon differentiation. In accordance with these findings treatment of differentiating cells with specific inhibitors of cholesterol biosynthesis was observed to block morphological differentiation in a reversible manner: addition of mevalonate restored differentiation. These results indicate a specific role for sterol synthetic processes during differentiation of neuroblastoma cells.

By  $^{125}\text{I}$ -EGF binding studies it was established that the number of EGF receptors in differentiating N1E-115 cells gradually decreases from 19,500 to 5,300 after 24 hr of serum withdrawal (publ.32). This decrease is accompanied by an increase in receptor affinity. It was subsequently demonstrated that both the EG-stimulatable ( $\text{Na}^+$ , $\text{K}^+$ ) ATPase activity (as measured by ouabain-sensitive  $^8\text{Rb}$  influx) and the amiloride-sensitive  $\text{Na}^+$  uptake ( $\text{Na}^+$ , $\text{H}^+$  exchange) are dependent on the number of occupied EGF receptors (publ.32). These results suggest that the observed loss of EGF receptors during differentiation is a necessary condition for irreversible (neuronal) differentiation. This hypothesis was further strengthened by the observation that treatment of differentiating N1E-115 cells with specific inhibitors of cholesterol biosynthesis blocked the decrease in the number of EGF receptor sites and prevented morphological differentiation. Although further experiments are needed to clarify the mechanisms controlling the number and affinity of EGF receptors during neuroblastoma differentiation, it is clear that the physico-chemical properties of the plasma membrane and their underlying metabolic processes are somehow involved.

To be able to investigate these mechanisms the growth of N1E-115 cells under serum-free defined conditions is an obligatory step. Although such a medium is available for Neuro-2A neuroblastoma cells, each different cell line has its specific requirements and conditions have to be adapted specifically. N1E-115 cells can now be subcultured in Dulbecco's modified Eagle's/Ham's F12 medium (1:1 v/v) supplemented with transferrin (10  $\mu\text{g}/\text{ml}$ ), EGF (250 ng/ml) and insulin (10  $\mu\text{g}/\text{ml}$ ). Additional coating of the culture dish with extracellular matrix components is necessary. Morphological differentiation can be induced by omission of insulin only, upon which cells stop dividing. These results offer new possibilities to study the molecular processes underlying neuroblastoma cell differentiation. Preliminary results were obtained by studying lipid synthesis under these conditions, and showed that there is a preferential increase in the rate of sterol synthesis as compared to other lipid classes, thus supporting the findings described above.

Finally, in relation to the findings concerning changes in sterol metabolism during morphological differentiation, experiments have been started employing the fluorescence photobleaching recovery (FPR) technique. As reported in 1981, fluorescence polarization measurements on isolated plasma membranes and their lipid extracts indicated a significant decrease in membrane fluidity four days after serum withdrawal. The obvious advantage of the FPR technique is the possibility it offers to make measurements on intact cells *in situ* or at different locations within one cell, e.g. neurite vs. cell body. Preliminary results using different lipid probes indicate a 50% decrease in lateral lipid mobility, which is already evident 24 hr after serum withdrawal.

#### 2b. EGF and NGF action in rat pheochromocytoma (PC12) cells

Nerve growth factor (NGF) induces morphological, electrical and bio-

chemical differentiation of PC12 cells. The cells acquire a phenotype strongly resembling neuronal cells and thus are used as an *in vitro* model system for the study of neuronal differentiation, and particularly for the way of action of NGF<sup>1</sup>. The first interaction of NGF with its target cells occurs through binding to specific high-affinity receptor sites at the cell surface (for a review see publ.29). We have recently demonstrated that NGF stimulates the Na<sup>+</sup>,K<sup>+</sup> pump within minutes after addition to PC12 cells<sup>2</sup> as well as to chick embryo dorsal root ganglion cells (publ.5), similar to the effects of EGF on the Na<sup>+</sup>,K<sup>+</sup> pump in other cell lines. In addition to NGF receptors, PC12 cells also carry distinct cell surface receptors for EGF, the number of which decreases dramatically during NGF-induced differentiation<sup>2</sup>. Despite the opposite ultimate biological effects of NGF and EGF, i.e. neuronal differentiation and cessation of cell division vs. growth stimulation<sup>2</sup>, the two factors display a number of similar effects as well. Among the common events evoked by NGF and EGF are the induction of ornithine decarboxylase and tyrosine hydroxylase, the enhancement of cellular adhesion, and the stimulation of choline and orthophosphate incorporation into macromolecules. To this list of common responses we can now add the activation of the Na<sup>+</sup>,K<sup>+</sup> pump, since EGF stimulated ouabain-sensitive <sup>86</sup>Rb influx in a similar way as NGF, although the kinetics of the responses differed markedly. NGF caused a persistently higher Na<sup>+</sup>,K<sup>+</sup> pump activity over a period of 50 min, whereas the EGF-mediated response reached a transient maximum after 20 min. In addition, it could be demonstrated that both NGF and EGF also stimulated Na<sup>+</sup> influx nearly two-fold after 20 min, which was completely inhibited by amiloride, suggesting the involvement of the electroneutral Na<sup>+</sup>,H<sup>+</sup> exchange system. The presence of a functional Na<sup>+</sup>,H<sup>+</sup> exchange mechanism in PC12 cells was experimentally established by H<sup>+</sup> efflux measurements and by the effects of weak acids on Na<sup>+</sup> influx. Finally, a possible explanation for these parallel effects of NGF and EGF was suggested by the observation that cell growth was significantly stimulated during the first 24 hr upon addition of NGF. Growth stimulation by EGF was smaller than by NGF. Amiloride completely inhibited it in both cases, while the ultimate NGF-mediated differentiation was unaffected. These results demonstrate that both NGF and EGF stimulate growth of PC12 cells initially, but prolonged incubation with NGF results in growth arrest and neuronal differentiation. This growth-stimulating activity of both NGF and EGF is mediated through a Na<sup>+</sup>,H<sup>+</sup> exchange mechanism (manuscript submitted).

1. Greene, L.A. and A.S. Tischler (1979) - Proc.Natl.Acad.Sci.USA 73, 2424-2428.

2. Huff, K.R., D. End and G. Guroff (1981) - J.Cell Biol. 88, 189-198.

### 2c. Tumour-promoting phorbol esters: tools in the study of differentiation and development

Compounds with tumour-promoting activity have been isolated from croton oil, a product of the seeds of *Croton tiglium* (Euphorbiaceae), and have been identified as esters of the macrocyclic diterpene alcohol phorbol in the late sixties. Since then phorbol esters and their mode of action have been extensively studied and reviewed<sup>1-4</sup>. Phorbol esters have also proved to be powerful tools in the study of development and

differentiation. Their tumour-promoting activity appears to be related generally to their capacity to derange normal development *in vivo* and *in vitro* in mammals, birds and invertebrates, suggesting that they interact with general mechanisms involved in the regulation of development and differentiation, or that they can replace yet unidentified endogenous modulating molecules. Their ability to interfere with normal development and differentiation may well be central to their action as tumour promoters (for reviews see <sup>5</sup> and publ.29). The capacity of phorbol esters to affect spontaneous or induced differentiation has been demonstrated for a great variety of normal and transformed cells. Although in most cell types phorbol esters will inhibit cell differentiation, in some cell systems they induce differentiation instead. This shows that the biological activity of tumour promoters is dependent on the target cell, the state of differentiation of both embryos and cultured cells probably being the determinative factor.

The rapid effects of phorbol esters on a variety of plasma membrane properties have prompted the search for specific membrane receptors. Initial attempts using the most active phorbol ester in the mouse skin carcinogenesis system, 12-0-tetradecanoyl phorbol-13-acetate (TPA), were hampered by the lipophilic nature of the TPA molecule, which leads to non-specific partitioning into membranes. A breakthrough was reached when [<sup>3</sup>H] phorbol 12,13-dibutyrate (PDBu) was used in the binding studies<sup>6</sup>. PDBu is active *in vivo* and *in vitro* but is much less lipophilic than TPA, and has been used to demonstrate the occurrence of specific high-affinity receptor sites for PDBu on plasma membranes of a variety of normal and transformed mammalian and avian cells and tissues, including cells of embryonic origin<sup>7</sup>.

As a first step in this project, <sup>3</sup>H-PDBu binding studies were undertaken with neuroblastoma (N1E-115) cells. It has become obvious that this system is not suitable for performing such studies on monolayer cultures because of high aspecific binding levels to the culture substratum (plastic). As a reference system murine 3T3 cells were used. As in other studies<sup>8</sup> two receptor classes could be distinguished: about  $5 \cdot 10^5$  receptors/cell of the high-affinity class ( $K_d = 3 \times 10^{-9}$  M) and about  $2 \cdot 10^6$  receptors/cell of the low-affinity class ( $K_d = 1.7 \times 10^{-7}$  M). Further studies will be undertaken to study PDBu binding characteristics in embryonic tumour cell lines, as well as the early effects of phorbol esters on plasma membrane properties.

1. Blumberg, P.M. (1980) - CRC Crit.Rev.Toxicol. 8, 153-197.
2. Blumberg, P.M. (1981) - CRC Crit.Rev.Toxicol. 8, 199-234.
3. Weinstein, I.B. (1981) - J.Supramol.Struct.Cell.Biochem. 17, 99-120.
4. Weinstein, I.B. *et al.* (1979) - J.Supramol.Struct. 12, 195-208.
5. Mufson, R.A. and I.B. Weinstein (1981) - In: Biochemistry of Cellular Regulation (ed. M.E. Buckingham) Vol.III, Development and Differentiation, CRC Press, Boca Raton, pp. 179-196.
6. Driedger, P.E. and P.M. Blumberg (1980) - Proc.Natl.Acad.Sci.USA 77, 567-571.
7. Shoyab, M. and G.J. Todaro (1980) - Nature 288, 451-455.
8. Collins, M. and E. Rozengurt (1982) - J.Cell.Physiol. 112, 42-50.

## 2d. Growth and differentiation of embryonal carcinoma cells

In view of the heterogeneity of differentiation observed in the PCC4 embryonal carcinoma cell line (see previous report, Sect. IV.H.3e) two other cell lines have been selected in an attempt to obtain more homogeneously differentiated cell populations. These are the PC13 cell line (courtesy of Dr. J. Heath, Oxford, U.K.) and the F9 cell line (courtesy of Dr. J. Wartiovaara, Helsinki, Finland), both of which from large, flat endoderm-like cells (END-cells) following exposure to retinoic acid ( $10^{-6}$  M). Routinely, EC cells are plated for five days in large tissue culture flasks in Dulbecco's modified Eagle's medium with 10% (v/v) fetal calf serum containing retinoic acid. Analysis of time-lapse films shows that in the first two cell cycles following retinoic acid addition the intermitotic times are the same as for the stem cells (12-14 hr), but that the duration of the third cycle is approximately doubled (30 hr). The cells are then replated at densities required for experimentation. Under these conditions F9 cells remain morphologically homogeneous for four days and PC13 cells for at least seven days. Both cell lines exhibit increases in EGF binding upon differentiation: from negligible binding in stem cells to approximately 15,000 and 35,000 receptors per cell in PC13<sub>END</sub> and F9<sub>END</sub>, respectively. Moreover, both cell lines become mitogenically responsive (increase in <sup>3</sup>H-thymidine incorporation and cell number) to EGF, insulin and medium conditioned by the respective stem cell populations, as was previously reported by Heath *et al.*<sup>1</sup> and Rizzino *et al.*<sup>2</sup>. In addition we have found that under conditions appropriate for growth stimulation EGF rapidly (within 30 min) stimulates the Na<sup>+</sup>,K<sup>+</sup> pump by about 40% in F9<sub>END</sub> cells, with a return to basal levels after 90 min. This stimulation is amiloride sensitive, indicating the involvement of a growth factor-activatable Na<sup>+</sup>,H<sup>+</sup> exchange mechanism, as found in neuroblastoma and pheochromocytoma cells (see 1a and 2b above). Such stimulation is not observed in the stem cell population. Work is in progress to characterize the Na<sup>+</sup>,H<sup>+</sup> exchange system in the plasma membrane of stem cells and END cells and to measure Na<sup>+</sup> influx directly, with the aim of establishing the basis for the differences in response to growth factors of the two cell types.

In addition, PC13 stem cells can be synchronized by mitotic shake-off, as they are obtained as large single cells in late G<sub>2</sub>/early mitosis. On a suitable substrate division takes place synchronously within 1-2 hr of plating. This offers the possibility of a more detailed study of cell cycle regulation in this embryonal carcinoma cell line.

1. Heath, J., S. Bell and A.R. Rees (1981) - *J. Cell Biol.* 91, 293-297.
2. Rizzino, A., L.S. Orne and J.E. De Larco (1983) - *Exp. Cell Res.* 143, 142-152.

## 2e. Derivation of pluripotent cell lines directly from early mouse embryos

An important contribution to the field of (mammalian) developmental biology are the recent findings of Martin<sup>1</sup> and Evans and Kaufman<sup>2</sup>, who demonstrated that it is possible to derive pluripotent cell lines directly from mouse blastocysts in culture. To acquire more experience with this system and to obtain such embryo-derived cell lines in this Laboratory a study was initiated in collaboration with Dr. J. Karasiewicz

(University of Warsaw). Mouse blastocysts (4½ days old) were obtained in phosphate-buffered saline containing antibiotics and subsequently transferred to Dulbecco's modified Eagle's medium (bicarbonate-buffered) with 10% (v/v) fetal calf serum in tissue culture dishes. Within three days the blastocysts had attached to the substrate, "hatched" and flattened, so that at least two morphologically different regions were distinguishable - a large area of extremely flattened trophectoderm cells and smaller central regions of cells protruding from the substrate: the egg cylinder. Development to this stage required neither medium conditioned by embryonal carcinoma cells<sup>1</sup> nor delayed implantation of the blastocysts used<sup>2</sup>. This observation was subsequently confirmed by Evans (personal communication). The egg cylinder, representing the inner cell mass, is then transferred to medium containing 10% (v/v) fetal calf serum + 10% (v/v) newborn calf serum on a substrate on which inactivated STO mouse fibroblasts are present as a feeder layer. Within a few days small cells begin to grow out from the egg cylinder. So far we have been unable to obtain a continuously proliferating culture of such cells. This is probably due to the condition of the STO feeder cells, which were inactivated by mitomycin C treatment rather than by the more usual procedure of X-irradiation. Work is in progress to solve this problem.

1. Martin, G.R. (1981) - Proc.Natl.Acad.Sci.USA 78, 7634-7638.
2. Evans, M.J. and M.H. Kaufman (1981) - Nature 292, 154-156.

#### *2f. Membrane dynamics in early mouse embryos*

In collaboration with Dr.J. Karasiewicz (University of Warsaw) this project was initiated in 1981 and was continued in 1982. A large series of fluorescence photobleaching recovery measurements was carried out to establish possible regional or temporal modulations in the lateral mobility properties of plasma membrane lipids in unfertilized, fertilized and dividing mouse eggs. No direct evidence was found for large alterations in these properties related to fertilization. A more detailed analysis of the data is being carried out at present.

#### *2g. Freeze-fracture electron microscopy of the mouse oocyte plasma membrane (in collaboration with J.G. Bluemink)*

Mammalian embryogenesis starts with the fertilized egg, yet most of its developmental potential is already acquired during the oocyte stage, when it is surrounded by follicle cells which penetrate the zona pellucida and supply the oocyte with considerable quantities of essential metabolites. If one is interested in processes involved in early mammalian development, ultrastructural and biochemical characterization of the pre-fertilization stages can be very relevant. After developing a device for freeze-fracturing mouse oocytes, the ultrastructural characterization of the plasma membrane was started.

The plasma membrane of follicle cell-enclosed oocytes possesses two prominent features: gap junction formation plaques and so-called rhombic particle arrays. Gap junction formation plaques, i.e. groups of 10-20 gap junctions varying dramatically in size (5-500 particles) and shape, are often situated in IMP-poor areas. In such plaques intramembranous par-

ticles (IMPs) are found which are structurally associated with the gap junctions. These IMPs are larger (12 nm) than the IMPs in the gap junction itself (10 nm), and could represent early stages of gap junction formation, as was proposed earlier by Decker<sup>1</sup>. The fact that gap junction formation plaques exist next to single gap junctions suggests that the diameter of follicle cell protrusions contacting the oolemma can vary greatly, i.e. from about 300 nm in single focal sites to about 5  $\mu$ m in multifocal contact sites.

The rhombic particle arrays consist of 25 particles on the average, the particle diameter is  $10.5 \pm 1.9$  nm, the mean particle distance is  $19.8 \pm 2.2$  nm, and the acute angles in the array are  $81.3 \pm 9.4^\circ$ . The arrays are sometimes situated in gap junction formation plaques. A rhombic pattern of IMPs was described earlier in excitable cells of *Planaria*<sup>2</sup>. The rhombic particle arrays found in the present study exactly match those found in *Planaria*.

In denuded oocytes some remnants of contact sites were found in low frequency, i.e. small single gap junctions. Rhombic particle arrays were found in the same frequency as in follicle cell-enclosed oocytes. This suggests that these structures are not of a gap-junctional nature and may reflect some other form of functional contact between the oocyte and the follicle cell. The fact that some single gap junctions were found in the denuded oocytes was probably due to incomplete defolliculation. Next to the gap junctions, tight junction-like structures were found which were never encountered in follicle cell-enclosed oocytes. This phenomenon was also obvious in defolliculated oocytes of *Xenopus laevis* and it may therefore be stated that defolliculation can lead to new plasma membrane properties, the functional significance of which is still obscure.

At present ultrastructural characterization of the plasma membrane of oocytes matured *in vitro* is being carried out in order to elucidate the possible stage-specificity of the structures described above.

1. Decker, R.S. (1976) - J.Cell Biol. 69, 669-685.
2. Quick, D.C. and R.G. Johnson (1977) - J.Ultrastr.Res. 60, 348-361.

### 3. Other projects

#### 3a. Ultrastructural aspects of rapid plasma membrane growth in mitotic neuroblastoma cells (in collaboration with J.G. Bluemink)

In recent years this Laboratory has studied the changes in structural, dynamic and transport properties of the plasma membrane during the cell cycle of mammalian cells. These properties are modulated during the cell cycle in a coordinated way, such that a set of unique plasma membrane features can be attributed to the various phases of the cell cycle. Evidence has been provided that these modulations in plasma membrane properties are related to the process of membrane growth during the cell cycle.

Measurements of cell surface area have shown that in Neuro-2A cells the rate of membrane growth is dependent on the phase of the cell cycle. The major increase in cell surface area, i.e. approximately 60%, occurs rapidly within a short period around mitosis. We have examined in more detail the (ultra)structural aspects of plasma membrane growth during

cytokinesis by time-lapse cinematography and scanning, thin section and freeze-fracture electron microscopy.

Alterations in cell morphology during the cell cycle, recorded by time-lapse cinematography under phase contrast, were analysed in more detail by scanning electron microscopy. In S-phase the cells are thinly spread out, firmly attached to the substratum with pseudopods, and show very few, if any, surface protrusions (microvilli, folds or blebs). During G<sub>2</sub>-phase the cells begin to retract, while the cell surface becomes elaborated with microvilli. Shortly before mitosis they rapidly round up, the number of microvilli increases and blebs become apparent. The cell surface is most highly elaborated with microvilli, folds and blebs while the cells go through cytokinesis and attain a dumbbell shape. In early G<sub>1</sub>-phase the daughter cells flatten, the cell surface smoothens and surface irregularities disappear. It is obvious from these observations that cytokinesis is associated with a rapid increase in cell surface/volume ratio after mitosis.

Details of the process of plasma membrane growth and of the resulting alterations in plasma membrane structure are provided by freeze-fracture electron microscopy. Mitotic or early post-mitotic cells show random occurrence of bleb-like patches and mounds in continuity with the P-face of the plasma membrane. These patches frequently show the contour of a bleb, are notably devoid of intramembranous particles (IMPs) and have a multilayered organization, as judged from their stepwise fracture behaviour. The IMP-free patches show both continuous transitions and rim-like discontinuities with the plasma membrane. These structural aspects are suggestive of the fusion and incorporation of multilamellar lipid vesicles into the plasma membrane. Further inspection of replicas for places where the fracture plane exposes the peripheral cytoplasm as well as the E-face of the plasma membrane shows vesicles and multivesicular bodies, some of which are continuous with the inner aspect of the plasma membrane. These vesicles also show stepwise changes in the level of the fracture plane and are devoid of IMPs, indicating that they are composed of multilamellar lipidic membranes.

Ultrathin sections of cells prepared with a method that minimizes lipid extraction provide further evidence for the fusion of electron-dense aggregated vesicles with the plasma membrane. The clusters of vesicles often share their 5 nm thick membrane and appear as multilamellar structures. As judged by their multilayered organization, their electron density and their sensitivity to lipid-extracting agents they are apparently of a purely lipidic nature.

These data are interpreted to show that rapid and extensive plasma membrane growth around mitosis takes place by the incorporation of multivesicular bodies from the underlying cytoplasm into the plasma membrane. These bodies exhibit a multilamellar structure, are IMP-free in freeze-fracture preparations, and show certain characteristics in ultrathin sections indicating that they represent purely lipidic membrane material. As a result of a process of membrane fusion and incorporation the plasma membrane during mitosis transiently acquires an extremely heterogeneous composition, IMP-free domains being inserted into areas with an aggregative IMP distribution. Under conditions where the cellular contractile network does not yet allow for complete relaxation and post-mitotic cell flattening, membrane lipid can be present in excess to what can be locally integrated. As a consequence blebs may transiently

form. Aldehyde fixation is thought to induce an artefactual, protruding, bleb-like appearance of the IMP domains.

We think that cell cycle-dependent modulation in membrane fluidity, brought about by the increase in the proportion of membrane lipid relative to other components, affects plasma membrane transport properties. Lipid insertion presumably is a prerequisite for protein insertion to follow later in the cell cycle. The results have been submitted for publication.

### 3b. Fluorescence photobleaching recovery method

The Fluorescence Photobleaching Recovery (FPR) assembly which has been set up in this Laboratory (see Progress Report 1979) is an important tool for measuring the lateral diffusion characteristics of proteins and lipids in biological membranes. The membrane component of interest is labelled fluorescently, and using laser optics a small area on the cell surface (1-2  $\mu\text{m}$  radius) is illuminated and the fluorescence intensity measured. After a short 1,500- to 10,000-fold increase in laser light intensity, which results in irreversible bleaching of a fraction of the fluorophores in the illuminated area, the recovery of fluorescence in this area is monitored again with the attenuated laser beam. Recovery can result from lateral diffusion, membrane flow, or a combination of the two. Although the theory which relates the kinetics of fluorescence recovery to the lateral diffusion coefficient and the mobile fraction of the labelled membrane component is well established<sup>1</sup>, the complexity of the mathematical expression does not permit straightforward determination of the parameters of interest. Recently a simplified mathematical expression has been worked out<sup>2</sup> which describes the recovery kinetics up to a very good approximation for a Gaussian laser profile under moderate conditions of bleaching. Based on this simplified expression we have developed a method (publ.37) which permits linearization of FPR curves without any *a priori* parameter assumptions. From the linear plot the extent of bleaching, mobile fraction and lateral diffusion coefficient can be determined unequivocally. Moreover, deviations from linearity are indicative of the presence of additional recovery processes such as membrane flow or multiple diffusion coefficients. Using second order regression analysis these deviations from linearity can be quantified, so that the method allows correction of the diffusion kinetics of a major diffusing component for the presence of alternative recovery processes. The usefulness of the method was tested on computer-simulated recovery curves, varying the contribution of membrane flow, multiple diffusion coefficients, and statistical noise due to counting errors. We are now using this method routinely for measurements on cells in culture.

1. Axelrod, D. *et al.* (1976) - *Biophys.J.* 16, 1315-1329.
2. Yguerabide, J., J.A. Schmidt and E.E. Yguerabide (1982) - *Biophys.J.* 40, 69-75.

## V. Other Research Projects

1. *Early reptilian development with special reference to the origin of the primordial germ cells (Chelonia mydas)* (P.D. Nieuwkoop; L.A. Sutasurya, Bandung)

This joint project (see previous report, Sect. V.1) was continued during a stay of P.D.N. of just over two months at the Institut Teknologi Bandung (Indonesia). The question of the endodermal or mesodermal origin of the primordial germ cells was approached in what is hoped will be a definitive series of experiments. Prospective lateral endoderm and lateral plate mesoderm were isolated from the caudal, unsegmented region of 8- to 12-somite embryos and cultured either separately or in combination on the chorio-allantoic membrane of host embryos of at least four weeks old. Cultivation was for about a week at 25°C. The experiments were hampered by a high rate of bacterial and fungal infection due to the fine dust originating from volcanic eruptions in the vicinity of Bandung.

## 2. Plasma membrane dynamics in early molluscan embryos (*Nassarius reticulatus*) (J.E. Speksnijder, Zoology, State Univ. of Utrecht; S.W. de Laat)

Earlier freeze-fracture studies have demonstrated that the plasma membrane of the egg of *Nassarius* shows regional differences in the numerical distribution of intramembranous particles correlated with the animal/vegetal polarity of the egg (see previous report, sect. V.3). As a logical extension, a study was initiated of the dynamic properties of the plasma membrane using the fluorescence photobleaching recovery method. Preliminary results indicate that the lateral mobility of the lipid probe di-C<sub>14</sub> is significantly higher in the plasma membrane of the polar lobe than in that of the animal hemisphere of the uncleaved egg. This difference persists through the first cleavage cycles up to the 8-cell stage. Apparently the polar lobe membrane possesses specific properties possibly correlated with the localization of morphogenetically active factors in it. The study will be continued in 1983.

## 3. Designing *in vitro* test systems for pre-screening of teratogens

Under the sponsorship of Shell Internationale Research Maatschappij B.V. and the Ministry of Volksgezondheid, Ruimtelijke Ordening en Milieubeheer (Public Health and Environment) a project was started, in collaboration with Dr. A.J. Durston, to develop *in vitro* test systems for screening potentially teratogenic substances. The necessity for this project originates from new legislation coming into force in the EEC which demands new chemicals to be tested for potential teratogenic hazard before they can be marketed. So far only extensive *in vivo* test systems are available and commonly used by industry and governmental agencies. Recent progress in the development of *in vitro* assay systems for mutagenicity and carcinogenicity, and the very high cost and time-consuming nature of *in vivo* assay systems for teratogenicity, make the development of *in vitro* (pre-screening) assay systems for teratogens desirable and possibly feasible.

Initially four different *in vitro* test systems were proposed in relation to the ability of substances 1. to inhibit or induce morphological differentiation in neuroblastoma (N1E-115) cells; 2. to affect cell growth rate by specific action on cell cycle parameters in neuroblastoma (Neuro-2A) cells in chemically defined serum-free medium; 3. to affect cell aggregation and morphogenetic organization in embryonal carcinoma cells; 4.

to affect growth rate, kinetics and differentiation of cellular slime moulds (*Dictyostelium discoideum*).

A list was compiled of substances categorized as probable teratogens, possible teratogens or unlikely teratogens. These substances were systematically tested for their effects as described above in a range of concentrations. Dose parameters were established; in the case of the mammalian cell systems these were the lowest dose resulting in 100% cell death, the highest dose at which no differences from control cells could be ascertained, and the lowest dose at which a specific effect occurred on the parameter being tested. To date these dose parameters have principally been determined in the first system (differentiating neuroblastoma (N1E-115) cells), where substances were scored positively if all three dose parameters could be determined. Failure to induce or inhibit differentiation resulted in a negative score. Of 55 substances tested so far in this system (42 probable/possible teratogens; 13 unlikely teratogens) we were unable to categorize four substances for reasons of solubility. Thirty-five teratogens scored positively and four negatively; four non-teratogens scored positively and eight negatively. Thus overall 42 out of 51 substances (84%) were correctly categorized in the neuroblastoma (N1E-115) cell system, a correlation level comparable to that observed in a different *in vitro* system<sup>1</sup>.

In the non-mammalian test system *Dictyostelium discoideum* substances were scored positively if the lowest effective dose in a given test (spore yield, morphogenesis) was below or equal to the no-effect dose (toxic level), and negatively if above. Some substances were non-testable on these criteria, since solubility became limiting before a toxic dose was reached. For these substances the same tests were repeated, but after 24 hr of incubation and cell growth in the presence of the test substance. The latter tests are now in progress and overall correlation with teratogenicity awaits the results.

In the studies undertaken so far and described above, results of a more general interest for *in vitro* toxicology were also obtained. In both of the mammalian cell systems (1. en 2.) a significantly higher concentration of the test substance was found to be required to induce the same effect in serum-containing medium than in serum-free medium. Since neuroblastoma (Neuro-2A) cells can multiply in chemically-defined serum-free medium (see also sect.IV.1c) at the same rate as in media containing fetal calf serum, this system was used to test this interesting observation systematically. For 10 out of 17 compounds tested, concentrations between 2 and 20 times higher were required for 50% inhibition of growth in serum-containing medium. Consequently the ranking of test substances for capability to inhibit growth was different for the two culture conditions. In addition, through analysis of time-lapse films and application of the modified "transition probability model"<sup>2</sup> growth inhibition induced by a variety of substances could be specifically attributed to cell death/detachment or delayed cell cycle progression. The technique of cell counting, which is generally used in growth inhibition experiments, does not permit this type of conclusion regarding the mechanism involved. Two papers describing these results in detail have been submitted.

1. Braun, A.G. *et al.* (1982) - Proc.Natl.Acad.Sci.USA 79, 2056-2060.

2. van Zoelen, E.J.J., P.T. van der Saag and S.W. de Laat (1981) - Exp.Cell Res. 131, 395-406.

4. *Membrane fluidity and the action of adrenocorticotrophic hormone (ACTH) on the brain synaptic plasma membrane (Rattus norvegicus)* (M. Hershkowitz, Weizmann Institute of Science, Rehovot, Israel; W.H. Gispen and H. Zwiers, Molecular Neurobiology, State Univ. of Utrecht; S.W. de Laat)

The adrenocorticotrophic polypeptide hormone ACTH has a broad spectrum of effects on brain metabolism and animal behaviour<sup>1,2</sup>. At the level of the synaptic plasma membrane the ACTH derivative ACTH<sub>1-24</sub> affects protein phosphorylation, adenyl cyclase activity and polyphosphoinositide metabolism. So far, however, no high-affinity receptors for ACTH could be demonstrated on synaptic plasma membranes (SPM). In this cooperative project possible direct effects on SPM lipid fluidity were analysed by a structure-activity study of the influence of ACTH derivatives on membrane lipid fluidity, as estimated by steady-state fluorescence polarization of the lipid analogue 1,6-diphenyl-1g 3,5-hexatriene (DPH). Seven ACTH derivatives were tested in all. The results have demonstrated that these derivatives can be subdivided into two categories, based on their ability to induce a rapid (15 sec) 10-20% increase in SPM lipid fluidity: ACTH derivatives containing the aminoacid sequence 5-11 (1-24, 1-16, 5-18, 1-39) are active in this respect, whereas the derivatives lacking all or part of this sequence are inactive (1-10, 4-9, 11-24). This structure-activity relationship corresponds well with the earlier observed effects on protein and lipid phosphorylation (although they occur in the absence of ATP), as well as with the effects of ACTH derivatives on grooming and avoidance behaviour in rats. This suggests that direct modulation of membrane lipid fluidity may be an early event in the action of ACTH. The results will be published.

1. De Wied, D. and W.H. Gispen (1977) - In: Peptides in Neurobiology (ed. H. Gainer) Plenum Press, New York, pp. 397-448.
2. Gispen, W.H. (1980) - Progr. Brain Res. 53, 193-206.

5. *Ageing and lipid fluidity of brain synaptic plasma membranes: effect of ACTH (Rattus norvegicus)* (M. Hershkowitz, Weizmann Institute of Science, Rehovot, Israel; W.H. Gispen and H. Zwiers, Molecular Neurobiology, State Univ. of Utrecht; S.W. de Laat)

As an extension of the previous project, possible age-dependent changes in SPM lipid fluidity and modification of the effect of ACTH were studied. To this end SPM were prepared from various regions of the brain of six- and 24 month-old rats. Steady-state fluorescence polarization measurements, using DPH as a probe, have indicated that irrespective of the origin of the SPM (forebrain, cortex or hippocampus) an approximately 10% increase in membrane lipid fluidity was observed with ageing; regional differences in SPM lipid fluidity were not significant. The active derivative ACTH<sub>1-24</sub> increased SPM lipid fluidity in all preparations, but more strongly in SPM from old rats. As a consequence the difference between "young" and "aged" preparations disappeared after treatment with ACTH<sub>1-24</sub>. The results also suggest that ACTH<sub>1-24</sub> is more effective on SPM from hippocampus. In correspondence with the results described under 4. above, ACTH<sub>1-10</sub> was inactive. These data indicate that ACTH<sub>1-24</sub> is capable of overcoming at least the effect of

ageing on brain synaptic membrane lipid fluidity. The results will be published.

6. *Lateral diffusion of plasma membrane components during the cell cycle (Tetrahymena)* (R.J. Hill, Univ. of Copenhagen, Copenhagen, Denmark; S.W. de Laat)

A collaborative study has been initiated to determine possible modulations of the lateral mobility properties of plasma membrane lipids and proteins during the cell cycle of *Tetrahymena*. After developing suitable experimental conditions for synchronization of *Tetrahymena* and introducing fluorescent lipid analogues into the plasma membrane, a series of measurements of their lateral diffusion properties was carried out with the fluorescence photobleaching recovery (FPR) technique. These experiments provided indications for an unexpected dependence of the lateral diffusion coefficient determined on the extent of bleaching, in particular for high rates of bleaching. We are analysing at present whether this finding is due to inherent limitations of the FPR method, which would set the allowable bleaching limit at 60%, or to experimental artefacts.

7. *Research carried out in the amphibian facility* (R. Verhoeff-de Fremery, F.J.M. Vervoordeldonk)

7a. *Recording of characteristics and quality of egg batches from individual females (Xenopus laevis)*

Recording of data (see previous report, sect. V.5a) was continued and a computer programme was made that will allow the tracing of females producing eggs with specially desired characteristics within the population.

7b. *Development of eggs subjected to excess gravity (Xenopus laevis)*

This project forms part of the work reported last year in sect. I.A.1.ii (excess-g test in connection with the ESA space project). A few of the experimental eggs (centrifuged at ca. 90 x g for 30 min) were fertilized and reared; they all completed metamorphosis. The larvae developed more slowly and the toads remained smaller than normal. All showed under-developed forelegs, both skeleton and musculature being very frail. The animals were killed when adult. Dissection did not reveal any internal abnormalities.

7c. *The regulation of reproduction and spawning (Pleurodeles waltl)*

From February to June and in October and November the effectiveness of Gn-RH injections in males and of HCG injections in females was tested (see previous report, sect. V.5b.ii). Spermatophores were deposited only sporadically. In cooperation with the work group Comparative Endocrinology of the University of Utrecht the examination of testes has been undertaken to establish possible abnormalities and to find possibilities of improving the functioning of the testes by means of hormone treatments. The results will be available early in 1983.

#### 7d. Functioning of the testes in older males (*Ambystoma mexicanum*)

Often older males (5 years or older) do not deposit spermatophores. Dr.E. Elkan, Mount Vernon Hospital, Northwood, England, examined a number of fixed testes histologically. He observed that with increasing age the testes become more lobulated and the number of non-spermatogenic areas and areas where spermatic tissue is replaced by fibrosis increases. It is not clear whether this phenomenon is characteristic of Axolotls reared at the H.L. only. Timely replacement of older by younger males seems a much better way to guarantee normal reproduction than to try and stimulate the remaining functional parts of older testes by means of hormone injections.

#### 7e. Tumours

The registry of Tumors in Lower Animals, National Museum of Natural History, Washington DC, U.S.A., diagnosed a piece of skin and underlying musculature from *Ambystoma mexicanum* as collagenous peritoneal polyps. Two greatly enlarged and irregularly shaped fat bodies from *Rana lessonae* contained embedded multiple granulomas.

### VI. Miscellaneous

1. Prof. Nieuwkoop continued work on the monograph on epigenesis and inductive interactions during early chordate development, being written jointly with Prof.A.G. Johnen and B. Albers (see previous report, publ. 30). Although the literature will be restricted to the last two decades it still is vast. The book will cover embryonic development up to the individuation of the organ anlagen, as well as the preparatory phases of oogenesis, oocyte maturation and fertilization. Preliminary drafts of various chapters were written.
2. Prof. R. Chandebais stayed at the Laboratory for one month for a final check of the manuscript and references of a monograph written in collaboration with Dr. J. Faber (publ.25).

### VII. Papers published and accepted for publication in 1982

#### Published

1. Bluemink, J.G. and J.-C. Beetschen - Observations ultrastructurales sur le syndrome ectodermique consécutif à l'effet maternel de la mutation *ac* chez le Pleurodèle. Arch.Anat.Microscop.Morphol.Exp. 71, 72 (1982).
2. Bluemink, J.G., W.J. Hage, S.W.de Laat, W.A.M.van Maurik and L.G.J. Tertoolen - Membrane blebbing as a transient manifestation of lipid insertion during the cell cycle. Cell.Biol.Internatn.Rep. 6, 657 (1982).
3. Boonstra, J., C.L. Mummery, E.J.J.van Zoelen, P.T.van der Saag and S.W.de Laat - Monovalent cation transport during the cell cycle (review). Anticancer Res. 2, 265-274 (1982).
4. Boonstra, J., S.A. Nelemans, A. Feijen, A. Bierman, E.J.J.van Zoelen, P.T.van der Saag and S.W.de Laat - Effect of fatty acids on plasma

- membrane lipid dynamics and cation permeability in neuroblastoma cells. *Biochim.Biophys.Acta* 692, 321-329 (1982).
5. Boonstra, J., S.D. Skaper and S. Varon - Regulation of Na<sup>+</sup>,K<sup>+</sup> pump activity by nerve growth factor in chick embryo dorsal root ganglion cells. *J.Cell.Physiol.* 113, 28-34 (1982).
  6. Dorresteijn, A.W.C., S.M. Bilinski, J.A.M.van den Biggelaar and J.G. Bluemink - The presence of gap junctions during early *Patella* embryogenesis: an electron microscopical study. *Dev.Biol.* 91, 397-401 (1982).
  7. Laat, S.W.de, J. Boonstra, W.H. Moolenaar, C.L. Mummery, P.T.van der Saag and E.J.J.van Zoelen - Cation transport and growth control in neuroblastoma cells in culture. In: *Membranes in Growth and Development*; eds. J.F. Hoffman, G.H. Giebisch and L. Bolis; New York, A.R. Liss; *Proc.Internatn.Conf. on Biol.Membranes*; Crans-sur Sierre, Switzerland, June 1981; *Progress in Clin.Biol.Res.* 91, 211-236 (1982).
  8. Laat, S.W.de and W.H. Moolenaar - De celmembraan, regelcentrum van groei en ontwikkeling. In: *Biofysica; levende Natuurkunde*; eds. M. Chamalaun and D. Stavenga; Amsterdam, *Uitg.Intermediair*; *Intermediair Bibliotheek*; p.13-29 (1982).
  9. Laat, S.W.de and P.T.van der Saag - Modulation of structure and function of the plasma membrane in the cell cycle of neuroblastoma cells. In: *Genetic Expression in the Cell Cycle*; eds. G.M. Padilla and K.S. McCarthy Sr., New York, Acad.Press; *Cell Biology, a Series of Monographs*; p.337-361 (1982).
  10. Laat, S.W.de and P.T.van der Saag - The plasma membrane as a regulatory site in growth and differentiation of neuroblastoma cells. *Internatn.Rev.Cytol.* 74, 1-54 (1982).
  11. Luckett, W.P. and W. Maier - Development of deciduous and permanent dentition in *Tarsius* and its phylogenetic significance. *Fol.Primatol.* 37, 1-36 (1982).
  12. Moolenaar, W.H. - Na<sup>+</sup>/H<sup>+</sup> exchange in the action of growth factors. In: *Ions, Cell Proliferation, and Cancer*; eds. A.L. Boynton, W.L. McKeehan and J.F. Whitfield; New York, Acad.Press; *Proc.Symp. Lake Placid, N.Y., July 1982*; p.151-162 (1982).
  13. Moolenaar, W.H., C.L. Mummery, P.T.van der Saag and S.W.de Laat - Rapid ionic events following growth stimulation of neuroblastoma cells. In: *Membranes in Tumour Growth*; eds. T. Galeotti, G. Neri and S. Papa; Amsterdam, Elsevier Biomed.Press; *Proc. Internatn. Workshop on Membranes in Tumour Growth*; Rome, June 1982; p.413-418 (1982).
  14. Moolenaar, W.H., Y. Yarden, S.W.de Laat and J. Schlessinger - Epidermal growth factor induces electrically silent Na<sup>+</sup> influx in human fibroblasts. *J.Biol.Chem.* 257, 8502-8506 (1982).
  15. Mummery, C.L., J. Boonstra, P.T.van der Saag and S.W.de Laat - Regulation of the cell cycle in neuroblastoma cells: the role of ion transport. *Cell Biol.Internatn.Rep.* 6, p.654 (1982).
  16. Mummery, C.L., J. Boonstra, P.T.van der Saag and S.W.de Laat - Modulations of Na<sup>+</sup> transport during the cell cycle of neuroblastoma cells. *J.Cell.Physiol.* 112, 27-34 (1982).

17. Nieuwkoop, P.D. - La détermination et la migration des cellules germinales dans le règne animal. *Ann.Biol.* 21, 37-51 (1982).
18. Paleček, J., G.A. Ubbels and J. Mácha - An immunocytochemical method for the visualization of tubulin-containing structures in the egg of *Xenopus laevis*. *Histochemistry* 76, 527-538 (1982).
19. Sugimoto, K., W.J. Hage and J.G. Bluemink - Gap junction formation between normal and reaggregated endoderm cells of *Xenopus laevis* neurulae. *Wilhelm Roux's Arch.Dev.Biol.* 191, 143-148 (1982).
20. Verhoeff-de Fremery, R. and F.J.M. Vervoordeldonk - Skin auto-grafts as markers in the toad (*Xenopus laevis*). *Laboratory Animals* 16, 156-158 (1982).
21. Yew, D.T. - Development of the eyes in Agnatha and Chondrichthyes (Elasmobranchia). *Anat.Anz.(Jena)* 151, 231-239 (1982).
22. Zoelen, E.J.J.van, L.G.J. Tertoolen, J. Boonstra, P.T.van der Saag and S.W.de Laat - Effect of external ATP on the plasma membrane permeability and (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity of mouse neuroblastoma cells. *Biochim.Biophys.Acta* 720, 223-234 (1982).

*Accepted for publication*

23. Bluemink, J.G., W.J. Hage; M.H.F.van den Hoef and W.J.A.G. Dictus - Freeze fracture electron microscopy of membrane changes in progesterone-induced maturing oocytes and eggs of *Xenopus laevis*. *Europ.J.Cell Biol.* 31, 85-93 (1983).
24. Boonstra, J., C.L. Mummery, E.J.J.van Zoelen, P.T.van der Saag and S.W.de Laat - Cation transport during the cell cycle of neuroblastoma cells. In: *Ions and Water in Biological Systems*; eds.V. Vasilescu et al.; London etc., Plenum Press; *Proc.Conf.on Ions and Water in Biological Systems*; Bucharest, Sept.1982.
25. Chandebois, R., in collaboration with J. Faber - Automation in Animal Development - A new Theory derived from the Concept of Cell Sociology. Basel, Karger; *Monographs in Developm.Biol.* 16 (1983).
26. Dorresteyn, A.W.C., H.A. Wagemaker, S.W.de Laat and J.A.M.van den Biggelaar - Dye-coupling between blastomeres in early embryos of *Patella vulgata* (Mollusca, Gastropoda): its relevance for cell determination. *W. Roux's Arch.Developm.Biol.*
27. Durston, A.J., C. Weijer and S.A.McDonald - Regulation of cell differentiation and multicellular organisation in the cellular slime mould *Dictyostelium discoideum*. *Europ.J.Cell Biol.*
28. Laat, S.W.de, J.G. Bluemink, J. Boonstra, C.L. Mummery, P.T.van der Saag and E.J.J.van Zoelen - Membrane fluidity in growth and differentiation of neuroblastoma cells. In: *Mechanisms of Alterations of Membrane Fluidity*; ed.M. Shinitzky; Boca Raton, CRC Press.
29. Laat, S.W.de, J. Boonstra, W.H. Moolenaar, C.L. Mummery, P.T.van der Saag and E.J.J.van Zoelen - The plasma membrane as the primary target for the action of growth factors and tumor promoters in development. In: *Development in Mammals*; ed.M.H. Johnson; Amsterdam, Elsevier/North-Holland Publ.; vol. 5.

30. Laat, S.W.de, L.G.J. Tertoolen, P.T. van der Saag and J.G. Bluemink - Quantitative analysis of modulations in numerical and lateral distribution of intramembrane particles during the cell cycle of neuroblastoma cells. *J. Cell Biol.* 96, 1047-1055 (1983).
31. Lawson, K.A. - Stage specificity in the mesenchyme requirement of rodent lung epithelium *in vitro*: a matter of growth control? *J.Embryol. exp.Morphol.* 74, 183-206 (1983).
32. Mummery, C.L., P.T. van der Saag and S.W.de Laat - Loss of EGF binding and cation transport response during differentiation of mouse neuroblastoma cells. *J.Cell.Biochem.* 21, 63-75 (1983).
33. Ubbels, G.A., T.G. Brom, H.P. Willemsen and J.J.H. van Nunen - The role of gravity in the establishment of the dorso-ventral axis in the developing amphibian embryo. In: *Space Biology with Emphasis on Cell and Developmental Biology*; eds. N. Longdon and O. Melita; Noordwijk, Europ.Space Agency (ESA) Scient.and Techn.Publ.; Proc.Workshop, Cologne, March 1983; p.77-82 (1983).
34. Ubbels, G.A., K. Hara, C.H. Koster and M.W. Kirschner - Evidence for a functional role of the cytoskeleton in determination of the dorso-ventral axis in *Xenopus laevis* eggs. *J.Embryol.exp.Morphol.*
35. Verhoeff-de Fremery, R. - Diseases in the amphibian facility of the Hubrecht Laboratory (*Ambystoma mexicanum*, *Xenopus laevis*, *Discoglossus pictus*, *Rana pipiens*, *Rana lessonae*, *Bombina orientalis*). In: *Comptes Rendus du Premier Colloque International de Pathologie des Reptiles et des Amphibiens*, 1982.
36. Zoelen, E.J.J. van, C.L. Mummery, J. Boonstra, P.T. van der Saag and S.W.de Laat - Membrane regulation of the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase during the neuroblastoma cell cycle; correlation with protein lateral mobility. *J.Cell.Biochem.* 21, 77-91 (1983).
37. Zoelen, E.J.J. van, L.G.J. Tertoolen and S.W.de Laat - Simple computer method for evaluation of lateral diffusion coefficients from fluorescence photobleaching recovery kinetics. *Biophys.J.* 42, 103-108 (1983).



# **Centraalbureau voor Schimmelcultures**

---

## **Progress Report 1982**

**Edited by G.S. de Hoog**

Oosterstraat 1, 3742 SK Baarn,  
The Netherlands

Yeast Division, Julianalaan 67a,  
2628 BC Delft, The Netherlands

## CONTENTS

Introduction	3
Scientific staff	4
General topics	4
Mycological taxonomy	5
Zygomycetes	5
Oomycetes	6
Ascomycetes and their anamorphs	6
Endomycetes and basidiomycetous yeasts	13
Basidiomycetes	16
Applied Mycology	18
Entomogenous fungi	18
Plant pathology	18
Medical mycology	18
Publications 1982	20
Articles	20
Abstracts	22

## Introduction

One of the major aims of the CBS is to conduct scientific research on the taxonomic groups represented in the fungus collection. In this era of increasing financial limitation, the continuation and stimulation of a high standard of research receives particular attention. Each year renewed emphasis is placed on whether and how the scientific output of our institute can be increased and improved.

Most CBS contributions to the taxonomy of the fungi have come from individual, dedicated researchers. The collection has provided an almost inexhaustible source of material for study. Stimulation of a research-centred attitude has resulted in an increasing number of taxonomic monographs: over the last 15 years the yearly productivity in taxonomic treatments has nearly duplicated. Nevertheless, in order to ensure a continued research output, both quantitative and qualitative, management should not only be directed towards individual action, but also towards the development of an efficient 'infrastructure'. Such an infrastructure is both material and mental. The infrastructure is gradually built up and cultivated through a regular exchange of ideas and research experience, and through the practical work associated with the collection, stimulated by a shared objective of improved maintenance. Individual scientific achievements contribute to a good infrastructure, which in turn provides a brood-cell for satisfactory taxonomic monographs. This sketches the ideal situation which we hope to achieve.

The care of the collection in its broadest sense is an investment into future specialized research. Over the last few years much attention has been paid to the improvement of our maintenance techniques. In order to minimize degeneration all strains are preserved in at least two different ways, that is on agar slants and lyophilized. An additional though infrequently used safeguard is the collection under mineral oil. Certain strains on agar slants may degenerate considerably after repeated transfer. This problem is most pressing in the Basidiomycetes, as these are often sterile and thus cannot be lyophilized. With the development of biotechnology, increasing numbers of strains are deposited which have characteristic properties or which have been genetically manipulated. On agar media such strains may lose the properties for which they were deposited in the collection. To date the CBS was not able to fulfil such demands particularly from industries and institutes for applied sciences. The CBS is one of the first institutes to acquire the status of International Depository for patented strains under the Budapest Treaty, and should therefore be able to guarantee strain property conservation over a thirty year period of maintenance.

In view of the above problems, we are preparing to introduce storage under liquid nitrogen. This is not a new method, but thus far there were too many drawbacks and risks for routine use on the large scale demanded for our collection. A constant supply of liquid nitrogen must be ensured in order to bridge discontinuities in supply; in addition a reliable alarm system is needed to control the level of nitrogen in the vivostats. Such equipment has only recently been brought on the market. Further, progress has been made with the storage of the fungal specimens. Traditionally these are stored in glass ampoules, which are expensive and hazardous. There is a substantial danger of explosion when an improperly sealed ampoule is removed from the nitrogen. The use of polypropylene drinking straws introduced by Elliott (*Trans. Br. mycol. Soc.* 67, 245-246, 1976), has solved this problem. The volume can now be reduced by a factor 10, the costs by a factor 300, and leaking straws do not explode, but tear.

Special care was taken with groups that grow or sporulate with difficulty in culture. We continued to experiment with water-cultures of Oomycetes. Adapted methods were sometimes used, simulating the conditions of the natural substrate, e.g. media with sterilized mealworms, mushrooms or with live nema-

todes; cultures were thus brought into an optimal condition prior to lyophilization. Some fungi grow only a few millimeters in a month, and already show considerable change on the isolation plate. Such strains are also dried on the natural substrate and *in vitro*, and slides are stored in addition to living cultures.

#### Scientific staff

(as from December 1st, 1982)

- H.A. van der Aa (monographic treatment of *Phyllosticta* and *Coniothyrium* and their teleomorphs; taxonomy of Coelomycetes in general; identification of Ascomycetes)
- J.A. von Arx (general mycological taxonomy, with emphasis on Ascomycetes and Melanconiales)
- A.W.A.M. de Cock (taxonomy of Oomycetes and other aquatic fungi)
- G.W. van Eijk (structural elucidation of metabolites, with emphasis on quinone pigments and carotenoids; comparative studies of sterols, fatty acids and volatiles)
- K.W. Gams (taxonomy of *Acremonium*, *Verticillium* and other phialidic Hyphomycetes; taxonomy of *Mortierella*; taxonomy and ecology of soil fungi in general; nomenclature)
- E.J. Hermanides-Nijhof (taxonomy of *Fusarium*, *Aureobasidium* and related fungi; documentation and administration of culture collection)
- G.S. de Hoog (taxonomy and character study of yeast-like fungi; taxonomy of little differentiated Hyphomycetes; identification of Dematiaceae; TEM)
- C.A.N. van Oorschot (thallic Hyphomycetes including *Chryso sporium*, *Monacrosporium* and *Dactylella*; taxonomy of the *Arthrobotrys* complex)
- R.A. Samson (*Penicillium*, *Aspergillus* and related fungi; taxonomy and application of entomogenous fungi; thermophilous fungi; industrial and food mycology; SEM)
- M.A.A. Schipper (Mucorales, currently with accent on the genus *Rhizopus*)
- J.A. Stalpers (Basidiomycetes, with emphasis on Aphyllorphales and anamorphs of Holobasidiomycetes; SEM)
- G.A. de Vries (human and animal mycology, allergenic fungi; Actinomycetes; hypogeous macromycetes)
- A.C.M. Weijman (chemotaxonomy; chromatographic profiling of fungi; GC-MS)

#### Yeast division

- L. Rodrigues de Miranda (basidiomycetous yeasts)
- M.Th. Smith (ascomycetous yeasts)
- D. Yarrow (*Saccharomyces* and related genera)

#### General topics

D. Yarrow sent information to be used in the book 'Yeasts: Characteristics and Identification' to J.A. Barnett (Norwich, UK). The contents of this book are devoted to two principal functions. Firstly, to facilitate the use of yeasts in industry and in the laboratory, as much information as practicable being included for each species. Secondly to the identification of yeasts, a photomicrograph and systematic description being given for each species in addition to a series of keys and tables.

Chapter 6 gives a tabulary summary of the physiological characteristics of the yeast species. In addition, Chapter 7 describes each species fully, though succinctly, and the descriptions are important for the book's role as a guide for identifying yeasts. Quick and reliable identification of yeasts can be important in industry and medicine for establishing the precise causes of undesirable contamination on the one hand, or of certain diseases on the other.

The book should make such identifications possible. Minimal lists of physiological tests and corresponding results are provided for each species. Each list gives the maximum discrimination of a species from all other yeasts described in the book.

Chapter 8 gives keys for identifying all known yeasts, except those of the genera *Cyniclomyces*, *Oosporidium* and *Malassezia*, which require special conditions for cultivation. In addition to a key to the species of all yeast genera, there are keys to particular groups of special interest to workers in medicine or various branches of industry. The smallest possible set of tests has been selected which can provide an identification of these groups. These include yeasts growing on hydrocarbons or methanol, clinical yeasts associated with food, wines, brewing and soft drinks.

In collaboration with W. Jülich (Rijksherbarium, Leiden) W. Gams completed a biennial review on fungal taxonomy for 'Progress in Botany'. Though other obligations prevented serious progress in the contribution by W. Gams to the international project 'Compendium genericum Hyphomycetum', the collected documentation of the literature was computerized.

A.C.M. Weijman and G.W. van Eijk studied the practical application of infusion bottles internally coated with agar medium, for both cultivation and sample preparation prior to biochemical analysis. All preparations were executed in a single bottle resulting in a simplified procedure. An application note was published in *Antonie van Leeuwenhoek*.

## Mycological taxonomy

### ZYGOMYCETES

The revision of all CBS strains of the genus *Rhizopus* was continued by M.A.A. Schipper. The study included morphological examination, establishment of growth responses to different temperatures and mating experiments. A paper was prepared on species with relatively small structures in cooperation with J.A. Stalpers, who performed the SEM studies. A second study on the larger species was also completed, and a third on the medium-sized strains is in preparation.

The number of accepted species is limited. The first group comprises *Rhizopus microsporus* with two varieties, *rhizopodiformis* and *oligosporus* and the closely related homothallic *Rh. homothallicus*. Several clearly distinguishable taxa within the group had been found to intermate freely in previous tests done by M.A.A. Schipper. W. Gauger (Lincoln, Nebraska, USA), specialist in the genetics and sexuality of fungi, agreed to germinate the zygospores to study the progeny. The highly interesting first results have prompted Dr. Gauger to apply for sabbatical leave in 1983/1984 to continue the project with M.A.A. Schipper, J.A. Stalpers and H. van den Ende (Plantenfysiologisch Laboratorium GU, Amsterdam) at the CBS.

The group with larger structures contains the heterothallic *Rhizopus stolonifer* with its variety *reflexus* and the homothallic *Rh. sexualis* with var. *americanus*.

The remaining strains form a complex around *Rh. oryzae*. More mating experiments will be performed in order to establish the taxonomic relationships.

Through the kindness of Prof. Dr. K. Hara, Prof. Dr. K. Furuya and Dr. T. Yokoyama, first descriptions were made accessible of several older Japanese *Rhizopus* species. The valuable help of these taxonomists is gratefully acknowledged.

In February abundant growth of *Mortierella simplex* was observed by W. Gams on the surface of a compost heap. This observation prompted a comparative study of the strains preserved in the collection. All turned out to be highly psychrophilic, a feature so far unknown in this genus. The location of an unusually large specimen of *Endogone lactiflua* by J. Daams (Kortenhoeft) offered an opportunity to reattempt the thus far unsuccessful cultivation of

this fungus. Each fragment plated out yielded a *Mortierella*, altogether four species amongst which the uncommon *M. gemmifera* and *M. cystojenkinii*, but not the desired *Endogone*.

## OOMYCETES

R.P.W.M. Jacobs revised the genus of aquatic Oomycetes, *Apodachlya*. The CBS strains were supplemented by strains received from R. Emerson's collection. Six species and two varieties had been described previously. Due to meagre protologues and non-existent type material, the status of certain taxa, particularly of *Apodachlya punctata*, *A. brachynema* and *A. seriata* had remained a point of discussion. After a detailed comparative investigation of the living strains the number of species was restricted to three.

The study of the genus *Pythium* was continued, mainly focussed on species with filamentous sporangia from aquatic environments. The occurrence of these fungi in such habitats suggests that they function as decomposer organisms of vascular plants or algae. In cooperation with the Laboratorium voor Aquatische Oecologie (Nijmegen) the phytaceous fungi associated with the decomposition of nymphaeids were studied. In addition to *Pythium marsipium* and *P. pleroticum*, a number of *Pythium* species were isolated with filamentous non-swollen sporangia. Under special culture conditions (in water under a day/night rhythm) sexual reproductive structures of *P. diclinum* and *P. apleroticum* were also obtained. However, several isolates designed as *Pythium* 'F', remained sterile. Within this group three types could be distinguished according to their temperature/growth relationships. As previous records of the species were rare and only one was so far represented in the CBS collection, a manuscript was prepared with detailed descriptions and short discussions.

## ASCOMYCETES AND THEIR ANAMORPHS

### *Coelomycetes*

The revision of the species described in the genus *Phyllosticta*, the main project being worked on by H.A. van der Aa, has undergone considerable progress. An extended manuscript is being prepared concerning the almost 3000 species which have to be excluded. Additional notes were written on the species belonging to *Phyllosticta* s.str., and the study of type and secondary collections from various origins was continued. Most of the species could be assigned to other well-known genera, or enlisted into synonymy with plurivorous species belonging to the genera *Phoma*, *Phomopsis*, *Asteromella* and *Coniothyrium*.

As a result of this investigation, only a few true *Phyllosticta* species remain, their identity requires classification. One of the elucidated species is *Phyllosticta owaniana*, of which more authentic material became available (B). An interesting species of *Phyllosticta*, isolated from leaves of diseased clove (*Eugenia aromatica*) was received from the Industrial Crop Research Station Tandjungkarang in Sumatara, Indonesia. This species will be included in a comparative study of several closely related species of *Phyllosticta* with small conidia, and is tentatively identified as *Phyllosticta eugeniae* Young.

New or critical species of *Phoma* are being investigated in a cooperative project with the Plantenziektenkundige Dienst at Wageningen. Among these is a species isolated by S. Vanev (guest worker) from *Trachystemon orientale* leaf spots in Bulgaria, a yellow pigmented strain isolated by H. Nielander (student, State University, Utrecht) from soil in the Netherlands, and some South African strains isolated from *Syringa* sp.

Several isolates of the pleomorphic species, *Phoma epicoccina* were studied by H.A. van der Aa. The *Epicoccum* state of this species is sometimes predominant, in which case the fungus cannot be distinguished from *Epicoccum purpurascens*. To study this phenomenon and to find a possible biochemical basis

for eventual distinction of the two taxa, isolation and lyophilization of additional strains was initiated.

The genus *Phomopsis* is another rather uniform coelomycetous genus with characteristic stromatic fruit-bodies. These may, however, be reduced if formed on leaves. Such forms have often been described as *Phyllosticta* species. For identification at species level fresh isolates are indispensable to solve sometimes very long standing problems in this field. Examples are *Phyllosticta iliciseda* Sacc. and *Ph. ilicina* Sacc., both occurring on leaf spots of *Quercus ilex*. The holotype specimen of each species was studied and compared with fresh collections made in Northern Italy. The species concerned proved to be identical, but different from the other *Quercus*-inhabiting species which were described recently by Butin (*Sydowia* 33, 18-28, 1980; *Eur. J. For. Path.* 11, 33-44, 1981). In this comparative study some new isolates of *Amphiportha leiphaemia* were also included. The anamorphs of this genus are *Phomopsis*-like, but recent authors preferred to classify them in a separate genus, *Amphicytostroma* Petrak. The completion of this study was augmented by the fortunate isolation of the type species, *A. tiliae*, from ascospores of the teleomorph, *Amphiportha hranicensis*, found on dead branches of *Tilia* in Baarn. Other *Phomopsis* species studied extensively include *Ph. subordinaria* and *Ph. oxalina*, which have several synonyms in *Phyllosticta* on *Plantago* and *Oxalis*, respectively; further *Phomopsis leptostromiformis* on *Lupinus* in O. Flevo-land and the *Phomopsis* anamorph of *Diaporthe vaccinii*, which was isolated by H.A. van der Aa from fruits of *Vaccinium vitis-idaea* imported from Poland.

H.A. van der Aa also continued the isolation and tentative description of fungi belonging to the genus *Coniothyrium* s.l. One of these received special attention since the fungus proved to be the cause of a disease of *Euphorbia* cultivars imported from Italy. The isolates produced a yellow pigment.

*Cyclothyrium* is another coelomycetous genus with one-celled, brown conidia arising by enteroblastic conidiogenesis. Species of this genus were formerly described in various genera with brown conidia, such as *Coniothyrium* and *Cytoplea*. *Phyllosticta juruana* also has to be transferred to this genus. A further undescribed species of *Cyclothyrium* was fortuitously found on the type collection of *Phyllosticta mimusopsidis* Henn., a fungus which has to be reclassified in *Phomopsis*.

In cooperation with the Plantenziektenkundige Dienst at Wageningen, all available new and old strains of *Libertella* from various trees were compared with strains received from K. Messner (Vienna, Austria), who had conducted a survey of *Libertella blepharis* on apple trees. The strains, which usually sporulate poorly even after a long period of incubation all proved to be very similar. The study has to be supplemented with inoculation experiments.

The anamorph of *Botryosphaeria stevensii*, collected from branches of *Fraxinus excelsior* at Maarsseveen, could be studied in various stages of development in vivo and in vitro. The conidia of this fungus remain hyaline and one-celled for a long time, in which condition the fungus has been described repeatedly under names such as *Discula macrosperma*. The conidia become brown and possibly 1-septate with maturity. In this mature state the fungus is known as *Diplodia mutila*. According to recent authors, this species is the type of the genus *Diplodia*, which is a heterogeneous assemblage of hundreds of species. The conidiogenesis of *D. mutila* is described as holoblastic (Sutton - *The Coelomycetes*, Kew, 1980), but in our fresh isolates enteroblastic conidiation was found, frequently with percurrent growth and consequent thickening of the conidiogenous cell apex.

On a specimen of *Phyllachora vetivericola*, collected by V. Agnihotrudu (Bangalore, India) and sent to the CBS for identification, a hitherto unknown *Linochora* conidial state was found.

S.G. Vanev (guest worker) carried out part of his taxonomic study of *Discozia* under the guidance of H.A. van der Aa. This coelomycetous genus con-

tains 74 described plant-inhabiting species. 1200 herbarium specimens from 33 herbaria were examined, supplemented with the few available CBS strains and some new isolates. Applying modern criteria of taxonomy of imperfect fungi the genus was reduced to 14 plurivorous and morphologically distinct species. One species will be described as new and 3 species have to be classified in other genera. Two manuscripts on *Discosia* are in preparation and one on the reclassification of *Discosia minima* in the genus *Mycocleptodiscus*, is in press. Dr. Vanev paid a short visit to the Commonwealth Mycological Institute (Kew, UK), to discuss the results of his investigations.

Under the guidance of H.A. van der Aa, Ms C. Jansen (student, State University, Utrecht), made a comparative study of the species of *Cytospora* from deciduous trees, especially those isolated from Rosaceae. The CBS collection has 40 strains, though many are poor or do not sporulate. These and several newly isolated strains were analyzed on different media under various conditions. In some cases herbarium specimens gave additional information on the morphology of the conidiomata and the sporulating structures. As a result of this study a diagnostic key will be made for the distinction of major groups of species, in some cases with keys down to species level.

G.W. van Eijk and H.J. Roeymans, in cooperation with G.H. Boerema (Plantenziektenkundige Dienst, Wageningen) investigated six isolates of *Ascochyta*, comprising five species and a variety, and a *Phoma* species for the production of the phytotoxin ascocochitine. The presence of this compound in *A. pisi*, *A. pisi* var. *foliicola* and *A. fabae* was established by means of thin-layer chromatography and ultraviolet-visible spectrophotometry. The formation of very small amounts of pachybasin and chrysophanol was demonstrated for each strain by GC-MS.

G.W. van Eijk and H.J. Roeymans continued a study of the pigment production by *Phoma exigua* var. *foveata*, the fungus causing tuber rot or 'gangrene' of potatoes. Several fractions obtained through thin-layer chromatography were analyzed with GC-MS. Aloe-emodin (omega-hydroxychrysophanol) was identified with TLC, GLC and MS. The 1-hydroxy-3-carboxyanthraquinone identified has not been reported previously as a natural substance. The trivial name pachybasic acid was attached to this compound. A further compound was identified as 1,8-dihydroxy-3-carboxyanthraquinone (rhein) and recognized as a new fungal metabolite. Earlier on in the course of this study omega-hydroxypachybasin had been recorded as a new fungal product from the same species.

#### *Hyphomycetes*

Connie A.N. van Oorschot continued a project initiated in 1981 on the taxonomy of predatory nematode-destroying fungi. A few species known to attack nematodes or rhizopods have in the literature been attributed to the genus *Tridentaria*. The authentic collection of the generic type *T. alba* (B), shows no remaining fungal material and the original description does not suffice to identify the fungus concerned. Some six species have been placed in *Tridentaria*, some with clear original descriptions and illustrations, but at present only a strain of *T. implicans* is available. The type material of the genus *Tricornispora* (UC), placed in synonymy with *Tridentaria* by recent authors, was found to be a coelomycete. It resembled *Eriosporella calami*, the type species of the monotypic genus *Eriosporella*, an isotype of which is maintained in the CBS herbarium. The fungi concerned are being investigated in cooperation with H.A. van der Aa.

The major topic of the research work carried out by C.A.N. van Oorschot encompasses the hyaline hyphomycetes which capture their prey by means of adhesive nets, branches or knobs, or with constricting or non-constricting rings. In addition to standard methods of cultivation and observation, different species of nematodes are added to cultures of available strains to induce the production of trapping devices. In this way the taxonomic importance of

these structures are weighed. By using different species of nematodes it is hoped that any tendency towards host specificity will be detected.

The morphology of the fungi on the host is compared to that of pure cultures on nutrient media. New strains were isolated from soil and humus baited with nematodes. A project was initiated with J.P. Latgé (Paris, France), who is working on the ecology and pathogenicity of nematode-trapping fungi with a view to their application in the biological control of wheat- and barley-attacking nematodes.

Many nematode-trapping species have been classified in *Trichothecium*, even though the type species, *T. roseum*, is non-predatory and shows distinctive retrogressive conidiogenesis. The revision of *Trichothecium* presently being undertaken also includes the genera *Geniculifera*, *Candelabrella* and *Duddingtonia* with which *Trichothecium* has been confused. The approximately sixty species currently or formerly attributed to *Arthrobotrys* are also being examined, live CBS strains being supplemented with herbarium material and fresh isolates where possible. The distinction between the *Arthrobotrys*, *Dactylella* and similar species, and true *Dactylaria* species is being carried out in cooperation with G.S. de Hoog. Within this group there has been much controversy with regard to genus delimitation.

The genus *Dactylaria* s. str. comprises hyphomycetes with pigmented, erect conidiophores, provided with scattered, cylindrical denticles in the apical region, and producing hyaline, septate conidia. In this sense about 20 species can be recognized, which are being studied by G.S. de Hoog in cooperation with G.L. Hennebert (Louvain-la-Neuve, Belgium). Most species are known from a few collections; many have been described only recently. Some new species were also collected. Studies of freshly sampled leaf litter and rotten pine needles proved that quite a few taxa of the genus are rather common. They may have been overlooked by earlier workers because they form only minute patches. In culture they grow slowly, often forming colonies of only a few millimeters in diameter.

In connection with the above research, the genus *Isthmologispora* was delimited from *Dactylaria* s. str.. A new species was described, which showed two types of chlamydospores, and a new combination in *Isthmologispora* was proposed.

In a joint paper by G.S. de Hoog, C.A.N. van Oorschot and T. Hijwegen (Vakgroep Fytopathologie, Wageningen), a new genus and species, *Dissoconium aciculare*, was described for a hyperparasite isolated from *Erysiphe* sp. The fungus showed a superficial resemblance to some species recently described as *Dactylaria*, but had an unusual conidium discharge mechanism. Pairs of conidia (1- and 2-celled) were apparently actively detached in a small droplet. As such a mechanism has not been described in any related hyphomycete, accommodation in a new genus was deemed necessary.

In this connection the genus *Cordana* was also studied. The leaf-parasitic members of this genus also discharge their conidia actively, though only after decreased vapour pressure. *Cordana* was briefly discussed in the above paper, and a key to the accepted species was provided.

A new thermophilic hyphomycete isolated from New Zealand soil, *Myceliophthora histoplasmodes*, was described in an article by C.A.N. van Oorschot, R.A. Samson and A.L.J. Cole (Christchurch, New Zealand). The papillate ornamentation of the conidia and a maximum growth temperature of 50-55°C distinguish *M. histoplasmodes* from the other species in the genus. Similar ornamentation is seen in the human pathogenic species, *Histoplasma capsulatum*, and in the *Chrysosporium* anamorph of *Renispora flavissima*; however, these do not have swollen conidiogenous cells.

M.A. Rachman (Aberdeen, Scotland) in his thesis on a dieback disease of *Pinus contorta*, found the causal agent to be an apparently undescribed species of *Ramichloridium*. The species was studied in detail by G.S. de Hoog; a joint manuscript was prepared. The article also deals with a *Stenella* species

collected by T. Boekhout (Rijksherbarium, Leiden) from rotten leaves in Colombia. This is one of the very few *Stenella* species with one-celled conidia and thus strongly resembles *Ramichloridium*, but it has a low incidence of long, pluriseptate conidia. Just before finishing the manuscript, a description of a very similar species, *S. cynanchi*, was published (Yen - Mycotaxon 16, 92, 1982), and comparison is thus required.

The monographic study of *Verticillium* and related hyphomycetes was continued by W. Gams. A revision of the most important fungicolous species (with major emphasis on those on cultivated mushrooms) was finished and published in two parts, in collaboration with A. van Zaayen (Proefstation voor de Champignoncultuur, Horst). The first part dealt with taxonomy, the second with pathogenicity. Subsequent work was mainly concerned with *Verticillium* species that parasitize nematodes. They were found to belong to the section *Prostrata* described by Gams in 1971. Most of the species described by Drechsler in the 1940s as *Acrostalagmus* and *Cephalosporium* are now available in pure culture, and the species delimitation is being established. Typification of these species is not yet settled. Parasitism to nematodes was studied in vitro using *Panagrellus redivivus*. So far only some of the most recent isolates have shown to be virulent. These fungi are of major interest as possible biological control agents of plantpathogenic nematodes in several countries, and several institutes were approached in order to obtain as many isolates as possible. An apparently new representative of this group was obtained from G.J. Bollen (Vakgroep Fytopathologie, Wageningen).

A problematic group of phialidic hyphomycetes, intermediate between *Acremonium* and *Phialophora*, was studied together with M.R. McGinnis (Chapel Hill, North Carolina, USA). A new genus, *Phialemonium*, will be described to include two new species of mainly medical origin and one saprophytic species.

The study of *Niesslia* with *Monocillium* anamorphs was continued by W. Gams with the examination of a few herbarium specimens. This work is now almost ready for publication. Although new species are continually encountered, the former genus now contains about 24 teleomorph and the latter genus about 12 additional anamorph taxa. Some of the anamorph taxa are obvious aggregates and are connected to several *Niesslia* teleomorphs. The anamorphs are correlated with a well-defined group of teleomorphs and thus the genus must be rather homogeneous. Nevertheless the delimitation of *Monocillium* from *Acremonium* is rather difficult. Surprisingly, a freshly collected specimen of *Trichosphaerella inaequalis*, which is closely related to *Niesslia*, did not form a comparable anamorph in culture.

A specialized parasite of spiders was studied jointly by W. Gams, G.S. de Hoog and R.A. Samson. It can be delimited from its closest relative, *Sporothrix rectidentata* by longer conidia and two kinds of conidiogenous cells with either polyblastic or phialidic (*Verticillium*-type) conidiogenesis. Both fungi are best accommodated in *Engyodontium*. As in *Aphanocladium* the conidia of all these species are liberated after the supporting structures have shrivelled, leaving a very thin, cobweb-like net with propagules.

A peculiar hyphomycete was collected by J. Webster (Exeter, UK) from rabbit dung that had been incubated at reduced moisture levels. It was characterized by partly penicillate and claw-shaped phialides; these were studied by W. Gams using SEM. A new genus, *Onychophora*, will be described for this fungus by W. Gams, P.J. Fisher and J. Webster.

The description of *Oidiodendron scytaloides* n. sp., which was long overdue, was finished by W. Gams in collaboration with B.E. Söderström (Lund, Sweden), a manuscript was submitted to 'Cryptogamie, Mycologie'.

From the work done by a former student, J. Veerkamp, on soil fungi from a maize field in tropical Colombia, a paper was distilled to describe new species in *Trichoderma*, *Rhinochloidiella* and *Mortierella*.

A new species of *Pyxidiophora* was isolated repeatedly by G. Jager (Instituut voor Bodemvruchtbaarheid, Haren) as a weak hyperparasite on sclerotia

of *Rhizoctonia solani*. W. Gams found that the fungus readily produced perithecia in culture, in addition to a *Gabarnaudia* anamorph with unusual chlamydospore-like secondary conidia. While attending the Mykologische Dreiländer-Tagung in Jenbach, Tyrol, Austria, several fungi were isolated. *Mycogone calospora*, parasitizing *Ramaria* species, and *Zignoella abietes* were cultured for the first time. The delimitation of the *Cylindrotrichum* anamorph is still problematic in the latter. During a mycological foray in the Belgian Ardennes, the previously unknown *Dipodascus* teleomorph of the very common *Geotrichum armillariae*, was collected repeatedly on *Armillaria mellea*. As yet asci have not been produced in vitro.

Under the guidance of W. Gams, H. Nielander (student, State University, Utrecht) took up a study of soil fungi that was initiated by W. Verkerke in 1977 at the 'Investigation of Farm Management', OBS farms at Nagele, North-East Polder. The aim of the project was to ascertain whether different agricultural methods affect the flora of saprophytic soil fungi. Three types of management have been maintained since 1979, viz. conventional, integrated and biodynamic agriculture. The inventory was mainly carried out by means of dilution plates. A comparison was made of potato and barley fields at the conventional and biodynamic farms. The data obtained do not support the assumption that under biodynamic agriculture the number of species is higher than in conventional fields, although a considerable number of species were isolated which had not been encountered in 1977. This work yielded a few new species which are to be published.

R.A. Samson continued his survey on the entomogenous fungi collected in tropical rain forests in cooperation with H.C. Evans (Kew, UK). After a first paper on the *Cordyceps* species and their anamorphs on ants, the taxa parasitizing the *Camponotus* complex (Formicidae) were studied and described in a subsequent article. Common representatives were *Cordyceps lloydii* and *C. unilateralis*. They proved to compose a variable species aggregate. The typical *C. lloydii* is always accompanied by a *Hymenostilbe* anamorph, while *C. unilateralis* has several associated anamorphs belonging to the form genera *Hirsutella* and *Desmidiospora*. Among the specimens collected in the tropical forests of South-America, four new species of *Gibellula* were encountered. These taxa were described and a new key to the accepted species of *Gibellula* was provided. In the series 'Notes on the entomogenous fungi from Ghana' the species of *Hirsutella* were treated in detail.

R.A. Samson and M.C. Rombach (guest worker) collected and studied the entomogenous fungal flora in caves in South-Limburg (Netherlands). *Stilbella kervellei*, *Hirsutella dipterigena*, *Tritirachium cinnamomeum*, and some *Beauveria* and *Paecilomyces* species were found. Except for *H. dipterigena*, pure cultures of all species could be obtained. *S. kervellei* was particularly interesting, because it shows morphological characters connecting the genera *Stilbella* and *Polycephalomyces*. In the caves *S. kervellei* and *H. dipterigena* were common and reached epizootic proportions on helemyzid flies.

Together with G.C. Soares (La Minière, France), R.A. Samson completed a manuscript on the entomopathogenic species of the hyphomycete genus *Tolypocladium*. Species of *Tolypocladium* are usually encountered as soil inhabitants, but recent studies on the fungal pathogens of mosquitoes revealed that *T. cylindrosporium* also occurs as a pathogen on mosquitoes in California (USA) and New Zealand. In addition, a new species of *Tolypocladium* was recently encountered on glowworms in caves in New Zealand.

The taxonomy of *Isaria dubia*, a hyphomycete parasitizing *Hepialus* larvae (Lepidoptera), was elucidated together with B.L. Brady (Kew, UK). This fungus is the anamorph of *Cordyceps gracilis* and it differs considerably from the recognized taxa in *Isaria*; therefore a new genus, *Paraisaria*, was proposed.

The taxonomic study on the thermophilous fungi was continued by R.A. Samson. An isolate sent by J.M. Upadhyay (New Orleans, Louisiana, USA), close to *Thermoascus aurantiacus*, proved to differ by having smooth ascospores.

Several isolates sent in by A.L.J. Cole (Christchurch, New Zealand) deviated from the known thermophilous species of *Thielavia*, *Chaetomium* and *Scytalidium* and were studied in detail.

The revision of the cleistothecial Ascomycetes was continued by R.A. Samson and D.W. Malloch (Toronto, Canada), who visited the CBS for this project in April. The generic concepts were discussed, and keys to the accepted genera were completed.

A peculiar sporodochial *Myrothecium*-like fungus from India was studied by G.S. de Hoog and Vasant Rao (Hyderabad, India). It was characterized by cupulate fruitbodies lined with large, hyaline setae. In a later stage the emerald green, ornamented hyphae surrounding the sporodochium formed small wefts around the setae. The fungus was described as a new species of *Myrothecium*.

G.S. de Hoog also prepared a manuscript on the taxonomic structure of the highly pleomorphic genus *Exophiala*. The anamorph life cycles were elucidated by the distinction of a micro- (yeast-like) and a macrocycle (hyphomycetous), each with several facultative ways of development. The currently used characters were evaluated, and a synoptic key to the species was provided.

The testing of resistance to cycloheximide in *Ceratocystis* was continued by G.S. de Hoog and R.J. Scheffer (Phytopathologisch Laboratorium Willie Commelin Scholten, Baarn). Nearly all species with *Chalara* anamorphs were found to be sensitive, whereas those with other anamorphs were resistant to or even stimulated by cycloheximide. The results favour a distinction of *Ceratocystis* and *Ophiostoma*, thus confirming earlier studies using biochemical characters (Weijman & de Hoog - Antonie van Leeuwenhoek 40, 353-360, 1975). A manuscript was prepared with an evaluation of these characters, and a number of new combinations in *Ophiostoma*.

A.C.M. Weijman, in cooperation with G.S. de Hoog, started a study of the carbohydrate composition of *Sporothrix* and allied genera, in continuation of earlier studies of *Ceratocystis* and *Ophiostoma*. Capillary gas chromatography was used in combination with mass spectrometry (GC-MS), which has proved to be a valuable tool for interpreting the complex profiles resulting from the analysis of whole-cell hydrolyzates. The form-genus *Sporothrix* comprises mainly anamorphs of *Ophiostoma* species, but basidiomycetes such as *Calocera* and allied genera also have *Sporothrix*-like conidiogenous structures. Morphologically similar *Sporothrix* species can thus have markedly different backgrounds. In fact, Kurata (Mycopathologia 76, 45-58, 1981) recently indicated that rhamnose of glycoprotein origin is a potential taxonomic marker, when he found two subgroups within the genus. First results of the present study have confirmed the taxonomic significance of rhamnose in this genus, and have also shown asco- and basidiomycetous affinities. The biochemical grouping is in remarkable accordance with the intuitive display of relationships given by de Hoog (Stud. Mycol. 7, 1-84, 1974), who was unable to give the differences found a taxonomic status on the basis of morphology alone. The question arises whether the genus should now be subdivided on the basis of biochemical criteria, as is currently done in the yeasts.

## ENDOMYCETES AND BASIDIOMYCETOUS YEASTS

G.S. de Hoog, J.A. von Arx and M.Th. Smith made a taxonomic study of *Geotrichum capitatum*. The species is morphologically characterized by the presence of cicatrized sympodial rhachids. The examination of the physiological properties of ten isolates showed all cultures were non-fermentative. Glucose, galactose, 1-sorbose (variable), glycerol, lactic acid, citric acid (variable) and ethanol were utilized; the strains grew at 37°C and were resistant to 0.1% cycloheximide. As to the growth on the beta-glucosides cellobiose, salicine and arbutine, two groups could be recognized: *G. capitatum*, and a second taxon, morphologically characterized by absence of rhachids and

presence of swollen cells. Both taxa are associated with human lung disorders. M.Th. Smith and G.S. de Hoog extended the study to other *Geotrichum* species in the course of a joint project on the taxonomy of yeast-like hyphomycetes.

The revision of the genus *Dekkera* and its anamorph *Brettanomyces*, both known as spoilage organisms in bottled wines and soft drinks, was continued by M.Th. Smith. Two sporogenous isolates from soft drinks differed morphologically as well as physiologically from the two accepted *Dekkera* species and will therefore be described in a new species. The physiological properties of 57 out of 63 strains were found to agree with the original description of *Brettanomyces (Dekkera) bruxellensis*. In this study, the latter did not grow on raffinose and inulin, whereas these test results were reported to be positive in the literature. Since our results were consistent, these discrepancies might be explained by differences in purity of chemicals used. The % G+C of DNA, calculated for 36 selected strains, ranged from 36.5% to 43.4%. The revision was continued with the study of the morphological characteristics.

The revision of the genus *Lipomyces* was continued by M.Th. Smith. It was initiated because some isolates from soils did not compare exactly with any of the five accepted species. The gross morphology and the ultrastructure of the ascospores was studied in cooperation with W. Batenburg-van de Vegte (Laboratorium voor Microbiologie, Delft). Four types of ascospore ornamentation could be recognized by the carbon replica technique. These types seem to coincide with ultrastructural differences observed in ultra-thin sections. Twenty-seven strains were examined physiologically. As discrepancies with the original descriptions were observed, the growth properties will be re-examined in order to establish the variation of the physiological characteristics.

G.W. van Eijk, in collaboration with M.Th. Smith, started a study on the chemical characters of selected strains of *Hanseniaspora valbeyensis* and *H. guilliermondii*. Capillary GC of volatile compounds showed that the gaschromatographic profiles can be handled as additional taxonomic characters. The gaschromatograms of the sterol fractions of two strains of each species also revealed recognizable differences. The fatty acid patterns are typically ascomycetous, i.e. the gaschromatograms are characterized by high relative proportions of palmitoleic acid (C16:1).

While enlisting new strains into the collection, D. Yarrow found some recently described species to be synonymous with earlier described yeasts. *Torulopsis ethanolitolerans* and its variety *minor* are identical to *Candida ethanolica*; *C. parapsilosis* var. *toleyensis* is *C. guilliermondii*; *T. enohii* is *C. boidinii*, and *C. oleophila* is *Yarrowia lipolytica*.

G.W. van Eijk and H.J. Roeymans reinvestigated the neutral fractions of selected *Rhodosporidium*, *Sporobolomyces* and *Sporidiobolus* species, using capillary gas chromatography-mass spectrometry (GC-MS). Distinct differences between several species were found which had not been detected by the ordinary gas chromatography technique (GLC). For example, on packed columns a compound was tentatively identified as fungisterol on the basis of the same relative retention time (RRT) for all strains. However, GC-MS revealed that fungisterol was absent in the four strains of *Rh. infirmo-miniatum*. The peak with the same RRT in GLC turned out to consist of two other sterols. One sterol was identified as episterol; the other, with a molecular ion of 398 was a dihydroergosterol derivative. The facts clearly demonstrate that identification of compounds based on chromatographic retention time alone can be erratic. GC-MS thus presents a marked criterion to discriminate between the sterol patterns of *Rh. infirmo-miniatum* and the other red yeasts under study. C29, C30 and C31 sterols were only detected in *Sporobolomyces* and *Sporidiobolus*. Therefore these sterols may be of taxonomic significance. Some of them could be identified on the basis of their retention times and mass spectra. All strains investigated produced the unsaturated hydrocarbon squalene and ergosterol as the major sterol.

L. Rodrigues de Miranda and A.C.M. Weijman extended their study on the

taxonomy of the ballistosporic genera *Bullera* and *Sporobolomyces* to several recently isolated strains of uncertain affinity. The combination of morphological and biochemical data enhances proper classification. *Bullera* was found to contain xylose in the cell walls, whereas in *Sporobolomyces* this compound was generally absent. There are, however, a number of *Bullera* and *Sporobolomyces* species which show one or two characteristics of the other genus. *Bullera* is redefined as a genus with unpigmented species which produce ballistospores with two planes of symmetry, and with a third characteristic, namely xylose in the cell wall; *Sporobolomyces* species produce carotenoid pigments, form ballistospores with only one plane of symmetry and have no xylose in the cell walls.

The delimitation of these two genera nevertheless remains problematic. Strains of *Bullera piricola* and an undescribed species produce ballistospores with one, and others ballistospores with two planes of symmetry. Three undescribed *Bullera* species produce an orange-red pigment and ballistospores with two planes of symmetry; while two of the species have xylose in their cell wall and one has not. An unpigmented species, described as *Sporobolomyces singularis*, is transferred to *Bullera*, but its cell walls do not contain xylose and the ballistospores have been described as asymmetrical. Another *Sporobolomyces* species produces a purple pigment and asymmetrical ballistospores, but the cell wall contains xylose.

A number of new *Bullera* isolates from leaves in British Columbia were investigated by L. Rodrigues de Miranda, and the data necessary for a standard description were gathered. A joint publication will be prepared with B.N. Johri (Bhopal, India).

R.G. Shivas (Armidale, Australia) sent a number of yeast strains, isolated from the leaves of two kinds of Australian shrubs. Among those isolates were five undescribed species, two will be classified in the genus *Cryptococcus*, two in *Sporobolomyces* and one in *Rhodotorula*. Two papers were prepared with L. Rodrigues de Miranda. Much time had to be spent on the assimilation tests because the growth of some of these species under standard conditions was very poor. The same tests performed by R.G. Shivas differed considerably; consequently they had to be repeated several times.

The investigation of the genus *Cryptococcus* by L. Rodrigues de Miranda was continued. Much time was spent on the search for a useful method of isolation of DNA. The method in use for DNA isolation from ascomycetous yeasts did not prove optimal for *Cryptococcus* and other basidiomycetous yeasts. After the disintegration of the cell wall, the DNA apparently quickly deteriorates by the enzyme DNase. In the method now used, the cell content is in contact with a high concentration of ureum which represses such enzymes actively. A french press was used for breaking the cell walls which are extremely tough. The cells are suspended in a lysing buffer, containing 20% sodium dodecyl sulphate, concentrated ureum solution and a 4,8 M phosphate buffer. The DNA is separated from polysaccharides and proteins by passing the cell wall-free contents through a column of hydroxylapatite and washing and eluting the DNA from the column by a phosphate buffer. The result is a reasonably clean DNA solution with a light absorption ratio of approximately 1 : 2 : 1 as measured with wavelengths 203-260 and 280 nm.

DNA was isolated from 28 strains of *Cryptococcus laurentii* var. *laurentii* and var. *flavescens* with the above described method. Most curves were regular, indicating low contamination. Most samples had several melting curves at 260 nm, and C + G percentages were calculated as follows:

% G+C

Cr. 1.	2208	43.7/45.2	Cr. 1.	5598	55.1/55.3
Cr. 1.	2174	46.5/46.7	Cr. 1.	4933	55.6/55.6/55.6
Cr. 1.	2548	47.3/47.7	Cr. 1.	5489	55.9/56.2

Cr. 1.	2993	48.7/48.8	Cr. 1.	5746	53.8/54.4/56.5
Cr. 1.	6578	48.9	Cr. 1.	4926	55.7/56.5
Cr. 1. v.fl.	5595	49.3/49.6	Cr. 1.	5297	53.9/54.8/57.1
Cr. 1. v.fl.	4256	50.0/50.0/50.0	Cr. 1. v.fl.	942	56.3/57.1
Cr. 1. v.fl.	5594	50.2/50.6	Cr. 1.	6476	56.3/56.3
Cr. 1. v.fl.	1732	50.2/51.3	Cr. 1.	6473	56.6/56.7
Cr. 1.	973	51.0	Cr. 1.	4919	56.0/57.3
Cr. 1.	6034	50.5/51.1/53.6	Cr. 1. v.fl.	5539	57.2/57.3
Cr. 1. v.fl.	2409	52.3/52.5	Cr. 1.	4918	56.9/57.0/57.5
Cr. 1. v.fl.	318	54.0/54.2	Cr. 1.	6474	57.7/59.2
Cr. 1.	139	55.0/56.9	Cr. 1.	6475	56.7/59.2

The values range from 43.7 to 59.2, which is quite unusual. The lowest value was found in strain CBS 2208; it differs from all others by its dry and folded, rather than mucous or glistening colonies. It forms pseudo- and true mycelium. This strain is therefore transferred to *Candida*. 16 strains have values between 54.0 and 57.5, with two exceptions (59.2). Nakase & Komagata (J. gen. appl. Microbiol. 17, 121-130, 1971) reported values of 56% and 59% for the type strain CBS 139; Rodrigues de Miranda found respective values of 55.0 and 56.9 for the same strains. In spite of differences in methodology, there is much agreement in the results. The values determined for the strains of var. *flavescens* range from 49.3 to 57.3. 5 of the 8 available strains have values between 50 and 52.5. The values of the other three strains correspond to those of var. *laurentii*. The values mentioned in the literature for strain CBS 2409 (var. *flavescens*) are 51.2 and 54 (Nakase & Komagata - J. gen. appl. Microbiol. 17, 121-130, 1971) and 58.0 (Storck et al. - J. Bact. 98, 1069-1072, 1969). The above results do not allow any clear conclusions to be drawn. In the near future DNA-DNA reassociation will be applied.

## BASIDIOMYCETES

The study on sclerotium-anamorphs of Basidiomycetes was continued by J.A. Stalpers. The type specimen of *Myriococcum praecox* Fr. (UPS), the generic type species, turned out to be identical with *Papulaspora polyspora* Hotson, the type species of *Minimedusa* Weresub & LeClair. The genus thus becomes a synonym of *Myriococcum*. It does not seem possible to base the classification of sclerotial cultures on merely sclerotial characters, such as presence of cuticle and cortex, the origin of the sclerotium-forming cells and the hyphoid or cellular nature of the medulla. Other characters of the mycelium, such as occurrence and distribution of clamp connections, the number of nuclei per cell, the branching pattern of 'runner hyphae', are needed to obtain a natural classification. As a working hypothesis, the following genera are accepted:

*Sclerotium* (teleomorph in *Typhula*): sclerotia with cortex, cuticle and hyphoid medulla, hyphae binucleate, clamps either constantly present or absent;

*Myriococcum* (teleomorph in *Athelia*): sclerotia with cortex, cuticle and hyphoid medulla, hyphae binucleate, clamps rare, sometimes absent, 'runner hyphae' with typical branches at right angles;

*Burgoa* (teleomorph in *Sistotrema*): sclerotia with or without indistinct cortex, without cuticle and with cellular medulla, brown, hyphae binucleate, clamps constantly present;

*Aegerita* (teleomorph in *Bulbillomyces*): sclerotia without cuticle and cortex, with cellular medulla, white, hyphae binucleate, clamps constantly present; '*Rhizoctonia*' anamorph of *Thanatophorus*: sclerotia with or without indistinct cortex, without cuticle, with hyphoid medulla, hyphae multinucleate, clamps absent.

J.A. Stalpers studied the type material of most of the 43 species described in

*Ptychogaster* and *Ceratomyces*. This is a heterogeneous assembly, which is also reflected by the diverse teleomorph genera (mainly polypores). The type species of *Ptychogaster* is *P. albus*, characterized by arthroconidia which later become thick-walled and chlamydospore-like; its teleomorph belongs to *Tyromyces* (= *Oligosporus*). The remaining species have either intercalary (teleomorphs in *Ganoderma* and *Inonotus*) or mainly terminal chlamydospores (*Abortiporus*, *Echinoporia* and *Granulobasidium*). The anamorph of *Laetiporus* was earlier classified in *Sporotrichum* and that of *Fistulina* in *Confistulina*. There has been some delay in the revision of *Sporotrichum* by J.A. Stalpers because in recent literature the synonymy of *S. pruinosum* Gilman & Abbott and *S. pulverulentum* Novobranova was not accepted. Though the supporting arguments are not convincing, proof to the contrary is required. Attempts to obtain anastomoses between several strains failed. L. Polonelli (Rome, Italy) offered to test the exoantigens by an immunodiffusion procedure and this is now in progress. A study into the carbohydrate composition of *Sporotrichum* and allied genera was initiated by A.C.M. Weijman in cooperation with J.A. Stalpers.

All type specimens of the species of *Corticium* described by G.H. Cunningham were received by J.A. Stalpers from Auckland, New Zealand, and about half were studied. Most of the names turned out to be synonymous of earlier described species, but others are good species which have to be transferred to other genera.

Together with W.M. Loerakker (Plantenziektenkundige Dienst, Wageningen), J.A. Stalpers investigated the cause of a fish-eye rot of pears. There had been two reports in the Netherlands, detected after several months of storage in cool boxes. The causal agent was obtained in culture and identified as *Butlerella eustacei*. The species is known from North America, but only on apples; the sole report on pears is from China. As the actual rot only became visible after several months of storage, a delayed germination of propagules (basidiospores of conidia) was suspected. A search for the organism on wood or litter in the orchard from which the pears originated was unsuccessful. However, the extremely dry summer of 1982 may well have prevented the formation of fruitbodies.

J.A. Stalpers also indexed all species described in *Rhizoctonia*. Together with P. van den Bogert (Leeuwarden), the CBS strains were screened and all multinucleate strains of the *Rh. solani*-complex were mated with tester strains of all anastomosis groups recognized thus far. Fresh isolates of all groups were obtained from the Plantenziektenkundige Dienst at Wageningen. Preliminary results indicated that all described anastomosis groups occur in the Netherlands, that at least some of these are morphologically distinct and that there are at least two more anastomosis groups. *Rhizoctonia* is an important genus in several respects. Some groups (e.g. *Rh. solani*, *Rh. cerealis* and *Rh. oryzae*) are responsible for considerable crop losses, while others are important mycobionts of orchids.

The study of the ontogeny of the hymenophoral trama of higher Hymenomyces was continued by J.A. Stalpers in collaboration with A.F.M. Reijnders (Amersfoort). All Aphyllophorales with poroid or hydroid hymenophores showed the same pattern. At first a sterile spine or ridge is formed, consisting of parallel to interwoven hyphae. Later a hymenium is formed, except at the apex which remains sterile. This was observed in genera such as *Trametes*, *Albatrellus*, *Boletopsis*, *Schizospora* and *Kneiffiella*. Species of Aphyllosporales which form ridges (e.g. *Cantharellus*, *Phlebia*) behave differently. These genera show strong growth in the subhymenium which forces the hymenium upward, and also form many new basidia. The subicular layer generally becomes stretched out and thinner. Boletales generally have a divergent hymenophoral trama. There is, however, much variation in the thickness of the mediostratum and the width and number of the basidium-forming hyphae.

F. Nerud (Prague, Czechoslovakia) worked one month at the CBS under the

guidance of G.W. van Eijk. Nine species of Basidiomycetes and two Ascomycetes were screened for the presence of ubiquinones (coenzyme Q system) by means of thin-layer chromatography, ultraviolet-visible spectroscopy and GC-MS. The results obtained so far indicate the presence of ubiquinones in seven Basidiomycetes and one Ascomycete. The study will be continued in cooperation with A.C.M. Weijman and M.A. Posthumus (Laboratorium voor Organische Chemie, Wageningen) to further evaluate mass spectrometric analysis of the coenzyme Q system in relation to taxonomic problems.

G.W. van Eijk, D.M.X. Donnelly (Dublin, Ireland) and J. Polonsky (Gif-sur-Yvette, France) isolated and identified toxic metabolites from *Heterobasidion annosum* and elucidated the structures of two substances isolated from the sporophores and aging cultures. The compounds, fomajorin-S and -D, were shown to be isocoumarin derivatives. The structures were determined from chemical and spectroscopic (infra-red, <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance and mass) data. Initial biosynthetic studies with the aid of feeding experiments (labelled acetate) indicated that the fomajorins originate from mevanolate via a protoilludylcation or its equivalent. Isocoumarins previously isolated from fungi, could be biosynthesized from polyketides.

G.W. van Eijk and H.J. Roeymans, in collaboration with J.A. Stalpers, continued the identification of crystalline material produced by members of the Basidiomycetes. The white crystalline needles present in cultures of *Coprinus impatiens* were identified as 1,4-dimethoxy-2,3,5,6-tetrachloro-benzene (= drosophilin A methyl ether) by means of GC-MS. The identity was established by synthesising the compound. G.W. van Eijk previously isolated the metabolite from *Mycena megaspora* (Agaricales) and from two species of *Fomes* (*Phellinus*) (Aphyllorales).

## Applied Mycology

### ENTOMOGENOUS FUNGI

M.C. Rombach (guest worker) studied mass-production methods of several species of entomopathogenic fungi in cooperation with R.A. Samson. A small mass-production unit for the white fly parasite, *Aschersonia aleyrodis*, was designed and is being extensively tested at Koppert BV (Berkel-Rodenrijs). The fungus was grown on both solid media (e.g. brown rice with additives) and in a so-called 'bi-phasic' production process. The latter involves a controlled drying of mycelium produced in liquid media. Under suitable conditions (light, temperature, air flow, etc.) conidia are produced in large quantities on the surface of the culture. Progress has been made with the improvement of quality and quantity of the conidia produced, mainly by adjusting the media for submerged fungal growth.

Experiments concerning the control of the greenhouse white fly *Trialeurodes vaporariorum* by *A. aleyrodis* were carried out in close cooperation with P.M.J. Ramakers (Proefstation voor Groente- en Fruitteelt onder Glas, Naaldwijk). Research was also done on the ubiquitous insect fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Particular strains of both fungi may be of value in the control of the black vine weevil (*Otiorrhynchus sulcatus*), an increasing nuisance in plant nurseries. *M. anisopliae* has been used in preliminary pathogenicity tests. Several batches were produced of a strain isolated from *O. sulcatus* originating from an insectary at Wageningen. Practical and commercial aspects of the control of *O. sulcatus* by *M. anisopliae* are under investigation.

### PLANT PATHOLOGY

In cooperation with the Delta Instituut (Yerseke), diseased plants of *Aster tripolium* were investigated by H.A. van der Aa. All the stem bases had been

severely attacked by *Botrytis cinerea*. Since this fungus is generally considered to be a weak parasite and the plants were all infected with leaf mining insect larvae, it was tentatively concluded that the disease is caused by a combination of factors, in which the fungal component is only secondary. A study of this complex disease, which at times is very destructive on the Dutch salt marshes, will be continued in the summer of 1983.

A mass spectrometric approach to detect gangrene of potato tubers, caused by *Phoma exigua* var. *foveata* was extended by A.C.M. Weijman, G.W. van Eijk and H.J. Roeymans, in cooperation with W. Windig and J. Haverkamp (FOM Instituut voor Atoom en Molecuulfysica, Amsterdam), P.J.D. Sackers (Hewlett-Packard Nederland, Amsterdam) and Ir. L.J. Turkensteen (Instituut voor Plantenziektenkundig Onderzoek, Wageningen). GC-MS, direct probe MS and pyrolysis-MS methods were studied in comparison with the currently used TLC approach. The Py-MS mainly detects differences in phenolic compounds produced by the two varieties *exigua* and *foveata* during pathogenesis. The other MS methods tested are based on the detection of the pigment pachybasin, which only occurs in var. *foveata*. GC-MS is a factor of 1000 more sensitive than TLC and is more chemically reliable. Ultimately, the methods can lead to automation and to a reduction of total expertise time.

#### MEDICAL MYCOLOGY

On June 19th, 1982, it was the 30th anniversary of the foundation of Medical Mycology Division at CBS by the TNO Advisory Committee for Medical Mycology. The new division was supported by the Health Organization (GO) of the Central National Board for Applied Scientific Research in the Netherlands (TNO). A major part of the work, essential for clinical diagnosis and therapy, comprises the investigation of clinical material, the identification of cultures and the verification of their etiological significance. This work often leads to taxonomic studies of which some examples follow.

G.A. de Vries and M.H.F. Luykx studied the morphological and biochemical characters of a *Cladosporium* isolate sent in by A.A. Padhye (Atlanta, Georgia, USA). The fungus had been isolated from breast tissue of a young Caribbean woman with a clinical picture comparable with breast carcinoma. Dark hyphae, typical of phaeohyphomycosis, were observed in the tissues. The isolate showed marked similarity to two isolates from cases of phaeohyphomycosis in the USA and in Nigeria, previously studied by G.A. de Vries and classified as *Cladosporium* sp. The poor enzymatic activity of the Atlanta isolate suggested a closer relationship to *Rhinocladiella* species which cause chromomycosis and occasionally phaeohyphomycosis. *Cladosporium carrionii*, which also causes chromomycosis and has the same poor enzymatic activities as *Rh. pedrosoi*, was most similar to the Atlanta strain. It has the same low temperature optimum for growth (about 30°C), but has shorter conidial chains and smaller conidia.

G.A. de Vries also made a taxonomic study of a hitherto undescribed *Phialophora* species isolated from a case of white grain mycetoma pedis (maduromycosis). The fungus was characterized by colonies dominated by thick-walled, brown chlamydospores and by brown and blue water-soluble pigments. Phialides only developed at a later stage on the drier parts of the colonies. A cream-coloured variant could be distinguished from the generally dark brown colonies. This new *Phialophora* will be published as *Ph. cyanescens* in a mycological contribution to a joint paper with H.A. Klokke (Dermatologische Kliniek, Groningen).

A fungus isolated from skin lesions was compared to the type strain of *Cyphellophora laciniata* which also originated from human skin. The new isolate showed well-developed phialides which produced almost straight conidia with up to five septa, in contrast to the indistinct phialides and usually strongly curved, 1(-2)-septate conidia of *C. laciniata*.

During the microscopic examination of one of the many sputum samples re-

ceived, M.H.F. Luykx observed very large, globose cells of *Aspergillus fumigatus*. The cells produced germ tubes with short hyphae all over the surface. A similar cell type had been seen by G.A. de Vries in 1959 in human adrenal tissue infected with *Aspergillus gracilis*. Such cells are unknown in sputum from patients with *A. fumigatus* infections.

The morphology and ornamentation of spores and conidia of allergenic fungi were studied by R.A. Samson in cooperation with G.T. Cole (Austin, Texas, USA). Using light- and scanning electron microscopy, the surface structures were examined, while thin sections and replicas were studied by TEM. The results of these studies were demonstrated in a poster session at the International Congress of Microbiology at Boston, USA, and also described in a chapter of a book on allergenic fungi.

G.A. de Vries described the genus *Cladosporium* for a Mould Atlas to be published by a Committee of European Allergologists.

The study of the keratinophilic mycoflora of the S. and E. Flevoland polders was continued by G.A. de Vries and M.C.C. Elders. For the first time a 'true' dermatophyte was isolated from the about 25 year-old polder O.-Flevoland: *Microsporium gypseum*. Through mating experiments it was shown to be the (-) type anamorph of *Nannizzia incurvata*.

## Publications 1982

### ARTICLES

Aa, H.A. van der and J.D. Janse - Bacteriën, Bacteria en Schimmels, Fungi. In W.M. Docters van Leeuwen: Gallenboek, 3rd ed., revised by A.A. Wiebes-Rijks, G. Houtman, pp. 49-70. Q.O. Thieme & Cie, Zutphen (1982).

Arx, J.A. von - On *Monilia sitophila* and some families of Ascomycetes. *Sydowia* 34, 13-29 (1981/1982).

Arx, J.A. von - Notes on *Microdochium* and *Idriella*. *Sydowia* 34, 30-38 (1981/1982).

Arx, J.A. von - *Faurelina indica* sp. n. *Sydowia* 34, 39-41 (1981/1982).

Arx, J.A. von - The genus *Dicyma*, its synonyms and related fungi. - *Proc. Kon. Ned. Akad. Wet., Ser. C*, 85, 21-28 (1982).

Arx, J.A. von - A key to the species of *Gelasinospora*. *Persoonia* 11, 443-449 (1982).

Arx, J.A. von, J.P. van der Walt and N.V.D.M. Liebenberg - On *Mauginiella scaettae*. *Sydowia* 34, 42-45 (1981/1982).

Arx, J.A. von, J.P. van der Walt and N.V.D.M. Liebenberg - The classification of *Taphrina* and other fungi with yeast-like cultural states. *Mycologia* 74, 285-196 (1982).

Boekhout, T. and W.A.M. Linnemans - Ultrastructure of mitosis in *Rhodospidium toruloides*. *Stud. Mycol.* 22, 23-28 (1982).

Cholil, A. and G.S. de Hoog - Variability in *Drechslera oryzae*. *Trans. Br. mycol. Soc.* 79, 491-495 (1982).

Cole, G.T. and R.A. Samson - Conidium and sporangiospore formation in pathogenic microfungi. In D.H. Howard (ed.): *The Pathogenic fungi: their biology, pathogenicity and detection*, vol. 1, pp. 437-524. Marcel Dekker Inc., New York (1982).

Constantinescu, O. - Studies in *Cercospora* and similar fungi. II. New combinations in *Cercospora* and *Mycovellosiella*. *Crypt., Mycol.* 3, 63-70 (1982).

Constantinescu, O. and H.A. van der Aa - *Phoma flavigena* sp. nov. from fresh water in Romania. *Trans. Br. mycol. Soc.* 79, 343-345 (1982).

Constantinescu, O. and R.A. Samson - *Triadelphia*, a pleomorphic genus of Hyphomycetes. *Mycotaxon*. 15, 472-486 (1982).

Donnelly, D.M.X., J. O'Reilly, J. Polonsky and G.W. van Eijk - Fomajorin S and D from *Fomes annosus* (Fr.) Cooke. *Tetrahedron Lett.* 23, 5451-5452 (1982).

Eijk, G.W. van and H.J. Roeymans - Distribution of carotenoids and sterols in relation to the taxonomy of *Taphrina* and *Protomyces*. *Antonie van Leeuwenhoek* 48, 257-264 (1982).

Eijk, G.W. van, H.J. Roeymans and A.C.M. Weijman - Biochemical characteristics of selected red yeasts. *Stud. Mycol.* 22, 39-49 (1982).

Evans, H.C. and R.A. Samson - *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems. I. The *Cephalotus* (Myrmicinae) complex. *Trans Br. mycol. Soc.* 79, 431-453 (1982).

Evans, H.C. and R.A. Samson - Entomogenous fungi from the Galapagos Islands. *Can. J. Bot.* 60, 2325-2333 (1982).

Gams, W. - Generic names for synanamorphs? *Mycotaxon* 15, 459-464 (1982).

Gams, W. and W. Jülich - Taxonomy and phylogeny of fungi. *Progr. Bot.* 44, 345-374 (1982).

Gams, W. and W. van Laar - The use of Solacol (validamycin) as a growth retardant in the isolation of soil fungi. *Neth. J. Pl. Path.* 88, 39-45 (1982).

Gams, W. and A. van Zaayen. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. *Neth. J. Pl. Path.* 88, 57-78 (1982).

Hedger, J.N., R.A. Samson and T. Basuki - *Scytalidium indonesiacum*, a new thermophilous hyphomycete from Indonesia. *Trans. Br. mycol. Soc.* 78, 364-366 (1982).

Hjortstam, K. and J.A. Stalpers - Notes on Corticiaceae (Basidiomycetes). XI. *Boidinia*, a new genus segregated from *Gloeocystidiellum*. *Mycotaxon* 14, 75-81 (1982).

Hoog, G.S. de - On the potentially pathogenic dematiaceous hyphomycetes. In D.H. Howard (ed.): *The pathogenic fungi: their biology, pathogenicity and detection*, vol. I, pp. 149-216. Marcel Dekker Inc., New York (1982).

Hoog, G.S. de - Morphology of anamorphs, I. *Rhodosporidium*. *Stud. Mycol.* 22, 3-9 (1982).

Hoog, G.S. de - Morphology of anamorphs, II. *Sporidiobolus* and *Sporobolomyces*. - *Stud. Mycol.* 22, 10-14 (1982).

Hoog, G.S. de and T. Boekhout - Teliospores, teliospore-mimics and chlamydo-spores. - *Stud. Mycol.* 22, 15-22 (1982).

Hoog, G.S. de and C. Rubio - A new dematiaceous fungus from human skin. *Sabouraudia* 20, 15-20 (1982).

Jacobs, R.P.W.M. - The genus *Apodachlya* - *Antonie van Leeuwenhoek* 48, 389-399 (1982).

Jacobs, R.P.W.M. - Pythiaceae fungi associated with the decomposition of *Nymphoides peltata*. *Antonie van Leeuwenhoek* 48, 433-445 (1982).

- Morgan-Jones, G. and W. Gams - Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloe typhina*, new taxa in one of two new sections of *Acremonium*. *Mycotaxon* 15, 311-318 (1982).
- Oorschot, C.A.N. van and G.S. de Hoog - A new species of *Anthopsis*. *Antonie van Leeuwenhoek* 48, 61-65 (1982).
- Payne, R.W., D. Yarrow and J.A. Barnett - The construction by computer of a diagnostic key to the genera of yeasts and other such groups of taxa. *J. gen. Microbiol.* 128, 1265-1277 (1982).
- Samson, R.A. and B.L. Brady - *Akanthomyces novoguineensis*, sp. nov. *Trans. Br. mycol. Soc.* 79, 571-572 (1982).
- Samson, R.A. and H.C. Evans - *Clathroconium*, a new helicosporous hyphomycete genus from spiders. *Can. J. Bot.* 60, 1577-1580 (1982).
- Samson, R.A. and H.C. Evans - Two new *Beauveria* species from South-America. *J. Invert. Pathol.* 39, 93-97 (1982).
- Samson, R.A., H.C. Evans and E.S. Hoekstra - Notes on entomogenous fungi from Ghana. VI. The genus *Cordyceps*. *Proc. Kon. Ned. Akad. Wet., Ser. C*, 85, 589-605 (1982).
- Samson, R.A. and C.W. McCoy - A new fungal pathogen of the scavenger mite, *Tydeus gloveri*. *J. Invert. Pathol.* 40, 216-220 (1982).
- Scholer, H.J., E. Müller and M.A.A. Schipper - *Mucorales*. In D.H. Howard (ed.): *Pathogenic fungi: their biology, pathogenicity and detection*, vol. I, pp. 9-59. Marcel Dekker Inc., New York (1982).
- Stalpers, J.A. - Het geslacht *Jaapia* - *Coolia* 25, 50-53 (1982).
- Stalpers, J.A. and W.M. Loerakker - *Laetisaria* and *Limonomyces* species (*Corticaceae*) causing pink diseases in turf grasses. *Can. J. Bot.* 60, 529-537 (1982).
- Stalpers, J.A. and A. van Zaayen - Raster-elektronenmicroscopie van champignonsporen. *Champignoncult.* 26, 496-499 (1982).
- Vanev, S.G. - *Discosia baarnensis* sp. nov. - *Trans. Br. mycol. Soc.* 79, 569-571 (1982).
- Vries, G.A. de - *Ascomycetes, Eurotiales, Sphaeriales and Dothideales*. In D.H. Howard (ed.): *The pathogenic fungi: their biology, pathogenicity and detection*, vol. I, pp. 81-111. Marcel Dekker Inc., New York (1982).
- Walt, J.P. van der, A.C.M. Weijman and J.A. von Arx - The anamorphic yeast genus *Myxozyma* gen. nov. *Sydowia* 34, 191-198 (1981/1982).
- Walt, J.P. van der, D. Yarrow, A. Opperman and L. Halland - *Pichia kodamae* sp. nov., a new homothallic yeast species. *J. gen. appl. Microbiol.* 28, 155-160 (1982).
- Weijman, A.C.M. and G.W. van Eijk - Use of a single infusion bottle to culture and prepare potentially hazardous microorganisms for biochemical analysis. *Culturing on solid media*. *Antonie van Leeuwenhoek* 48, 457-459 (1982).
- Weijman, A.C.M., I.J.A. Vlug and G.W. van Eijk - Carbohydrate patterns of selected *Rhodosporidium* and *Sporobolomyces* strains. *Stud. Mycol.* 22, 50-55 (1982).
- Windig, W. and J. Haverkamp - Pyrolysis mass spectrometry. I. *Rhodosporidium*. *Stud. Mycol.* 22, 56-59 (1982).

Windig, W. and G.S. de Hoog - Pyrolysis mass spectrometry. II. *Sporidiobolus* and related taxa. *Stud. Mycol.* 22, 60-64 (1982).

Zaayen, A. van and W. Gams - Contributions to the taxonomy and pathogenicity of fungicolous *Verticillium* species. II. Pathogenicity. - *Neth. J. Pl. Path.* 88, 143-154 (1982).

#### ABSTRACTS

Ramakers, P.M.C., M.C. Rombach and R.A. Samson - Application of the entomopathogenic fungus *Aschersonia aleyrodis* in an integrated control programme against glasshouse whitefly *Trialeurodes vaporariorum*. *Abstr. 3rd int. Colloq. Invert. Pathol.*, p. 99 (1982).

Rombach, M.C. and R.A. Samson - Small scale production of the entomopathogenic fungus *Aschersonia aleyrodis*. *Abstr. 3rd int. Colloq. Invert. Pathol.*, p. 229 (1982).

Samson, R.A. - Modern aspects of food mycology. *Antonie van Leeuwenhoek* 48: 413-414 (1982).

Samson, R.A. - Laboratory culture and maintenance of entomopathogenic fungi. *Proc. 3rd int. Colloq. Invert. Pathol.*, pp. 182-187 (1982).

Samson, R.A. - Schimmelkontaminatie van voedingsmiddelen. *Studiedag Hyg. Voedingsnijverh. Vlaamse Chem. Ver.*, Gent, pp. 41-50 (1982).

Samson, R.A. and H.C. Evans - New fungal pathogens of arthropods in tropical rain forests. *Abstr. 3rd int. Colloq. Invert. Pathol.*, p. 224 (1982).

Samson, R.A., M.C. Rombach, H.C. Evans and G. Riba - Comparative studies in vitro on various species of the entomopathogenic genus *Aschersonia*. *Abstr. 3rd int. Colloq. Invert. Pathol.*, p. 227 (1982).

Vries, G.A. de - Recent advances in medical mycology. - *Proc. simp. Dermatol.*, Sabta, pp. 1-11 (1982).

# **Institute for Ecological Research**

---

## **Progress Report 1982**



Instituut voor Oecologisch Onderzoek,  
Boterhoeksestraat 22, 6666 GA Heteren,  
The Netherlands

## CONTENTS

1. Task and function of the institute
2. Scientific staff
3. General summary (J.W. Woldendorp)
4. Heritability of bill dimensions in the Great Tit (A.J. van Noordwijk, P.L.M. Klerks)
5. Local survival of the breeding birds in a Coot population (A.J. Cavé, J. Visser)
6. International bird ringing research (A.C. Perdeck)
7. Effects of drought on the microclimate in grassland (Ph. Stoutjesdijk)
8. Seasonal variation of Olsen-P and organic P fractions at some sites in relatively old dune grasslands (S.R. Troelstra, M.A. van der Meulen, R. Wagenaar)
9. Comparison of developing populations of *Plantago lanceolata* in a hayfield and a pasture (J. Haeck, J.H. Mook)
10. Demography of a coastal type of *Plantago major* L., a preliminary report (C.W.P.M. Blom, J. van Heeswijk)
11. Variability in morphological characteristics of *Plantago lanceolata* (P. Slim, J. van der Toorn)
12. Germination of *Plantago major* seeds of different stages of maturity (R. Soekarjo)
13. Different K and P requirements for *Plantago major* ssp. *major* and *Plantago lanceolata*? (S.R. Troelstra, W. Smant, R. Wagenaar)
14. Growth of *Plantago lanceolata* L. in a permanently waterlogged soil system and the effect of the neighbouring wetland species *Carex disticha* L. (T. Blacquièrè)
15. The effect of ambient nitrate concentration on growth and nitrate uptake of two *Plantago* species - a summary (A.H.J. Freijsen, H. Otten)
16. Salt spray and its influence on the vegetation of the coastal dunes of Voorne and Goeree (the Netherlands) in relation to man-made changes in coastal morphology (J.C. Vulto, P.J.M. van der Aart)
17. Publications in 1982

## 1. Task and function of the institute

The institute was founded in 1954 by the Division of Sciences of the Royal Netherlands Academy of Arts and Sciences to carry out and promote terrestrial ecological research in a broad sense and to co-operate with other organizations engaged in such research. The headquarters of the institute were transferred in the summer of 1982 from Arnhem to Heteren. Part of the botanical research is done at a second seat in Oostvoorne. Field work is carried out in various parts of the Netherlands (Fig. 1).



Fig. 1. Location of the Institute for Ecological Research and its field-work sites.

1. Headquarters in Heteren
2. Department for Dune Research "Weevers' Duin"
3. National Park "De Hoge Veluwe", where most of the field-work on the Great Tit is done
4. Vlieland (additional field-work on the Great Tit)
5. Oosterhout (additional field-work on the Great Tit)
6. Liesbosch (additional field-work on the Great Tit)
7. Westeinderplassen, where most of the field-work on the Coot is done
8. Dunes of Goeree (additional field-work of the Department for Dune Research).

The institute is primarily concerned with two long-term research projects based on a multidisciplinary approach. Parts of these projects involve close collaboration with a number of university departments. Many graduate students participate in the institute's research programme as part of their studies.

The main theme of the research projects is the relationship between the properties of plants and animals and those of their habitat. In this respect special attention is paid to plants of grasslands, mainly *Plantago* species, and to birds, particularly the Great Tit and the Coot.

Although the institute's research programme is aimed in the first place at contributing to increased insight into general ecological problems, much of the information collected can be used in applied research on agriculture and nature management; this aspect is taken into account in the planning of the research projects and incidental applied research projects are carried out if additional funds become available. A number of government services make use of the results.

In addition, the institute administers the ringing of birds in the Netherlands. It is also the site of the Euring Data Bank, where all recoveries of birds ringed in Europe are compiled.

The institute is supervised by a committee appointed by the Division of Sciences of the Academy, and is financed by the Government.

## 2. Scientific staff

### Director

J.W. Woldendorp

### Population Biology of Birds

J.H. van Balen (Ecology)

J. den Boer-Hazewinkel

(Ecology, guest worker)

A.J. Cavé (Ecology)

P.J. Drent (Behavioural ecology)

J.A.L. Mertens (Eco-physiology)

A.J. van Noordwijk (Population genetics, guest worker)

A.C. Perdeck (Ecology and Bird Migration)

J.M. Tinbergen (Ecology)

K.R. Westerterp (Eco-physiology, BION/ZWO)

### Bird ringing Centre and

### Euring Data Bank

R.D. Wassenaar (Manager)

### Population Biology of Plants

P.J.M. van der Aart (Ecology)

M.J. Adriani (Ecology, guest worker)

Tj. Blacquièrre (Eco-physiology, guest worker BION/ZWO)

C.W.P.M. Blom (Ecology)

J.M.M. van Damme (Population genetics)

C. van Dijk (Plant-microorganism relationships)

A.H.J. Freijssen (Eco-physiology)

J.M. van Groenendael (Ecology, guest worker LH Wageningen)

F. de Haas (Eco-physiology, guest worker)

J. Haeck (Ecology)

R.H. Hengeveld (Ecology)

D. van der Laan (Ecology)

J.H. Mook (Ecology)

M. de Nooij (Plant-microorganism relationships)

A.J. Smit (Eco-physiology, guest worker)

R. Soekarjo (Eco-physiology)

Ph. Stoutjesdijk (Microclimatology)

J. van der Toorn (Ecology)

S.R. Troelstra (Soil science)

## 3. General summary (J.W. Woldendorp)

The two multidisciplinary research projects on the adaptations of birds and plants to their environment were continued in collaboration with the universities of Utrecht and Groningen. This report contains the preliminary results of parts of both projects. A contribution on salt transport in the dunes of Voorne (applied research) is also included.

In the project on 'Comparative population-ecological research on birds, with the Great Tit *Parus major* serving as a reference' a study was made on the heritability of bill dimensions in the Great Tit. High heritability values were found for this character and it was concluded that the genetic variation present in bill measurements would allow a rapid response to selection. However, there is still insufficient knowledge of the phenotypic changes in the bills of individuals in the various seasons. Therefore, no definite conclusions can be drawn concerning the existence of selection on bill size. Circumstantial evidence suggests, however, that this selection pressure is not very strong. This leads to the preliminary conclusion, that it is the absence of selection and not the absence of sufficient genetic variation which makes a study of micro-evolution of this aspect difficult.

The Coot *Fulica atra* has been studied for a number of years to compare certain aspects of its population biology with those of the Great Tit. In contrast to the latter species, the Coot is a partial migrant and a waterbird; it has, however, in common with the Great Tit a breeding territory. In studies of the Great Tit much attention has been paid to the effects of the severity of the winter on the survival of the breeding birds and on the consequences of this for the number of breeding pairs, and it seemed worthwhile to study similar effects in the Coot. It was found that in the latter species too, the survival of breeding birds depends on the severity of the winter, but that the change in breeding density from one season to the next is not related to this factor. A cyclic pattern was found to occur in the population size that was ascribed to changes in spacing behaviour. This hypothesis will be tested in future research.

Apart from the studies on the population biology of bird species the institute also administers the Dutch Ringing Scheme and the Euring Data Bank. In the bank recoveries from the 30 European ringing centres are collected. In the present report a survey is given of the numbers of birds recovered in the various European countries and of the numbers that have up till now been collected in the Euring Data Bank.

The project on 'Comparative research on demographic, physiological and genetic properties of grassland plants in relation to their specific place of occurrence' was continued in close collaboration with the Departments of Plant Physiology and Population Genetics of the University of Groningen and the Department of Plant Physiology of the University of Utrecht. In the project that till 1988 is subsidized by the Netherlands Organization for the Advancement of Pure Research, the five *Plantago* species occurring in the Netherlands are being studied. In the study of the environment of these species the effects of drought were investigated. After rain the soil surface in an open vegetation dries out quickly and there is an abrupt change in microclimatological conditions. In a closed vegetation a humid climate near the ground is maintained much longer. In the latter case the temperature near the soil surface is 1.5°C below that of the free air, while in open vegetation it may be 12 to 14°C higher. Short-term variations in temperature are much more frequent in the latter case than in a lush vegetation; this also applies to diurnal changes. In a dense vegetation there is a gradual change in microclimate during development of the vegetation and an abrupt and long-lasting effect of mowing. In the latter respect short open grassland is a more stable habitat.

As another aspect of the habitat of the *Plantago* species the seasonal variations in the various phosphorus-fractions of the soil were studied. A considerable spatial variation in all organic P-fractions was found but the seasonal variation, if any, is not spectacular. The latter is assumed to be due to the occurrence of steady state conditions between mineralization and plant uptake. In this way shifts in the mineralization/immobilization turnover rate can be masked.

In the demographic studies the population development of *P. lanceolata* was investigated in a pasture and a hayfield, that were both sown 15 years ago,

and which, apart from the management, were in all other respects alike. In both situations the *P. lanceolata* populations appeared to be still in a stage of development and it is assumed that the populations are spreading outwards from their initial place of settlement. This process is in a more advanced stage in the hayfield than in the pasture. The latter possibly originates from a comparatively higher survival of both adult plants and seedlings. The data so far obtained are consistent with the notion that relatively open hayfields on poor soil are the optimal habitat of *P. lanceolata*.

A preliminary discussion of the demography of a coastal type of *P. major* is given. The life-history characteristics of this type, that has the morphology of *P. major* ssp. *pleiosperma* and the low seed numbers per capsule of *P. m.* ssp. *major*, were studied at three different sites. No large differences in these characteristics were found. In the near future the demographic behaviour of this type will be compared with that of other *P. major* populations.

The study of the morphological characteristics of *P. lanceolata* populations was continued by comparing plants grown from seeds. The same trends were found as in the experiments with root cuttings which were reported on last year. Plants from open and low vegetation are predominantly characterized by short, narrow leaves and flat rosettes, those from dense and high vegetation possess long broad leaves and erect rosettes. The variances of these characters were higher in plants from seeds than in those from cuttings. From this result it is hypothesized that the adult plants surviving in the field are a selection from plants present in the seed. A study of selection phenomena in the field is in progress.

A comparison was made of the potassium and phosphorus requirements of *P. major* ssp. *major* and *P. lanceolata*. In preliminary waterculture experiments it was found that the yield of *P. major* decreased more strongly as a response to a decreasing K-availability than that of *P. lanceolata*. In shoots of *P. lanceolata* a decreasing potassium concentration was more compensated by an increasing sodium content than in those of *P. major*, where compensation by calcium was observed. A similar trend was also observed in plant material from the field. In this field material the potassium content of *P. major* was considerably higher than that of *P. lanceolata*. The data support those of earlier publications in which it was stated, that *P. major* shows a preference for a soil with a relatively high K-status. In water cultures *P. major* reacted more strongly to an increase in P-concentration than *P. lanceolata* and in field material of the former species generally higher P-contents were found. This leads to the preliminary conclusion that *P. major* has a relatively high P-requirement. This may be one of the reasons for the species to occur in sites where a more or less uninterrupted P-supply is guaranteed by adequate soil moisture conditions, since P-transport in the soil-root system is mainly by diffusion.

Considerable attention has been given to the growth of *P. lanceolata* in permanently waterlogged soils. Since this species forms hardly any aerenchyma under such conditions, it has to cope with anaerobic conditions in a different way. Some indications were obtained that oxygen supply takes place via the aerenchyma of neighbouring species such as *Carex nigra*. Also nitrate formation should take place under the influence of the latter species and thus explain the levels of nitrate-reductase in *P. lanceolata* which are higher than the basal level. Laboratory experiments to prove this hypothesis gave no unequivocal results. This was due to the technical problems of such experiments. Therefore, to elucidate the critical role of oxygen diffusion in wetland ecosystems and effects on nitrogen cycling, more information needs to be collected.

In a study of the effects of low ambient nitrate concentrations on uptake and growth of *P. major* and *P. lanceolata* it was found that the uptake rate of the former species was higher. This is in accordance with results from other authors which suggest that eutrophic species have a greater root absorption capacity than oligotrophic species. This hypothesis will be tested in further experiments on the uptake kinetics of both species.

In recent years an extensive study has been made of the influence of salt spray on the vegetation of the coastal dunes of Voorne and Goeree. This became necessary as the coastal morphology has changed considerably recently due to the influence of man and will change still more in the near future as plans exist to store mud from the harbour of Rotterdam in a huge basin off the coast of Voorne. This study shows that the composition of the vegetation has changed considerably due to diminished salt transport.

#### 4. Heritability of bill dimensions in the Great Tit (A.J. van Noordwijk, P.L.M. Klerks)

##### INTRODUCTION

Many tit species are found almost exclusively in either broadleaved or coniferous woods. Both in Europe and in North America there is a clear difference in bill morphology associated with habitat. Tits from broadleaved woods have relatively broad and short bills, while species from coniferous woods have relatively long and slender bills (Snow 1954; Lack 1966). The same difference is also found between populations within a species, in the Coal Tit *Parus ater* and the Blue Tit *Parus caeruleus* (Lack 1969). The long and slender bill found in coniferous species is thought to be advantageous because it improves access to insects between the needles and in the cones, that form a large part of the diet (Snow 1954; Lack 1966). It is not clear how selection in favour of the broad and short bills in broadleaved habitat operates.

The Great Tit is found in both coniferous and broadleaved woods, albeit in different densities. If the explanation for the between-species difference in bill morphology is valid, one expects that Great Tits are subjected to both opposing selective forces. In mixed habitats there may be differences between individuals, or even the same individuals may be undergoing the different selection regimes. It is therefore interesting to study the variation in bill

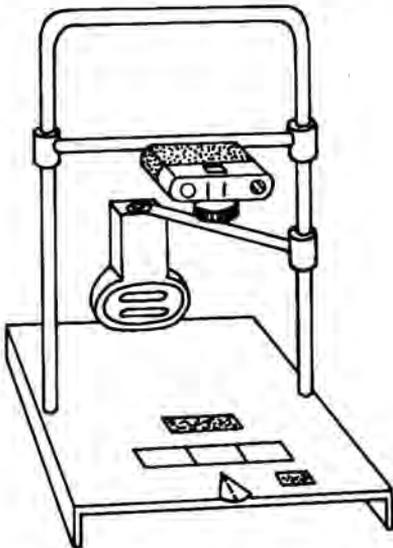


Fig. 4.1. The apparatus used for photographing bills in the field. The bill is held in the triangular gap in the front of the platform. Camera, flashlight, reference scale and identification stickers are shown.

morphology in a population inhabiting a mixed forest, and to establish the extent of its genetic variation. There are, however, a number of practical problems in measuring bills on live birds with sufficient accuracy.

## METHODS

Initial attempts to measure bills in the field with sliding callipers were abandoned, because they were too time consuming and lacking in reproducibility. Both problems were overcome by photographing the bills in the field together with a reference scale. Bills were held on a platform above which a camera and a flashlight were fixed (Fig. 4.1). Pictures were taken from above and from the side. Measurements were made in the laboratory using sliding callipers to the nearest 0.1 mm from projections of the negatives at 5 times life size.

This report will be limited to three bill measurements:

- 1) Length from the tip of the bill to the anterior edge of the nostril (mean of measurements from above and from the side).
- 2) Breadth at 6 mm from the tip.
- 3) Height at 6 mm from the tip.

For comparison with overall body size, measurements of tarsus length were also taken and included in the analyses. Measurements were made on breeding birds from the island of Vlieland in 1979 and on birds caught during inspections for roosting birds in the same population in December 1979. The fact that

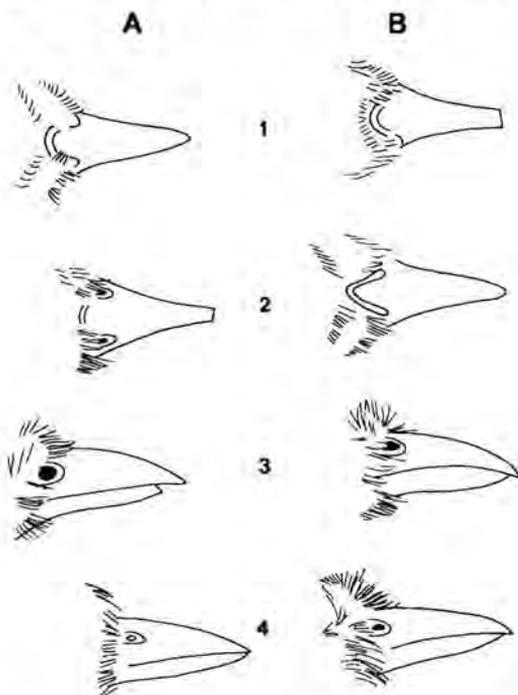


Fig. 4.2. Examples of abnormal bills of Great Tits encountered on Vlieland. Column A in the breeding season 1979 and column B in December 1979.

- 1) number B 347998, male born in 1976 (from above)
- 2) number B 347758, male born in 1974 (from above)
- 3) number B 383001, male born in 1976 (from aside)
- 4) number B 383099, male born in 1976 (from aside).

pedigrees are relatively well known for the tits in the Vlieland population makes it possible to establish the resemblance between relatives, from which inferences about the importance of genetic variation can be drawn (see van Noordwijk *et al.* 1980 and previous Progress Reports for a short introduction to quantitative genetics and the application of these methods to data from natural populations).

## RESULTS

Before going into the analyses of the data, it is worth mentioning that one of the most striking features of the data collected is that the bills of more than five per cent of the birds had clearly visible defects, such as broken tips or outgrown upper or lower mandibles (Fig. 4.2). Many of these individuals were retrapped later and were then usually found to have normal bills. Although the numbers are too small for a rigorous analysis, there is no indication that survival was affected by these bill defects. Moreover, the nestlings of individuals with clearly visible bill defects during the breeding season seemed to be in good condition.

Even though individuals with gross defects were left out of the data used for further analysis (15 out of 236 captures), there will be a number of cases where similar defects had partly healed at the time of observation and were not detected as abnormalities. Especially with fairly low numbers of observations such somewhat erratic measurements may have a considerable effect on the results. This is the main reason for excluding the 15 cases with the most extreme visible defects. These form a biologically meaningful aspect of the phenotypic variation in bill size and bill shape, but they must be dealt with separately due to the relatively small number of observations.

Some of the main results are summarized in Table 4.1. For each of the three bill measurements, the repeatability and three heritability estimates are given. For comparison the same parameters for tarsus length have been included. The results for tarsus length, with a high repeatability and a strong resemblance between full sibs, but with no parent-offspring resemblance, are typical for a situation with poor nestling growth (van Noordwijk 1982). There is, however, no difference in mean tarsus length between nestlings and parents which would have been expected. The fact that the full sib resemblance is high, while the parent-offspring resemblance is low must mean that the resemblance is caused by sharing of environments rather than sharing of genes.

The results for bill measurements show a different pattern, although the same individuals were used in both analyses. Both for bill length and breadth at 6 mm from the tip, we find that both parent-offspring heritability estimates are close together and that these are higher than the repeatability and lower than the resemblance based on full sibs. For bill height all four estimates are close together. Considering that no heritability is observed for tarsus length in the same sample, the heritability estimates for bill length of 0.5, for bill breadth of 0.7 and for height of 0.8 are surprisingly high.

## DISCUSSION

### *Methodological problems*

In this summary of results, several problems have so far been ignored. There are significant differences in the means between sexes, and between measurements made in the breeding season and in December, as well as a few similar problems. A more detailed account of these problems will be given elsewhere, together with the results for other primary and derived bill measurements (van Noordwijk and Klerks, in prep.).

Heritability estimates calculated separately for each sex, using data from one of the two periods only, are very similar to the overall results. In this respect there are two important differences between the repeatability and the

heritability estimates presented. In calculating repeatability estimates, the differences between the periods are included in the intra-individual variance, while the difference between sexes is included in the inter-individual variance. In the heritability estimates the difference in mean value between sexes is excluded from the analysis by combining the estimates for given combinations of sexes. It is important to note that there are no differences in variance between the sexes. In both parent-offspring estimates the number of cases where measurements on the parent and on the offspring were made in the same period is about equal to the number of cases where measurements were made in different periods. This means that the parent-offspring heritability estimates are relatively unbiased. The weakest aspect of these estimates is that they are based on a few year-classes and a single season.

*The difference between bill and tarsus*

The tarsus is a structure for which growth is completed very early (by about day 12 after hatching), whereas the bill reaches full adult size and shape about 6 weeks after hatching (O'Connor 1975). In this respect tarsus and bill represent extremes in the length of the growing period. There is also a difference in the fact that the bill is a horny structure that continually wears and grows, while the tarsus length does not change after growth is completed. In other words the repeatability of tarsus length is 1.0 apart from measurement error, whereas the repeatability for bill measurements includes both measurement error and real, biologically meaningful, variation. This allows us to give a speculative explanation for the difference in repeatability and heritability between tarsus and bill measurements. There are two points to be explained: 1) why are the heritability values for bill measurements higher than

Table 4.1. Repeatability and heritability estimates for bill measurements and tarsus length. Data from Vlieland 1979.

	repeatability (1)	heritability		'heritability'
		midparent (2)**	single parent (3)**	full sibs (4)**
Bill length	0.25	0.49*	0.48*	0.75*
Bill breadth (at 6 mm)	0.33*	0.68*	0.70*	0.92*
Bill height (at 6 mm)	0.76*	0.71*	0.92*	0.92*
Tarsus length	0.93*	-0.07	-0.03	0.92*
Approx. sample size	25	50	140	80

\*  $P < 0.05$

\*\* There is no evidence for heterogeneity among the estimates that were combined.

- (1) Repeatability for combined sexes. Values for females ( $n = 15$ ) are similar, values for male bill measurements ( $n = 10$ ) are lower.
- (2) Weighted mean of daughter-midparent and son-midparent estimates.
- (3) Weighted mean of mother-daughter, mother-son, father-daughter and father-son estimates.
- (4) Weighted mean of estimates based on intra-class correlations within sexes and product moment correlations for brother-sister combinations.

the repeatability and 2) why is the heritability for bill size higher than that for tarsus length?

Normally the repeatability is an upper limit for the heritability (Falconer 1960). This is not true, however, in the situation where the general environmental variance (*i.e.* environmental factors that are constant within, but variable between individuals) is less important than the systematic difference between the periods of measurement. In our case there is independent evidence for a difference between periods. The difference in mean values for the two periods is relatively large and significantly different from zero for bill length and bill breadth. Consequently, we may conclude that the general environmental variance is unimportant. This provides a sufficient explanation in answer to the first question. The second question may be answered from the observation that the environmental (*i.e.* non-genetic) variance in tarsus length will be the result of the environmental conditions experienced during the first 12 days after hatching, whereas the period during which the environment affects bill size is much longer, both during initial growth and during subsequent life. This makes it possible that the difference in environmental conditions experienced by the parents and by the offspring during the important period is much greater for tarsus length than for bill size.

There are two possible ways in which this may result in a poor parent-offspring resemblance, either the conditions during growth of the offspring may have been poor, so that the offspring do not grow to their full genetically programmed size, or this may have been the case for the parents. Van Noordwijk (1982) argued that the first of these possibilities is the more likely, because of fledglings in poor condition have a very small chance to become members of the breeding population. In the data for Vlieland, however, the nestlings do not have a lower mean tarsus length than the parents. Within the group of parents the tarsus length of the one year old birds is lower than that of the older birds. This does not explain why there is some resemblance in tarsus length between offspring and mothers, but not between offspring and fathers. At present our only acceptable hypothesis is, that the lack of heritability for tarsus length is mainly caused by the presence of a number of male breeding birds whose tarsus length has not reached its full genetically programmed size, while the longer period during which the environmental conditions have an effect on the bill phenotype is thought to be responsible for the absence of a similar effect on bill size and shape.

## CONCLUSIONS

The presence of genetic variation for bill measurements indicates that there is a potential for a rapid response to selection. This makes it feasible to study changes in bill size as a response to changes in environmental conditions. In other words the high heritability values (together with the observed amount of variation) suggest that the differences that are found between species and between populations could be brought about in a short time if there was strong selection. Before a more detailed study of the selection operating on bill size and shape can be undertaken, more knowledge of the phenotypic changes in the bills of individuals through the seasons is necessary. At present the anecdotal observations of the normal survival of individuals with gross abnormalities suggest that in this population selection for bill dimensions was not very strong. Therefore, no conclusions can be drawn with respect to relations between the amount of genetic variation and possible mechanisms that may exhaust or maintain this variation. This leads to the overall conclusion that it is the absence of selection and not the absence of sufficient genetic variation which makes a study of micro-evolution difficult.

## REFERENCES

- Falconer, D.S. (1960) - Introduction to quantitative genetics. Oliver and Boyd, Edinburgh & London.
- Lack, D. (1966) - Population studies of birds. Clarendon Press, Oxford.
- Lack, D. (1969) - Ecological isolation in birds. Blackwell, Oxford.
- Noordwijk, A.J. van (1982) - Variation in body weight of the Great Tit, heritability and condition. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk. 2e Reeks 79, Progress Report 1981 I.O.O., 9-12.
- Noordwijk, A.J. van, J.H. van Balen and W. Scharloo (1980) - Heritability of ecologically important characters in the Great Tit. *Ardea* 68, 193-203.
- O'Connor, R.J. (1975) - An adaptation for early growth in Tits *Parus* spp. *Ibis* 117, 523-526.
- Snow, D.W. (1954) - The habitats of Eurasian tits (*Parus* spp.). *Ibis* 96, 565-585.



Plate 1. The Coot *Fulica atra* is one of the two bird species under study by the institute. This non-passerine, partial migrant and water bird forms a good comparison with the intensively studied Great Tit *Parus major* (Photo J. Visser).

5. Local survival of the breeding birds in a Coot population (A.J. Cavé, J. Visser)

## INTRODUCTION

Since 1964 the population dynamics of the Coot *Fulica atra* have been studied by the second author. This study was originally a project of the Population Ecology Department. When, however, in 1981 the Bird Migration Department joined the project the aims were reconsidered and the scope widened. One of

the aims of the present study is to understand the annual variation in numbers of breeding birds in the study area.

Although the Coot is a partial migrant in the Netherlands, a considerable part of the population stays in the vicinity of the breeding area during the winter. The Coot forages in and around the water, and during severe winters many die of starvation (J. Visser 1978).

This makes it worthwhile to evaluate the effects of winter severity on the local survival of the breeding birds and to consider its consequences for the number of breeding birds.

## MATERIAL

At the study area 'Westeinderplassen' the breeding birds were marked by metal leg rings (commenced 1966), and, additionally by coloured leg rings (commenced 1972), or numbered neck collars (commenced 1981). In the following seasons these birds were identified either by recapture or by the observation of the coloured leg rings or neck collars. In the present paper both kinds of identification as well as the initial capture are called captures.

Only captures at or near the nest in the years 1967-1982 have been used.

## ANALYSIS

If two breeding seasons are considered a breeding bird of the first season can be present or absent in the second season.

If the absence is permanent, the bird could either have died or have emigrated permanently. Without further information it is impossible to distinguish between these two possibilities, and they have to be grouped together as 'died locally'.

If the absence is temporary, the bird may have emigrated temporarily. It is also possible that the bird did not breed in the study area in the second year, although it remained there. These possibilities also cannot be separated by considering only the captures near or at the nest, and they are therefore grouped together as 'absent' in contrast to birds that are 'present' in the second year. Absent and present birds are grouped together as 'survived locally', in contrast to 'died locally' birds.

The absence of a breeding bird could not be stated with certainty, since not all the breeding birds are caught during the breeding season. It is therefore necessary to determine the capture rate, *i.e.* the fraction, captured in a certain year, of the breeding birds present in that year. This was possible as the number of breeding pairs were counted accurately for each year of the study. This rate, called the capture rate of the birds present, was on average 0.37 for the males and 0.32 for the females.

Birds that bred in previous years and that are still alive (not emigrated permanently) may be present or absent in a certain year. Captures in following years show that such birds are alive in that year. Of these birds a fraction is caught in that same year. This fraction is called the capture rate of the locally surviving breeding birds. If the birds are regularly absent from the breeding population, this capture rate is lower than the capture rate of the birds present. The mean capture rate of the locally surviving males and females was 0.39 and 0.34, respectively. These values do not differ appreciably from the values 0.37 and 0.32 given above for the capture rates of males and females present. These figures reveal that locally surviving breeding birds are rarely, if at all, absent from the breeding-bird population.

If a bird, once it has bred in the study area, continues breeding there until it dies or emigrates permanently, the local survival rate may be calculated from the captures and recaptures of two successive breeding seasons. Considering two successive breeding seasons (year 1 and year 2), the local survival rate is the fraction breeding in year 2 of the birds that have bred in

year 1. This local survival rate can be calculated from the fraction recaptured in year 2 of those captured in year 1, by correcting this fraction for the capture rate of the birds present in year 2. The resulting annual local survival rates are given in Table 5.1.

Table 5.1. Annual estimates of the local survival rates of males and females separately and combined for the years 1967/68-1981/82. Study area 'Westeinderplassen'.

Year	Local survival rate			Number of ice-days*
	males	females	combined	
67/68	0.67	0.83	0.75	5.2
68/69	0.47	0.53	0.50	13.9
69/70	0.40	0.30	0.36	18.8
70/71	0.69	0.46	0.61	10.2
71/72	0.74	0.44	0.64	5.8
72/73	0.65	0.82	0.72	1.6
73/74	1.11	0.58	0.84	2.2
74/75	0.64	0.96	0.79	0.0
75/76	1.03	0.70	0.87	8.8
76/77	0.79	0.82	0.81	3.2
77/78	0.93	0.58	0.72	7.8
78/79	0.51	0.26	0.36	30.6
79/80	0.52	0.58	0.55	8.4
80/81	0.62	0.55	0.59	4.8
81/82	0.57	0.40	0.49	16.2

\* Mean number of ice-days in November-February of the 5 main weather stations in the Netherlands.

Having estimated the annual local survival rates, it becomes possible to relate these rates to winter severity. The regressions of the annual local survival rates of the males and females on the winter severity, measured by the mean number of ice days in the Netherlands (Table 5.1) are calculated. The following results are obtained:

1. there is a highly significant negative regression in the females (low local survival rates with hard winters).
2. there is a nearly significant negative regression in the males.
3. neither the intercepts, nor the slopes of the regression lines of the females and the males differ significantly.

Based on the latter finding combined annual survival rates for males and females are calculated (Table 5.1). The regression of these local survival rates on the number of ice days is highly significant (Fig. 5.1).

Since the settled breeding birds suffer in hard winters, it may be expected that the same is true for the potential first-breeders. However, the change in total breeding-bird numbers from one season to the next is not related to the

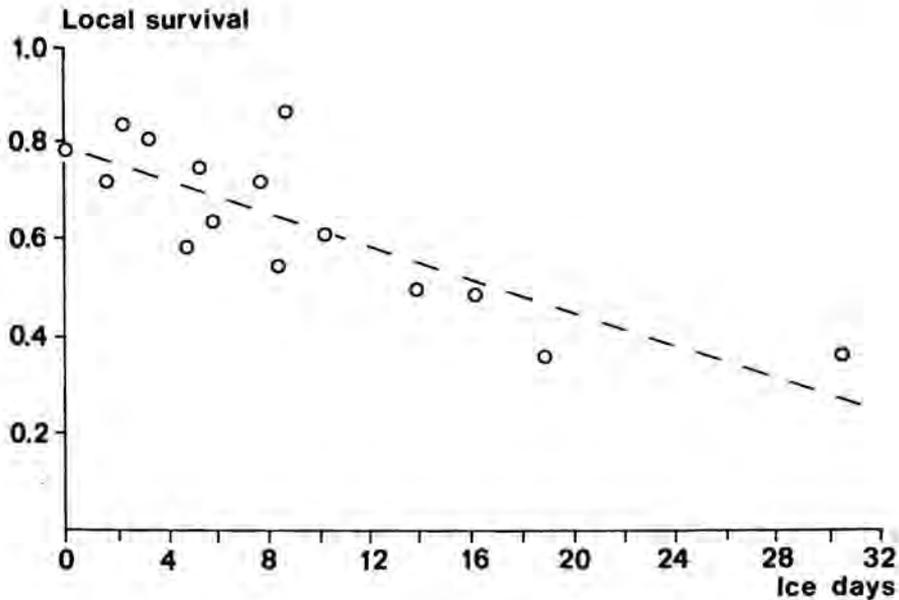


Fig. 5.1. Coot. The relationship between the mean number of ice days in November-February and the local survival rates of breeding birds (males and females combined) in the next season.

severity of the winter in between (Fig. 5.2), except perhaps for very severe winters (1978/1979, 30.6 ice days).

## DISCUSSION

This paper shows that the survival of the breeding birds depends on winter severity. It is remarkable, however, that the change of breeding-bird density from one season to the next is not related to winter severity, implying that the lost breeding birds are compensated for by new ones. This finding suggests that 1) there is an excess of potential breeding birds, and 2) competition for breeding territories is important.

Without further complications, limitation of the number of breeding pairs by territorial behaviour would result in a constant population. In the present case, however, the population density seems to follow a cyclic pattern (Fig. 5.3).

One suggestion for this pattern is that there are periodic changes in carrying capacity. There were no indications for this in our study area.

Another suggestion is that density-induced changes in territorial behaviour occur (Moss and Watson 1981).

Studies of the Great Tit (Drent 1983), and Steller Jay (Brown 1963) have shown that territorial birds are dominant over non-territorial birds. In conflicts, territorial birds win from non-territorials not only inside but also outside their territories. Non-territorial birds can only acquire a territory in areas not aggressively defended by territorial birds. New territories of Great Tit (Drent 1983), Western Gull (Ewald *et al.* 1980), and Sanderlings (Myers *et al.* 1979) are small when the number of potential occupants is large in relation to the area left free by the territorial birds. Holders of small ones try to enlarge their territories.

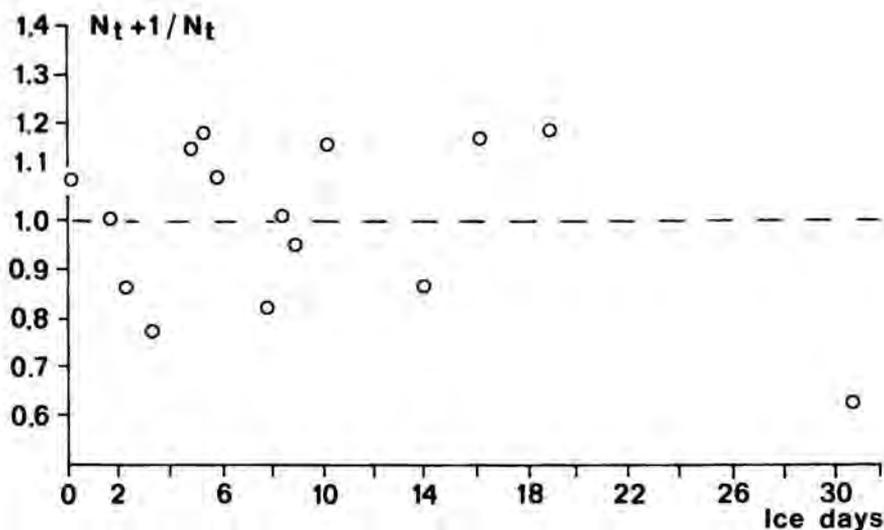


Fig. 5.2. Coot. The relationship between the mean number of ice days in November-February and the change in the number of breeding birds ( $N_{t+1}/N_t$ ) from year  $t$  to year  $t + 1$ .



Fig. 5.3. Coot. The number of breeding pairs at the study area in the years 1964-1982.

It is known (Drent, pers. comm.) that the attachment of the Great Tit to a certain site increases with the time spent there successfully. At the same time the bird becomes more aggressive in and around the territory. Consequently the possibilities of enlarging a small territory increase with the number of years that the bird is present (breeding experience).

The above sketched pattern of spacing behaviour may lead to a cyclic pattern in breeding-bird numbers. The underlying mechanism could be described as follows.

When the density is low the territories are large and the production of young is low. Provided that the number of candidates is higher than the number of territorial birds that have died, the latter are replaced by a larger number of new birds. Consequently, the breeding-bird population is larger than in the preceding year and produces more young. This results in a further increase of breeding-bird numbers and a further decrease in the mean territory size.

This increase in breeding-bird numbers continues until the density is so high and the territories so small that few, if any, candidates can join the breeding-bird population. As a result of the exclusion of new breeding birds the mean age (*i.e.* breeding experience) in the population increases: therefore, the territory holders are now more inclined to enlarge their territories, than during the period of population increase. At this stage of the cycle a large part of the territories that become free, are divided by neighbours and only a few new birds can enter the breeding-bird population.

This process continues until the birds no longer enlarge their territories at the expense of candidates and the population starts to increase again.

The main point in this hypothesis is that density depends on a changing spacing behaviour. In its turn the spacing behaviour depends on changes in the age-distribution (*i.e.* distribution of breeding experience). Changes in spacing behaviour underly also Chitty's (1967) hypothesis, postulating selection for aggressive genotypes at high density. Our hypothesis is analogous, but selection for aggressive genotypes is replaced by selection for individuals, which have acquired a large breeding experience. It is likely that variation in the survival of the breeding birds brought about by winter severity interferes with the cyclic pattern suggested, and hard winters possibly have synchronizing effects.

We hope to test the above hypothesis in the future and agree with the remark made by Watson and Moss (1964) that better information on spacing behaviour is a key to understanding population limitation in many vertebrates.

## REFERENCES

- Brown, J.L. (1963) - Aggressiveness, dominance and social organization in the Steller Jay. *The Condor* 65, 460-485.
- Chitty, D. (1967) - The natural selection of self-regulating behaviour in animal populations. *Ecol. Soc. Austr., Proc.* 2, 51-78.
- Drent, P. (1983) - The functional ethology of territoriality in the Great Tit *Parus major* L. Diss. RU Groningen.
- Ewald, P.W., G.L. Hunt and M. Warner (1980) - Territory size in Western Gulls: importance of intrusion pressure, defence investment, and vegetation structure. *Ecology* 61, 80-87.
- Moss, R. and A. Watson (1981) - Inherent changes in the aggressive behaviour of a fluctuating Red Grouse *Lagopus lagopus scoticus* population. *Ardea* 69, 113-119.
- Myers, I.P., P.G. Connors and E.A. Pitelka (1979) - Territory size in wintering Sanderlings: the effects of prey abundance and intruder density. *The Auk* 96, 551-561.

Visser, J. (1978) - Fat and protein metabolism and mortality in the Coot *Fulica atra*. *Ardea* 66, 173-183.

Watson, A. and R. Moss (1981) - Advances in our understanding of the population dynamics of Red Grouse from a recent fluctuation in numbers. *Ardea* 69, 103-111.

## 6. International bird ringing research (A.C. Perdeck)

Many questions in animal ecology can be solved only if the individual animal can be recognized. The obvious procedure is to mark the animal, and in birds the ringing of the leg is an easy way to do this. Ringing has further been stimulated by the fact that bird movements as such, i.e. migrations, are popular study objects. Because long distance movements are common, well-known international addresses have to be used on the rings, and an extensive administrative system is needed to keep accurate records.

This has led to the establishment of national ringing centres. These centres issue rings to field workers, keep files of all ringings and recoveries, and supply users and finders of rings with the history of the individual birds.

In the present century some 30 European centres have administered the ringing of at least 35 million birds and a million recoveries of ringed birds have been reported to them (for further details see Perdeck 1982). In the Netherlands the national ringing centre is part of the Institute for Ecological Research. The information at this centre is used on a large scale by Dutch universities and institutes for their pure and applied ecological research programmes. It was the first European centre that computerized the data, and developed an internationally standardized computer system that is now being introduced all over Europe (including the U.S.S.R.).

The Dutch ringing centre has held from the start a prominent place in the international organization of European ringing centres (EURING).

Since the founding of this body, the Dutch delegates have always expressed the opinion that an international centre for the management of all European ringing activities should have priority. For, as birds do not recognize fron-

Table 6.1. Number of birds recovered before 1983 according to country of ringing (in thousands).

<u>EC countries</u>		<u>Comecon countries</u>		<u>Remainder of Europe</u>	
United Kingdom	333(225)	USSR	60	Finland	83*
Netherlands	119(119)	Czechoslovakia	43	Sweden	55*
Denmark	106(64)	Germany (GDR)	20*	Norway	35
Germany (FRG)	100(77)	Poland	10*(4)	Switzerland	15(9)
France	60*(54)	Balkan states	10*	Spain	3
Belgium	60*(52)			Portugal	1*
Italy	26				
Total	804(591)		143(4)		192(9)
Grand total	1,139(604)				

\* rough estimate, no exact data available.

( ) available from the Euring Data Bank on 31-12-1982.

tiers, recoveries of a certain species ringed in one country should be complemented with recoveries of the same species ringed in all other countries.

Finally in 1975, following a Dutch proposal, EURING founded a common data-bank which is situated at our institute. The tasks of the Euring Data Bank are as follows:

1. Keeping a record of the number of birds ringed and recovered, in order to be able to inform research workers of the quantity of the material available for each species.
2. Collecting all records of recoveries of birds ringed in Europe in a computerized system.
3. Supplying data from the bank to institutes or persons for research purposes.

The Institute managed, to a great extent, to fulfil these tasks with its own means. However, to input older data, extra money was needed. A request for financial aid from the EEC was met with general approval by all committees concerned (both national and international), with the result that funds have become available to perform tasks concerning all the EEC countries.

The work is now in full progress, and the Euring Data Bank is increasingly used for research on bird ecology and conservation. A summary of the number of recoveries of birds ringed in Europe, together with the number available from the Euring Data Bank is given in Table 6.1.

## REFERENCE

Perdeck, A.C. (1982) - Bird-ringing in Europe. *Endeavour*, New Series 6, 27-33.

## 7. Effects of drought on the microclimate in grassland (Ph. Stoutjesdijk)

The microweather in the vegetation is closely coupled to the macroweather above it. Yet there is also an effect of past weather conditions, especially rain.

A bare (impervious) surface quickly dries after rain. As long as there is a film of water present it behaves like a wet surface. As soon as it dries out there is an abrupt change of temperature and humidity conditions on the surface and in the boundary layer adhering to it. The conditions on a soil surface are not quite as extreme but are very similar.

In a closed vegetation the situation changes drastically. The soil surface is sheltered from radiation and wind. The vegetation as a whole uses much more water than bare soil, once this surface has dried out. However, the vegetation draws its water from deeper layers of the soil.

Thus the soil surface stays humid and this, together with the shelter from wind and radiation and the water vapour produced by the vegetation, maintains a humid microclimate near the ground.

For how long, one is inclined to ask. A first impression is given by the scheme of Fig. 7.1. Here temperature and humidity conditions in several types of grassland are shown. The measurements were made in May after a week of dry sunny weather. The diagram shows the difference between the air temperature at 1 cm height in the vegetation and the air temperature at 1 m height above the vegetation. In the same way the difference between the saturation deficits ( $\Delta SD$ ) is displayed. The diagram also shows the difference between the vapour pressure in the vegetation and that in the free air ( $\Delta E$ ) and the absolute value of the saturation deficit in the vegetation (SD) and in the free air.

In heavily manured grassland (1) on wet soil the temperature is about 1.5°C lower than the free air temperature (19°C). The saturation deficit is about 1 mm Hg i.e. 9 mm lower than in free air.

More open grassland (2) is warmer and less humid but still the SD is lower than in free air.

Enclosed by the heavily manured grassland (1) there is a reserve of the original unmanured grassland of low productivity (3). A considerable part of the solar radiation reaches the ground and the moss layer is dry in spite of the waterlogged conditions in the upper soil layers. The microclimate at 1 cm is dry-warm, in great contrast to the manured grassland. The temperature is 12 to 14°C higher in (3) than in (1) and the SD is 12 to 20 times as high. This is an extreme example of how agricultural management can change microclimatic conditions.

In a vegetation of *Festuca ovina* (4) on high ground but still humid (water content of the upper 5 cm 36% by volume) the microclimate is dry and warm.

Finally there is dry short grassland on dry ground (5) in which the temperature and the SD are still higher.

*Plantago lanceolata* occurs over the whole range of conditions with the exception of the lush dense grassland (1). In absolute terms conditions in the lush grassland (1a) have not changed much shortly (36 hours) after the last rain. In the hayfield (2a) there is a clear decrease of the SD and T is somewhat higher.

In the unmanured grassland (3a) the conditions are now warm-humid, the moss layer is damp.

The dry grassland (4a, 5a) is still in the dry-warm sector or more correctly again, for in this type of grassland a warm-humid microclimate is found only shortly after rain and one day of dry sunny weather is sufficient to bring it back into the warm-dry sector.

The dense high grassland can maintain a high humidity at the 1 cm level even in prolonged dry periods.

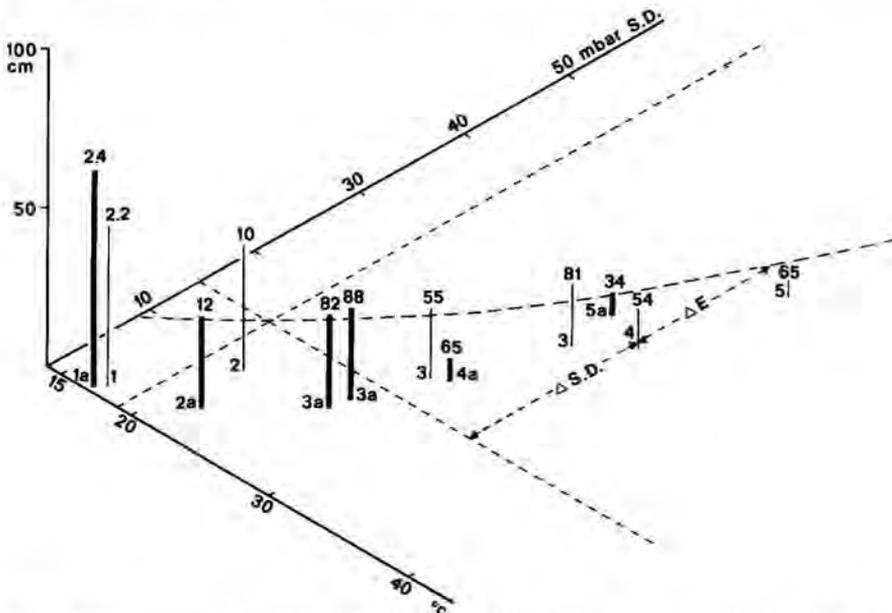


Fig. 7.1. Temperature and Saturation Deficit in different types of grassland. The length of the bars indicates the height of the vegetation, the number at the top of the bars gives the percentage of solar radiation transmitted. Thin bars: after dry period; heavy bars: shortly after rain. Further details in text.

The month of July 1982 was very dry, the only rain of importance fell within the first few days and on the 15th. On the 26-28th there were minor showers of which the effect on the 29th was not perceptible on soil or vegetation. On 3rd August temperature and humidity were measured in several grassland stands of between 25 and 70 cm high and a transmission between 3,8 and 6,8 per cent. The temperature excess,  $\Delta T$ , was between 2,6 and  $-2,0^{\circ}\text{C}$  and  $\Delta\text{SD}$  between  $-5,3$  and  $-12,4$  mm Hg.

It is significant to state that this was the case both in grassland where the root zone must have been within the reach of capillary water and those where the root zone was far above it. In the latter case the water content in the upper 5 cm of the soil ranged between 7,8 per cent and 14,4 per cent by volume. The soil composition varied between fine humous sand and sandy clay and the water content of the topsoil must have been close to the permanent wilting point.

At the time no measurements were made in the unmanured grassland (3) but its height (50 cm) and density (5% transmission) as well as measurements on other occasions leave little doubt that at this stage it can also maintain a high humidity in the vegetation.

As an outlook for future work we shall now consider the changeability of habitat conditions in a more general way.

In the open dry vegetations where high values of  $\Delta T$  occur there are short temperature fluctuations of several degrees with a time scale of seconds. There are longer term fluctuations due to large scale convection effects of moving clouds, these have a time scale of several minutes to half an hour. Again these fluctuations are strongest with high  $\Delta T$  values. Changes in air humidity are coupled with the temperature variations.

What has been said of the short term fluctuations also applies to the diurnal changes because nocturnal conditions do not differ greatly between the vegetations and so the diurnal variations are strongly coupled to the  $\Delta T$  values attained by day.

Of the long-term fluctuations the effect of drought in relation to vegetation structure has already been mentioned.

Another aspect is the slow change of habitat conditions during the development of the grassland and the abrupt and long-lasting effect of mowing. In this respect short open grassland is a more stable habitat than high, lush grassland.

## 8. Seasonal variation of Olsen-P and organic P fractions at some sites in relatively old dune grasslands (S.R. Troelstra, M.A. van der Meulen, R. Wagenaar)

### INTRODUCTION

Research on the organic phosphorus pool in coastal dune grasslands (Troelstra and van der Meulen 1979, 1980) was continued in 1980 and 1982. One of the objectives of this research was to establish possible seasonal variations in organic P fractions (Halm *et al.* 1972; Cole 1977; Bowman and Cole 1978) and in Olsen-P (Olsen *et al.* 1954).

In a previous paper (Troelstra and van der Meulen 1980) the plan was put forward to investigate the fluctuations of the labile organic P pool in fresh field material during the season and in a laboratory-incubation experiment, extracting complete soil cores or remoistened pretreated soil material without being dried previously. At a later stage, we decided to also investigate in fresh field material the possible fluctuation of the other organic fractions, *i. e.*, moderately labile, moderately resistant, and highly resistant.

## MATERIAL AND METHODS

The soil samples collected at site T4 during the 1978-1979 period of the nitrogen research program (Troelstra and Wagenaar 1980), were subjected to an organic P fractionation scheme as outlined by Bowman and Cole (1978). Except for the bicarbonate extraction (Olsen-P and labile organic P), ground samples were used.

In the following, all sampling procedures refer to the 0-10 cm soil layer. In March 1980, another location (T4-P) was selected in the Westduinen area (island of Goeree) in the immediate vicinity of site T4. Three grids (60 x 60 cm; subdivided into sixteen units of 15 cm square) were laid out adjacent to each other, in an L-shape (Fig. 8.1, A). Directly outside the area of the grids a bulk sample was taken for the analysis of site characteristics. On March 17 1980, grid III was sampled (24 soil cores) for aerobic incubation in the laboratory at 30°C and at field moisture content (present at sampling). The intact soil cores were put in polypropylene centrifuge tubes (closed with 20  $\mu$  polyester gauze, an open inner lid and a perforated outer lid, respectively), transported to the laboratory in a cooling box, and incubated for periods up to 30 weeks. The other two grids were used for sampling during the season and their exact position was marked. On each occasion the following samples were taken from corresponding adjacent grid units (Fig. 8.1, A): 1) 3 soil cores for P analyses (total of 6); 2) 3 soil cores for moisture content determinations (total of 6). Samples for P analyses were transported in a cooling box to the laboratory and stored overnight in a cold room. Moisture content determinations were started immediately upon arrival and the NaHCO<sub>3</sub> extractions were performed the following morning.

Dried and sieved soil material from sites T2 and T4 was also incubated aerobically in polypropylene centrifuge tubes at 30°C and 20 per cent moisture up to periods of 31 weeks (T4) or 15.5 months (T2). After incubation, the T2 samples were dried for a short time at 40°C before further analysis, whereas the T4 samples were extracted without previous drying. The samples of T2 and T4 were analyzed for all P fractions (Olsen-P, inorganic and organic P fractions; the complete results of the fractionations were reported elsewhere: Troelstra and van der Meulen 1980) and NaHCO<sub>3</sub>-P (Olsen-P and labile organic P), respectively. The incubation of the T4 samples was performed in triplicate.

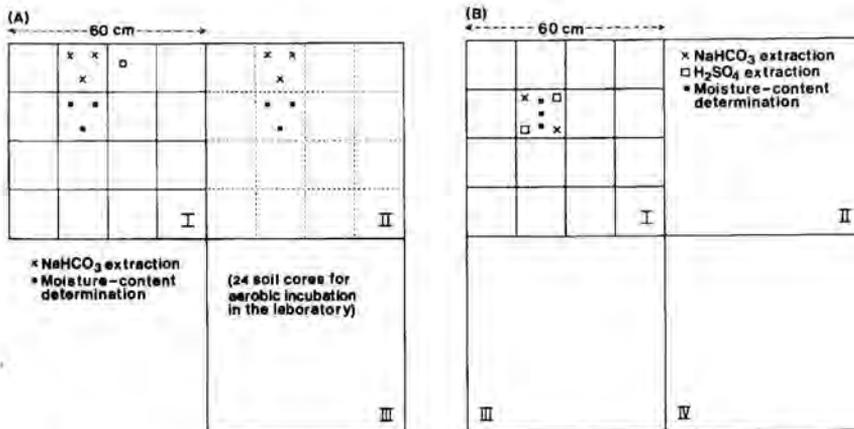


Fig. 8.1. Sampling procedures applied in the studies on organic P at site T4-P in the Westduinen (A) and at site HP in the Heveringen (B).

Since in some instances (entire soil cores or complete contents of centrifuge tubes) the use of a 1:20 soil: solution ratio is rather unpractical, a 1:5 (dry) soil: (extracting) solution was applied. Preliminary experiments with varying soil: solution ratios (1:20-1:5) and shaking periods (0.5 and 1 hr) indicated relatively small effects on the results. The general trend was slightly higher values of Olsen-P and labile organic P at the lower soil: solution ratios. We had adopted a shaking period of 1 h instead of 0.5 h; based on the results of the preliminary experiments, the maintenance of a 0.5 h shaking period is perhaps to be preferred. For our purpose, i.e., the detection of seasonal fluctuations, however, the modified technique can certainly be considered to be adequate. P concentrations were calculated as ppm P oven-dry weight, and allowance was made for the average moisture content on each sampling date or the moisture content after incubation (the 'effective' soil: solution ratios were always slightly lower than 1:5).

In March 1982, a site (HP) was selected for seasonal sampling in a relatively old dune area in the immediate proximity of the laboratory (Heveringen, Oostvoorne). Four grids (60 x 60 cm) were laid out in a square (Fig. 8.1, B) and their exact position marked. Directly outside the area of the grids bulk samples were taken for the analysis of general chemical characteristics of the site. On each occasion the following samples were taken from randomly selected corresponding grid units:

- 1) 2 soil cores for NaHCO<sub>3</sub> extraction (total of 8);
- 2) 2 soil cores for H<sub>2</sub>SO<sub>4</sub>/NaOH extraction (total of 8);
- 3) 3 soil cores for moisture-content determinations (total of 12).

Grids I/II and III/IV were always sampled on 2 successive days, respectively. In this sampling program a smaller gouge auger was used than in the previous studies and the samples were collected directly in 500-ml Erlenmeyer flasks and moisture content tins, respectively. Upon arrival at the laboratory, extraction procedures were immediately started using a (dry)soil: (extracting) solution ratio of 1:10 and a shaking period of 0.5 h. A first estimation of the moisture content was obtained by means of a sampling procedure performed 3-4 days prior to the sampling dates for the P analyses (4 soil cores taken directly outside the area of the 4 grids). Final P concentrations were calculated using the moisture percentages determined on each sampling date and for each grid.

For both extractions, preliminary experiments with varying soil: solution ratios indicated relatively small effects on the results. Following the H<sub>2</sub>SO<sub>4</sub> extraction, soil samples were quantitatively transferred to filter paper in a Buchner funnel and washed several times with a total of 700 ml of demineralized water and 25 ml of ethanol, after which the soil was dried at 70°C.

Table 8.1. Some selected soil properties (0-10 cm depth) of three sites in the Westduinen area (T2; T4; and T4-P) and one site in the Heveringen (HP).

Site	pH		% organic matter (loss-on-ignition)	ppm			
	H <sub>2</sub> O	KCl		total P	total organic P	labile organic P	Olsen P
T2	4.7	3.8	5.4 ± 0.68*	192 ± 12	153 ± 15	35.5 ± 5.7	6.8 ± 2.3
T4	4.4	3.4	5.0 ± 0.47	142 ± 12	119 ± 10	33.8 ± 5.4	6.2 ± 1.7
T4-P	4.2	3.3	5.3	145	118	34.1	6.3
HP	4.9	4.0	6.6	221	145	25.0	8.2

\* mean ± standard deviation (19 samples dates)

## RESULTS AND DISCUSSION

Site characteristics are shown in Table 8.1. Indeed, the properties of the neighbouring locations T4 and T4-P are very similar. Although the figures of sites T2 and T4 are comparable, there are distinct differences. Most values are higher for site T2 (the same holds for not-shown values of total N, exchangeable Ca and Mg, and 'effective' CEC), which is also a more productive site in respect of the above-ground biomass (Troelstra and Wagenaar 1980). Location HP in the Heveringen area resembles the three sites in the Westduinen. Sites HP and T2 are comparable with regard to pH, total P, and total organic P. The organic matter content was highest at location HP. However, when interpreting the figures, it should be borne in mind that the values shown in Table 8.1 refer to different intensities of sampling (number of soil cores, sample area), which are of importance in view of the small-scale spatial variability present (Troelstra 1978). During the season, the moisture content varies in a similar way at the 4 locations (Fig. 8.2; for site T2 see Fig. 7.5.1b in Troelstra and Wagenaar 1980).

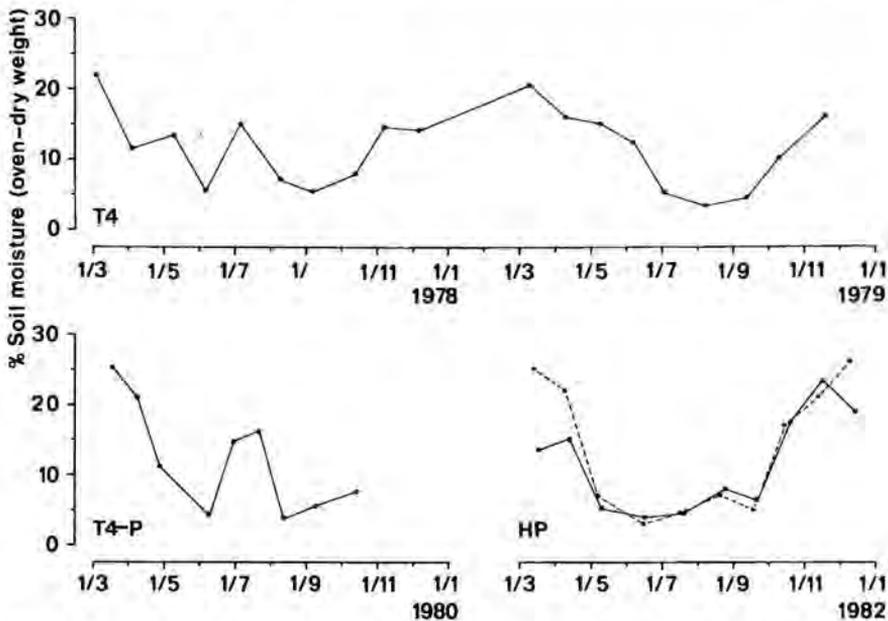


Fig. 8.2. Seasonal patterns of soil moisture content in the 0-10 cm soil layer at locations T4 and T4-P in the Westduinen, and at site HP in the Heveringen.

Fig. 8.3 shows the seasonal pattern of organic P fractions and Olsen-P at site T4 during 1978-1979. For site T2 the variation of Olsen-P and labile organic P was shown in a previous study (Fig. 7.6.1b in Troelstra and van der Meulen 1980). When looking at Fig. 8.3, we can restate our previous conclusion (Troelstra and van der Meulen 1980) and extend it to all organic P fractions. It appears that much of the observed fluctuation is probably spatial and seasonal variation of organic P fractions, if any, is not spectacular for the Westduinen area. When expressed as percentage of the sum of the organic P fractions, the following values were found for the 2-year period (mean  $\pm$  standard deviation): 32  $\pm$  4 (labile), 30  $\pm$  3 (moderately labile), 27  $\pm$  1 (moderately re-

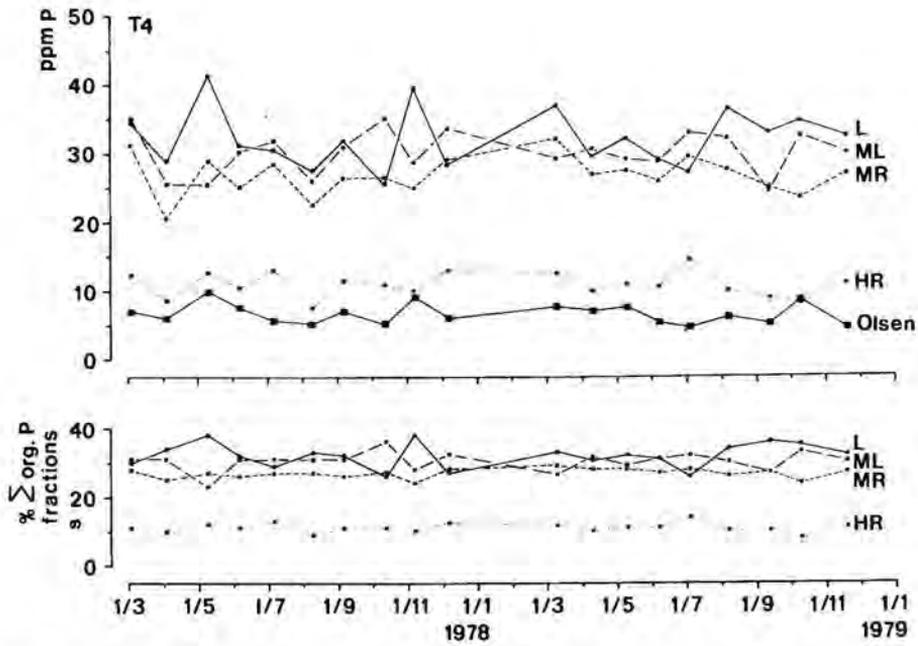


Fig. 8.3. Seasonal patterns of organic P fractions and Olsen-P at site T4 in the Westduinen (0-10 cm soil depth); L = labile, ML = moderately labile, MR = moderately resistant, and HR = highly resistant (organic P).

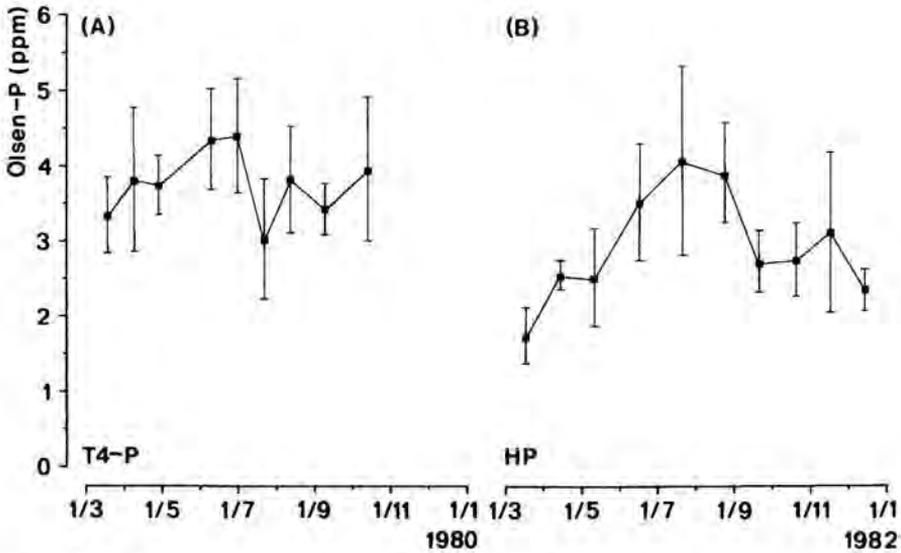


Fig. 8.4. Seasonal variation of Olsen-P in the 0-10 cm soil layer at location T4-P in the Westduinen and at site HP in the Heveringen.

sistant), and  $11 \pm 1$  (highly resistant). These values refer to pretreated (dried and sieved) soil material, whereas ground samples were used for the  $H_2SO_4/NaOH$  extractions.

The results of the bicarbonate extractions of fresh field material are shown in Figs. 8.4 and 8.5, A. At location T4-P, the Olsen-P levels were relatively constant and a distinct seasonal trend could hardly be detected (Fig. 8.4, A). On the other hand, at site HP a seasonal trend was observed with the highest levels occurring during the period July-August (Fig. 8.4, B). Labile organic P values showed a decreasing trend at site T4-P, whereas no seasonal pattern was found for location HP (Fig. 8.5, A). However, as to the decreasing pattern at T4-P, it should be mentioned that the separate trends for the grids I and II (Fig. 8.1, A) were rather different (results not shown). With respect to the expression of the over-all behaviour of the labile organic P pool, the picture in Fig. 8.5, A must therefore be seen as slightly distorted.

To a certain extent, the observed effects are in line with the results of the incubation in the laboratory for intact soil cores of site T4-P. During the incubation period of 217 days under nearly optimal conditions (*e.g.*, ca 20% soil moisture throughout the period), Olsen-P showed a 6-7 fold increase, whereas the labile organic P level remained relatively constant (Fig. 8.5, B). Under

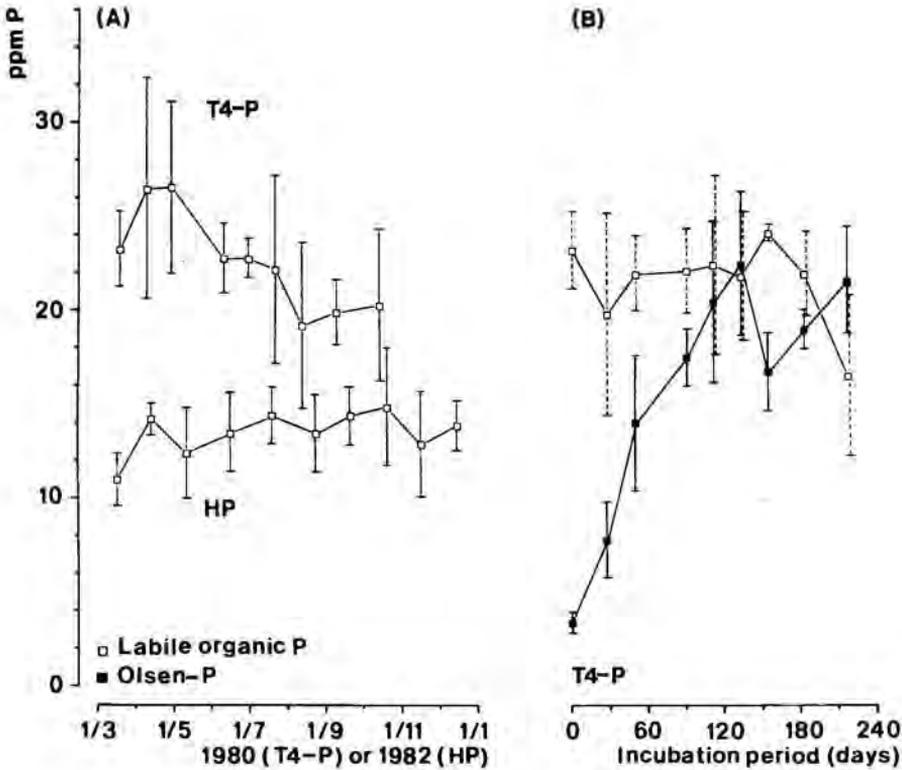


Fig. 8.5. Seasonal fluctuation of labile organic P in the 0-10 cm soil layer at site T4-P in the Westduinen and at site HP in the Heveringen (A), and time course of labile organic P and Olsen-P in intact soil cores (0-10 cm) from site T4-P during incubation at 30°C and at field moisture content (ca 20% dry weight; B).

natural field conditions one would expect something similar to happen, be it at a lower rate (applying the same reasoning as in the case of N mineralization experiments). In the field situation, however, the plant (or vegetation) takes up P from the 'available' pool, which in turn is replenished from organic and inorganic P fractions. Since the interactions within and between inorganic and organic P phases will be manifold and the mineralization of organic P does not necessarily have to proceed in the sequence highly resistant - moderately resistant - moderately labile - labile, one could raise the question whether P uptake by a vegetation must be expressed in a distinct seasonal fluctuation of, for instance, the labile organic P pool. Under the conditions of natural vegetations in temperate regions (low turnover rates), the possibility of partial or even over-all steady-state situations for all organic P fractions was suggested (Troelstra and van der Meulen 1980). The existence of more or less steady-state situations might explain the rather constant seasonal levels of labile organic P and even of Olsen-P in the field.

Only at site HP an increase of Olsen-P (as found in the laboratory with soil cores from site T4-P) was observed (Fig. 8.4). A partial explanation for the different effects at locations T4-P and HP may be found in the different moisture characteristics of the sites. In 1982, the conditions were clearly drier at site HP than at site T4-P during the 1980 season (Fig. 8.2). This could have resulted in better conditions for P uptake (diffusion of P to the root systems) at site T4-P as compared to the 1982 situation at location HP. Although a clear seasonal pattern was absent at site T4-P, it is remarkable that many of the mean values change qualitatively in a similar way as found for site HP. Both the strong decrease at location T4-P round about July and the decrease at site HP starting in about August coincide with better soil moisture conditions as compared to the preceding periods.

Although not very clear, the small decrease in labile organic P observed at site T4-P might be due to inadequate replenishment from the more resistant fractions. On the other hand, in the absence of plants and with favourable conditions for mineralization, the level of labile organic P remained relatively constant (incubation in the laboratory; Fig. 8.5, B).

When incubating pretreated (dried and sieved) soil material of site T4 in the laboratory at 30°C and 20 per cent moisture (dry soil), and performing the extractions without preceding drying, there was a strong increase and decrease of Olsen-P and labile organic P, respectively, during the first period of 28 days, after which both levels remained at about the same value (Fig. 8.6, A). With soil material from site T2 and short drying periods (ca 40°C) before the extractions, the same effect was found. But now Olsen-P continued to increase over a much longer period (188 days), whereas labile organic P, after an initial decrease, remained relatively constant up to a period of 264 days (Fig. 8.6, B). The low values of labile organic P, found for the two longest incubation periods, corresponded with relatively high values for the moderately labile organic P fraction (Fig. 7.6.4 in Troelstra and van der Meulen 1980). Remoistening of the pretreated material followed by immediate drying has a significant positive effect on the Olsen-P value (as shown in Fig. 8.6, B for  $t = 0$ ). This effect may be of importance in the field situation due to the occurrence of wetting/drying cycles during the season.

The results of the organic P fractionation at site HP are shown in Fig. 8.7. No clear seasonal fluctuation could be demonstrated in the different fractions. Expressing the values as percentages of the sum of the organic P fractions yielded relatively constant figures during the season (mean  $\pm$  standard deviation):  $11 \pm 1$  (labile),  $25 \pm 1$  (moderately labile),  $45 \pm 2$  (moderately resistant), and  $19 \pm 1$  (highly resistant).

The differences between T4 and HP are evident. At site T4 about equal proportions were found for the labile, moderately labile, and moderately resistant fractions. At location HP, the moderately resistant fraction comprised about half of the organic P pool, whereas the labile fraction was quantitatively the

least important. Research on the organic P fractions in 65 different habitats in the Netherlands, covering a wide range of soil types, has indicated that the labile organic P fractions tend to be proportionally more important at relatively low pH values (unpublished results). When comparing the two sites, however, it should be kept in mind that differences in technique were involved.

Fractionation of pretreated soil material of location HP yielded the following values (ppm P; percentages of the summed fractions between parentheses): 25.0 (21) labile, 39.9 (34) moderately labile, 35.4 (30) moderately resistant, and 16.0 (14) highly resistant. These figures refer to unground ( $\text{NaHCO}_3$  extraction) and ground samples (other extractions). Using unground soil samples only, the following values were found (in the same order): 25.0 (21), 30.2 (26), 43.5 (37), and 19.5 (16). Except for the labile fraction, the results of the pretreated material (unground samples only) are in agreement qualitatively with the seasonal data of Fig. 8.7. There are, however, also other differences involved pertaining to the procedures of drying and sieving. Anyhow, it is clear that the use of ground samples tend to over- and underestimate the moderately labile fraction and the resistant fractions, respectively.

As to the occurrence of seasonal fluctuations in the organic P fractions in dune grasslands, one must conclude that the effects, if any, are small and that it will be hard to establish such fluctuations definitely in a statistically reliable way due to substantial coefficients of variation of the organic P determinations themselves together with the spatial variation already present. Moreover, the shifts or turnover rates between organic P fractions and net mineral-

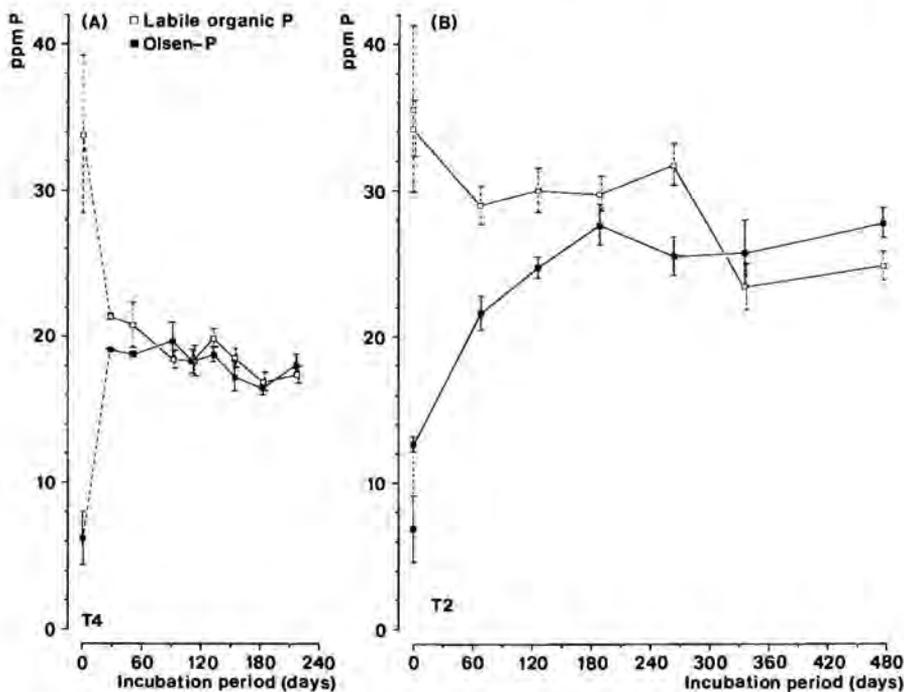


Fig. 8.6. Time course of labile organic P and Olsen-P in (dried and sieved) soil from locations T4 and T2 in the Westduinen (0-10 cm soil layer) during incubation at 30°C and 20% moisture (dry weight); following incubation, samples of T2 and T4 were extracted with and without a preceding short drying period, respectively.

ization/immobilization of the total organic P system are likely to be of more importance than possible fluctuations in the absolute amounts of the fractions.

REFERENCES

Bowman, R.A. and C.V. Cole (1978) - An exploratory method for the fractionation of organic phosphorus from grassland soils. *Soil Sci.* 125, 95-101.

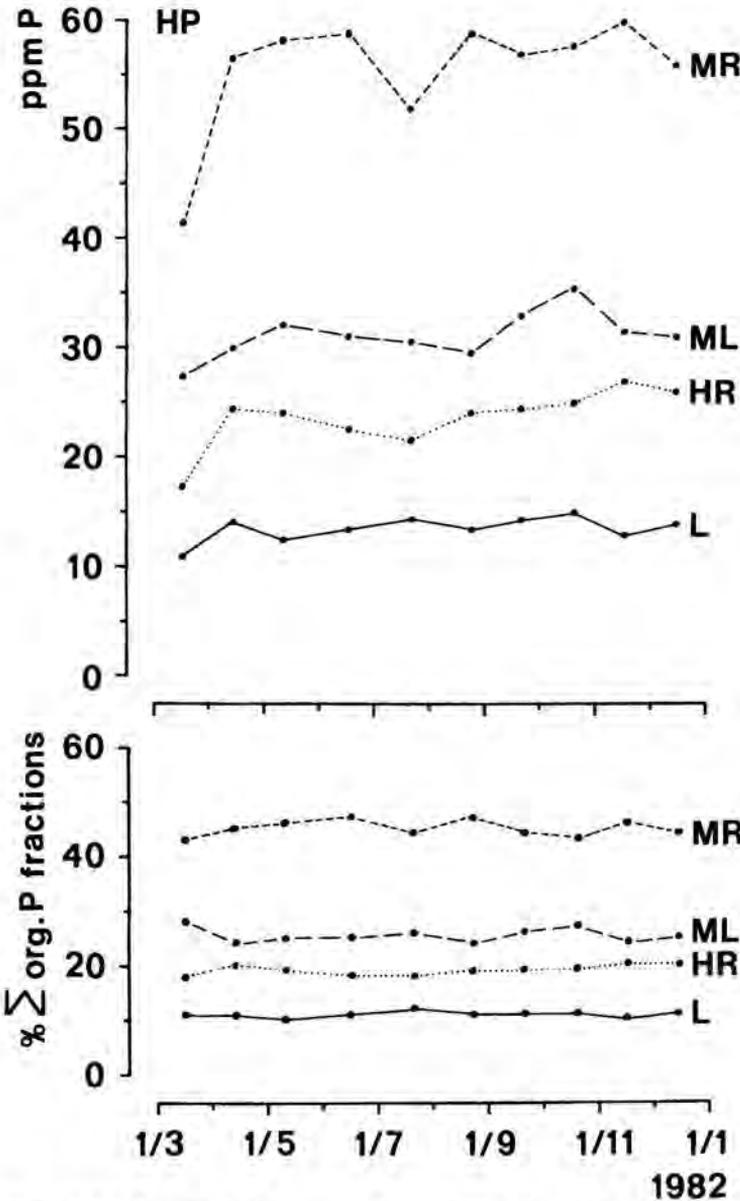


Fig. 8.7. Seasonal patterns of organic P fractions at site HP in the Heveringen (0-10 cm soil depth); L = labile, ML = moderately labile, MR = moderately resistant, and HR = highly resistant (organic P).

Cole, C.V., G.S. Innis and J.W.B. Stewart (1977) - Simulation of phosphorus cycling in semi-arid grasslands. *Ecology* 55, 1-15.

Halm, B.J., J.W.B. Stewart and R.L. Halstead (1972) - The phosphorus cycle in a native grassland ecosystem. In: Isotopes and radiation in soil-plant relationships including forestry. Proc. Symp. IAEA, 571-586.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean (1954) - Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939.

Troelstra, S.R. (1978) - Spatial variability of soil chemical properties in an older dune area (Westduinen) of Goeree. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 71, Progress Report 1977 I.O.O., 321-327.

Troelstra, S.R. and M.A. van der Meulen (1979) - Organic soil phosphorus fractions in an older dune area (Westduinen) on the island of Goeree. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, Progress Report 1978 I.O.O., 376-381.

Troelstra, S.R. and M.A. van der Meulen (1980) - Seasonal variation of labile organic phosphorus levels in a relatively old dune area (Westduinen) on the island of Goeree. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 75, Progress Report 1979 I.O.O., 40-46.

Troelstra, S.R. and R. Wagenaar (1980) - Seasonal patterns in the availability of mineral nitrogen in a relatively old dune area (Westduinen) on the island of Goeree. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 75, Progress Report 1979 I.O.O., 37-40.

#### 9. Comparison of developing populations of *Plantago lanceolata* in a hayfield and a pasture (J. Haeck, J.H. Mook)

It is not often possible to compare populations of grassland plants in semi-natural environments that differ only in long term management, but are alike in all other respects. In the Netherlands hayfields are mostly on the relatively poor soils and pastures on the richer soils. The possibility of studying the direct influence of management on the demography of plants is only present in sites where long term experiments are carried out. Van den Bergh (1979) has described an experiment like this in which a pasture was divided in plots with different levels of fertilizing and in which the management of part of the plots was changed to annual mowing. In twenty years the species composition of the vegetation developed in different ways in the various plots, revealing large fluctuations in the species composition. In the case of *P. lanceolata* populations of considerable density built up after 12-16 years in the hayfields that did not receive nitrogen fertiliser, but thereafter the densities declined. It could also be established that during this period genotypic differences appeared between the populations of pasture and hayfield, which were caused by selection (van der Toorn and ten Hove 1982). It was thought it would be of interest to study the population processes that lead to this fluctuation in density and this selection.

Similar waves in the population density may be expected in grasslands in which the fertilization is discontinued (Londo 1978). A possibility to study the rise-and-fall of a population of *P. lanceolata* arose in an experimental field of the Agricultural University of Wageningen (Department of Field Crops and Grassland Science), where a study is in progress of the development of the botanical composition of grassland after manuring has been stopped.

The field is located on sandy soil, with a low natural fertility, on an experimental farm at Achterberg (near Wageningen). In 1967 the farm was reparcelled and a parcel of 0.25 ha was resown with some species of grasses and *Trifolium*

*repens* to assist in the establishment of the grasses. Artificial fertiliser (NPK) was applied during three seasons, but in 1970 fertilization was stopped. Since 1971 half of the parcel has been grazed by yearling oxen and the other half mown in June. Details of the management and of the subsequent development of the botanical composition of the field are given in Wind (1980).

The dry weight of the standing crop originally consisted of up to 90 per cent of *Lolium perenne*. This grass diminished greatly in importance, while other grasses like *Poa pratensis* (sown) and *Agrostis tenuis* (not sown) and *Holcus lanatus* (not sown) became more important. Simultaneously the total yield dropped; this development was more pronounced in the mown than in the grazed part. Two species of *Plantago* have been observed in both halves of the parcel (Wind 1980). *P. major* was seen last in 1972, while in the same year *P. lanceolata* was found. This species became more numerous in later years. Since 1978 the demography of *P. lanceolata* has been studied in 10 experimental plots of 0.3 x 0.4 m<sup>2</sup> in the pasture and 15 in the hayfield. An analysis of the spatial distribution of this species in a grid of ¼ m<sup>2</sup> plots spaced 4 metres apart in 1978, showed that on the whole the vegetation of each part was homogeneous, although quantitative differences in the occurrence of plants on a restricted scale existed. The demographic plots were laid out along a transect in both parts and care was taken to situate the plots in such a way that situations with large differences in the density of *P. lanceolata* were covered. In later years (from 1981 onwards) a more detailed description of the vegetation was started.

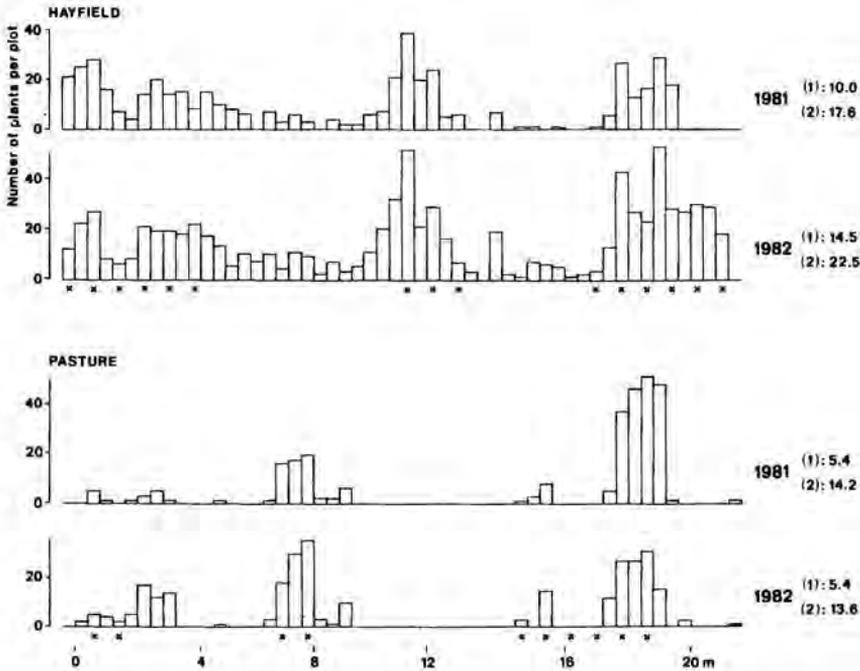


Fig. 9.1. Distribution of plants of *Plantago lanceolata* along a transect of approx. 20 m, divided in plots of 0.3 x 0.4 m<sup>2</sup> in parcels of grassland with different management. The demographic plots are indicated by a cross.  
 (1): mean density in all plots;  
 (2): mean density in demographic plots.

Among other observations the number of adult specimen of selected plant species in each of  $0.1 \times 0.1 \text{ m}^2$  plots in the total transect of  $0.3 \times$  approx.  $20 \text{ m}^2$  were noted. This was done according to a method used by Grubb (1980).

In the  $0.3 \times 0.4 \text{ m}^2$  plots used for the demographic work all stages of *P. lanceolata* were carefully mapped 3 times a year from 1978 onwards; also the plants were described in some detail (number of leaves, length and width of the largest leaf, stage of development, size and number of spikes). The fate of the individual plants was followed.

The data that were accumulated during the last five years have not been worked out in detail, but some preliminary results can be given here on the differences in demographic behaviour of *P. lanceolata* in the two situations. It was evident from the beginning that both populations were still in a stage of development, but a comparison of data from the description of the vegetations in the finer scale and the results of the demographic study, show that distinct differences exist between the two populations. The occurrence of *P. lanceolata* along the transects has been summarized in Fig. 9.1. The scale of representation has been coarsened by lumping together the data of  $0.1 \times 0.1 \text{ m}^2$  plots to plots of  $0.3 \times 0.4 \text{ m}^2$ . In this way it can be seen how the demographic plots are distributed with regard to the occurrence of the plants. It is evident that the species has established itself in certain restricted places and that it is in the process of spreading outwards from these sites. This process is in a much more advanced stage in the hayfield than in the pasture. Also it is evident that the overall density of adult plants increased from 1981 to 1982 in the hayfield, but not in the pasture. Although the development differs between the centres of distribution, the general population density is much greater in the hayfield than in the pasture.

It is difficult to say at this stage of analysis what causes the differences. The demographic data can lead to a first approximation, by showing in which stages of the plant the differences are brought about.

It should be pointed out that although the plots for demographic study are found in sites of high as well as low density, the mean density of these plots is higher than in total transect. It could therefore be that the findings from

Table 9.1. Number of plants per  $\text{m}^2$  of three categories of rosettes of *Plantago lanceolata* in two parts of an experimental field at Achterberg. In brackets: fraction of rosettes surviving till next year.

	hayfield			pasture		
	(1) rosettes sur- viving from previous year	(2) seedlings	(3) side rosettes	(1) rosettes sur- viving from previous year	(2) seedlings	(3) side rosettes
1978	115 (.90)	70 (.61)	8 (.43)	109 (.86)	175 (.39)	8 (.67)
1979	150 (.81)	67 (.50)	24 (.48)	168 (.81)	118 (.30)	14 (.76)
1980	167 (.87)	58 (.68)	23 (.39)	183 (.67)	21 (.52)	21 (.60)
1981	193 (.93)	225 (.38)	64 (.34)	147 (.68)	39 (.36)	13 (.22)
1982	286	102	65	118	28	18

these plots are biased, but this may be remedied by comparing what happens in low density and high density plots. Until now the analysis has been restricted to mean densities of three different types of plants, i.e. (1) rosettes that were also present in the preceding year, (2) seedlings or juveniles developed from seeds, and (3) newly developed side rosettes (Table 9.1). The number of plants in group (1) is given as they were present in spring (April-May) and groups (2) and (3) are summarized over the whole season. Apart from these densities the fraction is given of the different group of plants that survive to join group (1) in the next year. Thus, the plants given under (1) are composed of plants surviving from groups (1), (2) and (3) in the preceding year.

From Table 9.1 it can be seen that the high densities in the hayfield in 1981 and 1982 originate mainly from a very high survival of older plants and from a comparatively high survival of seedlings in all years. The rising density in the pasture in the early years is caused by the high densities of seedlings even though the survival was somewhat lower than in the hayfield. Because in later years the seedling densities in the pasture diminished and because survival of older plants was not very high, the overall density in the pasture also declined. It is probable that a more detailed analysis of the demographic data together with the available knowledge on the composition and structure of the vegetation in the transect, will lead to hypotheses on the influence of the structure of the vegetation which can be tested experimentally. In general the data are consistent with the notion that relatively open hayfields on poor soil are the optimal habitat of *P. lanceolata*, but the further development of the population has to be studied.

These observations stress the importance of long term demographic studies, that are combined with less detailed work on the general development of the vegetation and on the spatial distribution of plants in the vegetation.

#### REFERENCES

- Bergh, J.P. van der (1979) - Changes in the composition of mixed populations of grassland species, In: M.J.A. Werger (Ed.) - The study of vegetation. Junk, The Hague, 59-80.
- Grubb, P.J. (1980) - Communication in lecture Leyden University.
- Londo, G. (1978) - Over het gedrag in ruimte en tijd van *Taraxacum* en *Plantago*. *Gorteria* 9, 174-178.
- Toorn, J. van der and H.J. ten Hove (1982) - Variability of some leaf characters in *Plantago lanceolata*. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks* 79, Progress Report 1981 I.O.O., 45-51.
- Wind, K. (1980) - Botanische samenstelling van grasland bij extensivering van het gebruik. Vakgroep Landbouwplantenteelt en Graslandkunde, Mededeling 52, 1-19.

#### 10. Demography of a coastal type of *Plantago major* L., a preliminary report (C.W.P.M. Blom, J. van Heeswijk)

#### INTRODUCTION AND METHODS

This study was initiated as part of a multidisciplinary project on the relationship between demographic, physiological and genetic properties of *Plantago* species and the characteristics of their environment (van der Aart 1979).

Since 1980 a study on the demography of a coastal type of *P. major* has been carried out on plots (0.25 m x 0.50 m) at three different sites, namely Groene Strand (six plots) and Oostvoornse Meer (ten plots), both on the island of Voorne, and Kwade Hoek (six plots) on the island of Goeree. The number of

rosettes as well as the number of spikes per plot and their seed production were determined about five times a year during 1980, 1981 and 1982. Ten rosettes per plot per observation date were randomly selected in order to determine the average number of leaves and the length of the longest leaf per plant. From these data the approximate biomass was calculated using a method described by Noë and Blom (1982).

**RESULTS AND DISCUSSION**

In this report, the results of one or two representative plots per site are given as an example in Fig. 10.1 and 10.2. The biomass per plot in three successive years is shown in Fig. 10.1; the numbers of rosettes per plot are given in Fig. 10.2.

The maximum biomass in the plots at Groene Strand (G-2) was relatively stable each year, but considerable fluctuations within one year were found (Fig. 10.1). The plots at Kwade Hoek showed a decrease in biomass of *P. major*

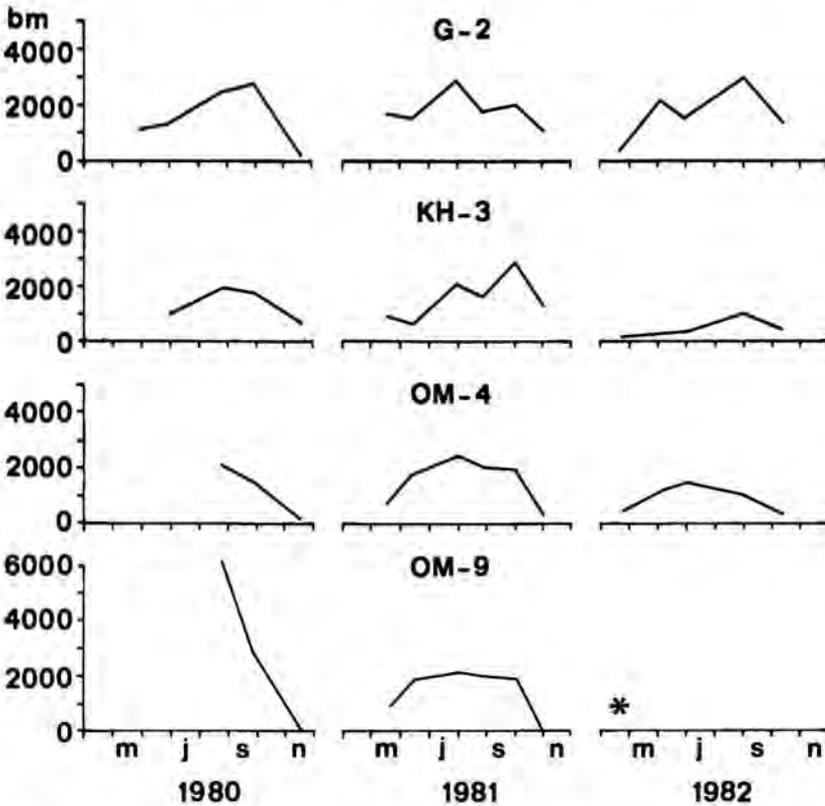


Fig. 10.1. The approximated biomass of a coastal type of *Plantago major* during three successive years on plots at Groene Strand (G-2) and Oostvoornse Meer (OM-4,9) on Voorne, and at Kwade Hoek (KH-3) on Goeree (the Netherlands). The biomass (bm) is given in approximated value (length of longest leaf × numbers of leaves per rosette) following the method of Noë and Blom (1982). \*: no plants present.

over the years (Fig. 10.1). Two examples are given from ten plots at Oostvoornse Meer, because some of the plots (*e.g.* OM-4) are situated on relatively high-lying sites, while others occur at relatively low-lying sites (*e.g.* OM-9), which are inundated in winter. Comparing the results of three successive years a slight decrease in biomass was found on the high-lying plots (Fig. 10.1, OM-4), whereas a dramatic decrease occurred on the wet plots (Fig. 10.1, OM-9).

The plots at Groene Strand show a small increase in the total number of rosettes per plot (Fig. 10.2), which was especially due to the establishment and low mortality of plants that emerged in 1981 and 1982. In general, a decrease in the total number of rosettes was found (Fig. 10.2), which was caused by a high mortality of plants present in 1980 and by a poor establishment of new plants in 1981 and 1982.

On the dry sites at Oostvoornse Meer only small differences in the total number of rosettes per plot were observed (Fig. 10.2, OM-4), whereas on the wet sites a significant decrease occurred (Fig. 10.2, OM-9). Because of the de-

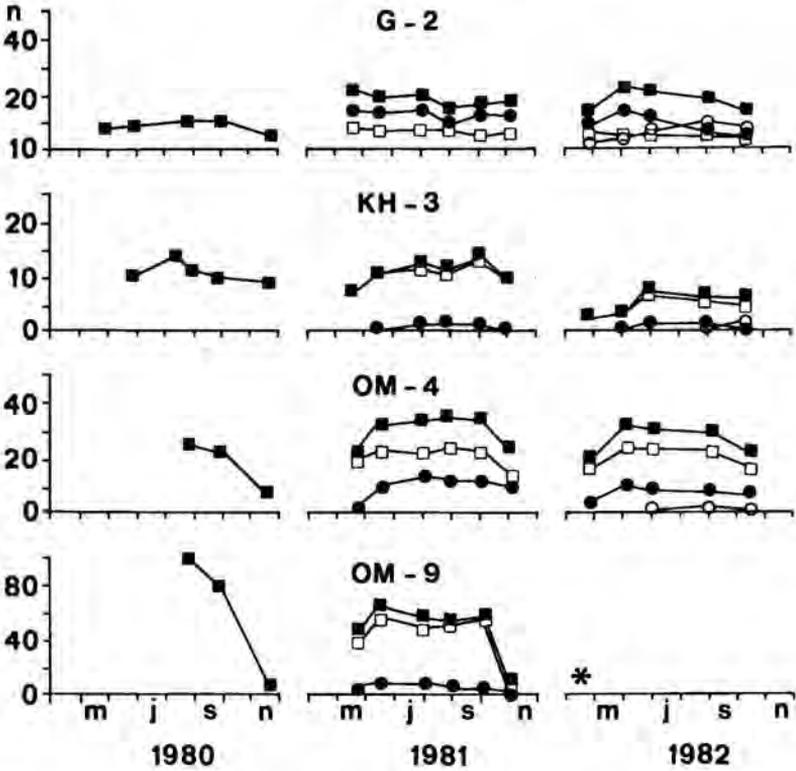


Fig. 10.2. The number of rosettes (*n*) of a coastal type of *P. major* during three successive years on plots at Groene Strand (G-2) and Oostvoornse Meer (OM-4,9) on Voorne, and Kwade Hoek (KH-3) on Goeree (the Netherlands). Total number of rosettes and cohorts per year are given. Solid squares : total number of rosettes  
Open squares : cohort of 1980  
Solid dots : cohort established in 1981  
Open dots : cohort established in 1982  
\* : no plants present.

crease in biomass of *P. major* on the dry plots at Oostvoornse Meer (Fig. 10.2, OM-4), the plants in 1982 were smaller than in 1980 and 1981. Interspecific competition is not very likely, but intraspecific interactions may occur on these plots. Experimental studies on these phenomena are in progress. The decrease in the numbers of rosettes of *P. major* at Kwade Hoek was probably due to the absence of open sites necessary for germination. At Kwade Hoek a dense grassy vegetation cover was observed. Here the declining biomass of *P. major* can be ascribed to the decrease in the total number of rosettes, which was also the case on the wet sites at Oostvoornse Meer (Fig. 10.1 and 10.2, OM-9).

In conclusion it can be stated that a fair uniformity exists in the studied life-history characteristics between *P. major* plants within each site. The differences in properties of individuals occurring in the various sites, however, are considerable.

As far as reproduction is concerned a relatively high seed production was observed at Kwade Hoek (between 500 and 2000 seeds per plot in 1981), while Oostvoornse Meer and Groene Strand showed a far lower seed production (between 40 and 800 and between 40 and 700 seeds per plot in 1981, respectively). No obvious relationship between seed production and establishment was found.

Based on the leaf, flower, fruit and capsule characteristics of the individuals, this coastal type of *P. major* belongs to the subspecies *pleiosperma* (cf. Mølgaard 1976). The mean number of seeds per capsule of this species at Kwade Hoek is  $8.83 \pm 0.49$ . At Groene Strand and at Oostvoornse Meer these values are  $7.03 \pm 0.26$  and  $6.57 \pm 0.25$ , respectively. These relatively low numbers of seeds per capsule contradict the suggestion of classifying this coastal type in the subspecies *pleiosperma*. In the near future genetic and population-ecological studies will probably lead to a more precise classification of the coastal type of *P. major*.

## REFERENCES

- Mølgaard, P. (1976) - *Plantago major* ssp. *major* and ssp. *pleiosperma* morphology, biology and ecology in Denmark. Bot. Tidsskrift 71, 31-56.
- Noë, R. and C.W.P.M. Blom (1982) - Occurrence of three *Plantago* species in coastal dune grasslands in relation to pore-volume and organic matter content of the soil. J. Appl. Ecol. 19, 177-182.
- Van der Aart, P.J.M. (1979) - A comparative multidisciplinary approach to the demography, physiology and genetics of plant species occurring in semi-natural grasslands. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, Progress Report 1978 I.O.O., 347-350.

### 11. Variability in morphological characteristics of *Plantago lanceolata* (P. Slim, J. van der Toorn)

It has been demonstrated by van der Toorn and ten Hove (1982) that *Plantago lanceolata* shows a morphological variability that can be correlated with vegetation structure, in the sense that plants with short leaves and a flat rosette are found primarily in pastures, whereas in hayfields plants have longer and more upright leaves. This difference persisted when root cuttings of adult plants collected in the field were reared under uniform conditions in the greenhouse. It was therefore concluded that these variations are genotypic.

In 1982 an experiment was started to study if and to what extent the same variability exists between plants derived from seeds. In this way it was thought possible to obtain information on whether the selective process in the vegetation resulting in the genotypic variation in adult plants, have played part in the past or are still active.

Table 11.1. Habitat data and plant characteristics (mean values and standard deviations - between brackets - of 50-days old seedlings) of 20 *P. lanceolata* populations.

Locality	Habitat data					Plant characteristics						
	Management	Soil type <sup>2</sup>	Vegetation height (cm)	Light trans- mission (%)	n	Cotyl length	Leaf length (mm)	Leaf width (mm)	Leaf number	Leaf angle (degrees) <sup>3</sup>	Leaf hairiness <sup>4</sup>	Flowering data <sup>5</sup>
Tielerwaard	Hayfield	C	33.9(5.8)	15(7)	20	33.8(6.1)	83.2(18.4)	10.9(2.5)	8.25(1.29)	155	.75(.72)	92(17)
Gameren	Hayfield	C	39.0(5.8)	15(8)	20	31.2(7.5)	85.8(16.1)	11.5(1.3)	7.65(1.27)	117	1.00(.79)	103(25)
Veerslootlanden <sup>1</sup>	Hayfield	P	15.1(.7)	48(11)	19	31.3(4.0)	84.8(10.1)	10.4(2.1)	6.95(.97)	100	.32(.48)	103(19)
Rheezermaten	Hayfield	P	28.9(3.7)	26(15)	19	30.3(5.1)	67.9( 8.9)	10.1(1.7)	8.16(1.17)	170	.79(.92)	103(23)
Pannerden-dijk	Pasture	SL	12.0(3.4)	51(16)	17	30.2(3.8)	81.1(14.9)	11.4(1.9)	6.88(.60)	115	1.82(.81)	101(24)
Ruitenbergr	Pasture	S	10.9(2.2)	62(17)	18	28.0(6.3)	66.3(11.9)	10.9(2.5)	7.39(1.20)	170	1.06(.94)	119(16)
Ossenwaard	Pasture	SL	32.2(7.3)	27(9)	15	27.3(5.3)	76.5(11.4)	11.5(1.9)	7.40(1.06)	125	1.67(.82)	97(17)
Heteren <sup>1</sup>	Hayfield	C	42.0(6.5)	27(12)	18	35.8(5.7)	99.2(10.6)	12.8(2.7)	7.55(1.20)	95	1.50(1.10)	105(23)
Achterbergr	Pasture	S	12.6(6.9)	60(24)	20	30.4(4.6)	84.7(13.3)	10.2(2.1)	8.20(1.20)	150	.85(.75)	97(19)
Uddel	Rough herbage	S	8.9(2.3)	67(13)	23	30.6(8.2)	72.0(15.8)	9.4(2.0)	7.96(1.58)	180	.78(.85)	115(25)
Westervoort	Rough herbage	S/C	22.0(4.4)	31(12)	11	28.5(8.7)	94.6(20.3)	11.4(1.8)	6.73(1.01)	100	1.18(.75)	103(21)
Nieuwcuyl	Pasture	S	8.8(1.9)	51(17)	19	30.0(7.2)	76.2(12.4)	10.9(2.5)	8.11(1.20)	170	.63(.60)	116(22)
Otterlo	Pasture	S	13.0(2.9)	52(14)	20	27.4(7.1)	84.8(14.8)	10.7(2.0)	7.65(1.23)	140	.80(.70)	101(19)
Westduinen <sup>1</sup>	Pasture	S	3.8(.9)	72(18)	17	25.5(5.7)	66.9(14.2)	9.1(1.4)	8.06(1.14)	180	1.29(.99)	110(15)
Oeffelt	Rough/Pasture	S	13.6(4.4)	64(22)	20	29.0(6.0)	67.2( 8.8)	10.2(2.1)	8.60(1.50)	175	1.55(.89)	104(18)
Bruuk <sup>1</sup>	Hayfield	PL	28.1(4.0)	36(13)	18	31.3(7.5)	84.0(18.4)	11.5(2.2)	7.94(1.26)	127	1.50(.92)	123(19)
Achterbergr	Hayfield	S	17.2(4.1)	59(26)	20	28.3(9.1)	72.4(17.9)	10.2(2.5)	7.50(1.28)	137	.85(.75)	102(21)
Heveringen	Rough/Pasture	S	8.3(3.4)	43(15)	19	33.5(5.8)	88.1(14.7)	9.6(1.7)	9.00(1.15)	125	.68(.75)	115(21)
Tienhoven	Pasture	S	11.5(1.9)	58(12)	18	30.5(5.8)	91.1(14.7)	11.6(2.0)	7.61(1.54)	125	1.67(.49)	104(23)
Junner Koeland <sup>1</sup>	Pasture	S	7.5(1.4)	69(12)	18	27.1(4.9)	58.4( 9.8)	10.0(2.0)	8.89(1.78)	180	1.61(.92)	122(14)

- 1) These localities were also used in the 1981 experiment
- 2) C = clay, S = sand, P = peat, PL = peaty loam, SL = sandy loam
- 3) Median value
- 4) See text
- 5) Days after germination

Table 11.2. Correlation coefficients for the mutual relation between plant characteristics, and for the relation between environmental variables and plant characteristics (population means).

	N	Cotyl length	Leaf length	Leaf width	Leaf number	Leaf angle <sup>1)</sup>	Flower. date	Leaf hairiness
Leaf length	20	+.632**	-	+.627**	-.397	-.841***	-.363	-.050
Light transmission (end of May, 1982)	20	-.541*	-.528*	-.582**	+.246	+.529*	+.412	+.084
Vegetation height (end of May, 1982)	20	+.487*	+.404	+.668**	-.191	-.454*	-.399	+.104

<sup>1)</sup> median value, \* p < .05, \*\* p < .01, \*\*\* p < .001.

Seeds from 20 field populations of *P. lanceolata* were collected. Only seeds with a diameter between 1.00-1.12 mm were used. The seeds were sown on the surface of pots filled with perlite covered with a thin layer of sand. Seedlings were thinned to one plant per pot in such a way that the pots contained plants of similar size and age (with respect to the day of germination). This selection was done in order to eliminate the influence of these characteristics on the morphology of the plants.

Twenty plants of each population were grown in a greenhouse at temperatures between 13°C (at night) and 24°C (by day). Light was supplemented during 7 hours a day, and Hoagland culture solution (diluted to 25 per cent of original concentration) was applied regularly.

The following characteristics were measured: length of cotyledons when fully grown, and when the plants were 50 days old, the number of leaves, the length and width of the third youngest leaf, the angle between the third and fourth leaf, and the hairiness of the third leaf according to the following scale: 0 = hairless, 1 = 1-3 hairs per cm<sup>2</sup>, 2 = 4-10 hairs per cm<sup>2</sup>, 3 = more than 10 hairs per cm<sup>2</sup>. The date of the start of flowering was also recorded.

To characterize the vegetations from which the seed populations originated the height of the vegetation and the percentage of light transmitted to one cm above the soil surface were measured in the original habitats at the end of May. For 10 populations light transmission data were also available from 1980.

The data are summarized in Table 11.1. Mean values and standard deviations are given, except in the case of the median leaf angle, because of skew frequency distributions. Correlations between the means of the various measurements are given in Table 11.2. Significant positive correlations exist between the length of leaf and cotyledon, and between leaf length and leaf width. A significant negative correlation was found between leaf length and

Table 11.3. Correlation coefficients between the percentage of light transmission in 1980 and 1982 (10 populations).

	Minimal transmission 1980	Transmission end of May 1982
Transmission (end of May, 1980)	+.828**	+.785**

\*\* p < .01.

the leaf angle. Thus, there appears to be a tendency that populations have either short and narrow leaved, flat rosettes, or long and relatively broad leaved erect rosettes. No significant correlations were found between leaf length and either the number of leaves, or the flowering date, or the leaf hairiness.

The significant correlations of the plant characteristics mentioned between vegetation height and light transmission, and the strong correlation that exists between light transmission and vegetation height ( $r = -0.873$ ,  $p < 0.001$ ), confirm that the former rosette type is found predominantly in open and low vegetation and the latter type in dense and high vegetations. Because the correlations for light transmission are slightly better than for vegetation height (except for leaf width), only those for light transmission will be used below.

Although the correlations of plant form and vegetation structure are in accordance with what one would expect to find if the plants were adapted to their environment, one could argue that the light situation is important during

Table 11.4. Frequency distribution of leaf angle and leaf length in the populations Heteren (He), Veerslootlanden (V), and Bruuk (B) from the 1982 and 1981 experiments.

Leaf angle class (degrees)	1982			1981		
	He	V	B	He	V	B
41-60	3	4				1
61-80	3	1				
81-100	4	5	4	1		1
101-120	1	1	5	4	6	2
121-140	3	2	3	8	5	5
141-160		4	2	8	11	12
161-180	4	2	4	1	1	1
Number	18	19	20	22	23	22
Mean	110	113	133	134	137	136
S <sup>2</sup>	1927.2	1980.3	979.7	547.6	231.0	665.6
F-value <sup>1</sup>	3.52**	8.57***	1.47	-	-	-

---

Leaf length class (mm)	1982			1981		
	He	V	B	He	V	B
21-30					1	1
31-40				2	10	11
41-40			1	7	9	6
51-60				8	3	4
61-70		1	4	2		
71-80	1	7	2	2		
81-90	5	5	6			
91-100	4	6	2	1		
101-110	5		1			
111-120	3		2			
Number	18	19	20	22	23	22
Mean	99	85	84	55	43	43
S <sup>2</sup>	112.4	102.0	338.6	201.6	42.5	74.5
F-value <sup>1</sup>	-	2.40*	4.55***	1.79	-	-

\*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$ ,

1) F-value =  $S^2_1/S^2_2$  in which  $S^2_1 > S^2_2$

the whole growing season and not only at the end of May. It seems likely that the minimal amount of light, reaching the soil during any period of the growing season, would be more important. Repeated light measurements are not available for 1982, but for 1980 they are available for 10 of the 20 populations under consideration. Table 11.3 shows that there is a strong correlation between the minimal light transmission and the transmission at the end of May as well as a strong correlation between the observations in May of both years, in spite of the fact that vegetation growth started early in 1980 and late in 1982. Therefore, we are satisfied that the figures reached in 1982 reflect correctly the characteristics of the different vegetations in both years and probably all other years, provided the management is unchanged.

The results from these experiments show the same trends as those in the experiment with root cuttings (van der Toorn and ten Hove 1982). In fact they give greater credence to them, as these new results are based on more populations from a larger geographic area.

It remains interesting to compare the results of both experiments more closely because they may provide indications about whether selection is still at work in the vegetation. It should be admitted that the exact test for the existence of selection between the seed stage and the adult stage would be to follow the actual germination and survival of plants in the field but a first approximation of the problem may be obtained by comparing the range of variation in seeds and in adult plants. This is done in Table 11.4 for three of the five populations used in both experiments. The other two populations were excluded from the comparison because the frequency distributions of their leaf angles were markedly skew, being too close to the value of  $180^\circ$  cannot be surpassed.

It can be seen from Table 11.4 that the variances of both the leaf angle and the leaf length of plants originating from seed (1982) tend to be greater than of plants originating from cuttings (1981). In four of six cases these differences are significant. This confirms the expectation that the adult plants surviving in the field are a selection from plants with different characteristics present in the seed. One would expect that seedlings transplanted in their own environment would survive better than those from other environments when transplanted in the same site. This was not confirmed by the findings of Anthonovics and Primack (1982), who did reciprocal seedling transplants with *P. lanceolata* in different localities. The results of their trials showed that there were either no or only slight indications that seedlings transplanted into their own site survived better than those from other provenances. The environment had a greater influence on survival than the genetical characteristics of the plants.

The present findings that marked morphological differences between populations exist and are already apparent at a very young stage (such as cotyl length) make further research necessary. Reciprocal transplantation experiments with some of the treated populations, in both the seedling and juvenile stage have been started to study selection phenomena in the field.

Table 11.5. Correlation coefficients between plant characteristics of 5 populations from the 1981 and 1982 experiments.

	Median leaf angle 1981	Mean leaf length 1981	
Median leaf angle 1982	+ .947	-	p < .02
Mean leaf length 1982	-	+ .990	p < .001

## REFERENCES

- Anthonovics, J. and R.B. Primack (1982) - Experimental ecological genetics in *Plantago*. VI. The demography of seedling transplants of *P. lanceolata*. *J. Ecol.* 70, 55-75.
- Toorn, J. van der and H.J. ten Hove (1982) - Variability of some leaf characteristics in *Plantago lanceolata*. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks* 79, Progress Report 1981, I.O.O., 45-51.

### 12. Germination of *Plantago major* seeds of different stages of maturity (R. Soekarjo)

## INTRODUCTION

Seeds of *P. major* will easily germinate at a temperature of 25°C. Below this temperature germination becomes increasingly difficult. There is usually no germination at 20°C or lower.

A period of cold treatment will lower the temperature threshold for germination (Sagar and Harper 1960). In an earlier report it has been stated that seeds subjected to a cold period show a second effect of this treatment: the rate of germination at a higher temperature (25°C) is markedly increased. At lower temperatures also, those seeds that can germinate, will do so in a short time. Consequences of these findings for the germination and establishment of this species in the field have also been discussed (Soekarjo 1982).

The question arose whether the need for a high temperature for the germination process was innate or that it developed gradually after the seeds had matured. Therefore, mature and immature seeds were germinated at different temperatures.

## MATERIAL AND METHODS

Spikes were harvested from plants grown outdoors. Within 5 days after harvest the seeds were put to germinate in petri dishes (9 cm diameter) with 6 ml of tap water on filter paper disks (Schleicher and Schull nr. 595). The values given in the graph are the mean values of 9 replicates of 10 seeds per petri dish. The seeds were allowed to germinate in an 8 hours light/16 hours darkness regime, under fluorescent light tubes (Philips TL 33) giving a light intensity of  $20 \pm 2 \text{ W.m}^{-2}$  at the level of the petri dishes.

The seeds were divided into three categories: 1. Brown seeds from completely brown spikes were considered to be mature (b in graph 12.1). 2. Seeds from brown spikes with green pedicels were used as an intermediate category. The colour of these seeds varied between brown and brownish green (bg in graph 12.1). Green seeds from completely green spikes were considered to be immature (g in graph 12.1).

The seeds were put at 25°C, 20°C and 15°C, respectively, for two weeks, after which the temperature was kept at 25°C for all groups during another two weeks.

## RESULTS AND DISCUSSION

The immature seeds became yellowish green during the first days in the petri dishes. The colour of the intermediate category of seeds shifted to brown in the same period. The development of a hard testa had not yet started in the green seeds.

The germination of the three categories of seed (Fig. 12.1) showed marked differences. The brown and brown-green seeds did not germinate at a tempera-

ture of 20°C and 15°C. Some of the green seeds, however, even germinated at 15°C, whereas 40 per cent of the population germinated at 20°C. The germination pattern of the intermediate category resembled that of the brown seeds rather than that of the green seeds.

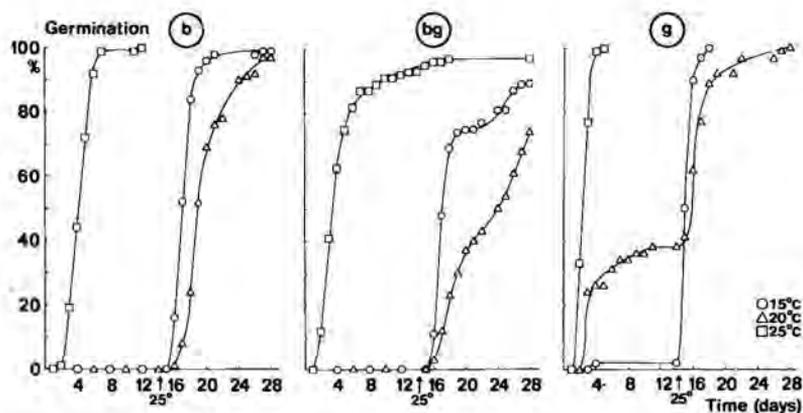


Fig. 12.1. Temperature effect on germination under short-day conditions of mature brown seeds (b), immature green seeds (g) and intermediate brown to brownish green seeds (bg) of *Plantago major*. The seeds that were kept at 15°C and 20°C were transferred to 25°C after two weeks.

From the results it is evident that the high temperature threshold for germination in *P. major* is present from the time of maturation of the seed. It is fully present in those seeds which have developed a hardened brown testa. Moreover, the rate of germination of the green seeds was higher than that of the other types under all treatments. The condition of the seeds requiring a high temperature for germination must be considered innate in the mature seed and must be classified as a type of primary dormancy (Vegis 1964). They do not germinate at a lower temperature except when this dormancy is broken by a period of cold treatment (Sagar and Harper 1960; Soekarjo 1982).

As *P. major* occurs in heavily trodden and grazed areas, damage to the immature spikes is likely to occur. Therefore, the fact that immature seeds are able to germinate immediately and at lower temperatures, may constitute an appreciable increase in germination potential.

## REFERENCES

- Sagar, G.R. and J.L. Harper (1960) - Factors affecting germination and early establishment of plantains. In: J.L. Harper (ed.) - Biology of weeds, Brit. Ecol. Symp. 1, 236-245.
- Soekarjo, R. (1982) - Effects of low temperature on the germination of seeds of *Plantago major*. Ver. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 79, Progress Report 1981, I.O.O., 51-55.
- Vegis, A. (1964) - Dormancy in higher plants. Ann. Rev. Plant Physiol. 15, 185-224.

### 13. Different K and P requirements for *Plantago major* ssp. *major* and *Plantago lanceolata*? (S.R. Troelstra, W. Smant, R. Wagenaar).

#### INTRODUCTION

Recent research on the soil chemistry of the natural habitats of *Plantago* species in various parts of the Netherlands has indicated a considerable degree of overlapping for both *P. major* ssp. *major* and *P. lanceolata* with regard to the range of soil chemical properties (Troelstra *et al.* 1981; van der Aart 1983). Mean values for properties of habitats of *P. major* were in most cases slightly higher, due mainly to the rather incidental occurrence of some extreme values. However, other functional aspects of 'nutrient availability' - such as transport conditions for nutrients (soil moisture), competition, rooting pattern, presence of root hairs and mycorrhizas - may be of more importance than the soil chemical index *per se*. In this respect it has to be recognized that the occurrence of *P. major* ssp. *major* tends to be more solitary (less or no competition involved) and on more compacted sites (which is probably a guarantee for relatively optimal transport conditions during the season) than is the case for *P. lanceolata*.

The inventory work on the ecology of the Dutch grassland plants by Kruyne *et al.* (1967) indicated a preference by *P. major* for a moderate to high P-status and for a relatively high K-status. On the other hand, the presence percentage *i.e.*, the percentage of fields per habitat class in which a species was found, of *P. lanceolata* increased with a decreasing P- and K-status of the soil.

In 1982, it was decided to set up some preliminary water-culture experiments with the species *P. major* ssp. *major* and *P. lanceolata* and with varying levels of P- and K-availability.

#### MATERIALS AND METHODS

Seeds of *P. major* ssp. *major* and *P. lanceolata* were germinated and allowed to grow in glass beads for ca 10 days. The seedlings were then grown for a further 10 days on the high treatment in question ( $\text{NO}_3/\text{NH}_4$  ratio 50/50), after which they were transferred to the different solutions.

The nutrient solutions were kept in 12-liter black polythene pots and continuously aerated. There were 6 plants per pot and the pH of the nutrient solutions was measured regularly and manually maintained at about 6 by additions of  $\text{H}_2\text{SO}_4$  0.02 N or  $\text{Ca}(\text{OH})_2 \pm 0.04$  N. The composition of the nutrient solutions is given in Table 13.1. Three  $\text{NO}_3/\text{NH}_4$  ratios were applied (100/0; 50/50; and 0/100) and dicyandiamide was added to the solutions ( $7.5 \text{ mg.l}^{-1}$ ) as a nitrification inhibitor.

Different levels of K-availability were introduced in the K experiment by varying the K/Na ratio: 5, 0.5, 0.2, and 0.03 ( $\Sigma (\text{K}+\text{Na}) = 3 \text{ meq.l}^{-1}$ ). P-levels in the P experiment were 0.75, 0.25, 0.10, and 0.05  $\text{meq. H}_2\text{PO}_4.\text{l}^{-1}$ ; during the experimental period an additional 0.5  $\text{meq.l}^{-1}$  was given to the highest treatment bringing the total up to 1.25  $\text{meq. H}_2\text{PO}_4.\text{l}^{-1}$ . Nutrient solutions were not renewed, but depletion of other elements than K or P was prevented by occasional replenishments during the experiment.

Plants were grown in the greenhouse (ca 23°C, daylight + artificial light; March-April and July-September period for K experiment and P experiment, respectively) and harvested 45 (K experiment) or 40 days (P experiment) after transfer to the treatment solutions. The plant material was rinsed with demineralized water and dried at 70°C for 48 hours. Dry weights were recorded for both shoots and roots after which the material was finely ground. Samples dried at 70°C were analyzed for total N and the constituents of the ionic balance as described elsewhere (Troelstra 1983).

Table 13.1. Composition of the nutrient solutions.\*

Ratio	meq. l <sup>-1</sup>								
	NH <sub>4</sub>	Na	K	Ca	Mg	H <sub>2</sub> PO <sub>4</sub>	NO <sub>3</sub>	Cl	SO <sub>4</sub>
(K experiment)									
100/0	-	0.5 + x**	2.5 - x**	4	1	0.5	4	1	2.5
50/50	2	0.5 + x	2.5 - x	4	1	0.5	2	2	5.5
0/100	4	0.5 + x	2.5 - x	4	1	0.5	-	4	7.5
-----									
(P experiment)									
100/0	-	-	2.5	4	1	0.75 - x***	4	1	1.75 + x***
50/50	2	-	2.5	4	1	0.75 - x	2	2	4.75 + x
0/100	4	-	2.5	4	1	0.75 - x	-	4	6.75 + x

\* micronutrients (mg.l<sup>-1</sup>): Fe(5), B(0.5), Mn(0.5), Zn(0.05), Mo(0.05), and Cu(0.02)

\*\* x = 0, 1.5, 2.0, 2.4

\*\*\* x = 0, 0.5, 0.65, 0.7

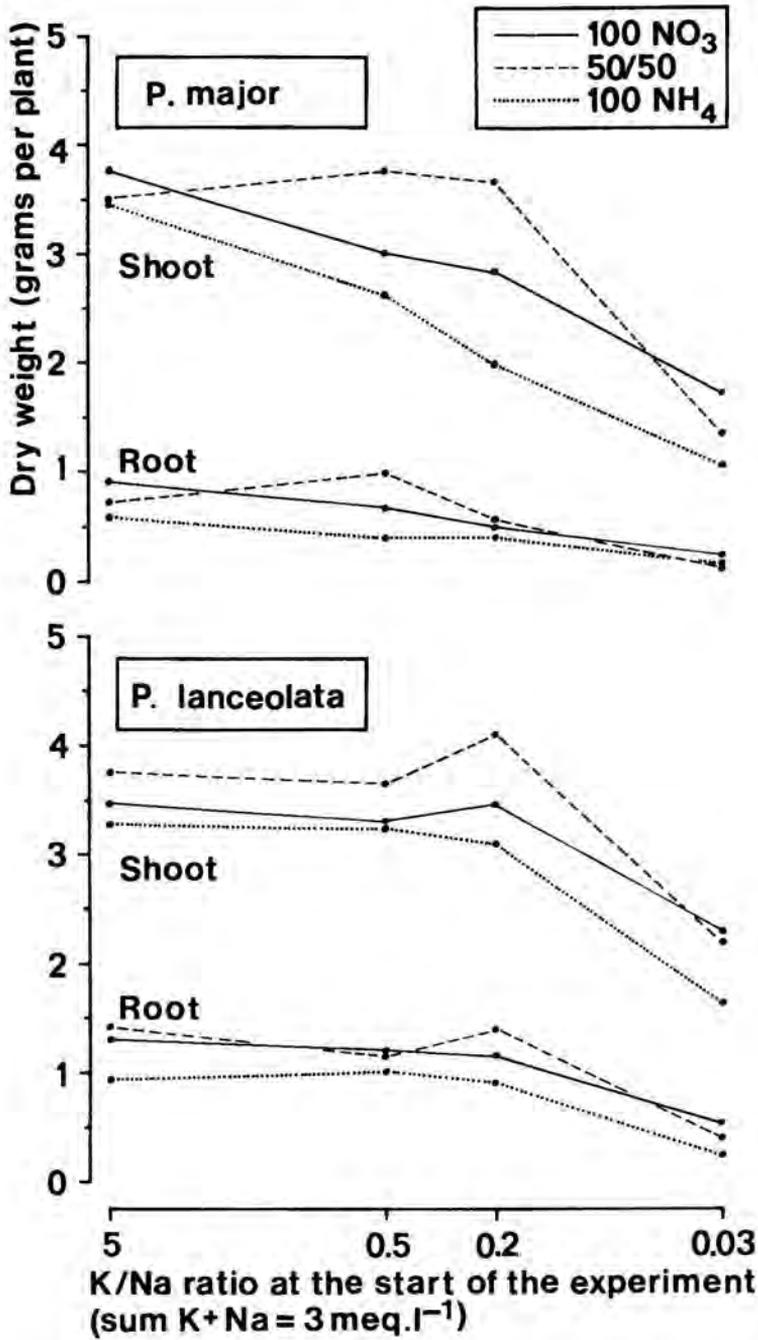


Fig. 13.1. Mean dry weights of shoots and roots of *Plantago major* and *Plantago lanceolata* in relation to K/Na ratio.

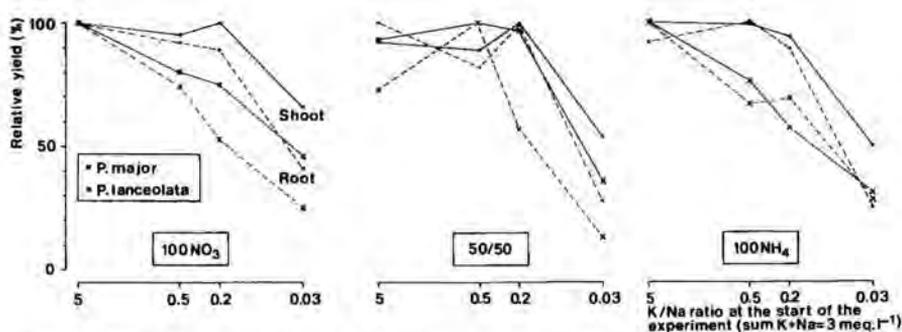


Fig. 13.2. Relative yields of shoots and roots of *Plantago major* and *Plantago lanceolata* in relation to K/Na ratio.

### RESULTS AND DISCUSSION

*K* experiment. Mean dry matter yields of the K experiment are given in Fig. 13.1. Although less pronounced in the 50/50 treatment, the yield of *P. major* decreased more strongly as a response to a decreasing K-availability in the medium than the yield of *P. lanceolata*. This is clearly demonstrated by the

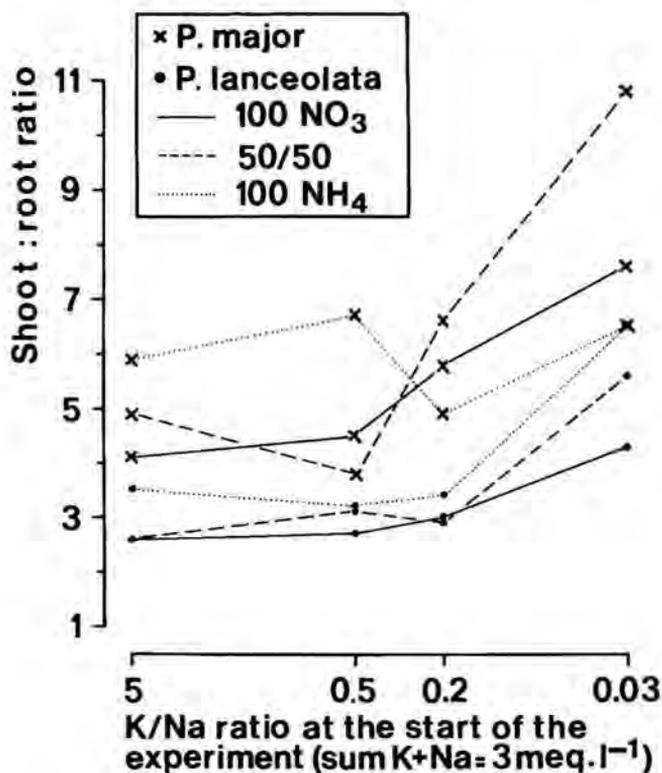


Fig. 13.3. Shoot: root ratios of *Plantago major* and *Plantago lanceolata* in relation to K/Na ratio.

relative yields of shoots and roots in Fig. 13.2. In general, the shoot: root ratios of *P. major* were higher, and, except for the lowest K/Na ratio, they were relatively constant for both species (Fig. 13.3). At the lowest K-level, the shoot: root ratios clearly increased. Plants of the low K treatments developed distinct K-deficiency symptoms.

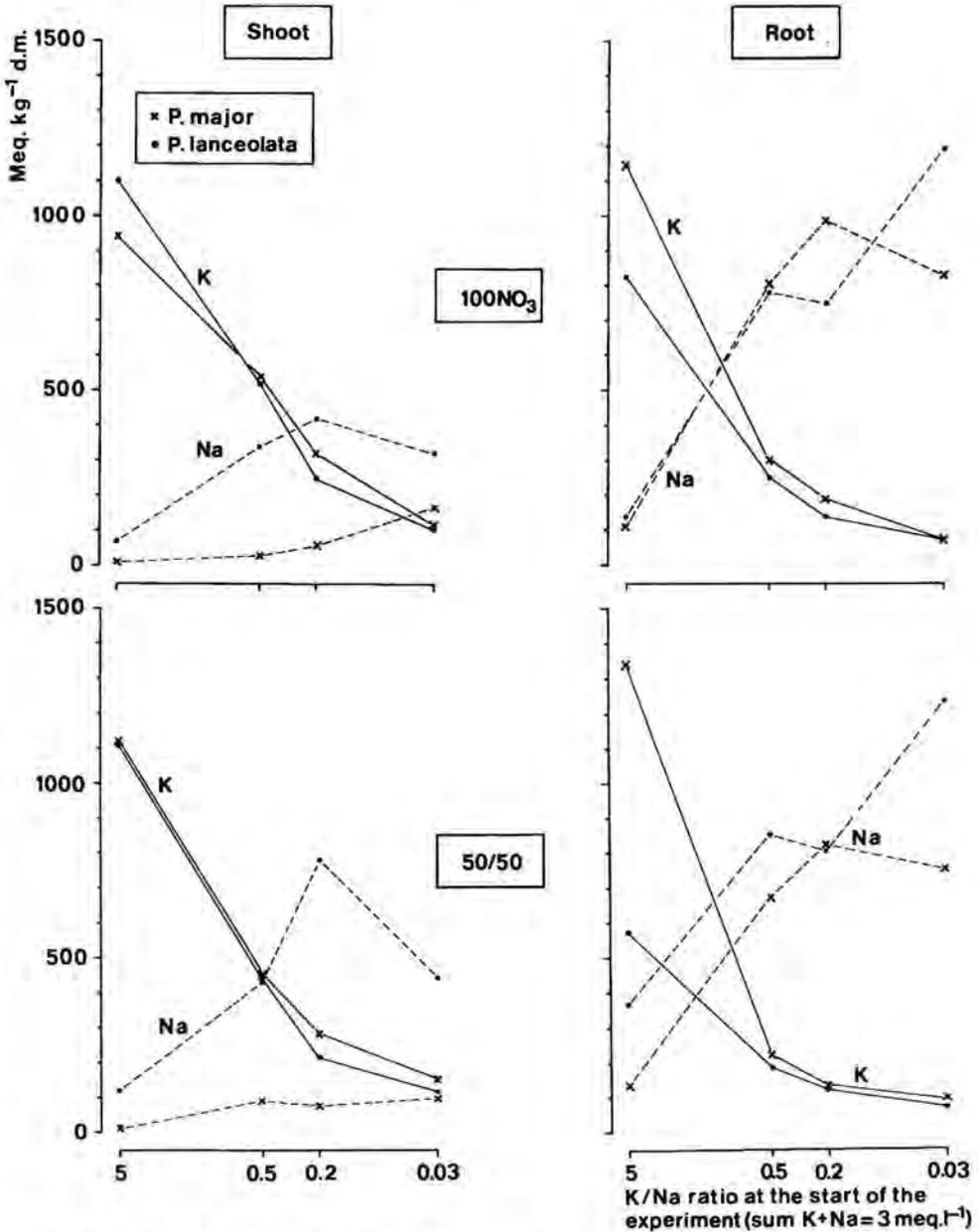


Fig. 13.4. Potassium and sodium contents in shoots and roots of *Plantago major* and *Plantago lanceolata* in relation to K/Na ratio.

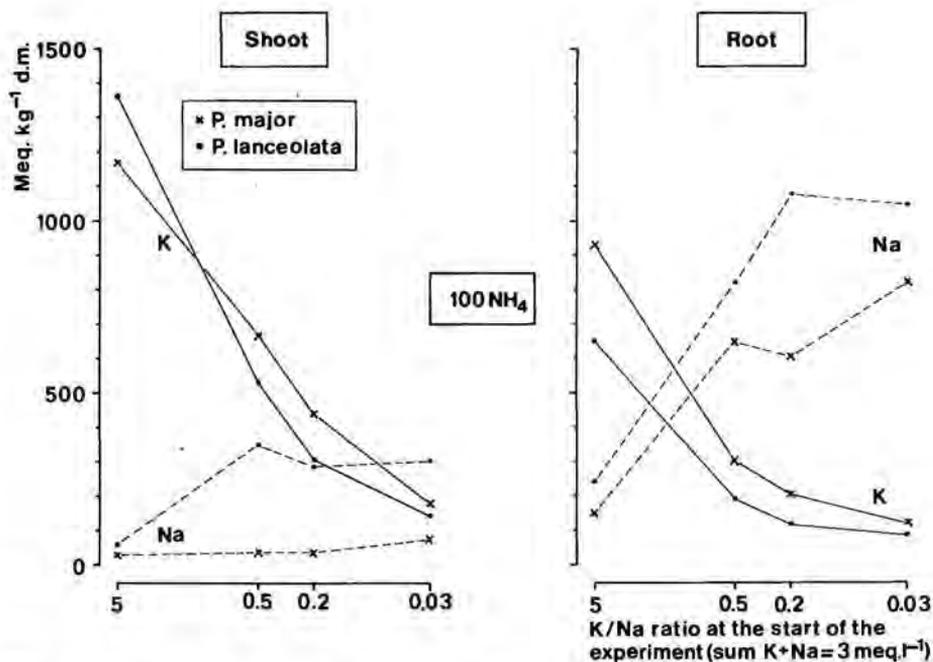


Fig. 13.4. Continued.

Regarding the effect of the K-Na replacement series on the K and Na contents of the plant material, some differences between the species are evident. In the shoot of *P. major* a decreasing K concentration in the ionic balance is much less compensated for by an increasing Na content than is the case of *P. lanceolata* (Fig. 13.4) and other *Plantago* species (*media*, *coronopus* and *maritima*; unpublished results). The decreasing K contents of *P. major* are met by increasing Ca contents (results not shown), rather than increasing Na contents. The picture of the roots is more or less the same for both species, showing a complete K-Na replacement (Fig. 13.4). Obviously, the relatively low Na contents of the shoot of *P. major* refer to differences between the species in rates of transport of Na from the root to the shoot and not to differences in uptake.

Indeed, plotting the ionic contents of field material of various locations in the Netherlands against each other, shows a sort of K-Na replacement in the leaves of *P. lanceolata* and relatively low Na contents in the leaves of *P. major* (Fig. 13.5). On the other hand, *P. major* shows some sort of K-Ca replacement, whereas no such relationship could be demonstrated for *P. lanceolata* (Fig. 13.6).

According to de Wit *et al.* (1963) plantain does not show a particular preference for any of the four cations. However, their results referred to *P. lanceolata* and in view of the present findings it is clear that this conclusion cannot be regarded to be generally valid for the genus *Plantago* and probably does not hold for *P. major*.

If the K contents of the leaves are plotted against the values for exchangeable K of the sites in question, it is noteworthy that the field material of *P. major* shows almost no concentrations of less than 400 meq. K.kg<sup>-1</sup>d.m., a level which certainly can be considered to be adequate (de Wit *et al.* 1963; Fig. 13.7). On the other hand, more than half of the samples of *P. lanceolata* were below this level and K contents < 200 meq. K.kg<sup>-1</sup>d.m. occurred, which may create a situation of specific K shortage (de Wit *et al.* 1963; van Tuil

1965). Obviously, the greater ability of *P. lanceolata* to substitute Na for K plays a role in this respect. A relatively high K content in plants generally having relatively high carboxylate (C-A) contents (*e.g.*, *P. major* and *P. media*; Troelstra and Smant 1979, 1980; Troelstra *et al.* 1981) may be of significance with respect to the internal mobility of carboxylates (van Tuil 1965).

As to the relatively high K contents of *P. major*, however, it remains to be seen whether these represent a direct causal relationship (pertaining to a possible higher K requirement for *P. major* as compared to *P. lanceolata*) or an indirect result of the more solitary occurrence of *P. major* (less or no competition, better transport of K). The present results, however, to some extent support the data of Kruyne *et al.* (1967) in the sense that *P. major* apparently shows a preference for a relatively high K-status.

*P. experiment.* Mean dry matter yields of the P experiment are shown in Fig. 13.8. Except for the 100 NH<sub>4</sub> situation, there is a tendency for *P. major* to react more strongly to an increase of the P concentration. Shoot dry matter of *P. major* clearly increases over the whole range of P concentrations, whereas the yields for *P. lanceolata* level off at the highest P level. In general, root dry matter decreases with an increasing P concentration. Starting at about the same low level of 2-3, the shoot: root ratio of *P. major* increases to a much higher value than that of *P. lanceolata* as the P concentration increases (Fig. 13.8). The relative yields show similar patterns for both species (Fig. 13.9).

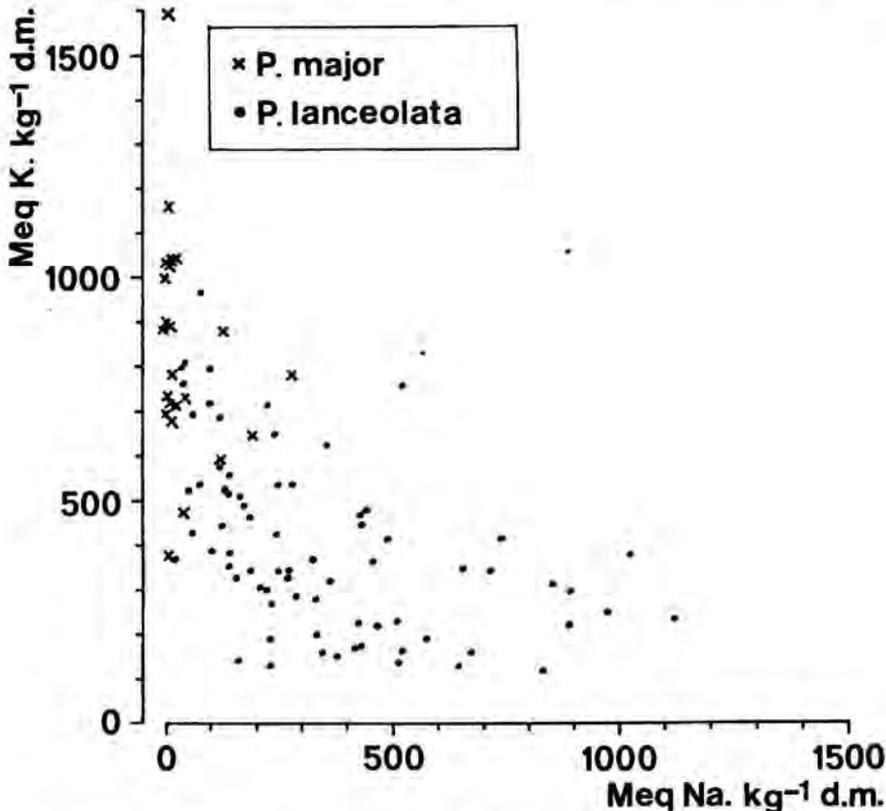


Fig. 13.5. The relationship between potassium and sodium contents in field samples (leaves) of *Plantago major* and *Plantago lanceolata* collected in different locations in the Netherlands.

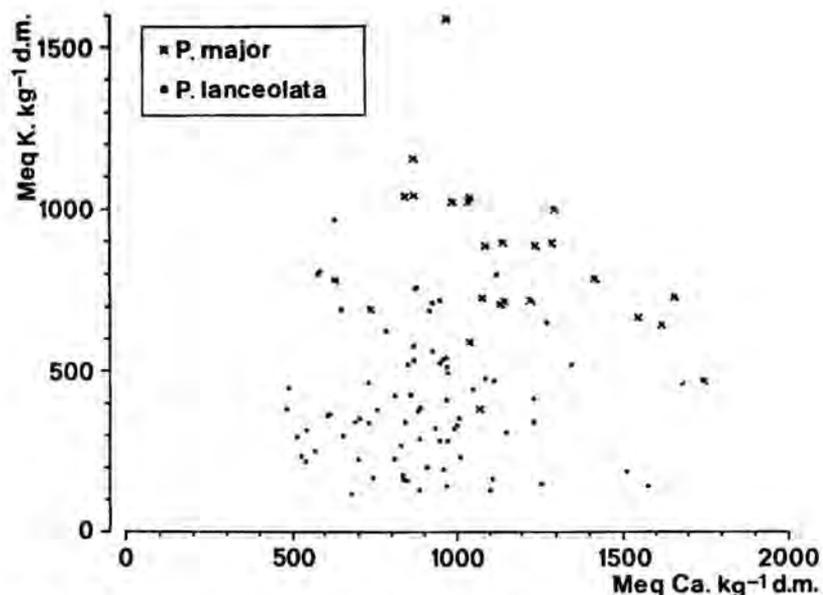


Fig. 13.6. The relationship between potassium and calcium contents in field samples (leaves) of *Plantago major* and *Plantago lanceolata* collected in different locations in the Netherlands.

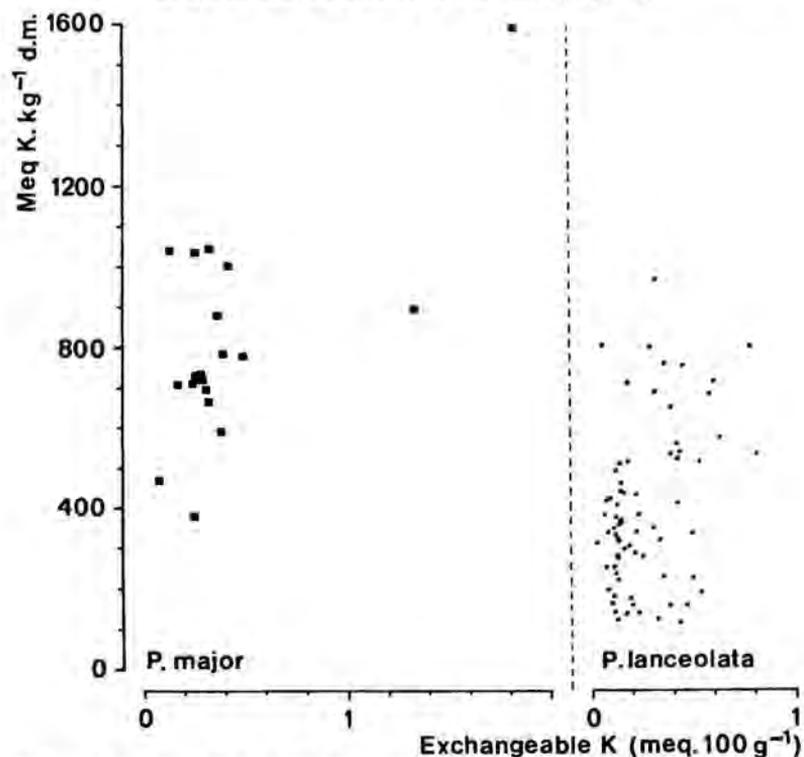


Fig. 13.7. The relationship between the K content in the leaves and the exchangeable K-value of the 0-10 cm soil layer for *Plantago major* and *Plantago lanceolata* in various locations in the Netherlands.

Only in the case of 100%  $\text{NO}_3$ , the relative yield of the shoot of *P. major* is clearly lower at lower P concentrations compared to the relative yield of *P. lanceolata*.

For both species, the P concentration in the dry matter leveled down to a minimum value of about 30 meq.  $\text{kg}^{-1}$  d.m. (Fig. 13.10) being much lower than the more or less optimum value of 70-80 meq.  $\text{kg}^{-1}$  d.m. mentioned by de Wit *et al.* (1963) for grass plants. Slight symptoms of P-deficiency were only visible in the lowest P treatment of the 50/50 series.

Plotting the P contents of the leaves of field material against the corresponding Olsen-P values of the natural sites reveals a clear difference between P.

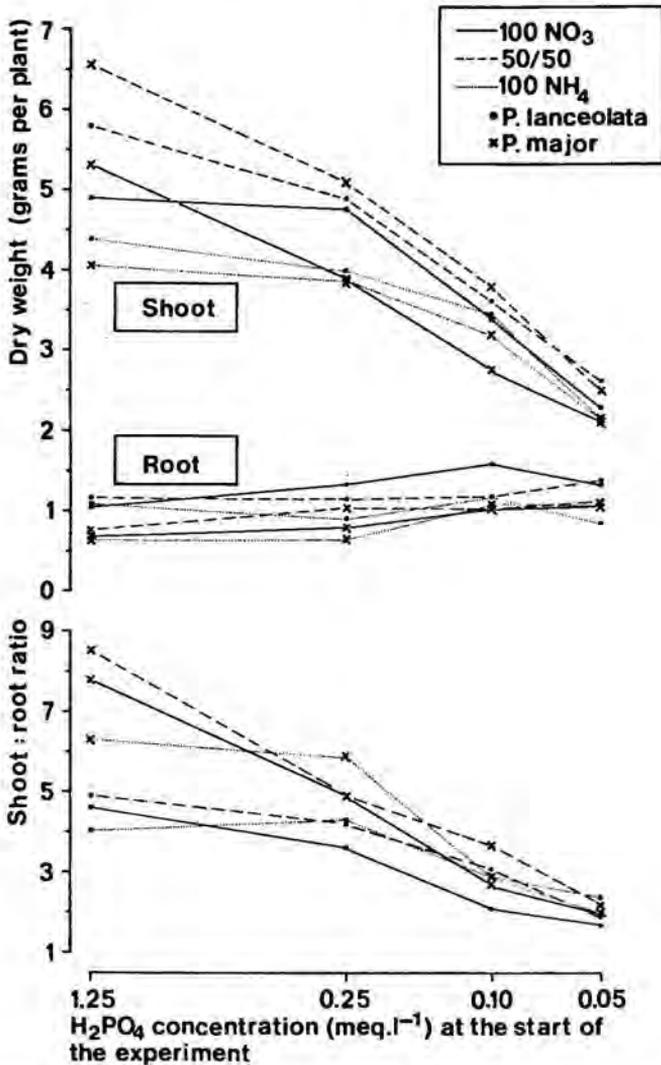


Fig. 13.8. Mean dry weights of shoots and roots and shoot: root ratios of *Plantago major* and *Plantago lanceolata* in relation to phosphorus concentration.

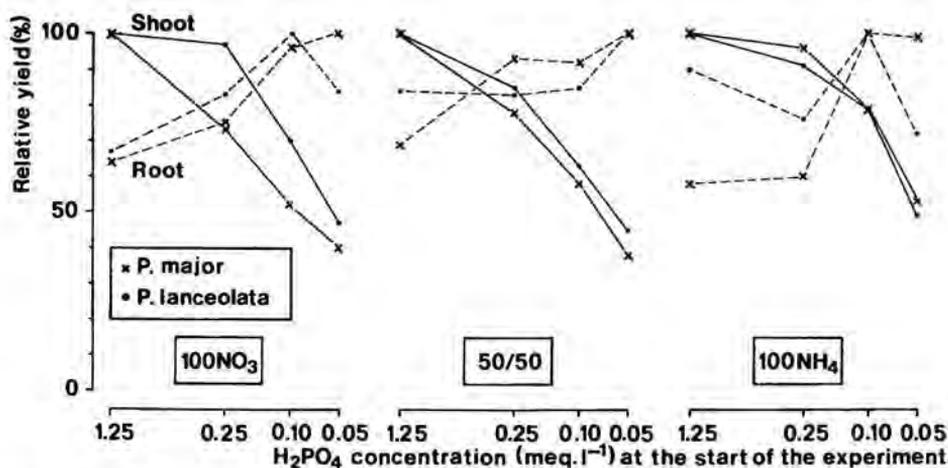


Fig. 13.9. Relative yields of shoots and roots of *Plantago major* and *Plantago lanceolata* in relation to phosphorus concentration.

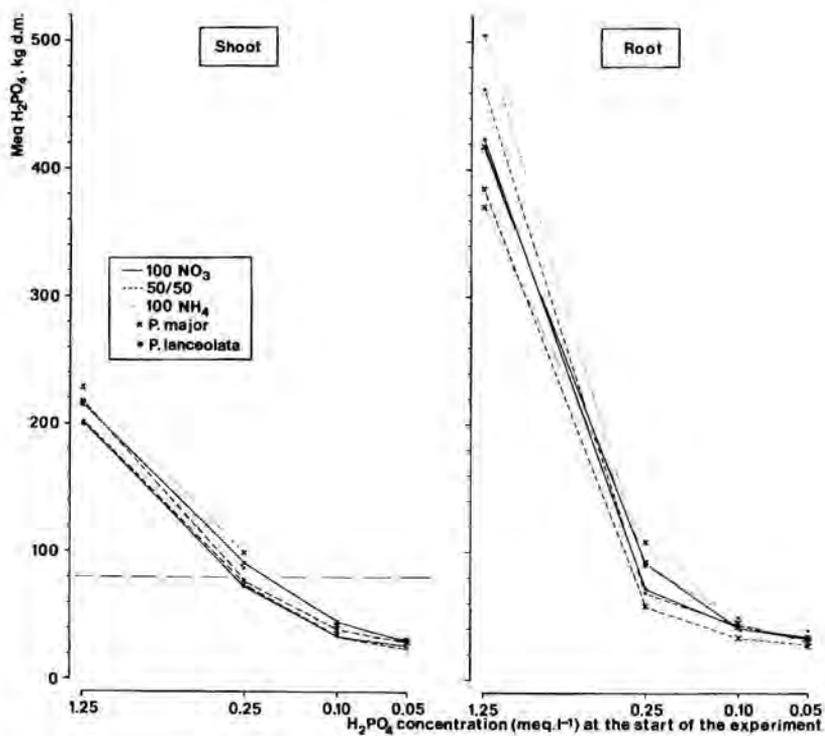


Fig. 13.10. Phosphorus contents in shoot and root samples of *Plantago major* and *Plantago lanceolata* in relation to the concentration of  $H_2PO_4$ . Broken line: adequate P supply according to de Wit *et al.* (1963).

*major* and *P. lanceolata* (Fig. 13.11). *P. major* is more often found at relatively high values of Olsen-P, and, at low levels of Olsen-P, the P concentration in the dry matter of *P. major* is relatively high. Moreover, it is remarkable that the low P concentration of 30 meq. kg<sup>-1</sup>d.m., as found in the present experiment, did not occur in field material of *P. major*. However, even values < 30 meq. H<sub>2</sub>PO<sub>4</sub>.kg<sup>-1</sup>d.m. were commonly found in field material of *P. lanceolata* (Fig. 13.11; and unpublished results).

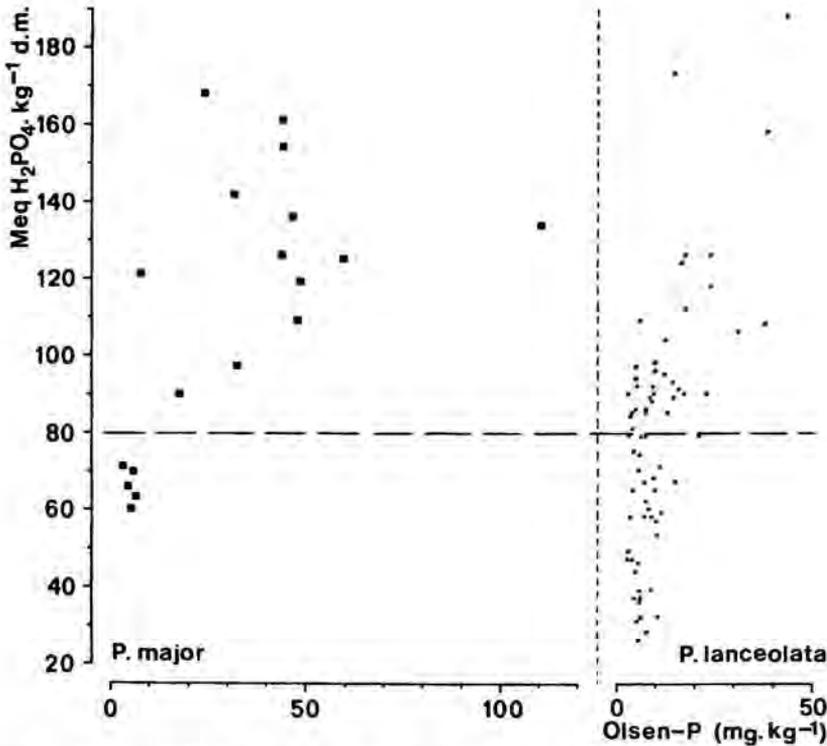


Fig. 13.11. The relationship between the P concentration in the leaves and the Olsen-P value of the 0-10 cm soil layer for *Plantago major* and *Plantago lanceolata* in various locations in the Netherlands. Broken line: adequate P supply according to de Wit et al. (1963).

Again, as in the case with K, the question can be raised as to the nature of the relatively high P contents of *P. major* in field material. Is one dealing with a direct or an indirect relationship? In view of the strongly positive relationship between shoot: root ratio and P-availability, a partial explanation could be that *P. major* has a relatively high P requirement. *P. major* is perhaps under certain circumstances unable to compete effectively for P, if the P availability is relatively low (caused by the presence of a dense vegetation rather than a low available P pool). This may well be one of the reasons for the species to occur in sites where a more or less uninterrupted P supply is guaranteed. Moreover, the adaptation of the species to more compacted sites or the occurrence in the field of relatively high shoot: root ratios, implies the necessity for the maintenance of an adequate P supply towards a limited root system. And since P transport in the soil-root system operates mainly by diffusion (Wild 1981), adequate soil moisture conditions are of paramount importance in this respect.

In view of the foregoing, the chemical composition of a very large specimen of *P. major* (above ground parts: 145 g d.m.; estimated shoot: root ratio > 15) on the verge of a tile-path in a neglected lawn is noteworthy:

meq(mmol). kg<sup>-1</sup>d.m. (leaves + spikes)

K	859	H <sub>2</sub> PO <sub>4</sub>	121	(C-A)	1239
Na	5	NO <sub>3</sub>	26	N <sub>org</sub>	1001
Ca	1173	Cl	292		
Mg	136	SO <sub>4</sub>	499		
NH <sub>4</sub>	4				

In spite of the very high biomass production and the high shoot: root ratio, the P content is still well above the adequacy level of 80 (de Wit *et al.* 1963) and the K concentration is also relatively high.

To gain more insight into the actual patterns of nutrient supply during the season in different *Plantago* sites, we plan to investigate the seasonal levels and fluctuations of mineral N, bicarbonate extractable P, exchangeable K, and soil moisture, for different *Plantago* species (*P. major* ssp. *major*, *P. major* ssp. *pleiosperma*, and *P. lanceolata*) and for different soil layers.

#### REFERENCES

- Aart, P.J.M. van der (1983) - Demographic, genetic, and ecophysiological variation in *Plantago major* and *Plantago lanceolata* in relation to vegetation type. In: Handbook of Vegetation Science, vol. 3, Population Structure of Vegetation (in press).
- Kruijne, A.A., D.M. de Vries and H. Mooi (1967) - Contribution to the ecology of the Dutch grassland plants. Agr. Res. Rep. 696, Pudoc Wageningen (in Dutch, with an English summary), 65 p.
- Troelstra, S.R. (1983) - Growth of *Plantago lanceolata* and *Plantago major* on a NO<sub>3</sub>/NH<sub>4</sub> medium and the estimation of the utilization of nitrate and ammonium from ionic-balance aspects. Plant and Soil 70, 183-197.
- Troelstra, S.R. and W. Smant (1979) - The ionic balance of some plant species from natural vegetations and its relation to nitrogen uptake and salt tolerance. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, Progress Report 1979 I.O.O., 381-389.
- Troelstra, S.R. and W. Smant (1980) - The ionic balance of some plant species from natural vegetations: comparison of plants grown in the greenhouse and the field. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 75, Progress Report 1979 I.O.O., 46-51.
- Troelstra, S.R., L. Sluimer, W. Smant, R. Wagenaar and M.A. van der Meulen (1981) - On the soil chemistry of natural habitats of *Plantago* species and *Hypochaeris radicata* in various parts of the Netherlands in relation to chemical composition of the plants. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 77, Progress Report I.O.O., 20-32.
- Tuil, H.D.W. van (1965) - Organic salts in plants in relation to nutrition and growth. Agr. Res. Rep. 657, Pudoc Wageningen, 83 p.
- Wild, A. (1981) - Mass flow and diffusion. In: D.J. Greenland and M.H.B. Hayes (Eds.). The chemistry of soil processes, pp. 37-80, Wiley.
- Wit, C.T. de, W. Dijkshoorn and J.C. Noggle (1963) - Ionic balance and growth of plants. Agr. Res. Rep. 69-15, Pudoc Wageningen, 68 p.

14. Growth of *Plantago lanceolata* in a permanently waterlogged soil system and the effect of the neighbouring wetland species *Carex disticha* (T. Blacquière)

INTRODUCTION (and some preliminary results)

Plants growing in permanently waterlogged soils have to solve several important problems:

1. The root area becomes anoxic, resulting in an oxygen deficiency for root respiration. Under anoxia a possibility may exist of using other terminal H-acceptors instead of oxygen, such as nitrate (Garcia-Novo & Crawford 1973). However, this can only play a minor role because of the lack of nitrate in waterlogged soils and in the tissue of most plants growing under natural conditions. Moreover, even if nitrate is abundant, its reduction can only consume a small part of the NADH-pool, produced in glycolysis to maintain adequate ATP levels in the cell.
2. When respiration in roots becomes greatly reduced, and the adaptation mentioned (1) does not occur, tolerance to the toxic products of fermentation, e.g. ethanol, or the avoidance of the synthesis of these products has to be developed. According to Crawford (1978), the extent of stimulation of the enzyme alcoholdehydrogenase and of alcohol accumulation under flooded conditions, are proportional to the sensitivity of the plant species to flooding. However, some tolerant species also accumulate ethanol, e.g. rice (John & Greenway 1976). In pea, a species sensitive to flooding, ethanol at concentrations even higher than those measured after flooding, did not affect growth (Jackson *et al.* 1982).
3. During anoxia the soil and rhizosphere are in a strongly reduced state, causing toxic effects of  $Fe^{++}$ ,  $Mn^{++}$ , gases such as  $H_2S$  and ethylene and organic acids, to which the plants have to be tolerant.

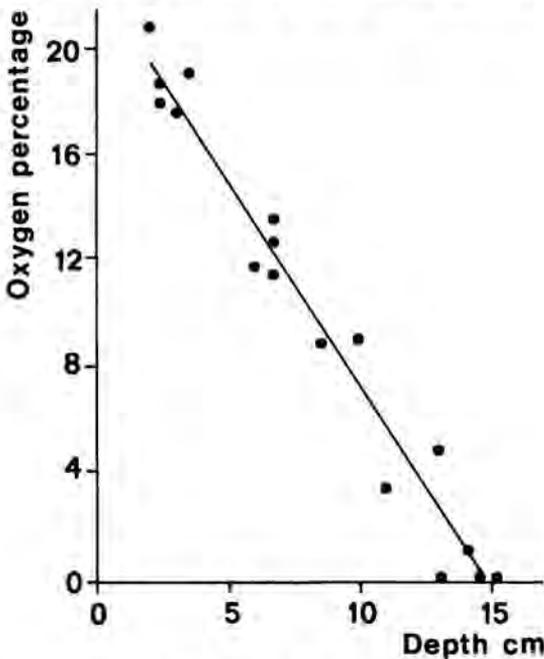


Fig. 14.1. Oxygen profile in the 15 cm top soil in Merrevliet (results of W. van Doorn).

The most important adaptation to wetland conditions is the capacity to form aerenchyma in stems, roots and rhizomes. The above mentioned problems can be largely avoided with aerenchyma; as oxygen for respiration can be transported to the roots, making fermentation unnecessary. The oxygen diffusion from the roots (Armstrong 1964) oxidizes the rhizosphere (Armstrong 1967).

After flooding, *Plantago* species develop adventitious roots, containing aerenchyma. In *P. lanceolata* however, growth of these roots was rather poor and aerenchyma development almost absent. The maximum rooting depth was 10 cm (W. van Doorn, pers. comm.).

*P. lanceolata* occurs on permanently flooded soils (e.g. Merrevliet, Vlaardingse Vlietlanden) and the roots penetrate the soil up to 15 cm. But, although it is flooded, in Merrevliet oxygen is available up to a depth of 15 cm (Fig. 14.1). In Merrevliet, A. Smit (unpublished results), isolated *P. lanceolata* plants from the rest of the vegetation by putting a PVC tube around their roots and removing the vegetation inside the cylinder. In this experimental setup the *P. lanceolata* plants died. The oxygen profile within the cylinder was much steeper than outside.

In an experiment by A. Smit and W. van Doorn, conducted in a greenhouse, *P. lanceolata* was cultured on sieved anoxic, flooded soil from the bottom of a ditch. The plants were grown in monoculture, in mixed culture with *Carex nigra* L. - an aerenchyma-containing bog species - or with artificial aeration. The summarized results of this experiment were:

1. There was no difference in dry-matter production of *P. lanceolata* in the three treatments.
2. The rooting depth of *P. lanceolata* was about 5 cm and was unaffected by being cultured with *C. nigra*. In pots with aeration *P. lanceolata* rooted to the level of the air inlet, i.e. about a depth of 12 cm.

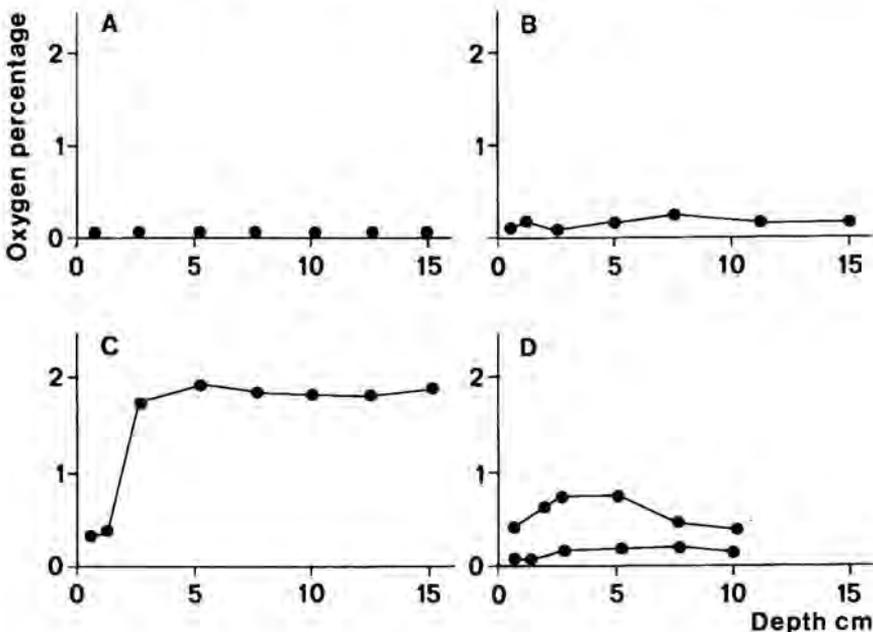


Fig. 14.2. Oxygen percentages in relation to depth. A: no vegetation; B: aeration only; C: *Carex nigra*; D: *P. lanceolata*; two pots (unpublished results of Smit and van Doorn).

- Oxygen in the soil columns was measured using a Clark type oxygen-sensor and an YSI oxygen monitor model 53. Some of the resulting profiles are plotted in Fig. 14.2. Pots without plants did not contain any oxygen, aeration gave little oxygen (bad penetration). *C. nigra* roots contributed some oxygen (= 2%), but *P. lanceolata* roots did likewise, although to a lesser depth.

## INUNDATION AND THE NITROGEN CYCLE

Further consequences of inundation and the resulting oxygen deficiency are changes in the nitrogen cycle in the soil. At decreasing oxygen tensions denitrification will increase, as long as nitrification takes place. At very low oxygen tensions nitrification will cease. Nitrogen then is mainly available in the form of the ammonium ion. *In vivo* activities of the enzyme nitratereductase were indeed very low in *P. lanceolata* in Merrevliet (Table 14.1), although they were higher than the basal level as determined by Smit & Woldendorp (1981). Nitrifiers in the rhizosphere of these *P. lanceolata* plants could not be detected. However, in plants with air spaces in the roots, or with an elaborate superficial rooting system (e.g. *Lychnis flos-cuculi*), the activities were much higher (Table 14.1). The following hypothesis was proposed (Fig. 14.3): Oxygen, transported via the aerenchyma system of bog plants, diffuses into the rhizosphere and favours nitrification. Due to the very dense root mat in Merrevliet, the rhizosphere of *P. lanceolata* can be partly oxidized to a level sufficiently high to permit some nitrification, resulting in a nitratereductase activity in the plants above the basal level. In wet reed stands and wet rice cultures nitrification has also been observed (Reddy 1982), possibly partly due to oxygen leakage. In the spring of 1982, nitrification was found to occur also in Merrevliet. *Nitromonas* and *Nitrobacter* were detected and *Nitrobacter* cells were accumulated by enrichment with nitrite.

In the present experiment the hypothesis was tested that:

- P. lanceolata* can maintain itself in wetlands due to the oxygen leakage from roots of neighbouring wetland species.
- P. lanceolata* partly uses nitrate as a nitrogen source in wetland conditions because nitrification in its rhizosphere is possible due to this oxygen leakage.

This paper describes an experiment to investigate the influence of oxygen leakage and growth in a mixed culture with a bog species on the growth and nitrate utilization of *P. lanceolata* under inundated conditions.

Table 14.1. Nitrate reductase activity in the leaf of some species at Merrevliet at different times. Activity expressed as micromoles nitrite evolved per hour per gram of dry matter. Numbers indicate mean  $\pm$  S.D.

Date	Species	Nitrate reductase activity	Species	Nitrate reductase activity
August 12	<i>P. lanceolata</i>	0.52 $\pm$ 0.16	<i>Rumex hydrolapathum</i>	3.40 $\pm$ 0.39
October 1	"	0.19 $\pm$ 0.16	<i>Phragmites australis</i>	0.67 $\pm$ 0.11
" 9	"	0.44 $\pm$ 0.22	<i>Carex distycha</i>	1.49 $\pm$ 0.11
November 5	"	0.62 $\pm$ 0.04	<i>Angelica sylvestris</i>	2.18 $\pm$ 0.54
December 10	"	0.13 $\pm$ 0.10	<i>Lychnis flos-cuculi</i>	4.80 $\pm$ 0.32

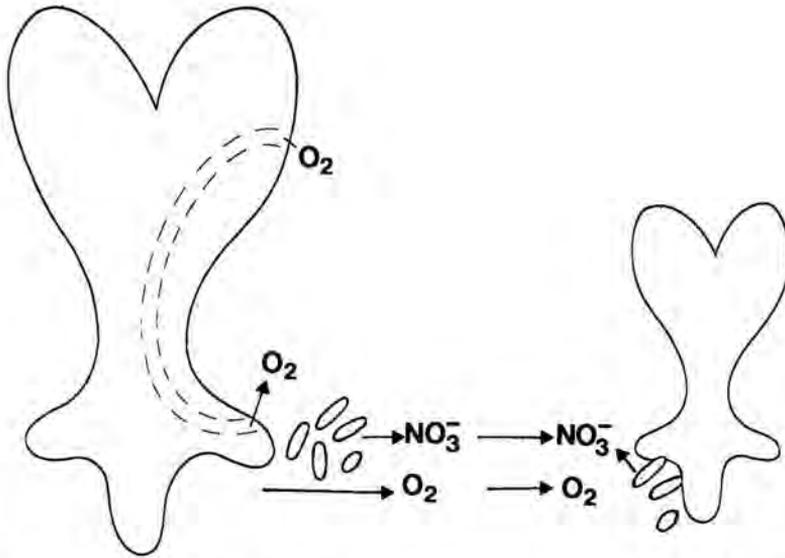


Fig. 14.3. Possible interactions between bog species and *P. lanceolata*; Oxygen leakage and nitrification.

#### MATERIALS AND METHODS

*Carex disticha* plants were collected at the edge of Merrevliet, cut into small units and recultured in a nutrient solution in perlite. After about two months the plants were placed in pots with soil and inundated. *P. lanceolata* seeds, harvested at Merrevliet, were sown on humid glass beads; when the seedlings

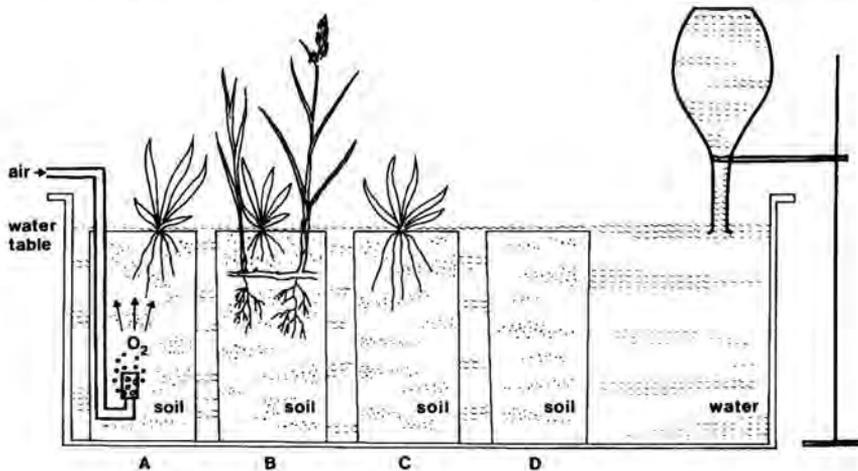


Fig. 14.4. Experimental design in which *P. lanceolata* was grown in two inundated soil mixtures (see text).

- A. *P. lanceolata* with aeration only
- B. *P. lanceolata* with *Carex disticha*
- C. *P. lanceolata* without aeration and *Carex disticha*
- D. Control without plants and aeration.

were two weeks old they were transferred to the inundated pots with and without *C. disticha*. In some of the pots the soil was aerated artificially. The water table was maintained at a constant level using the Mariotte principle. The treatments are summarized visually in Fig. 14.4. Two soil types were used in the experiments.

A. a mixture of peat and riversand (40/60 w/w), enriched with 5 g Soyabean protein (Reforma Vlaardingen) per pot.

B. sieved sludge from the bottom of a ditch.

Each pot was inoculated with a tea spoon of soil rich in nitrifiers. After prolonged growth during inundation the following parameters were measured:

- growth: number of leaves
  - leaf length
  - fresh weight of shoots and roots.
- *in vivo* nitrate reductase activity (Jaworski 1971).
- ammonium and nitrate contents of the soils.
- numbers of nitrifiers in the soil or rhizosphere. One gram of roots or soil was shaken for 1 hour in 1% pyrophosphate with glass beads. The most probable number of nitrifiers was determined according to Meiklejohn (1968). Tests for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were performed after Alexander & Clark (1965) with the Griess-Ilosvay reagents.
- pH of the soil, one part of soil diluted with 4 parts of water.
- Oxygen concentration in the soil, measured with an oxygen sensitive electrode.

## RESULTS

*The soil pH.* Upon inundation the pH of the soils dropped rapidly to a low level. At this point the *C. disticha* plants suffered and some died. After a period the pH of soil A stabilized around 6.5. The pH of soil B was about 7.

## PRODUCTION

The mean numbers of leaves, leaf length and freshweight of the harvested plants are listed in Table 14.2. Leaf length, and in soil B the number of leaves, were enhanced by artificial aeration. This also holds for the biomass of the plants. Mixed culturing with *C. disticha* did not affect these parameters. The top:root ratio was lowered by aeration (Table 14.3). This was not the case with the plants growing in mixed cultures. It was higher in soil B than soil A.

Table 14.2. Mean number of leaves (n), mean leaf length (l), the mean product of both (n x l) and mean fresh weight of *P. lanceolata* plants in the treatments of figure 4 on soil types A and B.

Treatment	n	l	n x l	fresh weight g/plant
A. 1. Monoculture	12.1 ± 2.5	13.0 ± 3.3	163 ± 68	17.0 ± 11.2
2. Mixed culture	-	-	-	-
3. Monoculture with aeration	11.6 ± 2.4	19.5 ± 3.9	229 ± 79	27.5 ± 18.9
B. 1. Monoculture	11.0 ± 2.8	12.8 ± 1.6	143 ± 54	-
2. Mixed culture	11.3 ± 3.7	16.6 ± 3.7	190 ± 81	12.6 ± 5.1
3. Monoculture with aeration	17.6 ± 5.8	23.7 ± 5.5	425 ± 184	52.3 ± 34.9

Table 14.3. Top-root ratio of *P. lanceolata* (means  $\pm$  S.D.)

Treatment	Soil type A	Soil type B
1. Monoculture	2.51 $\pm$ 0.67	5.62 $\pm$ 2.08
2. Mixed culture	-	4.66 $\pm$ 1.61
3. Monoculture with aeration	2.15 $\pm$ 0.87	2.99 $\pm$ 1.37

#### Nitrate reduction

The *in vivo* nitrate reductase activity is enhanced by aeration, but unaffected by growth in a mixed culture (Table 14.4).

Table 14.4. Nitrate reductase activity in *P. lanceolata*. Activity expressed as nanomoles nitrite evolved per hour per gram of dry matter, on a whole plant basis.

Treatment	Soil type A	Soil type B
1. Monoculture	352 $\pm$ 284	-
2. Mixed culture	-	302 $\pm$ 325
3. Monoculture with aeration	1142 $\pm$ 668	995 $\pm$ 644

#### Ammonium and nitrate contents of the soil

The ammonium content appears to be rather high. Nitrate was low in all treatments (Table 14.5).

Table 14.5. Ammonium and nitrate contents of the soils from different treatments; mg N / kg soil.

Treatment	Soil type A		Soil type B	
	ammonium	nitrate	ammonium	nitrate
1. Monoculture	72 $\pm$ 7	0.6 $\pm$ 0.1	131 $\pm$ 10	0.7 $\pm$ 0.3
2. Mixed culture	-	-	47 $\pm$ 29	0.7 $\pm$ 0.2
3. Monoculture with aeration	89 $\pm$ 14	0.2 $\pm$ 0.3	-	-

#### Number of nitrifiers

During the course of growth the numbers of nitrifiers were determined several times (Table 14.6). In all treatments rather high numbers were apparent. The numbers were higher per gram of soil than per gram of root with attached soil (rhizosphere).

Oxygen in the soil was measured with a Clark electrode and an oxygen analyser. Because of the high oxygen demand of the electrode, no valuable measurements were obtained.

## DISCUSSION

When the soil was aerated the production of *P. lanceolata* increased; this did not occur when grown in a mixed culture.

In a mixed culture, two opposite effects may interfere:

1. A (possible) positive interaction between roots of *C. disticha* and *P. lanceolata* (oxygen leakage).
2. Competition, both above and under-ground, between the two species.

The positive effect of roots of *C. disticha* was probably still rather small because of their limited root development. At Merrevliet the rootzone was deeper and the rooting very dense.

Competition undoubtedly has been an important factor in this experiment particularly competition below ground level. Above ground (light) competition was avoided.

In Merrevliet a large number of the plants in the experiment conducted by Smit (see introduction) died. This was not the case in our experiments. Probably the type of soil used is important, e.g. the fertility of the soil. Another possibility is that the plants at Merrevliet did not suffer from oxygen deficiency, but from one of the indirect effects of anoxia. Shortly after flooding, soils generally acidify strongly, liberating a lot of toxins. The death of the *P. lanceolata* plants might be caused by these toxins.

Nitrate reductase activity of the plants was increased only by aeration; otherwise the activities were about the same as measured at Merrevliet (Table 14.1), a little higher than the basal level. The number of nitrifiers tended also to be highest in aerated soils (Table 14.6). Surprisingly the number of nitrifiers were high in all treatments; consequently nitrifiers may survive rather long periods of anoxia (see Belser 1979).

Table 14.6. Most Probable Number of nitrifiers per gram of soil and per gram of root with attached soil particles (-numbers in brackets). Days were counted from start of inundation onward.

Day	Soil type A				Soil type B			
	1. monoculture		3. with aeration		1. monoculture		2. mixed cultures	
	NH <sub>4</sub> <sup>+</sup> -ox	NO <sub>2</sub> <sup>+</sup> -ox	NH <sub>4</sub> <sup>+</sup> -ox	NO <sub>2</sub> <sup>-</sup> -ox	NH <sub>4</sub> <sup>+</sup> -ox	NO <sub>2</sub> <sup>-</sup> -ox	NH <sub>4</sub> <sup>+</sup> -ox	NO <sub>2</sub> <sup>-</sup> -ox
93	-	-	-	-	28700	220	-	-
94	-	-	-	-	-	-	450	14400
99	(14400)	(900)	>57500	14400	-	-	-	-
	-	-	(>7200)	(900)	-	-	-	-
100	28700	900	>57500	3600	-	-	-	-
102	7200	7200	-	-	-	-	-	-
104	-	-	-	-	-	-	>57500	14400
							(1800)	(1800)
105	-	-	-	-	-	-	(14400)	(7200)

To elucidate the critical role of oxygen diffusion in wetland ecosystems and its effects on nitrogen cycling, some further information needs to be collected.

#### REFERENCES

- Alexander, M. and F.E. Clark (1965) - Nitrifying bacteria. In: C.A. Black (ed.): Methods of soil analysis. Ann. Soc. Agr. Madison Wisc. Part 2, 1477-1483.
- Armstrong, W. (1964) - Oxygen diffusion from the roots of some British bog species. *Nature* 204, 801-802.
- Armstrong, W. (1967) - The oxydizing effect of roots in waterlogged soils. *Physiol. Plant* 20, 920-926.
- Belser, L.W. (1979) - Population ecology of nitrifying bacteria. *Ann. Rev. Microbiol.* 33, 309-333.
- Crawford, R.M.M. (1978) - Metabolic adaptations to anoxia. In 'Plant Life in anaerobic environments' D.D. Hook & R.M.M. Crawford (Eds.). Ann. Arbor Science Publ. Inc. Michigan, 119-136.
- Garcia-Novo, F. and R.M.M. Crawford (1973) - Soil aeration, nitrate reduction and flooding tolerance in higher plants. *New Phytol.* 72, 1031-1039.
- Jackson, M.B., B. Herman and A. Goodenough (1982) - An examination of the importance of ethanol in causing injury to flooded plants. *Plant Cell Envir.* 5, 163-173.
- Jaworski, E.G. (1971) - Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Comm.* 43, 1274-1279.
- John, C.D. and H. Greenway (1976) - Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. *Austr. J. Pl. Physiol.* 3, 325-336.
- Meiklejohn, J. (1968) - Numbers of nitrifying bacteria in some Rhodesian soils under natural grass and improved pastures. *J. Appl. Ecol.* 5, 291-300.
- Reddy, K.R. (1982) - Nitrogen cycling in a flooded-soil ecosystem planted to rice (*Oryza sativa* L.) *Plant & Soil* 67, 209-220.
- Smit, A.J. and J.W. Woldendorp (1981) - Nitrate production in the rhizosphere of *Plantago* species. *Plant & Soil* 61, 43-52.

15. The effect of ambient nitrate concentration on growth and nitrate uptake of two *Plantago* species - a summary (A.H.J. Freijsen, H. Otten)

#### INTRODUCTION

In previous years a number of culture experiments were carried out in which the effect of a 25  $\mu\text{mol.l}^{-1}$  nitrate concentration on the performance of *Plantago* species was investigated. From these experiments and from similar experiments by other authors it became clear that only extremely low nitrate concentrations can restrict plant growth. When the nitrate concentration is low or average, sufficient nitrate is taken up to allow optimal plant growth. Short-term uptake experiments suggest that fairly high concentrations are required for optimal growth. In 1981 some methodological culture experiments were carried out to optimize culture at 10  $\mu\text{mol}$  nitrate per litre. An important prerequisite for culture at low concentrations is to avoid depletion at the root surface. In 1982 *Plantago lanceolata* L. and *P. major* L. *major* were grown at 7500

and 9.5  $\mu\text{mol}$  nitrate per litre. The object was to find different responses to the nutrient treatments and to relate these to the respective habitats. The habitats seem to be different with respect to nutrient availability. The rather poor and dry soils on which *P. lanceolata* is found, seem to offer a low and perhaps irregular availability of nutrients. *P. major* grows on soils which are characterized as ruderal. Stable soil moisture conditions guarantee a constant supply of nutrients and there is no competition.

## METHODS

Plants were grown in a flowing culture system under otherwise optimal conditions with nitrate concentrations of 7500 and 9.5  $\mu\text{mol}$  per litre. The relative growth rate and properties of the ionic balance were determined.

## RESULTS

*P. lanceolata* was more affected by the low nitrate concentration than *P. major*. The properties of *P. lanceolata* which were influenced, were: shoot biomass, leaf size, shoot nitrate concentration, and concentrations of some other ions. Because of a reduction of shoot growth the shoot: root ratio of *P. lanceolata* at the low nitrate concentration was lower than at the high concentration. Due to the reduction of shoot growth, the growth of the total plant was also slower. The concentration of nitrate in the shoot was reduced by 50 per cent at the low nitrate concentration; this decrease was counter-balanced by a conspicuous enlargement of the chloride concentration. The cause of these changes in *P. lanceolata* must be looked for in a 40 per cent reduction of nitrate uptake, i.e., the specific uptake rate diminished by 40 per cent. In *P. major*, however, there was only a very small reduction of the specific uptake rate. This meant that the extent of secondary changes in this species was also very restricted.

## DISCUSSION

Summarizing it may be said that *P. major* is more adapted to grow at a constant low concentration of nitrate than *P. lanceolata*. The literature suggests that eutrophic species can grow better with a suboptimal nutrient supply than oligotrophic species, because of a greater 'root absorption capacity' ( $= V_{\text{MAX}}$ ). It is conceivable that the quantity of uptake carrier in the root is decisive for the rate of uptake in the range of low concentrations (about 10  $\mu\text{mol.l}^{-1}$ ). Applying this theory, *P. major* grew better at 9.5  $\mu\text{mol}$  nitrate per litre than *P. lanceolata* because the former, being an eutrophic species, possesses more carrier than the latter, the oligotrophic species. This explanation will be tested in further experiments in which the kinetics of nitrate uptake of both *P. lanceolata* and *P. major* grown at low nitrate concentration will be investigated. The above mentioned explanation of the experimental results does not imply that *P. major* is also more suited to grow at still lower nitrate concentrations than 9.5  $\mu\text{mol.l}^{-1}$ . Possibly, the uptake rate at lower concentrations will be determined by the affinity of the carrier for nitrate. A better indication for the affinity than  $K_M$  seems to be the slope of the Michaelis-Menten curve. In future experiments this parameter will be measured to find out whether the oligotrophic *P. lanceolata* surpasses *P. major* with respect to this uptake parameter.



Plate 2. The dune grassland area 'Westduinen' (Island of Goeree) is the main growing site of Autumn Lady's Tresses *Spiranthes spiralis* in the Netherlands. In 1982 over 3000 flowering individuals were recorded. The photo shows individuals excavated *in situ* on 14 September 1982 (Photo C. van Dijk).

16. Salt spray and its influence on the vegetation of the coastal dunes of Voorne and Goeree (the Netherlands) in relation to man-made changes in coastal morphology (J.C. Vulto, P.J.M. van der Aart)

16.1. Introduction

Each year 10 million cubic meters of mud is dredged from the harbour area of Rotterdam. Plans exist to store the mud in a huge basin off the coast of Voorne (slufterdamproject). The dump is expected to cover an area of 700 ha and to rise 17 m above sea level. A construction of this size will have drastic environmental effects. One of these is a reduction in the amount of wind-transported sea-salt reaching the coastal dunes of Voorne.

To study the influence of salt spray on the various vegetation types of Voorne, the distribution in space and time of windborne sea-salt was studied in 1966 and 1967 (Sloet van Oldruitenborgh & Heeres 1969). Similar research was done by Vulto in 1973 and 1974 and Veelenturf in 1982 (Veelenturf 1982).

The wind speed and the salt supply on Voorne changed in 1966 due to the construction of the Maasvlakte and Brielse Gatdam. The coastal water has a higher salt content since the Haringvliet was closed in 1970.

From June 1973 till the end of 1974 the spatial and temporal distribution of the amount of supplied sea-salt has been recorded in the dune areas of Voorne and the northern part of Goeree (N-Goeree). There was a network of 60 measuring points in 5 transects mostly at right angles to the coast-line (Fig. 16.1). Measuring was done with a simple device: a 10 cm tube wrapped in filterpaper 1.5 m above ground.

The dune areas of Voorne and Goeree are situated south-west of Rotterdam in the northern Delta area where Rhine and Meuse flow into the North Sea. The area has distinct zones of relatively low dune-ridges and dune-slacks.

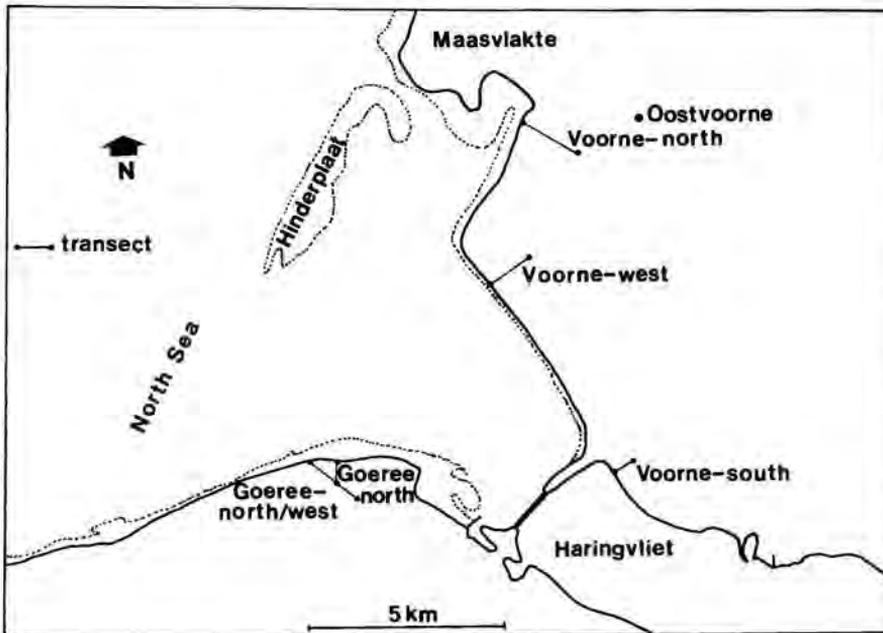


Fig. 16.1. Location of the research area and the position of the measuring transects.

On pioneer beach-dunes and the first dune ridge there are communities dominated by respectively *Elytrigia junceiformis* A. et D. Löve and *Ammophila arenaria* (L.) Link. Older dune ridges are covered with scrub dominated by either *Hippophaë rhamnoides* L., *Ligustrum vulgare* L. and *Crataegus monogyna* Jacq. The dune slacks are covered by herb vegetation or *Salix repens* L. or *Betula pendula* Roth. The dry dune-grasslands of the innerdunes are very rich in species. There is a large scale gradient from the coast-line to the inner dunes in wind speed and the salt content of the air.

## 16.2. Salt spray

The amount of sea-salt transported through the air and deposited by air and/or rainwater at a certain place is dependent on a great number of factors:

1. salt content of the coastal water
2. direction of the wind
3. wind velocity
4. distance to the high-water mark
5. height above sea-level
6. distance in the direction of the wind to geomorphological and vegetative barriers
7. precipitation
8. foliage

### 16.2.1. Salt content of the sea-water

In the Rhine/Meuse-estuary there are sharp fluctuations in the salt content of the water as a result of the large fluctuations in the river discharge. In the northern Delta area the fresh Rhine/Meuse water flows mainly via the Haringvliet and Nieuwe Waterweg into the North Sea. The discharge of the Meuse is usually small in comparison to that of the Rhine.

The average discharge of the Rhine is 2200 m<sup>3</sup>/s, discharges of more than 4000 m<sup>3</sup>/s are found on average 30 days a year, those of more than 6000 m<sup>3</sup>/s occur on average 5 days per year. Since the construction of the Haringvliet-dam the fluctuations in Cl<sup>-</sup>-content are determined to a great extent by the flow-opening of the Haringvliet-locks. The flow-opening is closely correlated to the Upper-Rhine discharge. The Cl<sup>-</sup>-content of the coastal water ranges from 1.7 g/l at a lock-opening of 875 m<sup>2</sup> to 18 g/l at an opening of less than 25 m<sup>2</sup>. When the lock-openings (less than 25 m<sup>2</sup>) and the river discharges (less than 1300 m<sup>3</sup>/s) are small the Cl<sup>-</sup>-content of the coastal water is high and the difference between the coastal water of Voorne and N-Goeree is slight (17 ± 0.5 g/l). However, when the discharge is large there is a significant salt gradient from the Haringvliet (less than 2 g/l) to the coasts of N-Voorne and N-Goeree (6 g/l). The Cl<sup>-</sup>-content of Haringvliet is low: 0.2 - 0.4 g/l.

### 16.2.2. Wind direction and wind velocity

Little salt is deposited by off-shore winds and winds with velocities below 5 m/s. Wind blowing at right angles to the coast-line have the greatest effect, whereas the salt deposit from wind from other directions is reduced by the cosinus of the angle between wind direction and the line at right angles to the coast ( $\beta_i$ ). The wind-value for a given point in the dune area was calculated by

$$w = \sum_{i=0}^{360} \sum_{t=0}^{336} t_i v_i \cos \beta_i \quad \text{on the assumptions above-mentioned}$$

where  $v_i$  = wind velocity in 0.5 m/s,  $t_i$  = time in hours over a 14-day period with wind direction  $i$ .

When the wind-value is equal ( $w = 2000$ ) the salt deposit at the highwaterline (HW-line) is lowest in S-Voorne (10 mg/jar/14 days) and highest in N-Goeree (60 mg), N-Voorne assumes an intermediate position (40 mg). The low value for S-Voorne is caused by the lower Cl<sup>-</sup>-content of the Haringvliet water.

From Table 16.1 it appears that there is a relation between windborne salt by air and the  $\text{Cl}^-$ -content of the coastal water.

16.2.3. Distribution of windborne sea-salt in time

In Fig. 16.2 the  $\text{Cl}^-$ -supply by air ( $\text{mg Cl}^-/\text{pot}/14 \text{ days}$ ) has been plotted for a number of points in transect N-Voorne for the period July 1973 till the end of December 1974. It appears that:

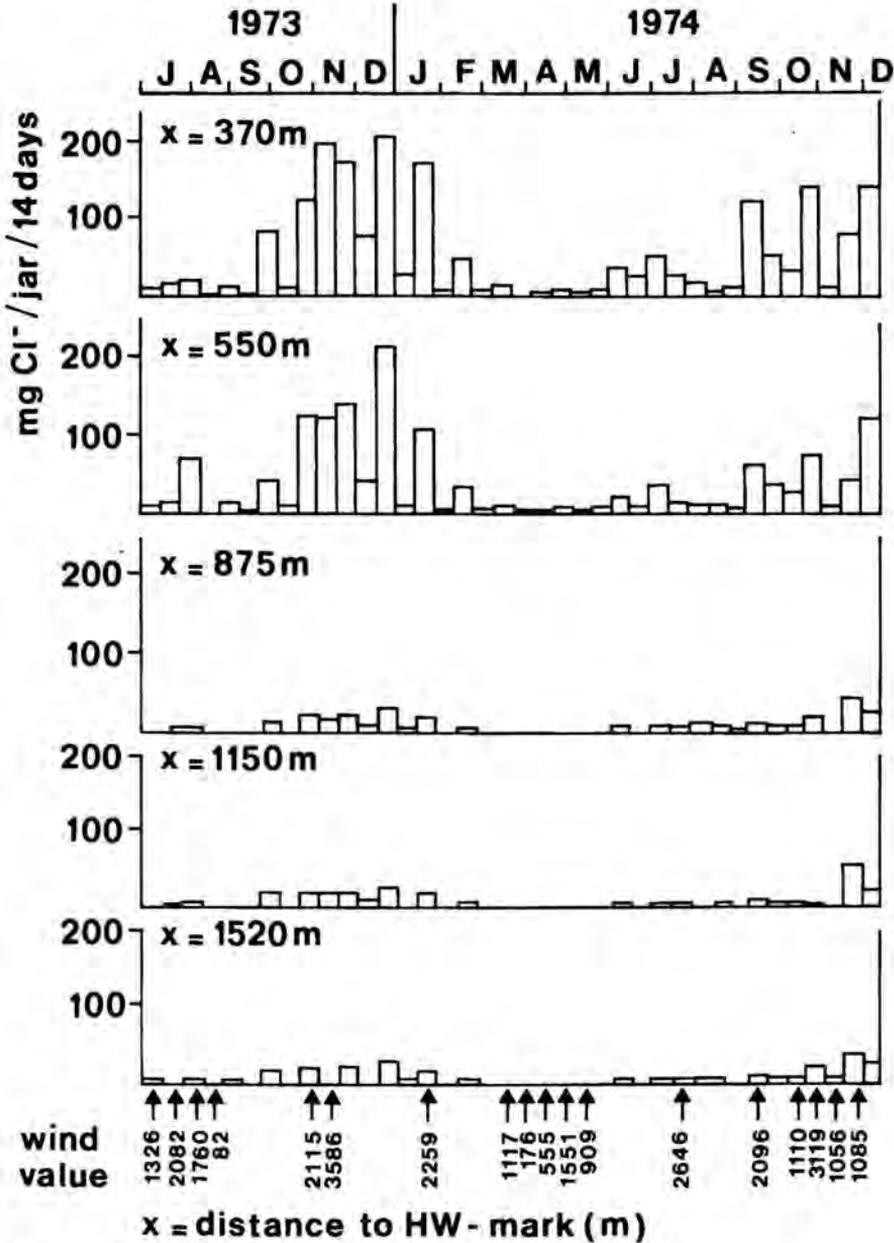


Fig. 16.2. The distribution of windborne sea-salt.

Table 16.1. Relation between salt deposit by air and  $\text{Cl}^-$ -content of the coastal water at different wind-values and distances (0 and 1 km) to the coast line.

Cl <sup>-</sup> -content (g/l) of the coastal water	quantity of sea-salt w = 2000	(mg Cl <sup>-</sup> /pot/14 days)			
		0	1		
S-Voorne	0.3	10	-	25	-
W-Voorne	2	60*	7	150	13
N-Voorne	3	40	15	160	24
N-Goeree	6	60	19	270	40

\* value deviating, point not on the narrow beach but on the first dune-ridge.

1. The  $\text{Cl}^-$ -supply fluctuates in time and is related to wind direction and wind velocity. The highest amount is deposited when the seawinds are strong (w larger than 2000); these occur from mid September up to the end of February and have a SW-, W-, NW- or N-direction.
2. Land inwards the absolute contents and the fluctuations decrease. On the beach the variation is great (8-210 mg  $\text{Cl}^-$ /jar/14 days). Further inland the content decreases gradually, and the maximum values are also lower. The variations of the salt-supply at a certain point are co-determined by geomorphological and vegetative barriers: e.g. at 1520 m, in spite of the greater distance to the coast-line, somewhat higher values occur than at 1150 and 875 m because the situation is higher and the vegetation more open (dune grassland).
3. By far the greatest part of the total salt deposited is within the first 750 m. On the beach (370 m) there is a supply of 1045 mg  $\text{Cl}^-$ /jar/year. This is about 5000 kg NaCl/ha/year.
4. Rare, extremely high salt supplies are very important: a gale with a wind-speed of more than 25 m/s in april 1973 deposited 85 mg  $\text{Cl}^-$ /jar at 1.5 km from the coast-line. This is 50 per cent of the annual supply for a point at this distance from the sea.

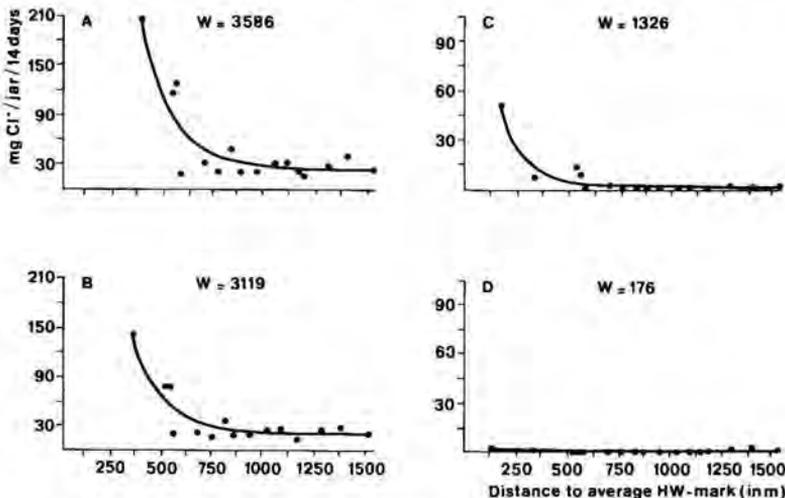


Fig. 16.3. Relation between salt deposition and distance from the sea for the Voorne-N transect at different wind-values.

#### 16.2.4. Distribution of windborne sea-salt in the area

The distribution of sea-salt in the area is determined by a number of processes that remove salts from the atmosphere: 1. removal under the influence of gravity ('fall out'); 2. capture by obstacles (e.g. a first dune ridge); 3. washing out by precipitation ('wash out'); 4. vertical mixing by diffusion (Junge & Gustafson 1957).

By the bursting of air-bubbles in the waves and the splashing drops in over-falling surf-waves the 'salt particles' are released into the air, and partly taken up by the air-current. As soon as this air-current crosses the shore the salt uptake stops abruptly and by subsidence the number of particles decreases land-inward. The larger particles subside first, the smaller ones are transported further inland.

In Fig. 16.3 for N-Voorne the amount of salt deposited is plotted versus the distance to the average HW-line for different wind-values. From this appears:

1. The amount of deposited salt decreases exponentially as the distance from the sea increases. The decline is very sharp in the first hundreds of meters (100-600 m dependent on the wind-value), where as a result of the subsidence of salt-particles  $\frac{1}{2}$  -  $\frac{2}{3}$  of the total air-borne salt is deposited. At about 1 km distance from the HW-line the amount of deposited salt reaches a more or less constant value (the 'basic concentration').
2. At increasing wind-values the amount of salt deposited is greater and the exponential decline sharper. This is probably because at higher wind speeds more larger salt particles are taken up by the air, however, above land these subside more rapidly than the smaller particles. The basic concentration is also increased.
3. At a low wind-value ( $w$  less than 1000) the decline is small. Already within 250 m from the average HW-line a basic level of less than 5 mg  $\text{Cl}^-/\text{jar}/14$  days is reached.
4. The discrepancy of the observed values compared to the calculated regression values is rather high. This is determined largely by differences in altitude and the influence of high vegetation. Geomorphological and vegetative barriers capture more salt and the wind speed also decreases locally.

In Fig. 16.4 the values are plotted for a period of high wind-value for four

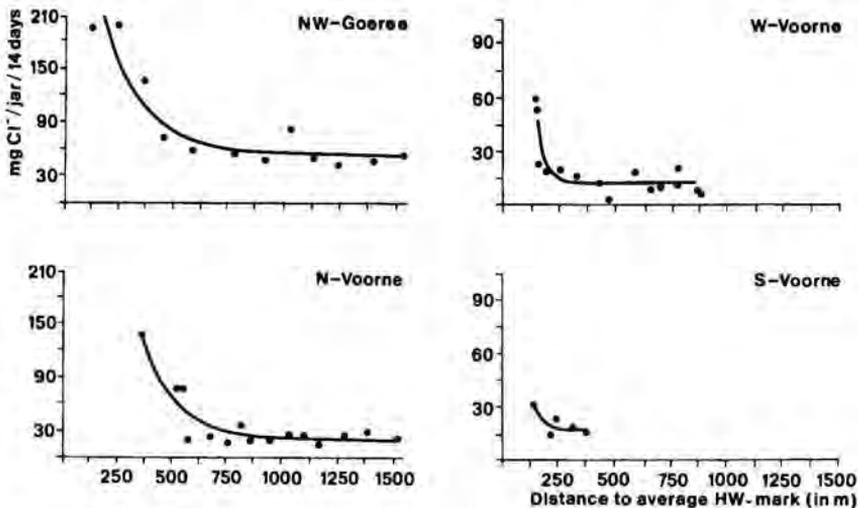


Fig. 16.4. Relation between salt deposition and distance from the sea for periods with a high wind-value in 4 different areas.

different areas. This shows that for all the areas the basic concentrations are different. When the wind-values are low the picture is the same, only the basic levels are much lower and the differences between the areas are smaller.

To acquire a clear insight into the impact of the Maasvlakte on the salt content of the air, the values of N-Voorne and N-Goeree are compared for a number of periods with the same wind-value (low, moderate, high) (Fig. 16.5). At equal wind-values the basic level in NW-Goeree is mostly a factor 2-3 greater than that in N-Voorne (at high wind-value 34 versus 11 mg Cl<sup>-</sup>/pot/14 days). This is probably the result of the salt being deposited on the Maasvlakte ('freshening'), slowing down of the wind above the Maasvlakte, a lower salt content of the coastal water, a smaller wave-height behind the Maasvlakte and Hinderplaat, and more salt being deposited on the relatively higher sea-wall.

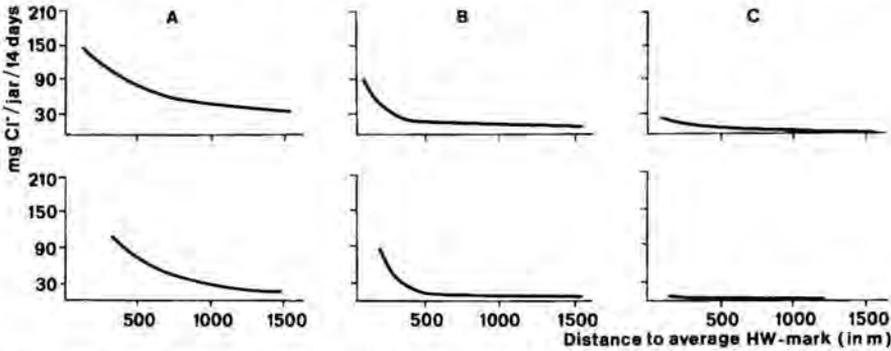


Fig. 16.5. A comparison of the relation between salt deposition and the distance from the sea between the transect Goeree-NW (top) and the transect Voorne-N (bottom) in periods with a high (A), a medium (B) and a low (C) wind-value.

In the Dutch coastal areas the precipitation has a marine origin (Leefflang 1938; Woudenberg 1960; Kooistra 1971). The amount of salts supplied by the precipitation is hard to separate from the amount deposited in dry form. An impression of this can be obtained by comparing the salt supply in 2 jars on the same spot with and without filter-paper. When it is assumed that the quantity of Cl<sup>-</sup> in a jar without filter-paper is zero in the absence of precipitation, it can be estimated that only 10-20 per cent of the total quantity of Cl<sup>-</sup> comes from the precipitation, both at low and high wind-values.

### 16.3. The significance of salt spray as an ecological factor in coastal dune areas

The significance of salt spray as an ecological factor in dune-ecosystems lies in the continuous supply of salts (ions) in the adjacent beach and dune environment (Ranwell 1975). These ions land on the soil-surface or on the vegetation and are transported further by precipitation. In the soil and groundwater the salts are often present in an easily available form to plants. Besides an indirect influence via the soil on plant growth there is also a direct influence by the deposit of salt on the leaves. Notably seedlings can be very sensitive for high salt-concentrations, symptoms of necrosis can occur.

On Voorne there are high Na<sup>+</sup>- and K<sup>+</sup>-contents in the soils of the *Elytrigia junceiformis* dunes and *Ammophila* dunes, that are mainly determined by the supply from the air. The exchangeable K<sup>+</sup>-content hardly decreases in the dry dunes, probably by a combination of a smaller K<sup>+</sup>-supply inland and the increase of the organic matter content with the ageing of the soil. The K<sup>+</sup> supplied from salt spray and released during the decay of organic matter is absorbed by formerly formed humus particles. In the dune slacks the Na<sup>+</sup>-content is relatively

high, also again by adsorptive binding to humus particles. Just as in the dry dunes the  $\text{Na}^+$ -content decreases land inward. In the dune slacks the  $\text{K}^+$ -content increases with the organic matter content and is bound relatively more strongly than the  $\text{Na}^+$ -content by a stronger adsorptive binding. In the dune area of Voorne salt spray is in this way of importance as an ecological factor in dune ecosystems (Adriani & van der Maarel 1968).

Sea-dune species (ecological species-group 3a) (Arnolds & van der Meijden 1975) e.g.: *Cakile maritima* L., *Ammophila arenaria* L., *Elymus arenarius* L. and *Festuca rubra* L. ssp. *arenaria*, and species from fresh/saline contact-environments (ecological species-group 3c) e.g.: *Glaux maritima* L., *Juncus gerardii* L., *Lotus tenuis* L., and *Odontites verna* L. in the Netherlands are bound to the first dune ridge, respectively occur exclusively in the outer dunes and on the higher parts of the salt marsh. In the outer dunes of Voorne the supply of salts via spray is great and that via the ground-water small.

#### 16.4. Salt spray in relation to changes in the coastal area of Voorne

In the past 30 years substantial man-made changes in the coastal area of Voorne and N-Goeree have taken place. In 1950 the Brielse Maas was dammed. The Maasvlakte was constructed in the early sixties. In 1970 the Haringvlietdam was completed. Now a plan has been drawn up to store harbour mud adjacent to the Maasvlakte. These completed and proposed works influence nature: direction and size of coastal currents, sedimentation/erosion, wind and wave-movement, agents which in the past have built up the coastal area of Voorne. The changes have had effects which are important to the salt spray.

Presumably the construction of the Maasvlakte has been an important factor in the decline of the salt-supply by air. Sea-salt will be deposited on the Maasvlakte and the wind speed will decrease. Compared to N-Goeree the salt-supply on N-Voorne is lower when the wind-value is the same (e.g.  $w = 2000$ : 40 resp. 60 mg  $\text{Cl}^-$ /jar/14 days).

Besides the construction of the Maasvlakte, the accretion of the Hinderplaat must also have been of importance as the wave-height has been influenced. In S-Voorne the salt supply from the air is much lower than in the other areas due to the sharp fall of the salt content of the Haringvlietwater and the situation of the transect behind the dam.

On N-Voorne due to the reduction in the supply of salt by air the quantity of salts in the soil of the outer and inner dunes has also been altered. Soil analyses from 1961 up to and including 1979 (data: Van der Laan) reveal that:

1. The  $\text{Cl}^-$ -content of the soil fluctuates strongly as result of the varying deposits of salt, discharge by rainfall and absorption by the vegetation, especially in the upper 10 cm; moreover, the contents in this layer can rise sharply by capillary rise of the soil-water and disiccation in dry periods (seasonal fluctuations).
2. The  $\text{Cl}^-$ -content decreases land inward both in the dry dunes and the dune slacks.
3. In the dune slacks the  $\text{Cl}^-$ -content of the soil seems to fall. Insufficient data are available for the dry dunes.

From ground-water analyses for the period 1966/74 it appears that:

1. Because salt-supply by seepage is not very plausible, the amount of  $\text{Cl}^-$  in the ground-water is determined mainly by the salt supply from the air via salt spray and/or rain-water.
2. The  $\text{Cl}^-$ -content of the ground-water decreases land inward.
3. The  $\text{Cl}^-$ -contents of the ground-water from 1970 onwards are lower than those in the previous periods.

The decline of the supply of salts from the air, which caused a lower salt content in the abiotic environment has possibly affected changes in the vegetation of N-Voorne:

1. Except for a small rise of the cover-percentage of *Juncus gerardii* since

1966/67 with respect to 1964/65 and the temporary appearance of some *Odontites verna*-individuals in 1976/77 (1976; an extremely dry year) the proportion of more or less halophytic plant species in a dune slack (Schaapenwei) practically not influenced by the Maasvlakte), has remained practically unchanged in the period 1964/81, and has certainly not decreased. On a dune slack near the Maasvlakte on N-Voorne (Vliegveld), however, the cover-percentage of these species has fallen sharply, e.g.: *Juncus gerardii*: 4a + 2m, resp. 3a + 2a, *Trifolium fragiferum* L.: 3a + +, *Glaux maritima*: 3a + totally disappeared resp. 4a + r, *Centaureum pulchellum* (G.W.) Druce: 2a resp. + + totally disappeared (unpublished data of Van der Laan).

2. From comparison of the flora-inventarisations of 1979 and 1962/64 (Boeken, Van der Laan & Oremus 1980) it appears that at 22 of 31 sections investigated the number of species and the rareness value has increased. In 9 sections, situated near the Maasvlakte (North-Voorne), these values have fallen. Between 1962 and 1979 on N-Voorne the number of species has decreased by 39 (104 species disappeared, 65 new species); the new species being more common in the Netherlands than the vanished species. In particular within two ecological species groups (3a = sea-dune species, 3c = species of fresh/saline contact-environments) the number of species has diminished.

In the dunes of N-Voorne significant changes have appeared in the flora and vegetation. An increasing recreation intensity would contribute to the changes at some sites. Air-pollution can play a part in the decrease of epiphytic lichens and mosses. Improvement of the sea-wall (first dune ridge) can have contributed to the decline of species on two sites in the first dune ridge. There are possibly small changes in level (and chemical composition) of the ground-water. The most important cause of the changes in the flora and vegetation that have occurred on N-Voorne, sheltered from north-westerly winds is probably the decreased supply of sea-salt from the air owing to the construction of the Maasvlakte and the Brielse Gatdam. Realisation of a planned huge basin for the storage of harbour mud adjacent to the Maasvlakte with a height of 17 m above sea level will further reduce the deposit of sea salt on the coastal dunes of Voorne. The effect on plant species composition and rareness value will be in line with those experienced on North-Voorne after the construction of the Maasvlakte.

A further reduction in plant species number and rareness value for the Voorne coastal dunes is to be foreseen.

#### 16.5. References

- Adriani, M.J. and E. van der Maarel (1968) - Voorne in de branding. Stichting Wetenschappelijk Duinonderzoek. De Volharding. Amsterdam.
- Arnolds, C.J.M. and R. van der Meijden (1975) - Standaardlijst van de Nederlandse flora. Rijksherbarium. Leiden, 1976.
- Boeken, M.M., D. van der Laan and P.A.I. Oremus (1980) - Changes in the flora of the Voorne coastal area. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 75, Progress Report 1979 I.O.O., 28-32.
- Junge, C.E. and P.E. Gustafson (1957) - On the distribution of sea salt over the United States and its removal by precipitation. *Tellus* 9 (2), 164-173.
- Kooistra, M.J. (1971) - De chemische samenstelling van de neerslag op Terschelling in het algemeen en de invloed hiervan op de vegetatie. *Ber. Fys. Geogr. Afd.* 4, 9-15. Geografisch Instituut Rijksuniversiteit Utrecht.
- Leeflang, K.W.H. (1938) - De chemische samenstelling van de neerslag in Nederland. *Chem. Weekbl.* 35, 658-664.
- Ranwell, D.S. (1975) - Ecology of salt marshes and sand dunes. Chapman and Hall. London.

Sloet van Oldruitenborgh, C.J.M. and E. Heeres (1969) - On the contribution of air-borne salt to the gradient character of the Voorne dune area. *Acta Bot. Neerl.* 18 (2), 315-324.

Veelenturf, P.W.M. (1982) - Salt spray. Vakgroep Fys. Geografie. Geografisch Instituut. Rijksuniversiteit Utrecht.

Woudenberg, J.P.M. (1960) - Het chloridegehalte van regenwater. Verslag K.N.M.I. V-67. De Bilt.

## 17. Publications in 1982

### SCIENTIFIC PUBLICATIONS

Akkermans, A.D.L. and C. van Dijk - On leguminous root-nodule symbioses with actinomycetes and *Rhizobium*. In: W.J. Broughton (Ed.) - Nitrogen fixation. Vol. I, Ecology, 57-103.

Balen, J.H. van, C.J.H. Booy, J.A. van Franeker and E.R. Osieck - Studies on hole-nesting birds in natural nest sites. I. Availability and occupation of natural nest sites. *Ardea* 70, 1-24.

Cavé, A.J. - Experiments on the use of the sun by Starlings in the discrimination of geographical locations for navigation. *Ardea* 70, 197-216.

Damme, J.M.M. van and W. van Delden - Gynodioecy in *Plantago lanceolata* L. I. Polymorphism for plasmon type. *Heredity* 49, 303-318.

Hengeveld, R. - Problems of scale in ecological research. Thesis, Leiden University, 132 pp.

Hengeveld, R. and J. Haeck - The distribution of abundance. I. Measurements. *J. of Biogeography* 9, 303-316.

Mook, J.H. and J. van der Toorn - The influence of environmental factors and management on stands of *Phragmites australis*. II. Effects on yield and its relationships with shoot density. *J. Appl. Ecol.* 19, 501-517.

Noë, R. and C.W.P.M. Blom - Occurrence of three *Plantago* species in coastal dune grasslands in relation to pore-volume and organic matter content of the soil. *J. Appl. Ecol.* 19, 177-182.

Oremus, P.A.I. - Growth and nodulation of *Hippophaë rhamnoides* L. in coastal sand dunes of the Netherlands. Thesis, Rijksuniversiteit Utrecht, 116 pp.

Perdeck, A.C. and C. Clason - Flyways of *Anatidae* ringed in the Netherlands. An analysis based on ringing recoveries. Proc. Sec. Techn. Meeting on Western Palearctic Migratory Bird Management, Paris 1979, 65-88.

Toorn, J. van der and J.H. Mook - The influence of environmental factors and management on stand of *Phragmites australis* L. I. Effects of burning, frost and insect damage on shoot density and shoot size. *J. Appl. Ecol.* 19, 477-499.

Toorn, J. van der and H.J. ten Hove - On the ecology of *Cotula coronopifolia* L. and *Ranunculus sceleratus* L. II. Experiments on germination, seed longevity and seedling survival. *Oecol. Plantarum* 3(17), 409-418.

Troelstra, S.R. and F. Berendse - Root CEC determinations to establish root biomasses of two plant species grown in mixtures. *Plant and Soil* 64, 277-281.

**PROGRESS REPORT 1981 (Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 79, 1982)**

Blom, C.W.P.M. and H.A.M. Jongeneel - Further studies on the population ecology of *Plantago maritima*, 41-45.

Freijisen, A.H.J. and H. Otten - The effect of stirring speed on nitrate uptake and growth of *Plantago*, 65-70.

Goede, P. de - The survival of juvenile Great Tits in the first two weeks after fledging, 7-9.

Haeck, J., P.J.M. van der Aart, H. Dorenbosch, E. van der Maarel and O. van Tongeren - The occurrence of *Plantago* species in ordinated Dutch plant communities, 26-33.

Jansen, K. and J. van Groenendael - The effects of density, seed size and time of germination on the yield and size distribution of *Plantago lanceolata* L., 34-41.

Mertens, J.A.L. - Loss of heat at night by Great Tits during egg laying and incubation, 12-17.

Mook, J.H. - The effects of self-thinning in populations of reed shoots (*Phragmites australis*), 70-74.

Noordwijk, A.J. van - Variation in body weight of the Great Tit, heritability and condition, 9-12.

Oremus, P.A.I. - Growth and nodulation of *Hippophaë rhamnoides* L., 74-80.

Perdeck, A.C. and G. Speek - A radar study of the dependence of migration intensity on expected ground speed, cloudiness and temperature, 17-26.

Soekarjo, R. - Effects of low temperature on the germination of seeds of *Plantago major*, 51-55.

Stoutjesdijk, Ph. - Spectral composition of light in grassland, 33-34.

Toorn, J. van der and H.J. ten Hove - Variability of some leaf characters in *Plantago lanceolata*, 45-51.

Troelstra, S.R. - The growth and chemical composition of *Plantago lanceolata* on a  $\text{NO}_3/\text{NH}_4$  nutrient solution; the estimation of nitrate and ammonium utilization from ionic balance aspects, 55-65.

**OTHER PUBLICATIONS**

Aart, P.J.M. van der - De Zeepe-duinen van Schouwen, *Natuurbehoud* 13, 10-12.

Adriani, M.J. - De kustlijn van Zuid Holland. *Zuidhollands Landschap* 2, 4-5.

Adriani, M.J. - Vervuild havenslib, hoe nu verder? *Zuidhollands Landschap* 4, 7-8.

Blom, C.W.P.M. - De relatie tussen oecofysiologie en populatiedichtheid bij planten. *Vakblad voor Biologen* 62, 454-457.

Haeck, J. - Over de mol, over z'n leven, en over de manier om daar een eind aan te maken. *Huid en Haar* 1, 204-211.

Hengeveld, R., H. van Biezen and H. Becker - Aspecten van statistisch gedrag van diversiteitsmaten. *Vakblad voor Biologen* 62, 230-234.

Laan, D. van der - De zeevering van Voorne noordelijk of zuidelijk van het Oostvoornse meer? Een kwestie van fatsoen! *Zuidhollands Landschap* 1, 8-9.

Mabelis, A.A. and H. Turin - De invertebraten fauna van de Zuidlimburgse kalkgraslanden: Beheer. *Nat. Hist. Maandblad* 71, 199-206.

Mook, J.H. - Populatie-oecologisch onderzoek aan riet. Vakblad voor Biologen 62, 400-402.

Mook, J.H. and J. van der Toorn - Pioniersplanten: vegetatie-ontwikkeling in een IJsselmeerpolder. Natuur en Techniek 50, 418-437.

Oremus, P.A.I. - Groei en nodulatie van de Duindoorn. Vakblad voor Biologen 62, 96-99.

Perdeck, A.C. - Met ringen op reis. De ontraadseling van de vogeltrek. Natuur en Techniek 50, 366-389.

Perdeck, A.C. - Zugvögel auf den Spur. Umschau 81, 745-748.

Perdeck, A.C. - Bird-ringing in Europe. Endeavour, New Series 6, 27-33.

Toorn, J. van der - Invloed van beschadigingen op de groei van riet en vegetatie-ontwikkeling in de IJsselmeerpolders. Vakblad voor Biologen 62, 394-397.



# **Limnological Institute**

---

## **Progress Report 1982**

**Edited by S. Parma and R.D. Gulati**



'Vijverhof', Rijksstraatweg 6,  
3631 AC Nieuwersluis, The Netherlands

Tjeukemeer Laboratory, De Akkers 47,  
8536 VD Oosterzee, The Netherlands

## CONTENTS

1. Introduction 4
  - 1.1. History of the Institute 4
  - 1.2. Organization of the Institute 5
  - 1.3. Research programme 7
  - 1.4. Silver Jubilee 7
2. 'Vijverhof' Laboratory 7
  - 2.1. Workgroup 'Primary and Secondary Production' 7
    - 2.1.1. Introduction 7
    - 2.1.2. Aquatic macrophytes 8
    - 2.1.3. Periphyton 11
    - 2.1.4. Zooplankton 14
    - 2.1.5. *Chaoborus flavicans* 16
    - 2.1.6. Biological water quality assessment of 'boezem' waters and polder waters 18
  - 2.2. Workgroup 'Mineralization of Organic Matter' 18
    - 2.2.1. Introduction 18
    - 2.2.2. Aerobic mineralization 20
    - 2.2.3. Nitrogen cycle 22
    - 2.2.4. Anaerobic mineralization and production 23
    - 2.2.5. Sedimentary flux of major phytoplankton groups 25
  - 2.3. Project 'Carbon Cycle in Lake Vechten': studies relating to the transport of particulate material 28
    - 2.3.1. Introduction 28
    - 2.3.2. Seston, epipelon and sedimentation 29
    - 2.3.3. Phytoplankton periodicity and sediment trap recoveries 33
    - 2.3.4. Spatial distribution of epipelon during summer stratification 35
  - 2.4. The Loosdrecht Lakes Restoration Project 36
    - 2.4.1. Introduction 36
    - 2.4.2. Hydrology 37
    - 2.4.3. Exchange of phosphorus compounds between sediment and water 39
    - 2.4.4. Phytoplankton pigment composition 43
    - 2.4.5. Phytoplankton species composition 44
    - 2.4.6. Primary production of phytoplankton 45
    - 2.4.7. Macrophytes 47
    - 2.4.8. Seston - epipelon interrelationships 48
    - 2.4.9. Zooplankton grazing 49
  - 2.5. Project 'Polder Research' 52
3. Tjeukemeer Laboratory 54
  - 3.1. General Introduction 54
  - 3.2. Workgroup 'Algology' 54
    - 3.2.1. Introduction 54
    - 3.2.2. Phytoplankton dynamics in the Tjeukemeer (Project A 1) 55
    - 3.2.3. Nutrient dynamics in the Tjeukemeer (Project A 2) 55
    - 3.2.4. Monitoring physical factors (Project A 3) 59
    - 3.2.5. Availability of nutrients (Project A 6) 59
    - 3.2.6. Bioassay experiments (Project A 7) 63

- 3.2.7. *Laboratory model of algal periodicity in the Tjeukemeer*  
(Project A 8) 64
- 3.3. *Workgroup 'Foodchain and Production Studies'* 65
  - 3.3.1. *Introduction* 65
  - 3.3.2. *A discrete event-oriented simulation model of the subsystem 'fish and its food organisms' in the Tjeukemeer* (Project V 1) 65
  - 3.3.3. *Population densities, population structure and biomass of copepods and herbivorous cladocerans in the Tjeukemeer* (Project V 2) 66
  - 3.3.4. *Autecology of *Leptodora kindtii* in the Tjeukemeer* (Project V 3) 67
  - 3.3.5. *Autecology of *Neomysis integer** (Project V 4) 67
  - 3.3.6. *Population dynamics and production of chironomid larvae* (Project V 5) 67
  - 3.3.7. *Ecology of smelt (*Osmerus eperlanus*), perch (*Perca fluviatilis*) and pikeperch (*Stizostedion lucioperca*) larvae* (Project V 6) 67
  - 3.3.8. *Ecology of 0<sup>+</sup> fish in the Tjeukemeer* (Project V 7) 67
  - 3.3.9. *Ecology of I<sup>+</sup> and older bream (*Abramis brama*) in the Tjeukemeer* (Project V 8) 68
  - 3.3.10. *Ecology of I<sup>+</sup> and older bream (*Abramis brama*) in the Langweerder Wielen* (Project V 9) 68
  - 3.3.11. *Ecology of I<sup>+</sup> and older white bream (*Blicca björkna*) and roach (*Rutilus rutilus*) in the Tjeukemeer* (Project V 10) 68
  - 3.3.12. *Ecology of the eel (*Anguilla anguilla*) in the Tjeukemeer* (Project V 11) 69
  - 3.3.13. *Population structure, growth and feeding of I<sup>+</sup> and older pikeperch (*Stizostedion lucioperca*) and perch (*Perca fluviatilis*) in the Tjeukemeer* (Project V 12) 70
  - 3.3.14. *Experimental feeding of eel (*Anguilla anguilla*) and bream (*Abramis brama*)* (Project V 13) 70
  - 3.3.15. *Ecology of 0<sup>+</sup> fish in the Frisian lakes* (Project V 14) 70
  - 3.3.16. *Effects of the Bergum Power Station on zooplankton and 0<sup>+</sup> fish in the Bergumermeer* (Project V 15) 70
  - 3.3.17. *The effects of experimental removal of bream in the Morra* (Project V 16) 70
  - 3.3.18. *Distribution of cyprinids in the Tjeukemeer* (Project V 17) 71
- 4. *Publications* 71
  - 4.1. *Papers published in 1982* 71
  - 4.2. *Papers in press* 74
  - 4.3. *Internal Reports* 75
  - 4.4. *Student and Trainee Reports* 75
- 5. *Acknowledgements* 76

## 1. Introduction

### 1.1. HISTORY OF THE INSTITUTE

The concerted effort of the Division of Natural Sciences of the Royal Netherlands Academy of Arts and Sciences and the Ministry of Science and Education to strengthen the position of ecological research in the Netherlands resulted in the establishment of three institutes: two involved in aquatic ecology, namely, the 'Hydrobiological Institute' at Nieuwersluis and the 'Division Delta-Research of the Hydrobiological Institute' at Yerseke and of the 'Institute for Ecological Investigation' at Arnhem, concentrating on problems of a terrestrial ecological character. The 'Hydrobiological Institute' founded in 1957 filled a gap that existed in biological and chemical research of the abundantly present freshwater systems in the Netherlands. Due to the separate development of the two institutes



The Loosdrecht Lakes. On the foreground a pattern of alternating banks and ditches. See for detailed description section 2.4.1. (Photo Studio Koppelman, Maarssen.)

for hydrobiological research their names were changed in 1968 to: 'Limnological Institute' and 'Delta Institute for Hydrobiological Research', respectively.

The Limnological Institute has two departments (Fig. 1), viz. 1) The 'Vijverhof' Laboratory at Nieuwersluis (Utrecht), at the original site of the Institute; 2) the Tjeukemeer Laboratory at Oosterzee (Friesland), started in 1966 as a field station of the International Biological Programme but gradually transformed into a well-equipped department now accommodated in a laboratory built in 1976.

Dr. Miss M.F.E. Nicolai (1957-1960) was the first director of the Institute. After her death Dr. H.L. Golterman succeeded her and was in 1972 joined by Dr. R. Soekarjo. Both directors resigned in 1978 in which year Dr. S. Parma took over as director.

## 1.2. ORGANIZATION OF THE INSTITUTE

The Institute is financed primarily by the Ministry of Science and Education by means of funds allotted to the Royal Netherlands Academy of Arts and Sciences.

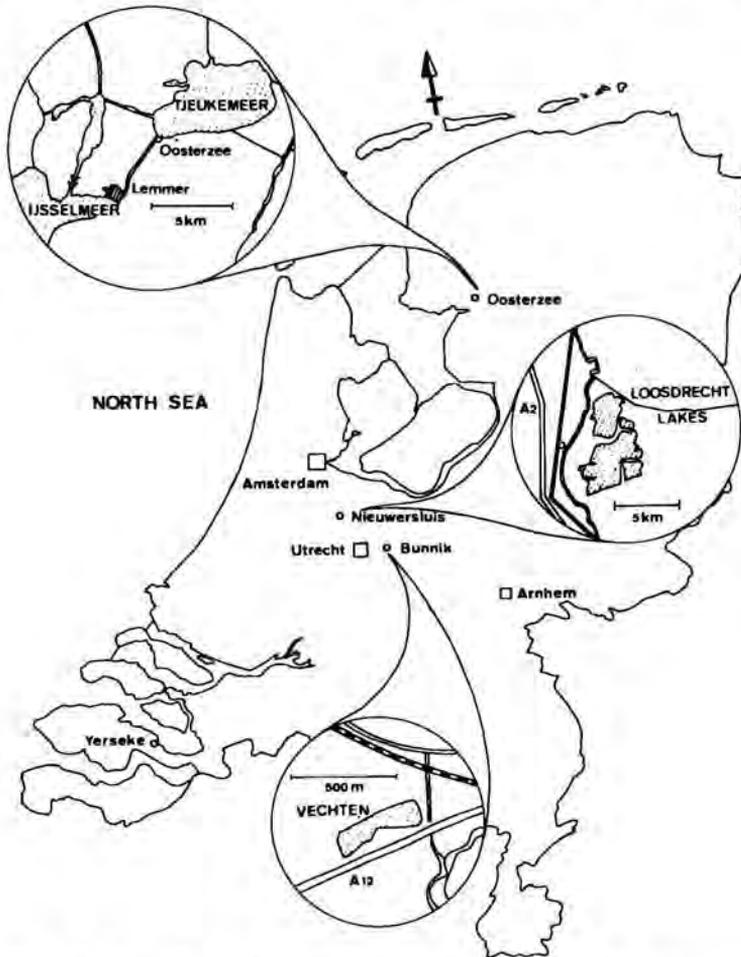


Fig. 1. Map of the Netherlands showing the study areas of the Limnological Institute, Lake Vechten and Loosdrecht Lakes research at 'Vijverhof' Laboratory, Nieuwersluis. Tjeukemeer research at Tjeukemeer Laboratory, Oosterzee.

Besides, research is done on a contract base, financed by other ministries and organizations.

The permanent strength of the Institute in 1982 was 41 full time places (45 persons). Twenty seven persons were working at the 'Vijverhof' Laboratory at Nieuwersluis and 18 at the Tjeukemeer Laboratory at Oosterzee, including staff members at both the laboratories. In addition, 14 guest-workers were financed via the Ministry of Housing, Physical Planning and the Environment, the Ministry of Economic Affairs, the STORA, the LaSOM, ZWO and the Beijerinck-Pop-

Table 1. Research scheme in 1982; g = guest worker, s = student or trainee

#### 'VIJVERHOF' LABORATORY, NIEUWERSLUIJ

##### *Workgroup 'Primary and Secondary Production'*

Dr. R.D. Gulati (leader)	R. van Keulen	Dr. P. Pomogyi (g)
Dr. E.P.H. Best	G. Postema	T. Burger-Wiersma (s)
Dr. H.J. Gons	K. Siewertsen	M. Hokken (s)
Drs. W.A. de Kloet	P.J. Boesewinkel-de	J. Heringa (s)
Drs. B.Z. Salomé	Bruyn (g)	P. Heutz (s)
Drs. J.E. Weekenstroo	Drs. P.J. den Oude (g)	J.C. Kromkamp (s)
J.H.A. Dassen	Drs. J.T. Meulemans (g)	H. Scholten (s)

##### *Workgroup 'Mineralization of Organic Matter'*

Dr. Th.E. Cappenberg (leader)	M.J. Bär-Gilissen	Drs. B.F.M. Kal (g)
Drs. J.J. Olie	E.M.J. Dekkers	M.J. van Dam (s)
Dr. C.L.M. Steenbergen	C.A. Hordijk	C.P.C.M. Hagens (s)
Drs. H. Verdouw	H.J. Korthals	J.T.G. Mathijssen (s)
Ing. A.G. Wisselo	Drs. P.C.M. Boers (g)	C. de Vlieger (s)
	Drs. J.W.Th. Bongers (g)	M.J. Westhoff (s)

##### *Project 'Polder Research'*

Dr. S. Parma (leader)	Ir. R. Veeningen (g)
-----------------------	----------------------

#### TJEUKEMEER LABORATORY, OOSTERZEE

##### *Workgroup 'Algology'*

Dr. J.R. Moed (leader)	J. Voerman	G. Werlemark (g)
Dr. H. de Haan	Ing. G.J. Schrottenboer	J. Bakker (s)
Th. de Boer	(g)	J. Bloem (s)
H.L. Hoogveld	G. Semplonius (g)	J.K. van Hoewijk (s)
H.A. Kramer	Ing. P.J. Timmer (g)	P.J. Waterlander (s)
	Drs. J.F. van Weerden (g)	

##### *Workgroup 'Foodchain and Production Studies'*

Dr. J. Vijverberg (leader)	S.J. Swart	A. Kamstra (s)
Drs. W.L.T. van Densen	S. Barelds (g)	F.L. van der Lugt (s)
Drs. E.H.R.R. Lammens	Drs. A.F. Richter (g)	M.A. van der Meer (s)
Drs. H.W. de Nie	Drs. G.A.A. Schoon (g)	J.H.A. Mol (s)
N. van Benthem	A. de Bakker (s)	W. Rotman (s)
Th.H. Frank	M.A. Faasse (s)	G.B. Schuilenburg (s)
A.G. Frank-Landman	E.M. Foekema (s)	R. Verbrugge (s)
P.J. Mac Gillavry	J. Geursen (s)	J.G.M. van der Wijst (s)
L. Lemsma	A. Heinen (s)	B. Zech (s)

ping Fund. Also 29 students took part in the scientific programme in 1982 (Table 1). The general service departments covering the administration, library, photography and ships and workshop consisted of 13 members.

### 1.3. RESEARCH PROGRAMME

The research programme at the 'Vijverhof' Laboratory is split up into two workgroups, viz. 'Primary and Secondary Production' (Section 2.1) and 'Mineralization of Organic Matter' (Section 2.2). For more than 20 years the main research efforts were focussed on the ecology of the stratified Lake Vechten, but recently studies in the shallow hypertrophic Loosdrecht Lakes were strongly intensified. Besides their own research topics the two workgroups cooperate in two projects in these lake systems, namely 'Carbon cycle in Lake Vechten' (Section 2.3) and a joint research project of eight institutes 'The Loosdrecht Lakes Restoration Project' for which E. van Liere was recently appointed as research coordinator (Section 2.4).

A third more or less independent research project, 'Oxygen budgets of ditches', is financed by the Ministry of Public Health and the Environment. It should be a basis for the management of polder waters - typical Dutch freshwater systems (Section 2.5).

The Tjeukemeer Laboratory also has two workgroups, viz. 'Algology' (Section 3.2) and 'Food Chain and Production Studies' (Section 3.3). Both groups are working mainly on the Tjeukemeer, but also other Frisian lakes are included in their research programmes.

The work of the Institute is based on five-year plans of studies which are carried out after approval of the Board of Trustees installed by the Royal Netherlands Academy of Arts and Sciences.

The Institute organizes training programmes for students from the Dutch universities. Training, research and boarding facilities are also offered to students and scientists from abroad.

### 1.4. SILVER JUBILEE

In 1982 the Institute celebrated her 25th anniversary. The most festive way of commemorating this event seemed to us the compilation of our more recent works in a special volume of *Hydrobiologia*: 'Studies on Lake Vechten and Tjeukemeer, The Netherlands' (Developments in Hydrobiology 11, reprinted from *Hydrobiologia*, vol. 95, 1982).

In honour of the 25th anniversary of both the Limnological Institute and the Delta Institute of Hydrobiological Research, the Biological Council organized a national symposium on aquatic ecosystem research. Our Institute presented a retrospection of our recent investigations on the carbon budget studies in Lake Vechten compared with other lakes and of the fish populations in relation to their food organisms in the Tjeukemeer.

## 2. 'Vijverhof' Laboratory

### 2.1. WORKGROUP 'PRIMARY AND SECONDARY PRODUCTION'

#### 2.1.1. Introduction (R.D. Gulati)

The main objective of the workgroup is to carry out research on production and metabolic processes of plants and animals in freshwater lakes of different trophic level, particularly in regard to changes accompanying eutrophication. The approach of the research is both a fundamental and a holistic one with emphasis on the ecosystem. Some studies are being carried out in close cooperation with the workgroup Mineralization of Organic Matter (Section 2.2).

The main aspects of studies being actively pursued are:

- distribution, composition, abundance and production metabolism of macrophytes, periphyton and epipelon in the littoral region of lakes;
- phytoplankton composition, abundance and production in the limnetic region of lakes;
- phytoplankton (seston) - zooplankton interrelationships, particularly those concerning herbivore-zooplankton grazing and role of zooplankton excretion products in stimulating phytoplankton production; and
- role of *Chaoborus flavicans* in the food chain.

The workgroup was engaged in three projects during 1982.

- Lake Vechten ecosystem research;
- Project Carbon Cycle (Section 2.3);
- The Loosdrecht Lakes Restoration Project (Section 2.4).

P.J. den Oude (Section 2.1.6) joined the workgroup for two years starting September 1982. The main aim of his study is to explore the possibility of employing ecological parameters to define water quality. For this den Oude will do an extensive literature research for a critical evaluation of the existing limnological data, both published and unpublished, in the Netherlands.

**2.1.2. Aquatic macrophytes** (E.P.H. Best, J.H.A. Dassen, Limnological Institute; P. Pomogyi, Kesztey, Hungary; J.J. Boon, FOM-Institute, Amsterdam; and J.T. Meulemans, Department of Aquatic Ecology, Amsterdam and Limnological Institute)

In Lake Vechten the number of plant species and the distribution of the main vegetation types and their biomass are being monitored since 1972 to quantify the changes in species composition and biomass, and the role of plants in the nutrient cycles of the lake.

For *Ceratophyllum demersum*, a predominant submerged species, a growth model is being developed (Progress Report, 1981), based mainly on  $^{14}\text{C}$ -assimilation as a parameter for photosynthesis. Respiration was calculated tentatively from the chemical composition of the plant material on a daily basis; since direct measurements give more insight into daily changes Infrared Gas Analysis is being attempted.

**Biochemical aspects of photosynthesis in submerged aquatics** (E.P.H. Best)

In shallow waters under circumstances of temporarily high light intensity, high temperature and high oxygen concentration submerged aquatics may switch rapidly from the  $\text{C}_3$ -carbon to the  $\text{C}_4$ -carbon fixation pathway and back to  $\text{C}_3$  under  $\text{C}_3$ -favourable environmental conditions. For *Elodea* sp. it was demonstrated earlier that although this species possesses several  $\text{C}_4$ -decarboxylating enzymes in sufficient quantities to sustain  $\text{C}_4$ -activity, the typical  $\text{C}_4$ -enzyme pyruvate-Pi-dikinase was absent. Induction of  $\text{C}_4$ -activity in *Ceratophyllum* was attempted under carbon limitation. For this, the  $^{14}\text{C}$ -distribution was compared in the early photosynthetic products formed both in medium to which bicarbonate was added ( $40 \text{ mg C l}^{-1}$ ) and to which it was not added; the labelling was done at pH 8.1 an hour after acclimation. The bicarbonate-poor condition is supposed to mimic the condition in unstirred layers around the plant leaves. If  $\text{C}_4$ -activity occurs the  $\text{C}_4$ -carboxylic acids aspartic acid and malic acid are expected to increase rapidly. The photosynthetic products were extracted with 80% boiling ethanol and water, and separated using chromatography following M.D. Hatch and co-workers (H.S. Johnson & M.D. Hatch, 1969, The  $\text{C}_4$ -dicarboxylic acid pathway of photosynthesis; identification of intermediates and products and quantitative evidence for the route of carbon flow; *Biochem. J.* 144: 127-134). The four preliminary runs are discussed here (Fig. 2). The aspartic acid and glucose/fructose could not be separated, but malic acid could be.

Although levels of malate detected were high in both plants grown in bicarbonate-rich and bicarbonate-poor water, they did not increase in time. The results con-

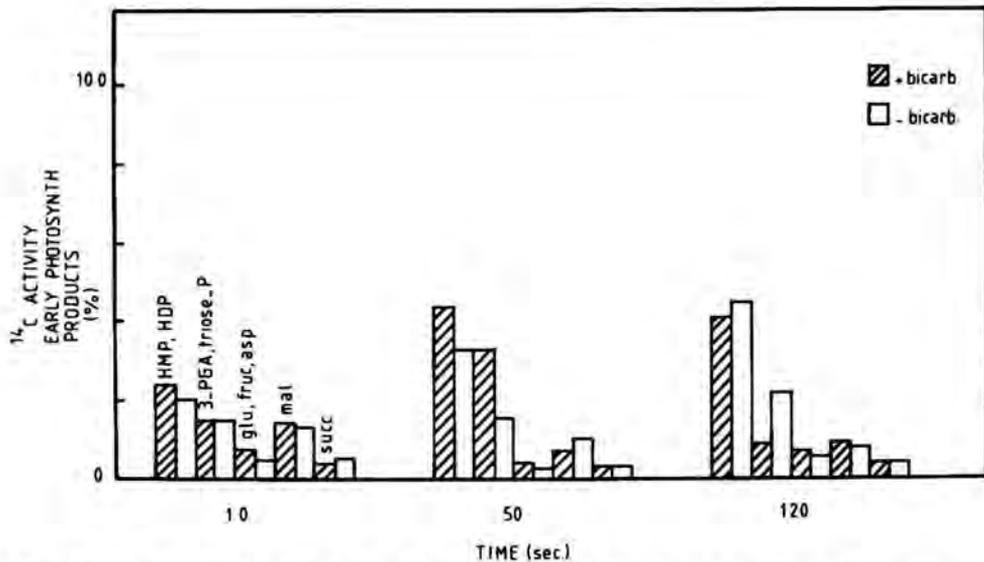


Fig. 2. The  $^{14}\text{C}$ -distribution in the early photosynthetic products of *Ceratophyllum* plants immersed for different periods in M-medium modified with  $^{14}\text{C}$ -labelled bicarbonate. Chromatograms were developed with isobutanol-formic acid-water (306 : 50 : 100) for 20 h and ethylacetate-pyridine-water (400 : 50 : 100) for 16-18 h. The radioactivity was located and quantified with a thin-layer chromatogram scanner (Berthold). Recovery varied from 63 to 77%. asp., aspartic acid; fruc, fructose; glu, glucose; HDP, hexose diphosphate; HMP, hexose monophosphate, mal, malic acid; PGA, phosphoglyceric acid; succ., succinic acid.

firm the earlier data that *Ceratophyllum* despite high levels of malate does not exhibit other  $\text{C}_4$ -characteristics. On the other hand, one-hour acclimation may be too short to induce  $\text{C}_4$ -photosynthesis. The ecological relevance of  $\text{C}_4$ -photosynthesis induced only after longer acclimation periods, would be very small.

*The relation between age and nutrient release from Ceratophyllum demersum* (P. Pomogyi, Kesztyel, Hungary; E.P.H. Best, J.H.A. Dassen, Limnological Institute; and J.J. Boon, FOM-Institute, Amsterdam)

Decay of aquatic plants in autumn is generally considered a substantial nutrient source in a lake. However, the growth period of plants passes gradually into the decay period of nutrient leaching. The extent and quality of nutrients leached, and their role in the ecosystems are largely unknown. Therefore, the relation between age and leakage of nutrients (C, N, P) from *Ceratophyllum* was studied. The plants were cultured in the open air for 163 days. The nutrient leakage was compared in living and previously killed plant material after incubation of the plants with their tightly adhering periphyton for 72 hours under aerobic conditions. Nutrient leakage from living and dead plant material differed in several respects. Phosphorus, and to less extent nitrogen, leaked faster from the dead than from the living plants (range 9.1-80.8% initial plant-P, range 4.1-32.1% initial plant-N); leakage of carbon was almost equal in both cases (range 1.8-15.8% initial plant-C). The nutrients were released in the order  $\text{P} > \text{N} > \text{C}$ .

Pyrolysis mass spectrometry showed that in the course of the developmental cycle main changes in the chemical plant composition concern the plant's carbohydrates; this supports our earlier data on the seasonal changes in carbohydrates

(starch, glucose, fructose, sucrose, stachyose). Mass spectra of living and dead plant material differed mainly in the loss of nitrogen-containing compounds from the dead plant material (Fig. 3). The effect of the age dependent, continuous leaching of nutrients from the submerged macrophytes on the nutrient recycling in e.g. Lake Vechten has still to be evaluated.

*Seasonal changes in biomass and production of the emergent macrophytes and their periphyton in Lake Maarsseveen (J.T. Meulemans)*

This study is supported by a three year grant from the Foundation for Biological Research in the Netherlands (BION), a subsidiary of the Netherlands Organization for the Advancement of Pure Research (ZWO). It is a cooperative project of the Department of Aquatic Ecology, University of Amsterdam and the Limnological Institute.

Its aim is to quantify the role of the reed-periphyton community in the C, N and P cycles of Lake Maarsseveen and to study the interrelationships between both components of the community. The study concerns the biomass, production and mineralization aspects of both reed and its periphyton.

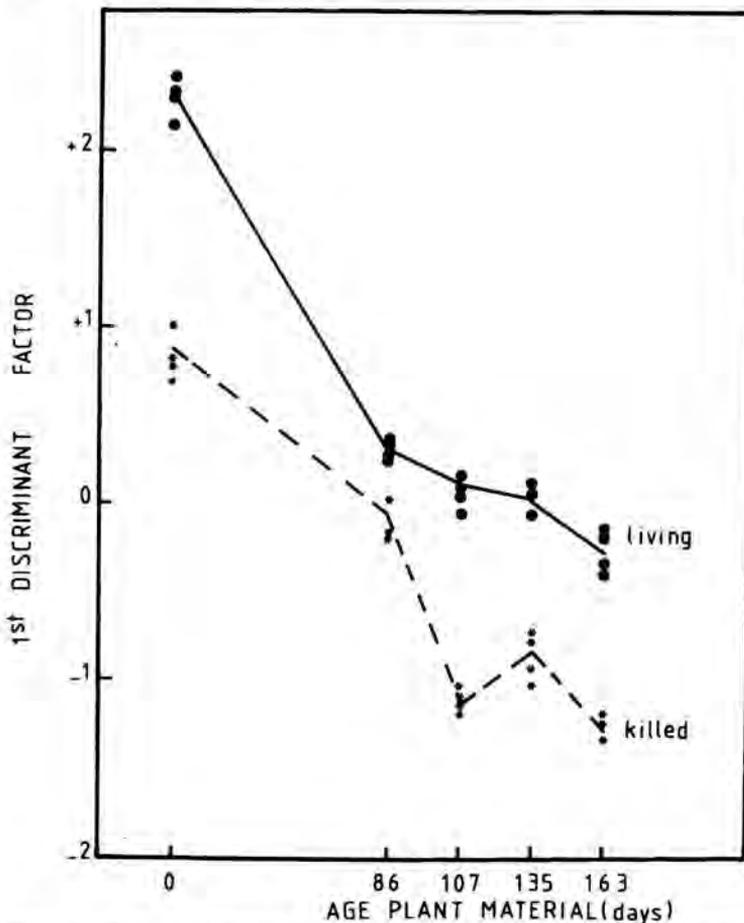


Fig. 3. Discriminant analysis of pyrolysis mass spectrometry spectra of living and dead *Ceratophyllum* plant material. Values of +2 indicate protein richness, and -2 polysaccharide richness.

The seasonal changes in the periphyton biomass adhering to dead reed stems were compared at the shore-side, the center and the lake-side of a reed-belt. The biomass values measured in winter and spring were high, but decreased in summer (maximum and minimum values being  $49.52 \pm 5.61$  and  $5.22 \pm 2.88$  g ash-free dry weight  $m^{-2}$  substratum respectively). The biomass at the shore-side of the reed-belt decreased further to  $0.54 \pm 0.26$  g ash-free dry weight  $m^{-2}$  substratum in November and December.

In winter the periphyton is dominated by diatoms (*Diatoma*, *Fragilaria* and *Ta-bellaria* species). In summer these genera are replaced by *Achnanthes*, *Gompho-nema* and *Synedra* species. From pigment-analysis the share of diatoms, green and red algae to the periphyton could be measured. Chlorophyll-*a* and pigments specific for diatoms (fucoxanthin) and green algae (chlorophyll-*b*) were separated using thin layer chromatography. From the ratios fucoxanthin/chlorophyll-*a* and chlorophyll-*b*/chlorophyll-*a* the percentages chlorophyll-*a* derived from diatoms, green and red algae were calculated. At the SW-side of the lake the peri-phyton mainly consisted of diatoms, especially at the shore-side and the center of the reed-belt. At the NE-side, however, more green algae occurred.

Incubators were developed and tested for measuring the *in situ* production of periphyton (oxygen exchange method).

The response of the carbon fixation rate to light was studied in the laboratory ( $^{14}C$ -method). Periphyton was carefully scraped from the reed stems and diluted with filtered lake water. Bottles were filled with 100 ml of the diluted periphyton and incubated with  $NaH^{14}CO_3$  ( $0.02 \mu Ci^{14}C ml^{-1}$ ) for 3 hours at light intensities ranging from 0 to  $1500 \mu E m^{-2} \cdot s^{-1}$ . In summer inhibition of the C-fixation rate under high light intensity was lower than found in spring and late summer. In the latter period periphyton was adapted to low light intensity (strong attenuation of light under a well-developed leaf canopy). The 'shade-adapted' periphyton showing almost no photo-inhibition in summer under high light intensities may be limited by factors other than light, i.e. nutrients. These experiments will be continued in 1983.

### 2.1.3. Periphyton (H.J. Gons, R. van Keulen, T. Burger-Wiersma, J.C. Kromkamp)

Studies on epipelton in Lake Vechten were carried out in cooperation with the workgroup 'Mineralization of Organic Matter' (Section 2.3).

#### Comparison of epipelton in Lake Vechten and Lake Maarsseveen I (J.C. Kromkamp)

Lake Vechten (area, 4.7 ha; maximum depth, 12 m) and Lake Maarsseveen I (70 ha; 32 m) have simple morphometry and are characterized as meso-eutrophic and mesotrophic, respectively. In both lakes epipelton was collected quantitatively from the bottom between the zone of the submerged macrophytes and the thermocline depth. The temporal and spatial distributions of the epipelton in the lakes were similar, biomass increasing markedly with depth, and exhibiting a pronounced peak during the mid-summer temperature maximum (Fig. 4). The mass of epipelton in Lake Maarsseveen was considerably higher than that in Lake Vechten, both on total dry weight and on organic weight basis; also, the epipelton in the former lake had a higher chlorophyll-*a* concentration. However, areal oxygen uptake in the lakes was comparable. It is not yet established if the differences between the two lakes are due mainly to those in limnetic production or to those in morphometry such as the depth of water column and slope length.

#### Ecology of *Nitzschia palea* (T. Burger-Wiersma)

Maximum densities of *Nitzschia palea* in batch cultures varied between  $6 \times 10^6$  and  $2 \times 10^9$  cells  $l^{-1}$ , and were independent of light intensity. The variations were large, possibly because the cultures were only partly synchronized. Thus,

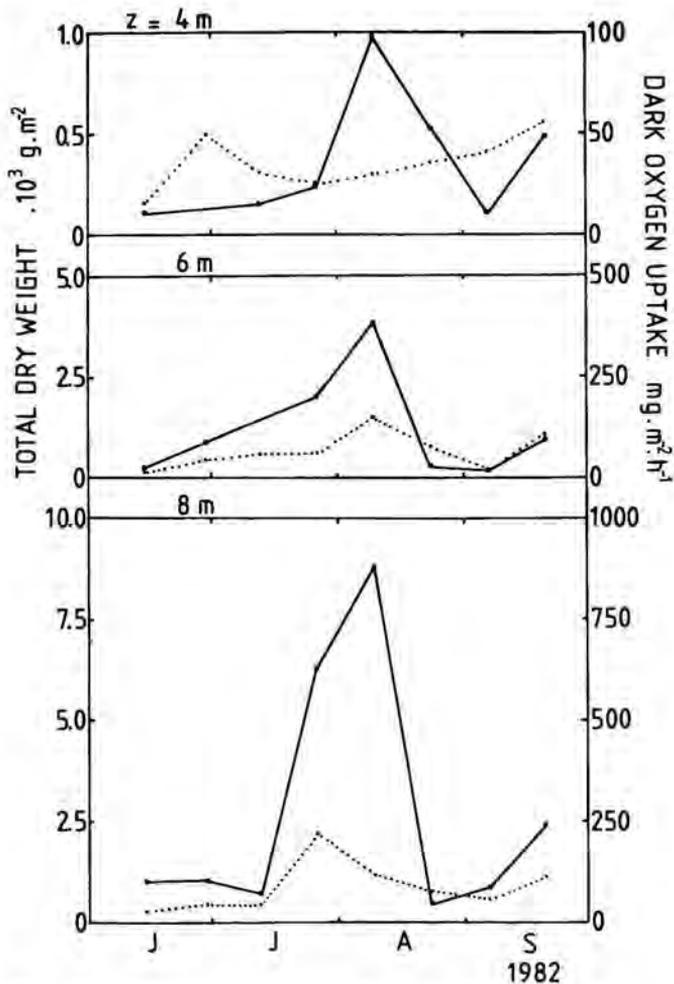


Fig. 4. Distribution of epipelagic total dry weight (solid lines) and dark oxygen uptake (dotted lines) in Lake Maarsseveen I.

the increase in growth rate during the exponential phase could be attributed mainly to an increase in the number of cell divisions in the first part of the light period.

The relatively low maximum density in batch cultures may be due to production of an auto-inhibitor since enrichment of the culture medium with extra nutrients did not raise the cell density, nor did the addition of chelating substances or vitamins produce any effect.

A linear, inverse relationship was found between the concentration of inhibitor and the maximum cell density (Fig. 5). The production of auto-inhibitor occurred during the growth phase of the algae but stopped when the stationary phase was reached. So, prolonged growth in the stationary phase did not affect the maximum cell density after the next inoculation. It did, however, change the growth pattern. Cells from the early stationary phase grew quicker and showed a shorter lag phase than those from the late stationary phase which had a longer lag phase and a lower maximum growth rate. The presence of auto-inhibitor, in about 10% of the maximum concentration, also reduced the maximum growth rate.

The maximum growth rates, 0.47, 0.58 and 0.63 d<sup>-1</sup>, at light intensities of 7, 25 en 100 W.m<sup>-2</sup>, respectively, did not differ significantly.

Nitrate concentrations increased with aging of the cultures. The concentrations of up to 0.3 mg.l<sup>-1</sup> NO<sub>2</sub>-N in the bioassays did not inhibit the growth of *N. palea*. The nitrite was, therefore, not considered to be a possible auto-inhibitor. Moreover, nitrite production did not stop in the stationary phase, and depended on light intensity.

The inhibitor does not appear to influence the photosynthetic capacity. Cells suspended in freshly-prepared medium showed no photosynthesis during the first 10 to 30 minutes. In comparable suspensions with inhibitor the photosynthesis started directly after exposure. Also in diluted suspensions without inhibitor, the photosynthesis began at the start of the experiment; the photosynthetic capacity was nine-fold that in the concentrated suspensions.

The role of changes in irradiation in producing the effects found in the photosynthesis experiments was not examined. The adaptation of cells to changes could be predicted from the combined results of adaptation effects found with growth experiments and of a photosynthesis-light curve, obtained earlier. Probably the enzymatic processes adapt more quickly to changing light conditions than the photochemical processes.

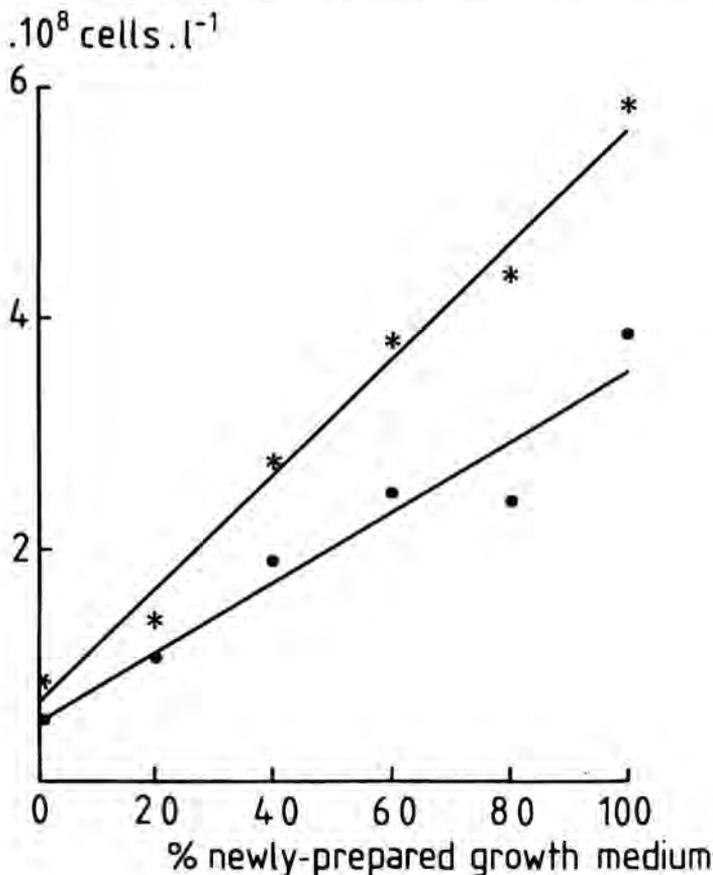


Fig. 5. Relationship between inhibitor concentration and maximum population density of *Nitzschia palea* grown in Erlenmeyer flasks (asterisks) and in infusion bottles (dots).

Addition of organic substrate to an inhibited non-axenic bath-culture, incubated at  $7 \text{ W.m}^{-2}$ , did not result in renewed growth of *N. palea*. The bacteria in the culture showed a higher affinity for the substrate compared with the algae and within 24 hours probably all of the organic substrate was consumed.

Attempts to obtain an axenic culture of *N. palea* failed, possibly because an old and thus inactive stock of antibiotics was used.

*N. palea* was able to grow under anaerobic conditions at a light intensity as low as  $0.9 \text{ W.m}^{-2}$ , reaching maximum densities of  $3 \times 10^8 \text{ cells l}^{-1}$ , which value is one-third of the one commonly found. No growth was found in the dark whether or not organic substrate was present. In low light, addition of acetate, glucose or yeast extract inhibited growth under anaerobic conditions, but lactate and casein seemed to stimulate growth.

#### 2.1.4. Zooplankton (R.D. Gulati, K. Siewertsen, G. Postema, J. Heringa)

##### *Phytoplankton-zooplankton interactions* (R.D. Gulati, K. Siewertsen, G. Postema)

The influence of zooplankton excretion products, particularly the nutrients  $\text{NH}_4 - \text{N}$  and  $\text{PO}_4 - \text{P}$ , on phytoplankton primary production, namely the carbon assimilation, was studied in laboratory bioassays. The production rates were measured in controls, and compared with those in a) the Lake Vechten water enriched with zooplankton excretion products and b) in lake water to which P and N were added in concentrations comparable with those of the enrichments named under a). The composition of water in the control and experimental vessels was as follows: 1) controls, zooplankton-free ( $150 \mu\text{m}$ ) lake water and membrane-filtered lake water ( $0.45 \mu\text{m}$ ) in ratios varying from 9:1 to 4:1; 2) excretion-enriched, zooplankton-free lake water and water containing the zooplankton excretion products in the same ratios as under 1); and 3) P and N enriched, zooplankton-free lake water and membrane-filtered lake water containing P and N in the same ratios as under 1). The P and N concentrations in the excretion enriched and in P and N enriched waters were the same.

In the period April-August 10 experiments were done followed by one each in October and November. But for the last two months the water temperature prevalent in the lake was used. Both during the acclimation to nutrient enrichment and the subsequent primary production measurements a constant light intensity of  $20 - 30 \text{ W.m}^{-2}$  was used. The primary production rates were measured at varying intervals starting one day after nutrient addition and continuing up to 14 days after the addition; the number of measurements in the intervening period also differed from experiment to experiment. The seston biomass as organic carbon was also measured both at the start and at the termination of the experiment, in order to compare it with the primary production data. The reduction in  $\text{PO}_4 - \text{P}$  and  $\text{NH}_4 - \text{N}$  concentration was also monitored both in the control and in experimental vessels, in order to get information on changes in P:C and N:C ratios in the seston produced and the likely effect of these ratios on the specific production rates.

The manner of nutrient addition may also affect the production patterns of phytoplankton. To examine this, the addition of excretion products in October and November was done in three ways: adding the excretion products a) in one stretch at the start of the experiment; b) manually but equally spread over the experimental period; and c) a regulated, continual addition by means of a peristaltic pump.

##### *Effect of excretion products* (R.D. Gulati, K. Siewertsen)

The effect of nutrient addition on phytoplankton production, irrespective of enrichment with the excretion products or with N and P was discernible only after a time lag of about two days. This confirms the preliminary observations during 1981.

The data on the seasonal changes in the effect of excretion products on the

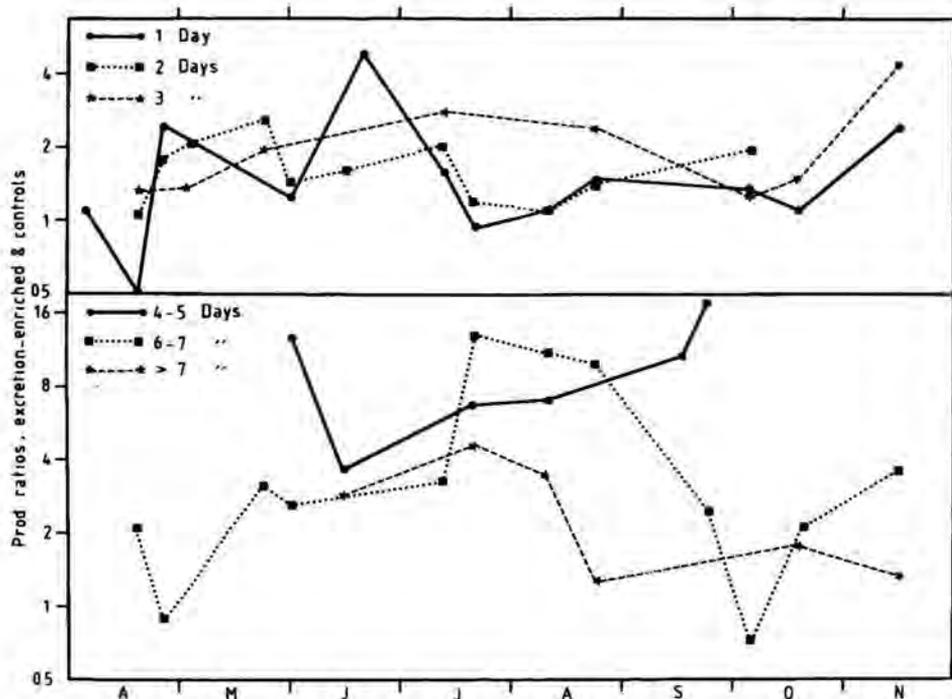


Fig. 6. Seasonal changes in the phytoplankton primary production ratios between Lake Vechten water samples enriched with zooplankton excretion products and controls, on different days following the enrichment.

primary production rates are summarized in Fig. 6. The most pronounced effect of enrichment using the zooplankton excretion products after one day of acclimation, was in June when the production ratio excretion-enriched: control was 4. In the subsequent summer period, one day acclimation did not produce any marked effects, but in the measurements two or more days after acclimation the stimulatory effect of excretion products was clear. Samples incubated after acclimation longer than 4-5 days in the excretion products assimilated  $^{14}\text{C}$  at a rate that was up to an order of magnitude higher than in the controls (see bottom Fig. 6).

The specific primary production rates ( $\text{mg C mg}^{-1}$  sestonic  $\text{C d}^{-1}$ ), however, did not increase markedly (Table 2). The P : C ratios in the organic material produced were significantly higher in the enrichment samples, more so in the P and N enriched samples. However, the regression relationship between specific primary production (Y) and P : C ratio (X) in the total seston revealed a better

Table 2. Means of specific primary production (SPP) and phosphorus : carbon (P : C) ratio. SD in parentheses.

Nature of sample	SPP	P : C ratio	n
Control	0.17 (0.10)	0.0004 (0.004)	9
Excretion-enrichment	0.21 (0.17)	0.014 (0.012)	13
P and N enrichment	0.24 (0.15)	0.036 (0.042)	8

relationship for excretion-enriched ( $P < 0.05$ ) than for N and P enriched samples ( $P > 0.05$ ). This was also true when the specific production was regressed on the N : C ratio. It is likely that the excretion products contain other compounds which are responsible for the differences in the relationship between the ratios and specific primary products.

Both, from the October and November data (Table 3) it is clear that addition of nutrients in one lot leads to higher seston mass and lower P : C and N : C ratios than when the nutrients are supplied daily or continually. Thus a regular supply of nutrients may reduce the chances of an early nutrient limitation. This last perhaps operates in the natural situation in which zooplankton may continually deliver nutrients in low amounts which can thus sustain a steady production over longer periods.

Table 3. Changes in phosphorus : carbon (P : C) and nitrogen : carbon (N : C) ratios in seston produced in the enrichment experiments during October-November, after a 14-day experiment.

Date	Ratio (%)	Nutrient addition			
		None (control)	In one lot (on day 1)	Once daily	Continuous
18 Oct.	P : C	0.12	0.22	0.33	0.50
	N : C	2.81	2.13	3.35	5.98
15 Nov.	P : C	0.07	0.15	0.22	0.27
	N : C	5.18	2.91	4.01	5.10

#### Energy flow and recycling based on $^{14}\text{C}$ -metabolism of phytoplankton and zooplankton (J. Heringa)

In six laboratory experiments zooplankton from Lake Vechten was fed  $^{14}\text{C}$ -labelled *Chlorella vulgaris*. A carbon budget was constructed by following the  $^{14}\text{C}$ -activity during 68 hours in the phytoplankton (*Chlorella* sp.), zooplankton, dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC).

The rate of  $^{14}\text{C}$ -accumulation in the animals is a measure for the uptake from phytoplankton. The  $\text{DO}^{14}\text{C}$  appearing is due to excretion from both zooplankton and phytoplankton and egestion from zooplankton. The  $\text{DI}^{14}\text{C}$  changes relate to phytoplankton and zooplankton respiration.

Several methodological problems came to light; a quantitative analysis of the carbon and energy flows within the mentioned compartments was difficult. One serious and unresolved problem was the loss of about 30% of the radioactivity during 68 hours. The activity in the DOC compartments fluctuated strongly in time and between the replicates. This may be attributed to differences in metabolism of bacteria adhering to zooplankton. The estimates of assimilation and respiration are, however, generally within the range of direct measurements reported by others.

#### 2.1.5. *Chaoborus flavicans* (J.E. Weekenstroo, P. Heutz)

The larval population (4th instar) of *Chaoborus flavicans* in Lake Vechten was monitored intensively during 1967-1969. Seasonal fluctuations showed a consistent pattern, namely a population maximum in late autumn followed by a gradual decrease through winter and spring to a mid-summer minimum. The mean annual population in the lake ranged from 35 to 50 million individuals. Based on this, together with the year-round presence and predacious character of the larvae, it was assumed that *Chaoborus* plays an important role in the energy flow in the

lake's ecosystem and in regulating zooplankton population structure. The present study is aimed at confirming the assumptions.

Bottom sampling, started in the summer of 1981, was continued, and an assessment of population energy requirements was attempted, by measuring respiratory metabolism of the 4th instars, and by estimating the total larval production. Further, factors causing mortality of the 4th instars in winter were investigated. The depletion of storage compounds may play a role.

The autumn population in 1982 of 55 million individuals in the lake indicates an increase of c. 300 percent compared to that of 1981 and a recovery to c. 80 percent of the population level in 1967-1969. The fluctuations in the population structure of the 4th instar appear to be more sensitive to changes in percentual mortality than to those in oviposition. For example, mortality due to predation by copepods of especially the first and second instars is likely to be the main factor determining the size of autumn populations.

Respiration was measured at four temperatures, 5, 10, 15 and 20°C, using both fed and unfed animals. Pooled means indicated significant differences in the oxygen consumption rates at 5 and 10°C and at 15 and 20°C, but not at 10 and 15°C (Fig. 7). Estimates of population respiration, based on respiratory data, benthic

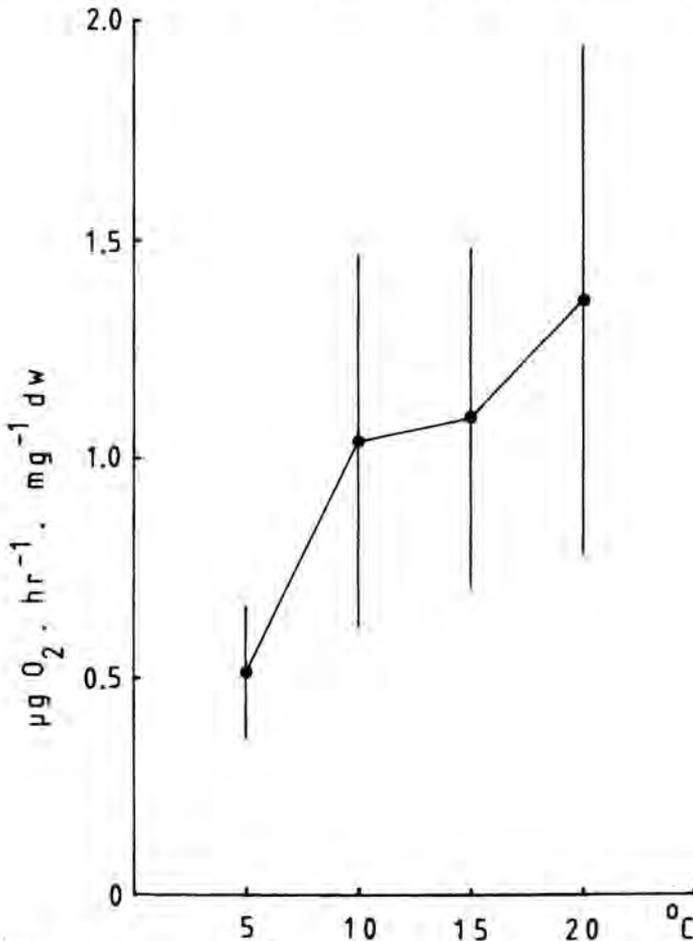


Fig. 7. The oxygen consumption rates of the 4th instar larvae of *Chaoborus flavicans*.

larval densities and percentage migrating population were: in 1982, 2.5 kg C compared with, e.g. in 1968, 5.7 kg C. The production calculations are based on a preliminary model. The total larval production thus calculated was 20 kg C in 1982 and 42 kg C in 1968. About 70 percent of the calculated production could be explained from field data. The remaining 30 percent may be the production of younger larval stages. This latter, in the absence of field data on densities, may not be accurate. Total population energy requirement thus estimated was 22.5 kg C in 1982 and 45 kg C in 1968. This is the best estimate so far, in the absence of data on anaerobic respiration and respiration of young larval stages.

Compared with annual production of zooplankton ( $> 85 \mu$ , c. 1500 kg C), *Chaoborus* energy requirements appear to be low, namely 1.5 percent in 1982 and 3.0 percent in 1968. So, the role of *Chaoborus* larvae in the energy flow of Lake Vechten seems to be very limited. Nevertheless, the regulation of zooplankton by *Chaoborus* larvae might be important in periods when both the respiratory activity and production of the larval population are high.

#### 2.1.6. Biological water quality assessment of 'boezem' waters and polder waters (P.J. den Oude)

This project was started in September 1982 and is financed by the STORA (the Foundation for Applied Waste-water Research).

In the Netherlands the Pollution of Surface Water Act of 1970 opened up many legal ways of combatting water pollution and of water quality control. However, assessment of water quality got an ecological basis only recently, in the Second I.M.P. 'Water' (a longterm indicative programme, 1980-1984, adjunctive to the Act).

In water quality research in the Netherlands, the attention is mainly focussed on the structure parameters, especially those concerning ecological diversity or saprobic indices or both. Recently some local water control agencies have attempted to assess the water quality and to evaluate the applicability of functional parameters in relation to community structure. This limnological approach is based mainly on the well-known system of Casper & Karbe. However, an ecological classification of the aquatic ecosystems is yet non-existent.

The main objectives of this pilot study are: (a) to select adequate structure and process parameters in order to describe the 'boezem'- and polder waters ecologically; besides theoretical clarity, simplicity and applicability are the important pre-requisites; (b) to apply these selected parameters to the existing data sets of aquatic environments and to categorize aquatic ecosystems by processing these data by computer-based cluster analysis; and (c) to advise on the nature of subsequent limnological investigations to complete the ecological classification such that it will be a useful tool in water quality management.

The study is phased as follows: (a) a literature survey (now in progress) and selection of existing data sets on the aquatic environments; for this, besides the manual and on-line searching, the relevant research institutes and government agencies were contacted; (b) a computer-based cluster analysis; and (c) evaluation of the results.

## 2.2. WORKGROUP 'MINERALIZATION OF ORGANIC MATTER'

### 2.2.1. Introduction (Th.E. Cappenberg)

The aim of the workgroup is to study the role of functional processes in the cycling of elements in the aquatic ecosystem. Fig. 8 schematically represents the interrelationships between the carbon, phosphorus and sulphur cycling processes, indicating their role in the mineralization of the photosynthetically formed organic matter by successive usage by bacterial groups and their various electron acceptors.

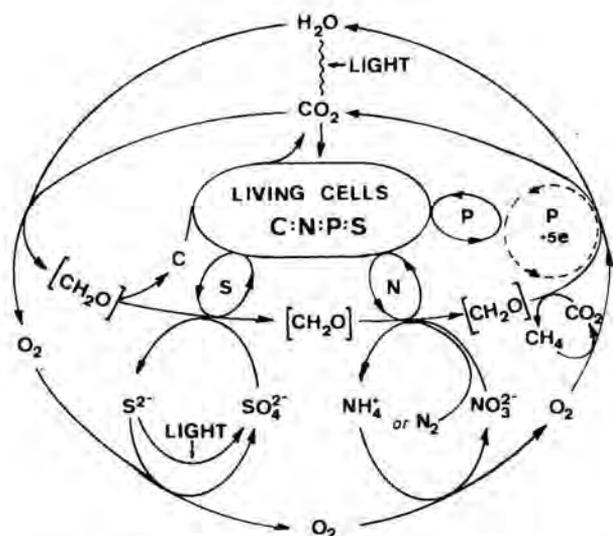


Fig. 8. Schematic representation of the interrelationships between the carbon-, nitrogen-, sulphur- and phosphorus-cycling processes in aquatic ecosystems.

The workgroup is involved in two main research objects, i.e. stratifying Lake Veichten and the shallow hypertrophic Loosdrecht Lakes (Section 2.4).

As part of an integrated ecosystem approach to the limnological problems in Lake Veichten (Section 2.3) the workgroup investigates the dynamics of the cycling of C, N and S in the limnetic region and in the sediments. Emphasis is laid on aerobic and anaerobic mineralization of organic compounds, produced during primary production, to their inorganic species. Particular attention is paid to kinetic aspects of these mineralization processes, using radioisotopic and chromatographic techniques in order to calculate the turnover rates and investigate the breakdown pathways. In addition, the fluxes of the various chemical compounds are being studied. This includes the exchange and diffusion processes as well as sedimentation rates for calculating the carbon, nitrogen and sulphur budgets. A general mathematical electron-acceptor model is being developed for a stratifying lake-ecosystem in collaboration with the Delft Hydraulics Laboratory, Environmental Hydraulics Branch (Ir. J.G.C. Smits).

The study in the Loosdrecht Lakes concerns the quantitative aspects of exchange, diffusion and turnover of phosphorus compounds in the sediment and between the sediment and the overlying water in relation to the loading of phosphorus (Loosdrecht Lakes Restoration Project, Section 2.4). In 1984, supply-water from the Amsterdam-Rhine Canal will be treated to remove phosphorus before it is let into the lakes. This is likely to reduce the external phosphorus loading by an order of magnitude. The study involves also quantification of the internal phosphorus loading. The project involves a study of

- the release rates of P-components between the sediments and the overlying water of the lake and availability of compounds released for autotrophic growth; the mechanisms of exchange processes will be studied using several types of sediment and P-concentrations in the overlying water;
- hydrology, hydrodynamics and physico-chemical characteristics of the lakes before and after the inlet of the treated water from the Amsterdam-Rhine Canal. Special attention will be paid to separate P-balances of the various lakes and the impact of vertical and horizontal ground-water inflow and outflow in these balances;

- spatial and temporal variations of suspended particulate matter in the Loosdrecht Lakes in relation to wind effects, the mineralization of P-compounds from the sediment and resuspended particulate organic matter at the mud-water interface.

The studies form a part of a coordinated and integrated project relating to the Loosdrecht Lakes restoration. The main objective is to find ways and means for better management of shallow hypertrophic lakes by reducing eutrophication in general and in the Loosdrecht Lakes in particular.

Parts of the research efforts of the workgroup are reported in the sections 2.3 (Carbon cycle in Lake Vechten) and 2.4 (The Loosdrecht Lakes Restoration Project).

#### 2.2.2. *Aerobic mineralization* (J.J. Olie, M.J. Bär-Gilissen)

Aerobic mineralization, i.e. uptake of photosynthetic excretion products by heterotrophic bacteria, respiration of the seston and microbial breakdown of detritus form an essential part of the carbon cycle in Lake Vechten. Vertical changes in the rates of C-fixation and excretion, chlorophyll-*a* concentration, phytoplankton species composition and bacterial carbon mineralization were investigated in the pelagic zone of the eastern depression of Lake Vechten for 3 successive years. Special attention was given to the  $^{14}\text{C}$ -technique for measuring extracellular release by phytoplankton and aerobic carbon mineralization (Blaauboer, 1982; Blaauboer *et al.*, 1982; Olie and Cappenberg, 1982; Olie *et al.*, 1982).

Two new methods were investigated for the *in vivo* concentration of seston in sediment traps and for using the diffusion chambers in the kinetics of primary production and aerobic mineralization.

As indicated by Olie and Cappenberg (1982) and Olie *et al.* (1982) concentration by continuous centrifuging did not damage phototrophic seston during spring. However, fragile phytoplankton species present during stratification are seriously damaged by this treatment. *In vivo* concentration by sediment traps should avoid the excessive and time-consuming handling of the samples. The concentrating effect of the trap was investigated throughout the year at one depth. Free water samples were taken with regular intervals using a Friedinger sampler. Synchronously the trap was exchanged with a new one. From both samples Lugol fixed phytoplankton was counted and  $^{14}\text{C}$ -fixation measured.

In April the trap population was dominated by centric diatoms giving a high production compared with the population in the water outside the trap. During stratification green algae dominated the trap population with a still larger production than during spring. The sampling depth was changed from 5 to 6.6 m. In summer when pennate diatoms and *Asterionella* were abundant in the traps these again were more productive than the population outside. From August on the concentration effect of the trap was absent, so the phytoplankton counts and primary production of the population in the trap were comparable with those of the population outside. The algal succession agreed with the findings of Blaauboer (1982). Variability in the rates of primary production and of extracellular release mostly exceeded 10% of the mean. This is due to zooplankton grazing. Since filtering over a 120  $\mu\text{m}$  screen removed large detritus particles in addition to zooplankton it was omitted from the procedure. The extracellular release of the outside population exceeded that of the trap due to the appearance of algae like *Mallomonas* which excreted more actively and to damage by the more actively grazing zooplankton.

In conclusion, the trap concentrates the blooming species which, when alive, are held in suspension by turbulence. This gives rise to a highly productive, probably growing population inside the trap during collection time. However, in autumn the trap population does not differ significantly from the outside population in number and primary productivity, due to increasing turbulence.

In the study of the carbon cycle of Lake Vechten the  $^{14}\text{C}$ -technique is being used so far for the estimation of phytoplankton primary production and zooplank-

ton grazing. Olie and Cappenberg (1982) measured  $^{14}\text{C}$  loss to follow the course of the aerobic mineralization, namely during seston respiration and microbial breakdown of detritus. In seston metabolism all organisms are involved, i.e. algae using organic carbon formed during photosynthesis, bacteria utilizing the algal extracellular products and zooplankton  $< 125 \mu\text{m}$  grazing on algae and bacteria. It is assumed that during 4 hours incubation the seston labelling is confined mainly to algae. In the microbial breakdown of detritus no distinction can be made between bacteria, protozoa and zooplankton  $< 125 \mu\text{m}$  in utilization of detritus nor between the particulate organic  $^{14}\text{C}$  of detritus and heterotrophic bacteria. As assumed for seston respiration the  $^{14}\text{CO}_2$  production mainly originates from bacteria and decomposing labelled remains of mainly algae. Incubations were started directly after primary production experiments. The results suggest a rapid turnover of labelled organics used for  $^{14}\text{CO}_2$  production in seston respiration and for microbial breakdown of detritus. In the time series experiments the  $^{14}\text{C}$  loss in organic carbon due to seston respiration was maximal in the hour in dark following 4 hours light incubation.

For investigating the process kinetics a diffusion chamber connected to a fraction collector was used. By studying the  $\text{DI}^{14}\text{C}$  concentrations in the fractions it is possible to measure input and output rates of  $\text{CO}_2$  from the organisms without destroying the sample. Therefore, it is possible to measure primary production and aerobic mineralization synchronously. It is, however, impossible to eliminate the internal recycling of  $\text{CO}_2$  within algae and within the diffusion chamber. Attempts are being made to fractionate the remaining organic carbon pool into carbohydrates, lipids, low weight organics and proteins to determine the pools that contribute to  $\text{CO}_2$  production.

Longer incubation times can be used without nutrient depletion. On the other hand, fouling of membranes by microorganisms is a problem and stirring of the chamber could damage algae. The effects of membranes, pump rates, the use of different C-compounds and stirring on diffusion were studied. The diffusion chamber is made of perspex and closed at the bottom by a polycarbonate filter holder (Fig. 9). On the top an extension of the same perspex tube is made (diffusion reservoir) so that a filter and rubber ring can be fitted in between. The chamber is stirred magnetically. The reservoir is closed by a lid having an inlet and an outlet, both powered by the LKB multiperspex pump. The inlet is connected to the main supply, the outlet to the fraction collector. Loss of  $^{14}\text{CO}_2$  from the fractions during storage is prevented by raising the pH to 10-11 by adding 1N NaOH through a LKB varioperspex pump. The fractions are assayed for total label and for organic carbon.

Diffusion of  $\text{DI}^{14}\text{C}$ ,  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -acetate was studied with three membranes viz. polycarbonate  $0.2 \mu\text{m}$  (Nuclepore), cellulose nitrate  $0.2 \mu\text{m}$  (Sartorius) and cellulose dialysis membrane  $5.10^{-3} \mu\text{m}$  (Visking). In general,  $\text{DI}^{14}\text{C}$  diffuses more rapidly than  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -acetate, while  $^{14}\text{C}$ -glucose diffuses somewhat more rapidly than  $^{14}\text{C}$ -acetate. In case of  $\text{DI}^{14}\text{C}$  at pH 7 a substantial amount is present as  $\text{CO}_2$ , therefore diffusion of  $\text{DI}^{14}\text{C}$  is determined more by  $\text{CO}_2$  than by  $\text{HCO}_3^-$ . This is due to diffusion of ions under electrically neutral transfer across membranes, i.e. either co-diffusion of oppositely charged ions or counter-diffusion of identically charged ions. In case of  $^{14}\text{C}$ -acetate the acetic acid is completely dissociated at pH 7 and the acetate diffusion is restricted to the aforementioned conditions. The pore size and structure determines to a great extent the diffusion of a given compound. Nuclepore filters with small variance in pore size and well defined pore structure bring about the shortest equilibrium time for all the studied compounds, Sartorius filters being the next. The  $5 \times 10^{-3} \mu\text{m}$  pore size of dialysis membranes greatly retard the diffusion. By stirring the reservoir an improvement factor of 2.7 in equilibrium time was achieved, the adherent water layers on top of the filter prolonging the diffusion track compared with batch incubations. The percent fixation observed in the diffusion chamber is high if the samples are diluted. In case of concentrated samples taken from chemostat cultures of algae, the reverse was true, probably due to the light limitation.

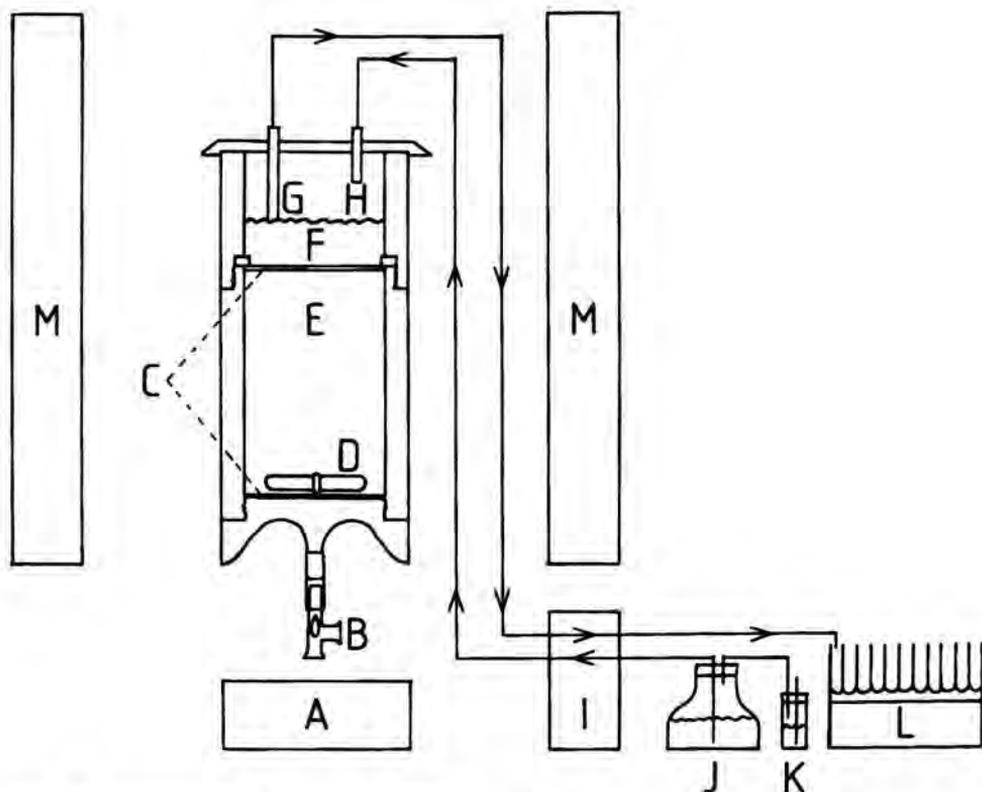


Fig. 9. Design of diffusion chamber

- |                                |  |
|--------------------------------|--|
| A magnetic stirrer             | H inlet from main reservoir                  |
| B 3-way stopcock               | I LKB multiperspex pump with double pumphead |
| C membrane filters             | J main reservoir                             |
| D magnet                       | K CO <sub>2</sub> -trap                      |
| E diffusion chamber            | L fraction collector                         |
| F diffusion reservoir          | M light source: TL fluorescent tubes         |
| G outlet to fraction collector |  |

### 2.2.3. Nitrogen cycle (H. Verdouw, E.M.J. Dekkers)

The studies on the mass-balance of nitrogen and on the temporal and spatial variations of sedimentation helped to quantify the processes relating to the nitrogen cycle in Lake Vechten (Verdouw and Dekkers, 1982 a, b). The study of nitrogen dynamics in the hypolimnion and sediments was started by measuring the turnover rates of ammonia using a <sup>15</sup>N-isotope dilution technique. The nitrogen fluxes, sedimentation and eddy diffusion were calculated. This approach is expected to clarify to some extent the complex system of ammonia production, consumption, and diffusion in relation to the nitrogen input by sedimentation.

The dynamics of the transport processes involved in the nitrogen cycle in the hypolimnion was evaluated. Two water layers are distinguished: 5 - 7.2 m and 7.2 - 9.6 m. Input and output of particulate matter were measured fortnightly by sediment traps at 5, 7.2 and 9.6 m in the centre of the eastern depression in Lake Vechten. Concentration profiles of all dissolved nitrogen compounds were measured monthly for calculating the concentration gradients at the boundaries of the mentioned layers. The water temperature profiles were measured at 0.5 m inter-

vals. In collaboration with the Delft Hydraulics Laboratory, a calculation of vertical dispersion coefficients in the hypolimnion was attempted. The goal of this project is to understand the flux of dissolved matter, for developing a general electron-acceptor model in stratifying lakes. These calculations are still in progress. A complication in Lake Vechten is the inflow of cold groundwater which sometimes disturbs the temperature-structure of the hypolimnion, thus interfering with the estimated dispersion coefficients.

Another uncertain factor is the exchange between water and sediment in the two layers. The bottom areas in these layers of 5–7.2 m and 7.2–9.6 m are about 40 and 60 percent, respectively, of their total areas. The input and output data for the layers have to be interpreted with great care, since the fate of sedimented material on the bottom is yet unknown: mineralization, resulting in nutrient regeneration within the layer, or further transport along the bottom of the lake for which strong evidence exists, but which cannot as yet be quantified.

#### 2.2.4. Anaerobic mineralization and production (Th.E. Cappenberg, C.L.M. Steenbergen, C.A. Hordijk, H.J. Korthals, J.T.G. Mathijssen, C.P.C.M. Hagenars).

Sedimentation and breakdown kinetics of organic matter were studied in the anaerobic hypolimnion and sediments of Lake Vechten focussing on the turnover and exchange rates of carbon compounds (Cappenberg and Verdouw, 1982). The breakdown of algal cell walls appeared to limit the rate of mineralization in the sediments. Molecular diffusion across the sediment-water interface of acetate, the most important breakdown product, could not account for the observed turnover rates of acetate to carbon dioxide and methane (Cappenberg *et al.*, 1982). In the hypolimnion and anaerobic sediments decreasing sulphate concentrations coincide with increasing numbers of sulphate-reducing bacteria, forming a concentration gradient of hydrogen sulphide. The rate of bacterial sulphate reduction is being quantified with the  $^{35}\text{S}$ -radiotracer technique and calculated from mathematical models.

Lower fatty acids and methane play an important role in the aerobic and anaerobic decomposition of organic matter in Lake Vechten; the quantification of their turnover and diffusion may help to explain some aspects of the carbon-cycling in the lake. Anaerobic sediment from the deepest parts was incubated *in vitro* to determine methane production; the production was estimated as 7–8 mMol  $\text{CH}_4\text{m}^{-2}\cdot\text{d}^{-1}$ , with the highest rates at 3–7 cm depth, i.e. just below the zone of sulphate reduction (0–3 cm). This confirms earlier work in which the highest numbers of acetate-fermenting methanogenic bacteria were observed at 4–6 cm together with the highest turnover rate constants of acetate (Cappenberg and Verdouw, 1982). Methane produced in the anaerobic sediment enters the water column by molecular diffusion and by bubble ebullition.

Molecular diffusion was quantified by measuring the methane concentration in the upper few cm of the sediment. The profiles showed a steep increase from 250  $\mu\text{M}$  to 1.6 mM (i.e. above saturation level) at a depth of 6 cm, for which bubble formation at this horizon might be an explanation. The methane flux was calculated using Fick's law as modified for sediment diffusion; it varied between 1 and 4 mMol  $\text{CH}_4\text{m}^{-2}\cdot\text{d}^{-1}$ . High methane production during the stratification period resulted in saturated concentrations and formation of bubbles in sediments below the sulphate reduction zone. Bubble ebullition proved to be a major factor; it was quantified by measurement *in situ* of the total flux of methane to the water column, using inverted calibrated cylinders suspended 0.5 m above the sediment surface, and amounted to 3–4 mMol  $\text{CH}_4\text{m}^{-2}\cdot\text{d}^{-1}$  (Table 4).

Lower fatty acids, which play an important role in the dynamics of organic matter in the ecosystem, were directly determined in water samples by gas chromatography using a 10% NPGA-column and formic acid saturated carrier gas for acetate profiles. An analytical procedure for determining acetate, formate and lactate in one single run was modified for quantifying these lower fatty acids

Table 4. Methane production and flux from the sediment of Lake Vechten in the last part of the stratification period (August - October).

Methane production ( $\text{mM CH}_4 \text{ m}^{-2} \cdot \text{d}^{-1}$ )		Methane flux ( $\text{mM CH}_4 \text{ m}^{-2} \cdot \text{d}^{-1}$ )	
batches (0 - 10 cm)	subcores	bubbles	diffusion
7 - 8	4 - 9	3 - 4	0.5 - 4

in the water column. The analysis involved filtration of the water samples, ion exchanging, concentration by freeze-drying, derivatization with 4-bromo-methyl-7-methoxy-coumarine and HPLC-fluorescence detection. Significant losses of fatty acids occurred only during concentration by freeze drying ( $40 \pm 10\%$ ). However, these losses were linear and independent of volume (0 - 30 ml) freeze-dried and concentration (0 - 10  $\mu\text{M}$ ).

Highest concentrations of acetate and formate are likely to be found in the water layers just above the sediment, as a result of sulphate reduction and flux from the sediment. The concentration of acetate and formate in these layers was in the range of 5 to 10  $\mu\text{M}$ . In the water layers in the upper part of the lake these concentrations decreased to about 5  $\mu\text{M}$ . In the circulation period some accumulation of lactate and acetate was found below 8 m depth (Fig. 10). However, no long term accumulation of acetate was found in the stratification period, whereas the lactate concentration was reduced below the detection limit of about 2  $\mu\text{M}$  (Hordijk and Cappenberg, 1983). Over the whole year concentrations of  $\text{C}_3$  -  $\text{C}_7$  fatty acids were below the 1  $\mu\text{M}$ -level.

The occurrence of photosynthetic sulphur bacteria in the meta- and hypolimnion of Lake Vechten was described, and productivity of these bacteria in relation to light intensity and sulphide concentration was discussed (Steenbergen, 1982; Steenbergen and Korthals, 1982). The rate of sulphide oxidation was estimated

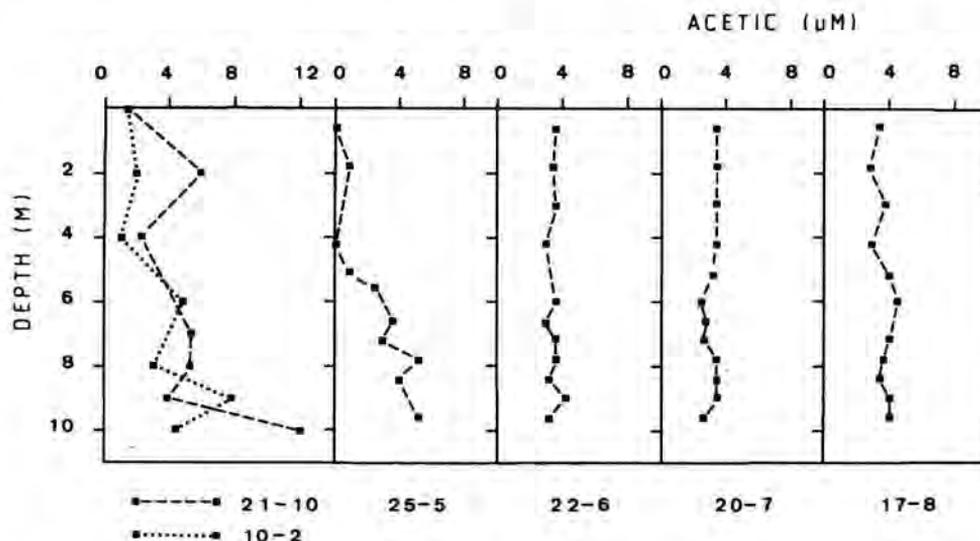


Fig. 10. Acetic acid concentration profiles in the open water phase of Lake Vechten, measured by High Performance Liquid Chromatography (HPLC) and Gas Liquid Chromatography (GLC), before (21-10-1982, 10-02-1982) and during stratification (others).

from the average rate of anoxygenic photosynthesis, resulting in a great discrepancy between the sulphide supply by diffusion and its calculated oxidation rate. This suggests, that besides the possibility that sulphide is not the only electron donor available for the phototrophs, sulphide production from inorganic sulphur compounds may take place in these water layers. Experiments with  $^{35}\text{S}$ -labelled compounds will be needed to obtain conclusive evidence for the above assumptions.

2.2.5. Sedimentary flux of major phytoplankton groups (C.L.M. Steenbergen, H.J. Korthals)

The periodicity of major dominant algal groups in Lake Vechten (Blaauboer, 1982; Steenbergen and Verdouw, 1982) and its relation to vertical and horizontal

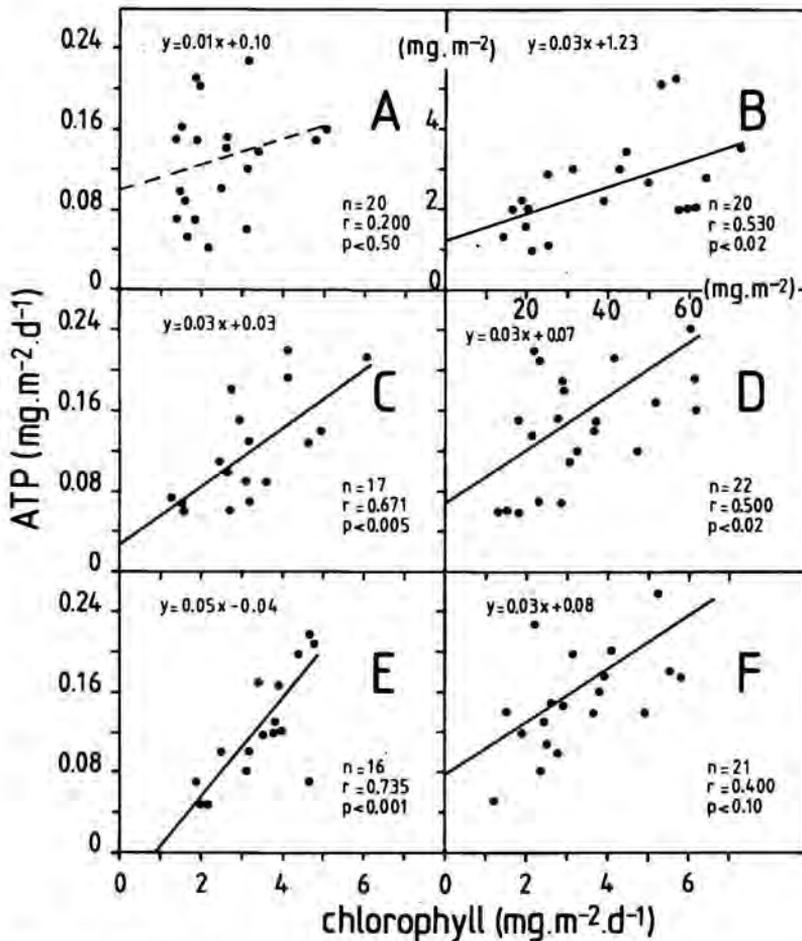


Fig. 11. The regressions of (y =) ATP sedimentation rate against sedimentation rate of (x =) chlorophyllous pigments for sediment traps suspended in the pelagial at depths of 5 m (A), 7.2 m (C) and 9.6 (E) and for traps suspended in the littoral at depths of 4 m (D) and 5 m (F). In (B) the correlation between (y =) ATP and (x =) chlorophyllous pigment concentration in the upper 5 - m water column samples is depicted. Note changed notations on coordinates.

transport of particulate matter is dealt with in section 2.3. Here the sinking losses of major algal groups in the epilimnion of Lake Vechten are quantified using sediment trapping and phytoplankton pigment analysis (Steenbergen, 1982; Steenbergen and Korthals, 1982). Loss of algae from the trophogenic zone of lakes occurs due to grazing by animals including protozoa, decomposition by bacteria and by sedimentation. The last-named process governs the amount of material reaching the hypolimnion where it is mineralized anaerobically (Cappenberg and Verdouw, 1982).

The volume of material collected by traps varies considerably between trapping periods as well as between the traps suspended in the pelagial and those in the littoral zone (Verdouw and Dekkers, 1982). Qualitatively the trapped material comprises viable algae, algal remains and debris, the composition reflecting broadly the phytoplankton periodicity (Section 2.3).

Both sedimented material and seston can be characterized by their contents of ATP and chlorophyll and compared (Fig. 11). The linear regression correlation between ATP and chlorophyll content of seston in the upper 5-m water column is significant ( $P < 0.02$ ) (Fig. 11B). Except for the material trapped at 5 m in the pelagial zone, where zooplankton is likely to interfere, ATP and chloro-

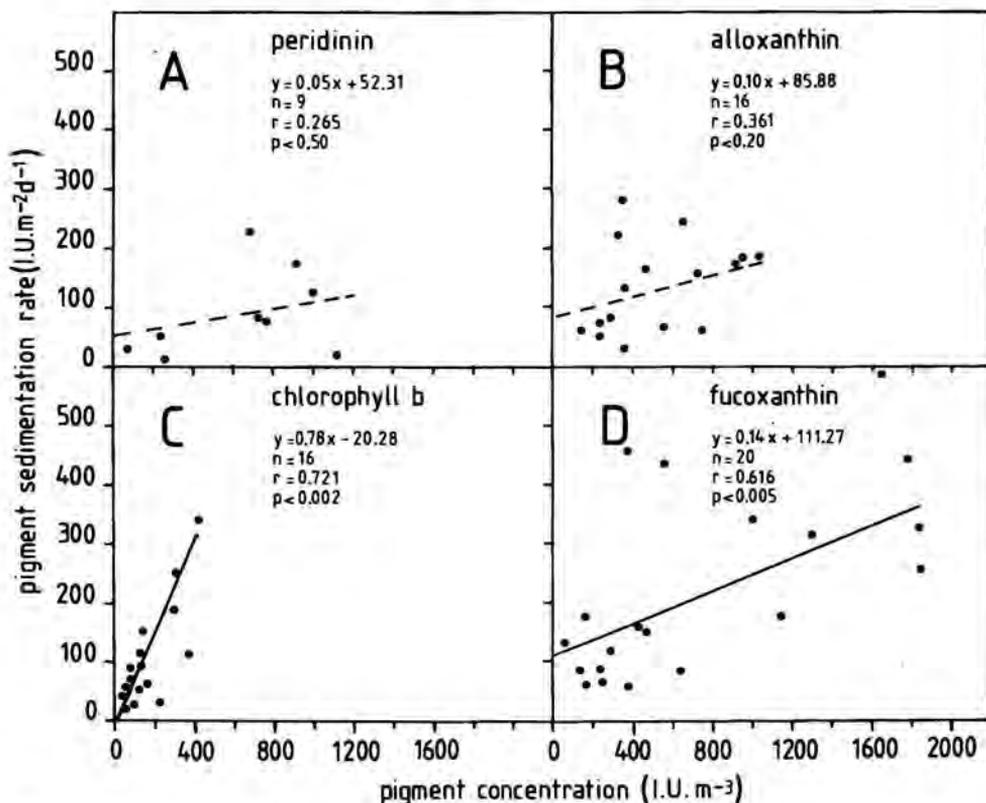


Fig. 12. Relationships between mean daily increment in the 5-m pelagial trap and mean concentration in the upper 5-m water column of specific photosynthetic pigments associated with major algal groups i.e. peridinin (A) with Dinophyceae; alloxanthin (B) with Cryptophyceae; chlorophyll-b with Chlorophyta and fucoxanthin (D) with Bacillariophyceae and Chrysophyceae. Pigment amounts are given in arbitrary integrator units (I.U.).

phyll contents of traps are significantly correlated and compare favourably with the seston data (Fig. 11). Thus, besides the organic detritus, intact algae may form the bulk of the entrapped biological material.

The various algal groups differed sharply in their sinking behaviour (Fig. 12). A significantly higher amount of pigment relative to that in the seston can be attributed to passive sedimentation or a movement directed downward. Apparently this holds good for chlorophyll-*b* and fucoxanthin (Fig. 12C, D), which are associated with green algal species and diatoms respectively. Particularly the crop and flux estimates for chlorophyll-*b* (i.e. green algae) agree very closely (Fig. 12C). So, sedimentation accounts for the removal of the major portion of

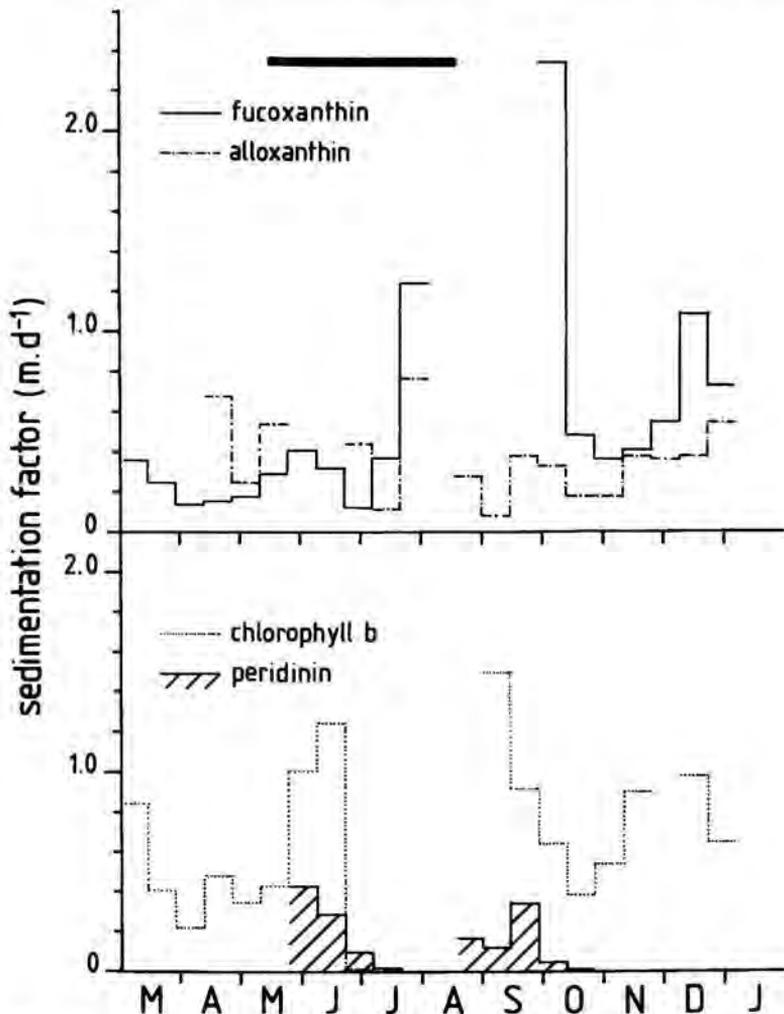


Fig. 13. Seasonal changes in apparent effective sinking velocity for specific photosynthetic pigments associated with major algal groups (cf. legend of Fig. 12) expressed as arbitrary integrator units (I.U.) and calculated from mean daily sinking rates in the 5-m pelagial trap and mean concentrations in the upper 5-m water column. Horizontal bar at the top indicates the period of relative thermal stability in the 3-5 m water stratum.

the green algal populations (Section 2.3). The opposite, i.e. no significant relative increase in trap pigment concentration, may be due to the algae that avoid the traps, are consumed by animals, or to the algae that died and decomposed after entering the traps because of a lack of downward movement. Apparently, one or more of these explanations are valid for motile algal groups as the dinoflagellates, containing peridinin, and the Cryptophyceae containing the specific pigment alloxanthin (Fig. 12A, B).

Recoveries of peridinin are an order of magnitude lower than the potential maximum concentration in the standing crop (Fig. 12A). Thus these organisms contribute only marginally to the sedimentary flux of phytoplankton.

Apparent effective sinking velocities (i.e. sedimentation factor) vary considerably with time and with the kind of algae (Fig. 13). Generally they are high during the period of low turbulence in the epilimnion during summer, which allows the passive sinking of the cells. The autumn and winter maxima of sinking velocity cannot be explained easily. In this respect one has to be cautious in interpreting quantitative aspects of sediment trap catches collected in turbulent conditions.

## 2.3. PROJECT 'CARBON CYCLE IN LAKE VECHTEN': STUDIES RELATING TO THE TRANSPORT OF PARTICULATE MATERIAL

(H.J. Gons, J.C. Kromkamp - Workgroup Primary and Secondary Production; C.L.M. Steenbergen, H. Verdouw - Workgroup Mineralization of Organic Matter)

### 2.3.1. Introduction

Lake Vechten was created in 1941 by excavating superficial sand layers, needed for the construction of a highway. The sandpit (surface area, 4.7 ha; maximal depth, 11.9 m; mean depth, 6 m) has no surface in- and outflowing streams and is thermally stratified from early May to early November. It has a well developed littoral region. During the stagnation period an anaerobic zone extends to the 5 - 6 m isobath. The phytoplankton composition points to a slightly eutrophic environment.

The lake has formed a permanent research object of the Limnological Institute since 1960. The limnological studies up to 1975 were aimed mainly at understanding the separate physical, chemical and biological characteristics of the lake. Evidently the processes, involving the three main properties of an ecosystem, i.e. the energy flow, the mineralization and nutrient recycling, and the population regulation, cannot proceed in isolation. In view of this there was a strong desirability for both a coordinated and an integrated approach to limnological problems in Lake Vechten. This new approach forms the basis of the present studies, in progress since 1976.

An account of this work was given in *Developments in Hydrobiology 11*, 1982 (Section 4: Publications).

The present research of the two workgroups is partly focussed on an integrated study of the carbon cycle in Lake Vechten. The results on the annual carbon budget of the lake were summarized (Progress Report 1980). From the comparison of carbon fluxes with changes in the organic carbon pools, three major research topics emerged:

- zooplankton food supply, mainly in April-June;
- origin of bacterial substrates with respect to mineralization, in both aerobic conditions in the littoral and limnetic regions, and anaerobic conditions in the deep strata;
- vertical and horizontal transport of particulate matter in relation to these problems.

At first the transport of particulates was investigated mainly in the hypolimnion, in order to compare this and the anaerobic mineralization with the productivity in the lake. In this case sediment traps may yield more realistic data than traps suspended in the turbulent epilimnion.

Recent research in the littoral revealed that import of sestonic matter from the limnetic region is important in the dynamics of epiphyton and epipelon. Quantification of organic matter indicated that decomposition following its deposition in the littoral is very important on lake scale. Since submerged vegetation covers only a small area, mainly epipellic organisms contribute to decomposition.

In lakes with a steep and sandy bottom, like Lake Vechten, accumulation of matter in the littoral is only temporary, so its seasonal changes can be quantified. Also, the concentration and composition of the particles in water and deposited matter can be compared. Moreover, the use of sediment traps in estimating deposition onto the sediments of the upper strata may be evaluated.

In February 1982 the first integrated, biweekly study of seasonal changes in epipelon, seston and sedimentation was started. The parameters are: seston in the 0-5 m layer, epipelon at 3, 4 and 5 m depths in the south-eastern part, and material collected in sediment traps at 5 m depth in the central part of the lake and at 4 and 5 m depth near the sampling sites of epipelon in the south-eastern part.

Horizontal variations in the distribution of the epipelon were measured at 3 and 5 m depth along three additional transects from June to September.

### 2.3.2. Seston, epipelon and sedimentation

For seasonal changes in the sestonic matter (Fig. 14A) different periods may be distinguished. In May the summer stratification becomes stable and a 'clear water phase' marks a transition in phytoplankton from spring to summer. A second period of low phytoplankton concentration occurs at the end of August with a distinct change in the phytoplankton composition (see below).

The seasonal changes in the seston concentration, expressed as total or organic dry weight are apparently low in comparison with changes in epipelon concentrations (Fig. 14A, B).

The epipelon, like seston (Fig. 14B), has a transition period in May and one at the end of August. Compared with earlier years, the epipelon concentrations at the end of 1982 were very high. The epipellic organic matter at 4-5 m depth was similar to that of seston; its dry weight, however, was manifold higher than in open water. This was also true for carbonates.

Comparison of seston and epipelon shows that the epipelon, on its decrease, is not resuspended in the water column. This confirms the hypothesis that the flow of limnetically produced particulates to the littoral sediments is one way. Following a wind-induced stirring up the epipelon shifts downwards along the slope to reach the anaerobic sediment. During this shift the chemical composition of the epipelon changes only slightly; apparently both fine-sized lighter particles and the heavier ones containing calcium carbonate are stirred up and transported.

During the early stratification period wind influence is limited to 3-4 m depth, since the density gradient prevents mixing; invariably, the epipelon accumulation is highest during these months. In 1982 by mid-August the epilimnion became isothermal, and the rapid decrease of the total epipellic matter was largely due to wind-induced mixing.

Besides hydrodynamics, limnetic productivity mainly determines the concentrations in the littoral sediments. The scale difference in the amounts of seston and epipelon indicates high turn-over rates in the open water. Thereby production just about compensates for the rapid sinking.

The low percentage of organic matter in the epipelon reflects the history of its mineralization before and after deposition. Presuming the minerals in the ash, e.g. silicate from diatom frustules, to be inert, the share of inorganic particulates produced in the limnetic region and accumulating in the littoral at 4-5 m depth can be estimated during June-August, when the epipelon is sheltered from wind influence. The amount thus estimated was more than that based on primary production in the previous years.

The sedimentation (Fig. 14C) was measured using perspex tubes with aspect

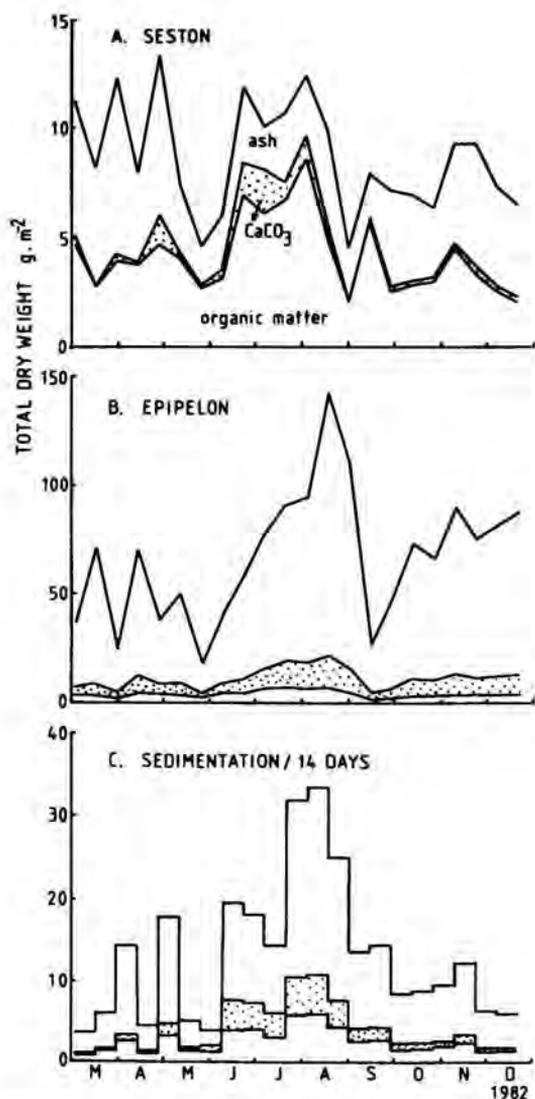


Fig. 14. Seasonal changes in the concentrations of seston (0-5 m depth), and epipelon (4-5 m depth) and the sedimentation in the littoral region (4-5 m depth).

ratio (height : diameter) of 5 as traps. These traps may give 'gross sedimentation', since particles, once collected, are not likely to escape. This is contrary to the littoral bottom, which may be visualized as a sediment trap with aspect ratio of zero. Hence, the material on the bottom does not always represent an integration of the particles reaching it, as water movements may cause displacement of the particles. Thus the changes in epipellic matter in March-May and September-December mostly were not comparable with the sedimentation measurements. During the first half of the summer stratification period, because of reduced turbulence, the manner of collection of material in sediment traps and on the bottom seemed similar: from mid-June to mid-August the matter collected in

sediment traps in the littoral averaged  $24 \text{ g.m}^{-2} \cdot (14 \text{ d})^{-1}$ , and the increase in the epilimnion was  $21 \text{ g.m}^{-2} \cdot (14 \text{ d})^{-1}$ , both values being total dry weight expressed on lake basis. These rates mainly apply to inorganic matter. The percentage organic matter in the sediment traps was higher than that in the epilimnion. This is because the epilimnetic particles were in a later stage of mineralization than the material collected for 14 days in the traps.

The sedimentation rates at 5 m depth in the littoral zone and in the open water (Fig. 15) were compared. Sedimentation rates near the shore were higher in the period of limited circulation in the lake, which was also found in previous years, in the hypolimnion. This suggests either a high sedimentation rate or a strongly localized transport of particulate matter along the littoral bottom. Highest sedimentation rates were found in the littoral zone at 4 and 5 m depths in the period when the thermocline was still descending but had not yet reached the depth of the trap. Possibly, the increasing turbulence at the bottom 'above' the traps caused resuspension of epilimnion. In deeper regions, where temperature gradients persisted, this material settled down. After the thermocline sank to a depth below the trap the sedimentation rates decreased, turbulence preventing sinking. This was observed for the traps at 4 and 5 m. The resuspension of bottom material did not result in a significant increase of sedimentation rates in the open water (Fig. 15).

The relations between seston concentration in the open water (0-5 m) and sedimentation rate in the open water and the littoral zone at 5 m depth were examined (Fig. 16). In the open water a strong correlation exists, but for the trap in the littoral the correlation is weak. If transport of particulate matter along the bottom is supposed, the weak correlation in the littoral is not surprising, as the actual seston concentrations in that region, especially near the catching area of the trap, were not measured.

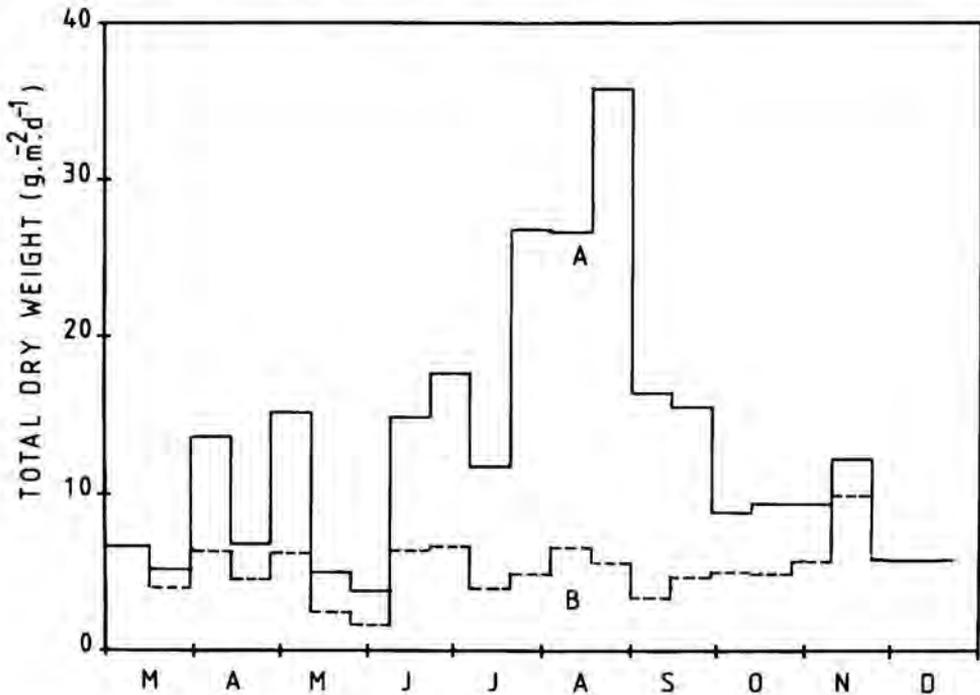


Fig. 15. Sedimentation rate at 5 m depth in the littoral region (A) and in the open water (B).

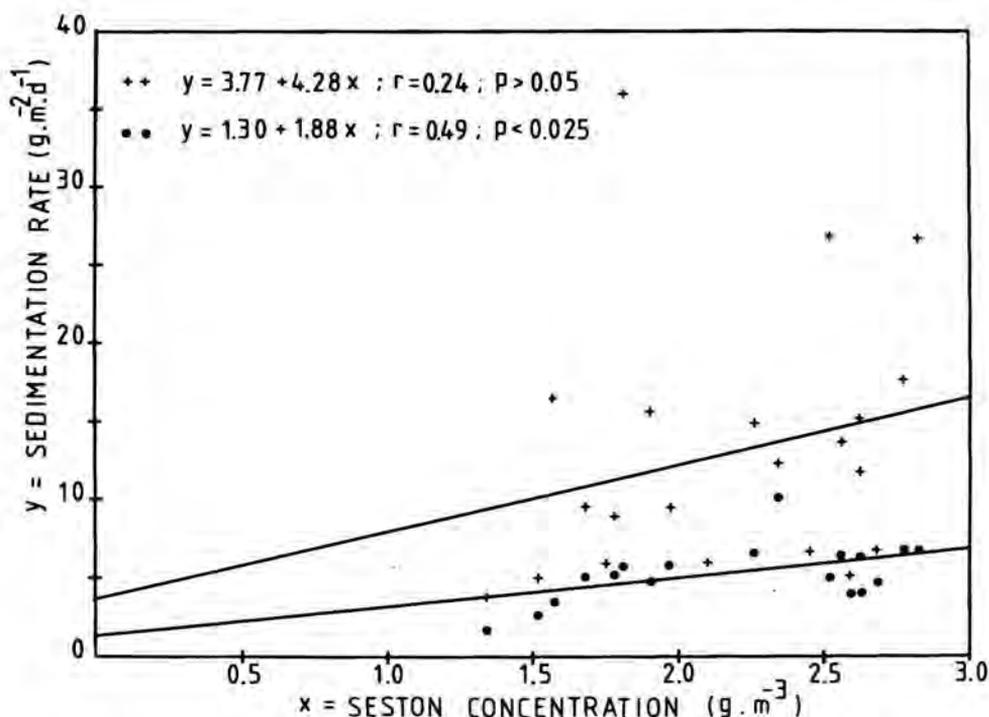


Fig. 16. Correlations between concentration in the 0-5 m stratum in the open water and sedimentation rates at 5 m depth of seston dry weight in the open water (dots) and in the littoral zone (crosses).

The relation between seston concentration and sedimentation rate may be described by an apparent sedimentation factor  $F$ , i.e. sedimentation rate ( $\text{g.m}^{-2}.\text{d}^{-1}$ ) =  $F$  ( $\text{m}.\text{d}^{-1}$ ) x total seston above the trap ( $\text{g.m}^{-3}$ ). From the data in Table 5 it is clear that the constituents of the seston sink at different rates. Carbonates have the highest sinking velocities and chlorophyll and ATP the lowest. However, sedimentation factors of organic constituents are likely to be influenced by mineralization in the traps, leading to their being underestimated in the material trapped.

Table 5. Sedimentation factors ( $F$ ) for components of the material collected at 5 m depth in the open water of Lake Vechten.  $n$  = number of collecting periods

Component	$F$ ( $\text{m}.\text{d}^{-1}$ ) Mean and S.D.	$n$
Total dry weight	$2.45 \pm 0.72$	22
Organic matter	$1.20 \pm 0.44$	22
Organic-C	$0.96 \pm 0.34$	21
$\text{CO}_3\text{-C}$	$5.75 \pm 2.30$	21
Chlorophyll	$0.37 \pm 0.18$	18
ATP	$0.26 \pm 0.24$	20

### 2.3.3. Phytoplankton periodicity and sediment trap recoveries

The periodicity of major algal groups dominant in the epilimnion, and recoveries of algae in traps at 5 m in the pelagic and littoral zones were studied. For this, the concentrations of 'signature' pigments were measured using chromatographic methods. These pigments are: fucoxanthin, associated with Bacillariophyceae and Crysophyceae; alloxanthin, representing Cryptophyceae; chlorophyll-*b*, specific for Chlorophyta and Euglenophyceae; and peridinin, indicating the presence and abundance of Dinophyceae (Fig. 17).

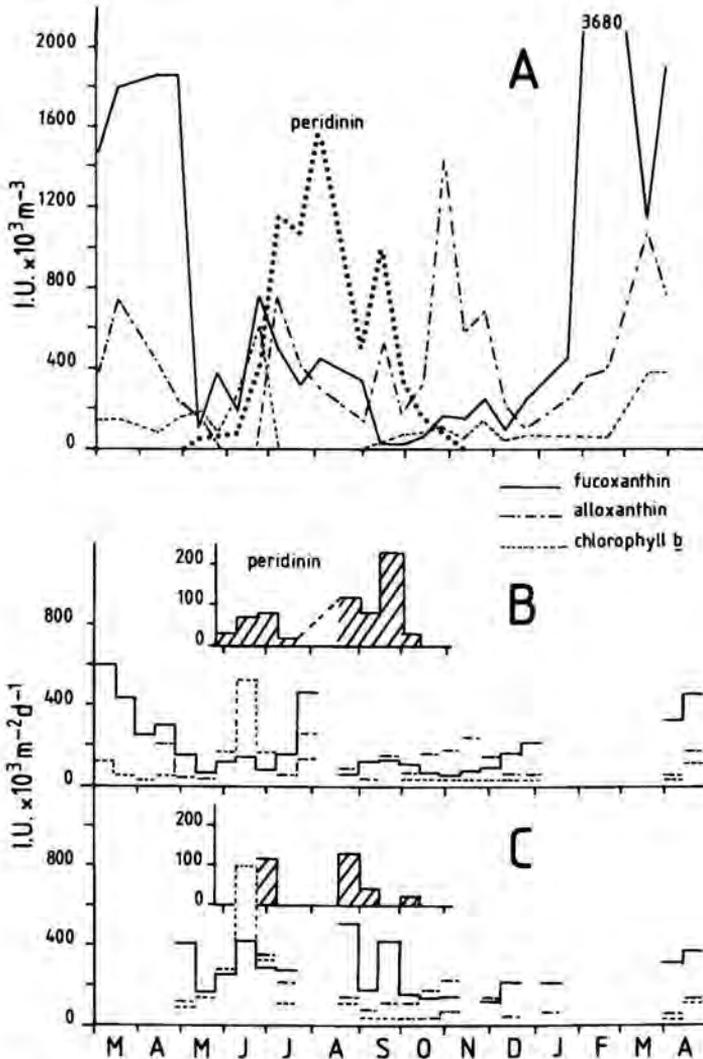


Fig. 17. A. Seasonal changes in the concentrations of signature pigments, expressed in arbitrary integrator units (I.U.), associated with specific algal groups (see text) in mixed samples from the upper 5-m water column; B and C, seasonal fluctuations in trapping rates of signature pigments at a depth of 5 m in the pelagic and the littoral zone respectively. Inset: trapping rate of peridinin; note change in vertical scale.

The seasonal changes in pigment concentrations (Fig. 17A) correspond to the succession of major algal groups in the lake. The early vernal maximum of fucoxanthin was associated with a bloom of *Stephanodiscus* spp. that collapsed rapidly after the onset of thermal stratification. In May phytoplankton densities were low, a common feature that coincides with the annual grazing maximum of herbivorous zooplankton in the lake. Subsequent peaks of fucoxanthin in early summer were related to *Dinobryon divergens*, *Asterionella formosa* and several Centricae.

The numbers and biovolume of Euglenophyceae being of minor importance in the lake, chlorophyll-*b* would be associated mainly with Chlorophyta, especially to Chlorococcales. Its maximum in June was related to colonial green algae (e.g. *Oocystis* spp.). Although present throughout the year, alloxanthin exhibited its maximum in autumn. This coincided with the deepening of the epilimnion and increased epilimnetic turbulence. Recruitment of *Cryptomonas* cells from the metalimnetic phototrophic assemblage which is gradually broken down seems a reasonable explanation for this bloom.

Among Dinophyceae, *Ceratium hirundinella* is important in Lake Vechten. Its seasonal cycle is reflected in the changes in peridinin concentration (Fig. 17A). The population increases exponentially between May and August, declining suddenly in August but recovering somewhat by mid-September, after which it gradually disappeared.

Preliminary data on the *Ceratium* population show that its rapid decline is due to mass encystment. High numbers of cysts were encountered in previous years in both epiphyton and epipelton. The cysts hibernate in the sediment and provide

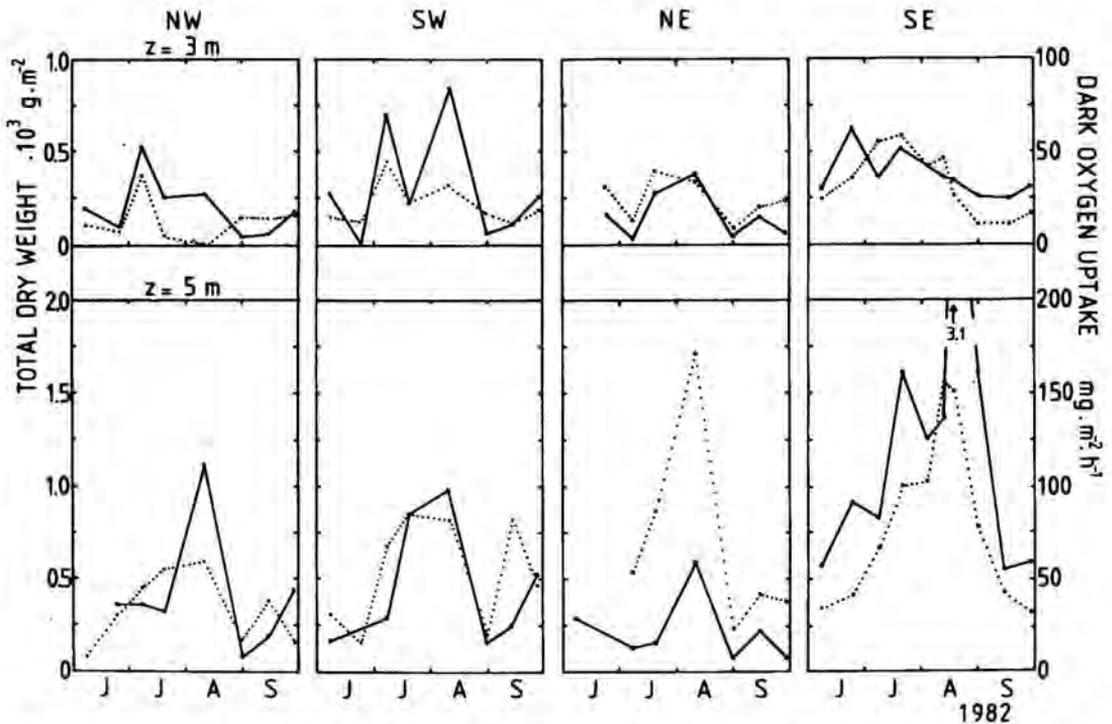


Fig. 18. Distribution of epipellic matter (solid line) and oxygen uptake in the dark (dotted line) along transects through the western (NW and SW) and eastern (NE and SE) depressions of Lake Vechten.

inoculum for subsequent growth. How these cysts escape mineralization is unknown.

Filamentous blue-green algae, particularly *Anabaena spiroides* were observed in early summer. Their biomass seemed unimportant compared with that of the *Ceratium* population. Their 'signature' pigment myxoxanthophyll was found only occasionally in trace amounts.

Although various algal groups differed sharply in sinking behaviour (Section 2.2.5; Fig. 12) the pigment composition of material in the traps broadly reflected the algal periodicity (Fig. 17A, B). Maximum recovery rates of fucoxanthin and chlorophyll-*b* generally coincided with peaks in abundance. This was not true for peridinin and alloxanthin. The failure to collect in the traps quantities of these pigments which are proportional to their concentrations in the water column may have several causes (Section 2.2.5). Apparently, motility of *Ceratium* and *Cryptomonas* spp. may be important in this respect. In addition, the delicate cryptomonads may break down and decompose rapidly in the traps.

Except for peridinin the amount of 'signature' pigments in the littoral traps (Fig. 17C), particularly in summer, was 2 to 3 times that in catches in the pelagic zone (Fig. 17B). The occasional presence of peridinin in the littoral traps may be because of the vertical migration behaviour of *Ceratium*. One can visualize that the density of *Ceratium* cells at 6-7 m, to which depth they can migrate freely, is highest.

That catches of fucoxanthin, alloxanthin and chlorophyll-*b* are higher in the littoral traps than in the pelagic region is difficult to explain. That, besides planktonic, benthic diatoms and benthic green algal species are found in the littoral zone needs verification by microscopic analysis. Benthic and sedimented planktonic forms will be stirred up during moderate wind stress from the shallow sediments. Subsequently, this material may sink to the deeper strata.

Analysis of benthic forms in sediment traps may give clues to the hypothesis of the downward transport of material along the bottom in the littoral zone. High trap catches, particularly in the south-eastern littoral region (Fig. 18), may be explained by the dominant wind-driven circulation. Sedimentation of transported particles is favoured by locally less turbulent conditions.

#### 2.3.4. Spatial distribution of epipelton during summer stratification

In the previous years the epipelton from almost all samples came from the transect in the south-eastern part of the lake. Only in April and May 1981 the observations were made at the north-eastern side. The chemical composition differed considerably between the two sides. The material from the NE-transect had the higher percentage of organic matter, with material in a less advanced state of mineralization than that from the SE side.

In the summer of 1982 epipellic dry weight and oxygen uptake in the dark by epipelton were measured at 3 and 5 m both in the eastern and western basins (Fig. 18). Generally, dry weight was highest in SE and lowest in NE; oxygen uptake was also highest in SE but lowest in NW.

The redistribution of particulates in Lake Vechten varies horizontally both in a quantitative and a qualitative sense. The chemical composition in SE and NE differed even more than in 1981. The percentage organic matter at the NE station was twice as high as at the SE station, both at 3 and at 5 m depth. Also the chlorophyll content per unit organic matter was higher at the NE transect. On an areal basis the chlorophyll-*a* content at both stations was comparable.

The sinking velocities of the epipelton measured in the laboratory confirmed that particles containing organic matter of low caloric value were the last to settle. The areal oxygen uptake rates at the NE and SE sites are comparable despite differences in the concentrations of the total epipellic matter. This may be explained as follows: in the deposition of easily degradable organic matter the two lake parts are similar, but for the lighter particles, in advanced stage of decomposition, the environment in the SE is more favourable for settling.

In the western depression, both the concentrations of epilimon and the areal oxygen uptake were low. This may be because the slope of the bottom at the NW and SW is much steeper than in the eastern lake parts. Furthermore, the horizontal differences may be due to circulation patterns in the lake. In Lake Vechten, however, these patterns are virtually unknown. For quantitative studies relating to the cycling of carbon and other nutrients, these horizontal differences in the decomposition of limnetically produced organic matter deserve special attention.

## 2.4. THE LOOSDRECHT LAKES RESTORATION PROJECT

### 2.4.1. Introduction (L. van Liere)

The Loosdrecht Lakes originated by mining peat from bogs. Peat was excavated from ditches and stacked into banks for drying. This resulted in a pattern of alternating banks ('legakkers') and ditches ('trekgaten') (Photograph on p. 4). By wind and concomitant wave action the banks were greatly eroded creating a series of smaller and larger lakes partially separated by remnants of former dykes (Fig. 19). Later, also sand was excavated and deposited elsewhere in the lakes, partly to create islands. The drinking water reservoir in the northern part of the Loosdrecht Lakes is not connected to the rest of the lakes. It receives groundwater from the Polder Bethune south of the Loosdrecht Lakes via a supply canal. Lake Loenderveen, bordering the drinking water reservoir, is also planned to be converted into a storage basin.

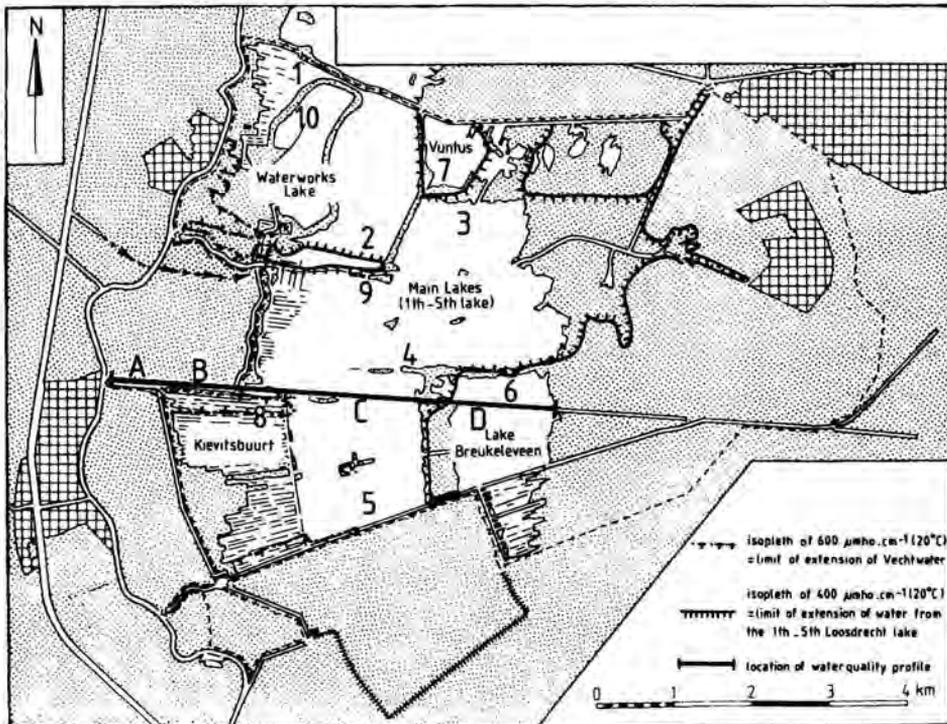


Fig. 19. Map of the Loosdrecht Lakes area. Numbers 1-10 are sampling stations. Isopleths of electrical conductivity ( $\mu\text{mho}\cdot\text{cm}^{-1}$  at 20°C) in September 1982. A-D, see Fig. 20.

The Loosdrecht Lakes receive water from the eastern part of the catchment area where groundwater migrates upward, and lose water to the Polder Bethune which has a lower water level. Seepage to this polder is large. In order to maintain a constant water level in the lakes for recreation purposes, water is supplied from the Polder Bethune and from the nearby river Vecht. This hydrological situation created a lake system known for its clear water and its richness in flora and fauna.

However, the upwelling groundwater from the east was increasingly used for other purposes so that more water from the polluted river Vecht was let into the lakes. Moreover, the expansion of surrounding inhabited areas resulted in discharge of untreated sewage into the lakes. The 'explosion' of recreation activities further deteriorated the lake system severely. In the course of the years the phytoplankton biomass has reached high levels, attenuating the underwater light irradiance such that growth of higher plants is limited and even large fields of Characeans have disappeared. The lakes now range from eutrophic to grossly contaminated depending on location and nutrient loading.

In the near future the water supply from the river Vecht will be stopped and instead dephosphorized water from the Amsterdam-Rhine Canal (ARC) will be let into the lakes. Also the surrounding inhabited areas nowadays treat their sewage and transport effluents elsewhere. These management measures are likely to reduce the annual external phosphorus load by an order of magnitude.

The process of restoration will be studied by eight institutes, namely, the Municipal Waterworks of Amsterdam, the Provincial Water Authority of Utrecht, the Research Institute for Nature Management, The Microbiological Laboratory of the University of Amsterdam, the National Institute for Water Supply, the Delft Hydraulics Laboratory, the Department of Hydro-geology and Geographical Hydrology of the Free University, and the Limnological Institute.

The aspects to be studied include: hydrology of surface- and groundwaters, material balances of the several compartments, exchange processes of phosphorus across the sediment/water interface, the physico-chemical situation, primary and secondary production relating to phytoplankton and zooplankton, physiological parameters of phytoplankton, dynamics of epipelon, macrophyte studies etc. The results of the experimental and field studies will be integrated by mathematical modelling, among others to simulate effects of supplementary management measures. Although the various institutes contribute substantially to the research programme the Dutch authorities have subsidized a number of the projects.

The progress of the work done in 1982 at the Limnological Institute related to the collecting of base line information is presented in the next paragraphs.

#### 2.4.2. Hydrology (B.F.M. Kal, P.C.M. Boers - Workgroup Mineralization of Organic Matter)

The aims of this research project are:

- establishing balances for water, P, N, and Cl not only for the whole system on an annual basis, but also for subsystems for periods of one month or shorter to provide a basis for better understanding of water quality;
- modelling hydrochemical budgets and water quality, the latter in connection with the research on biological and sediment properties of the lake-system.

The Department of Hydro-geology and Geographical Hydrology of the Free University rendered assistance for analysis of water samples and computer facilities.

In September observations on water quality and properties of sediment were made to collect base line data before inlet of ARC-water. In this period the accumulated effect of supplied amounts of polluted water of the river Vecht was at its maximum. Electrical conductivity (e.c.) of water, measured in the field, is fairly proportional to the content of total dissolved solids. In the Loosdrecht area total dissolved solids strongly decline in the range of Vecht-water, water from the main Loosdrecht Lakes, rain water and upwards seeping groundwater. Therefore, e.c. data are suitable to trace the dispersion of Vecht-water into the Loosdrecht Lakes, and through these lakes into other lakes and into the smaller

water-ways crossing the adjacent land. Vecht-water enters the area mainly through two sluices at the west side. Its course can be followed using the e.c. values ( $\geq 600 \mu\text{mho}\cdot\text{cm}^{-1}$  at  $20^\circ\text{C}$ ) only during its passage through the Kievitsbuurt (Fig. 19). It quickly mixes with the water in the main Loosdrecht Lakes. Penetration of this water (e.c.  $\geq 400 \mu\text{mho}\cdot\text{cm}^{-1}$  at  $20^\circ\text{C}$ ) far into the eastern area is facilitated by occasional pumping in summer which supplies lake water to the rural area north and east. Apparently, less water from the main lakes penetrates into the more isolated Lake Vuntus and Lake Breukeleveen.

The P and N concentrations in the surface water decrease strikingly more than the e.c. from the points of Vecht-water entrance to the main lakes (Fig. 20). That is because in the lake water diluting the inflowing Vecht-water concentrations of these nutrients are much lower than concentrations of the more conservative, e.c.-determining ions; this reflects the nutrient-fixing capability of the system. Total concentrations of P and N tend to be higher in Lake Vuntus and especially in Lake Breukeleveen than in the main lakes. Thus, the Vecht-water is not the decisive factor in explaining the hypertrophic nature of Lake Breukeleveen and Lake Vuntus.

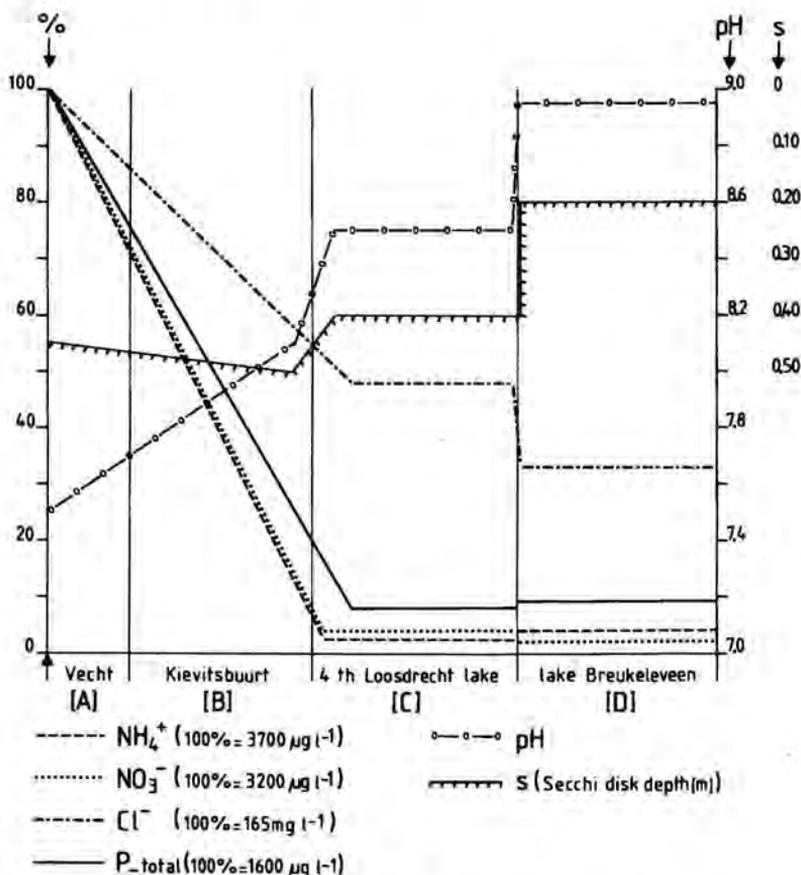


Fig. 20. Schematic diagram of the distribution of nutrients (P, N), chloride, pH and light climate from the entering-point of Vecht-water in the Loosdrecht Lakes to Lake Breukeleveen. A - D as in Fig. 19.

Besides the mapping of water quality by means of e.c.-values data on meteorology, water management and groundwater heads need to be collected for establishing monthly water balances. Also water quality data of surface water from 1975 onwards are being put on magnetic tape. The present hydrological network is extended with three recording level-gauges (in the main Lakes, Lake Vuntus and Lake Breukeveen) and with a recording wind gauge.

**2.4.3. Exchange of phosphorus compounds between sediment and water**  
 (P.C.M. Boers, J.W.Th. Bongers, A.G. Wisselo - Workgroup Mineralization of Organic Matter)

Lake restoration is often retarded by internal phosphorus loading caused by release of the accumulated phosphorus from the sediments. The aim of this project is to gain insight into the mechanisms of exchange processes of phosphorus compounds between sediments and overlying water in the Loosdrecht Lakes, and to quantify these processes.

The variations in sediment composition and structure are large. Sediments can consist of sand, peat, gyttja and mixtures. This makes the Jenkin mud sampler unsuitable for taking undisturbed mud cores. To overcome this problem a hand driven mud sampler was constructed.

The local differences in sediment structure also call for a sediment map which can provide insight into the processes causing phosphorus accumulation in the sediment and can greatly help to choose sampling stations for future work. For preparing such a map about 40 samples were taken, covering the whole lake

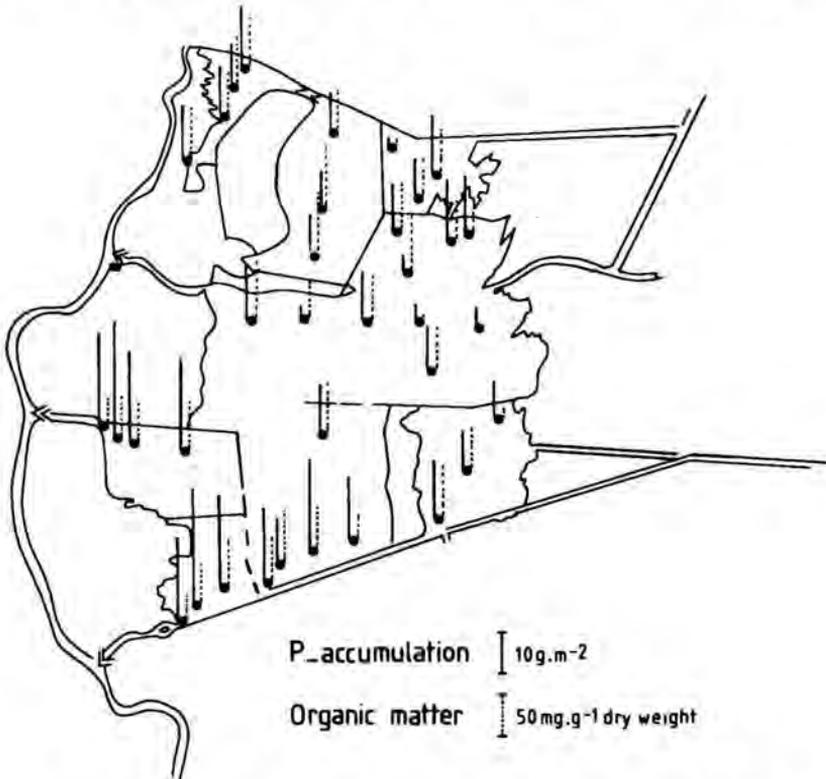


Fig. 21. Phosphorus accumulation in sediments (0 - 18 cm) in the Loosdrecht Lakes.

Table 6. Composition of a sediment core at station 11 (see Fig. 19).  
All concentrations in mg.(g dry weight)<sup>-1</sup>, except water (%)

Depth (cm)	H <sub>2</sub> O (%)	Org. matter	Org. C	N	CaCO <sub>3</sub>	Fe	Al	Ca	P
0 - 2	92	570	270	14	28	20	8.8	26	1.09
2 - 4	90	720	390	18	22	16	8.9	26	0.69
4 - 6	90	780	420	19	21	15	9.5	24	0.54
6 - 10	89	770	430	19	25	15	9.2	24	0.45
10 - 14	90	870	490	22	34	12	3.8	23	0.38
14 - 18	90	930	530	26	38	11	0	20	0.43

area. These samples were divided into fractions of 0-2, 2-4, 4-6, 6-10, 10-14 and 14-18 cm to determine the water content, organic matter and total phosphorus (Table 6). Interestingly, both the water content and amounts of organic matter are high, with the latter having a positive gradient downwards. The relatively high concentration of organic matter in the deeper segments is due to the peat layers.

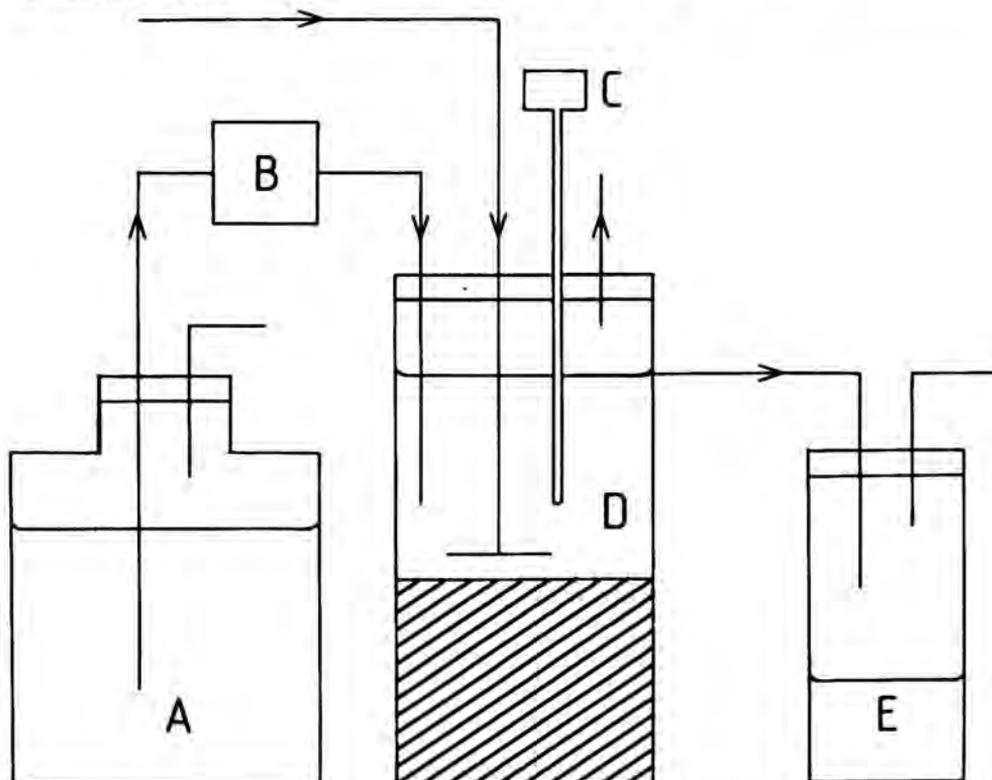


Fig. 22. Flow-chart of continuous phosphorus release reactor.  
A medium reservoir  
B peristaltic pump  
C pH-electrode  
D exchange compartment, top: water, bottom: sediment  
E effluent

From the water and phosphorus contents of the samples, the phosphorus content of the upper 18 cm was calculated and plotted (Fig. 21). Apparently phosphorus accumulation does not differ except at Kievitsbuurt near the inlet of phosphorus-rich Vechtwater where accumulation was high, and at some sampling stations with a sandy sediment where accumulation was low.

The phosphorus release rate of sediment cores was studied in the laboratory using a continuous phosphorus release reactor (Fig. 22). The reactor vessel is the polyacrylate inner core of the mud sampler. The reactor is kept at *c.* 20°C, the pH is monitored and the water flow rate is kept at about 10 ml.h<sup>-1</sup>. This results in a retention time of one day. At regular intervals the water pumped out of the vessel is sampled to determine soluble, reactive phosphorus (SRP). From two consecutive determinations with a known time interval, dilution rate and the dimensions of the reactor vessel, the phosphorus release rate can be calculated. For a typical example of such a release rate see Fig. 23. The results of experiments with cores taken from several sampling stations are given in Table 7. These results indicate that the release rates are mostly low compared with the mean external phosphorus loading (5.5 mg P m<sup>-2</sup>.d<sup>-1</sup>), but the rates are in the same order of magnitude as the expected external loading in the future (0.6 mg P m<sup>-2</sup>.d<sup>-1</sup>). The cores from the Kievitsbuurt, however, have high P release rates.

The phosphorus fractions in the sediments were determined following Hieltjes & Lijklema (J. Env. Qual. 9, 402-407, 1980). Extraction with NH<sub>4</sub>Cl gives 'loosely bound inorganic phosphorus', extraction with NaOH gives 'Fe- and Al-bound phosphorus' and that with HCl gives 'Ca-bound phosphorus'; the remainder phosphorus is called 'non-extractable phosphorus' and consists of phosphorus incorporated in silicate crystals and organic phosphorus compounds. However, it should be emphasized that the results of the extractions are determined by the technique used and no agreement exists about the extraction procedure. Another problem with this technique is the co-extraction of large amounts of brown humic acids with NaOH. These acids make a direct SRP determination cumbersome and contain considerable amounts of phosphorus. These humic acids can be removed by precipitation with acids followed by filtration, but the structure of the humic acids is altered during the process of extraction and precipitation, and therefore it is impossible to prevent changes in their phosphorus content. The phosphorus, co-precipitated with the humic acids is called 'humic acid bound phosphorus'.

A typical example of the results obtained with this extraction procedure is presented in Table 8. Generally, the sum of the NH<sub>4</sub>Cl- and NaOH-fractions is considered as 'exchangeable'. This seems not to be compatible with the low re-

Table 7. Phosphorus release rates of sediment cores in the first 30 days of an experiment.

Station*	Date of sampling	Release rate (mg P m <sup>-2</sup> .d <sup>-1</sup> )
4	09-06-82	0.4
6	04-08-82	0.4
12	15-09-82	15.0
10	29-09-82	6.7
3	27-10-82	0.8
9	27-10-82	0.5

\* for the location of the sampling stations see Fig. 19.

Table 8. Various phosphorus fractions in a sediment core (ppm)

Depth (cm)	P in sed.	NH <sub>4</sub> Cl-P	NaOH-P	Humic acid-P	HCl-P	not-extractable P
0-2	829	30	163	247	192	206
2-4	660	-*	151	217	181	122
4-6	547	-	138	161	108	147
6-10	492	-	98	107	96	180
10-14	471	-	128	130	78	152
14-18	480	-	86	142	93	168

\* = not determined

lease rates observed, which might be due to the high amounts of humic acid bound phosphorus.

The present research is focussed on the following aspects:

- chemical and physical properties of the sediment phosphorus determined by chemical extractions, with special attention to organic phosphorus compounds and phosphorus release experiments with anion exchange resins;
- biologically available sediment phosphorus and algal bioassays;
- completion of the sediment map by determinations of elemental composition (C, N, Fe, Mn, Ca, Al) of a selected number of sediment samples;

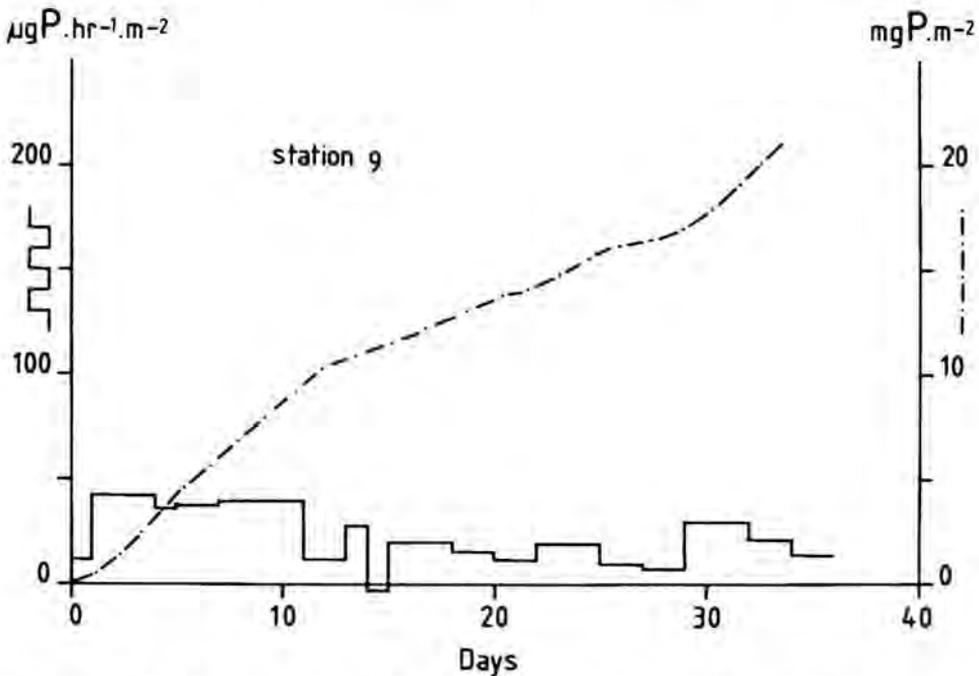


Fig. 23. Phosphorus release behaviour of sediment cores from station 9.

- continuation of the phosphorus release experiments, in which the phosphorus concentrations in the pore water will be also measured and
- phosphorus compounds and electron acceptors in the sediment pore waters.

2.4.4. *Phytoplankton pigment composition* (C.L.M. Steenbergen, H.J. Korthals, C. de Vlieger, M.J. Westhoff - Workgroup Mineralization of Organic Matter)

The study on phytoplankton pigment composition in the Loosdrecht Lakes, started in 1981 (Westhoff, 1982), was continued through autumn, winter and spring of 1982 (De Vlieger, 1982). The main purpose of the study was to collect base line information on periodicity and relative abundance of the major algal groups in view of the planned reduction of the external phosphorus loading rates in the Loosdrecht Lakes.

Two-dimensional ascending paper chromatography was used to separate pigment fractions. The presence of major algal types could be established from the presence of specific pigment fractions. The components chlorophyll-*b* and lutein indicate the presence of Chlorophyta and Euglenophyta. Chlorophyll-*c* and fucoxanthin point to the presence of Bacillariophyceae and Chrysophyceae. Zeaxanthin and myxoxanthophyll mark the presence of Cyanophyceae. Peridinin, that is specific for the occurrence of Dinophyceae, was found occasionally in trace amounts indicating that this algal group was of minor importance. From the amounts of the specific pigment fractions the relative contribution of the major algal types to the total phytoplankton community can be estimated (Fig. 24).

The microscopic observations (Section 2.4.5) indicate that Euglenophyta and Chrysophyta were of minor importance. So, Chlorophyta, Bacillariophyceae and especially Cyanophyceae are the major primary producers in the Loosdrecht Lakes. Various representatives of these three groups are present throughout the year in varying quantities. Cyanophyceae were particularly abundant dur-

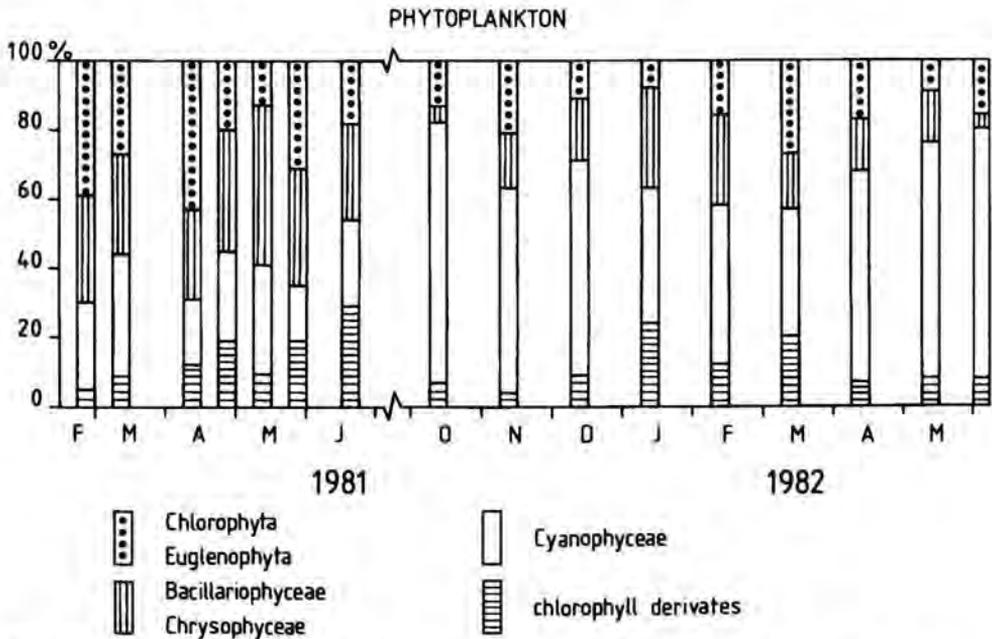


Fig. 24. Seasonal changes in the relative contribution of phytoplankton groups and chlorophyll breakdown products to total light absorption. Mean of results of stations 3 and 9, located in the Loosdrecht Lakes (see Fig. 19).

ing the autumn of 1981, when they accounted for about 70 percent of the total light absorption in the extracted samples (Fig. 24). In winter their share in the light absorption decreased gradually to about 35 percent and Bacillariophyceae and, to a lesser extent, Chlorophyta became relatively more important in the phytoplankton community. From April 1982 onwards Cyanophyceae again dominated the phytoplankton (Fig. 25). Their share in light absorption was two to three times that in the spring of 1981. This remarkable high density of Cyanophyceae observed during the spring in 1982 was also found in the rest of the year particularly during the late summer period.

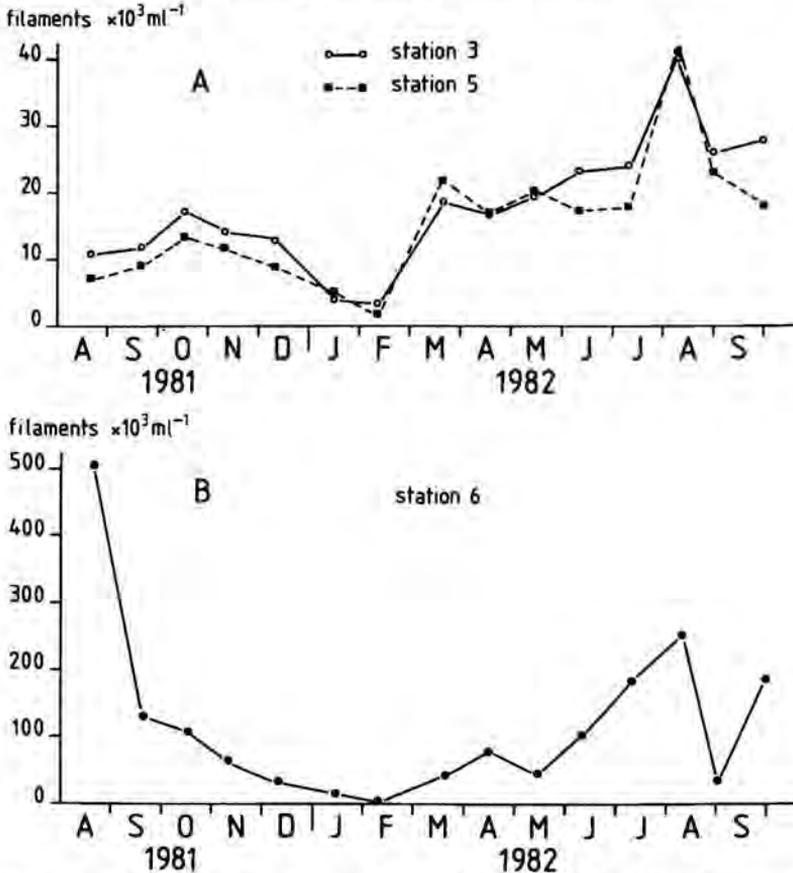


Fig. 25. Seasonal changes of filamentous Cyanophyceae densities at three sampling stations in the Loosdrecht Lakes.

#### 2.4.5. Phytoplankton species composition (P.J. Boesewinkel-de Bruyn, B.Z. Salomé - Workgroup Primary and Secondary Production)

Phytoplankton densities in the Loosdrecht Lakes were counted at five stations (see Fig. 19 for sampling stations). The study is financed by the Beyerinck-Popping Fund.

Samples were taken monthly from August 1981 through September 1982 with a Friedinger sampler from surface to 1 m depth, fixed with Lugol and counted with an inverted microscope (magnification 250 x) using the Utermöhl technique. Usually at least 100 specimens of each taxon were counted.

Filamentous blue-green algae (Cyanophyceae) were mostly thin forms c. 2  $\mu\text{m}$  thick and ca 200  $\mu\text{m}$  in mean length. They were abundant but generally could not be assigned to a genus. A small percentage only of these threads undoubtedly belonged to *Lyngbya*. Among the broader filaments *Oscillatoria redekei* and *Aphanizomenon flos-aquae* reached high densities. The Cyanophyceae at stations 3 and 5 reached a concentration of about 3,000 filaments  $\text{ml}^{-1}$  in February, rising to 40,000  $\text{ml}^{-1}$  in August (Fig. 25). In the more isolated Lake Breukeleveen (station 6) densities were much higher, varying from 6,000  $\text{ml}^{-1}$  in February to 256,000  $\text{ml}^{-1}$  in August 1982 and 500,000  $\text{ml}^{-1}$  in August 1981. At stations 3 and 5 the densities in 1981 and 1982 differed markedly, those in September 1982 being twice as high as those in August and September 1981 (see also section 2.4.4).

Compared with numbers of blue-green algae, the other algal groups, especially Chrysophyceae and Euglenophyta were represented poorly. Genera as *Scenedesmus*, *Pediastrum*, *Dinobryon*, *Phacus*, *Euglena*, *Trachelomonas*, *Asterionella* and *Diatoma* were scarce at station 6, but were encountered regularly at stations 3 and 5. The larger Centricae, on the other hand, reached high densities at station 6; they peaked in March and, at stations 3 and 5, in September also. *Melosira* sp. exhibited a peak in September in Lake Breukeleveen.

Samples from stations 8 and 9 were also studied, namely those taken on 23 September 1981, and on 18 March, 12 May and 1 September 1982. Both, densities of the frequently encountered genera and the total number of genera at different stations were compared. Chlorophyta were more abundant at station 8, but Cyanophyceae relatively less than at stations 3 and 5. Station 9 appears to be more or less intermediate between stations 3 and 5 on one hand, and station 8 on the other.

Concluding, Lake Breukeleveen, which is the most isolated among the lakes investigated, differs strikingly from the other lakes; blooms of blue-greens persist during the greater part of the year, but the densities of other algae are comparatively low. The stations 3 and 5 show hardly any differences. Station 8 differs in having less blue-greens but more Chlorophyta.

#### 2.4.6. Primary production of phytoplankton (W.A. de Kloet - Workgroup Primary and Secondary Production)

The phytoplankton production study in the Loosdrecht Lakes, started in 1981, was continued. The  $^{14}\text{C}$ - and the  $\text{O}_2$ -techniques for measuring production were investigated.

##### Comparison of the $^{14}\text{C}$ - and $\text{O}_2$ -techniques

The  $^{14}\text{C}$ -technique was examined comparing the photosynthetic quotient (PQ) i.e. ratio mol  $\text{O}_2$  production :  $\text{CO}_2$  fixation. The high production rates in the Loosdrecht Lakes (Fig. 26) facilitated a comparison of the techniques. Production rates were measured both *in vitro* and *in situ* to compare the techniques (Table 9). It was assumed that the  $^{14}\text{C}$ -technique measured net primary production using an average incubation time of 3 hours. The average PQ ( $n=12$ ) was 1.45.

Table 9. Means and ranges of photosynthetic quotient (PQ) in the Loosdrecht Lakes ( $n$  = number of experiments).

Incubation	Light conditions	n	PQ	
			range	mean
<i>in vitro</i>	13W33 Philips, 15 $\text{W.m}^2$	6	1.15-1.71	1.44
<i>in vitro</i>	13W33 Philips, 30 $\text{W.m}^2$	2	1.22-1.77	1.50
<i>in situ</i>	0.25 m depth, 25-100 $\text{W.m}^2$	2	1.32-1.65	1.49
<i>in situ</i>	1.0 m depth, 3-5 $\text{W.m}^2$	2	1.23-1.54	1.39

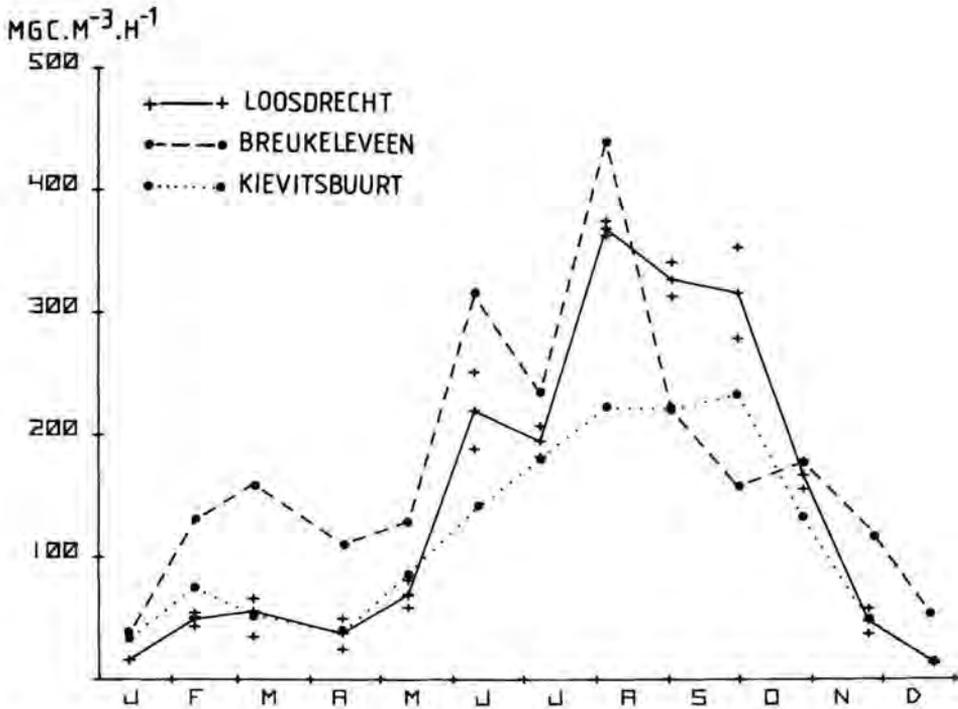


Fig. 26. *In vitro* phytoplankton production rates in the Loosdrecht Lakes.

In future both  $O_2$  and  $^{14}C$  but especially the latter technique will be employed, since the PQ value of 1.45 was high compared with reported values ranging between 1.0 and 1.4.

#### Production studies

The Loosdrecht Lakes, including Lake Breukeleveen, were sampled at four stations at four-weekly intervals.  $^{14}C$ -incubations were done *in vitro* for 3 hours using *in situ* temperature but a constant light intensity of c.  $15 W.m^{-2}$  (white light 13W33 Philips). From 9 June onwards, the production rates were measured *in vitro* using the  $O_2$  technique. Incubation conditions were the same as mentioned before, but light intensity was raised to  $30-35 W.m^{-2}$ . The PQ of 1.45 was used to transform the  $O_2$ -production rates into C-fixation rates as already mentioned.

The carbon fixation rates in the main Loosdrecht Lakes (stations 3 and 9 and until June 1982 station 5, see Fig. 19) ranged from 14 on 22 December to  $370 mg C m^{-3}.h^{-1}$  on August 4, the mean for the period being  $144 mg C m^{-3}.h^{-1}$  (Fig. 26). The rates in Lake Breukeleveen ranged from 36 to  $440 mg C m^{-3}.h^{-1}$ , on 13 January and 4 August respectively. These rates are 2-3 times higher than those in the main Loosdrecht Lakes in the period November 1981 - May 1982. During this period a spring maximum was observed in March. However, as the phytoplankton production rates raised sharply from June in both lakes, the rates in the two lakes were comparable but the values found during September in Lake Breukeleveen were even lower than those in the main Loosdrecht Lakes. During winter and in the spring the relatively higher phytoplankton production rates in Lake Breukeleveen most likely resulted in higher seston biomass and lower transparency than in the main Loosdrecht Lakes. In this period the Secchi

depth (S) was 30 - 45 cm and the vertical extinction coefficient ( $\epsilon_V$ , 400 - 700 nm) 4.4 - 5.1  $m^{-1}$  in Lake Breukeleveen compared with S-values of 50 - 85 cm and  $\epsilon_V$ -values of 2.4 - 4.3  $m^{-1}$  in the main Loosdrecht Lakes.

The production rates in the Kievitsbuurt (station 8) were during November 1981 - May 1982 comparable with those in the main lake and lower during the period of high production (June-October (Fig. 26)).

O<sub>2</sub>-production rates were also measured *in situ* on seven dates between May and November (Table 10) at station 9 (Fig. 19) at 0, 25, 50, 100, 150 and 200 cm depths. In May and November the water was more clear than in the preceding period. Consequently, the compensation depth ( $Z_{comp}$ ) was greater than the depth of the photogenic layer ( $Z_{1\% I_0}$ ). Thus, the total net production was higher on a clear, sunny day, e.g. on 26 May, than when the seston concentration was high, e.g. on 19 August. A production maximum was observed on 15 September.

Table 10. The *in situ* production rates in the upper 2 m and light transmission at station 9 in the Loosdrecht Lakes. BP, gross production; NP, net production; R, dark uptake of O<sub>2</sub>; -, not measured (see further in text).

Date	Temp. °C	Radiation J.cm <sup>-2</sup> .d <sup>-1</sup>	ΣBP mgC m <sup>-2</sup> .h <sup>-1</sup>	ΣNP mgC m <sup>-2</sup> .h <sup>-1</sup>	R %BP	NP <sub>max</sub> mgC m <sup>-3</sup> .h <sup>-1</sup>	I <sub>max</sub> %I <sub>0</sub>	Z <sub>comp</sub> cm	ε <sub>V</sub>	Z <sub>1%I<sub>0</sub></sub> cm
18/5	17	1337	-	93	-	114	35	190	3.0	155
26/5	23	2865	211	171	19	264	27	150	4.0	115
24/6	-	1141	112	32.5	70	132	50	95	5.5	90
19/8	17	2077	221	112	49	372	-	75	-	-
15/9	18	1434	335	191	43	417	47	100	4.6	100
13/10	12	355	155	78	50	210	70	100	4.9	95
10/11	-	182	73	50	32	89	50	160	3.7	125

The validity of the *in vitro* production rates was tested by comparing the rates with those collected *in situ* under comparable light intensity (Fig. 27). The differences between the two rates were not significant ( $p < 0.05$ ).

#### 2.4.7. Macrophytes (E.H.P. Best, J.H.A. Dassen - Workgroup Primary and Secondary Production).

To get an insight into the species number and the vegetation types present before the restoration starts, a survey of the aquatic plants was made. The survey, started in 1980 with submerged and floating-leaved species, was now completed by including the emergent macrophytes. These surveys will be repeated every 5 years.

In order to predict the possibilities for recolonization of the whole area and growth by submerged and floating-leaved aquatic plants, a seed bank inventory was started. Bottom samples were taken a) at sites where according to the literature submerged plants formerly occurred, b) in front of the zone of emergent plants and c) at the main inlets through which boats might transport seeds or other propagules. In all samples characean oogonia were present, although particularly in the main Loosdrecht Lakes these plants at present are absent. It has to be tested whether or not these oogonia, and also the other seeds present in the samples, are viable, since data on the germination potential of aquatic macrophyte seeds are virtually lacking.

In this mainly eutrophic area light and temperature will probably limit growth

of macrophytes. A study of the under-water light climate in the littoral zone was started, as it is expected to improve during the restoration. Light extinction coefficients were measured bi-weekly at the sites already colonized and those likely to be colonized in the near future. The extinction coefficients for the solar irradiation (400 - 700 nm) were calculated for 1 m depth, a normal colonization depth for macrophytes. Since this study was started in July nothing can be said so far about spring time, which is more important for the growth of macrophytes. However, during September and early October, when the plants are stocking their reserves, less than 2 percent of the surface radiation reached 1 m depth in the main Loosdrecht Lakes whereas in the Loenderveen Lake and the Drinking Water Reservoir light reaching 1 m was always more than 2 percent. The minimum light required to sustain growth in submerged plants is generally accepted to be 2 percent of the incoming radiation.

#### 2.4.8. Seston - epipelon interrelationships (H.J. Gons, R. van Keulen - Workgroup Primary and Secondary Production)

Epipelon was quantitatively collected from a stretch of sandy bottom in the central part of the Loosdrecht Lakes. The chemical characteristics, algal species composition and dark oxygen uptake rates of epipelon and seston were compared. The percentages of organic matter and carbonate in the epipelon and seston were similar, but the C : N ratios of organic matter in seston were consistently lower than those in epipelon. Filamentous blue-green algae, mainly *Lyngbya* sp., predominated both the phytoplankton and the epipellic algae, with chroococcal blue-greens being encountered only in the epipelon and filamentous green algae only

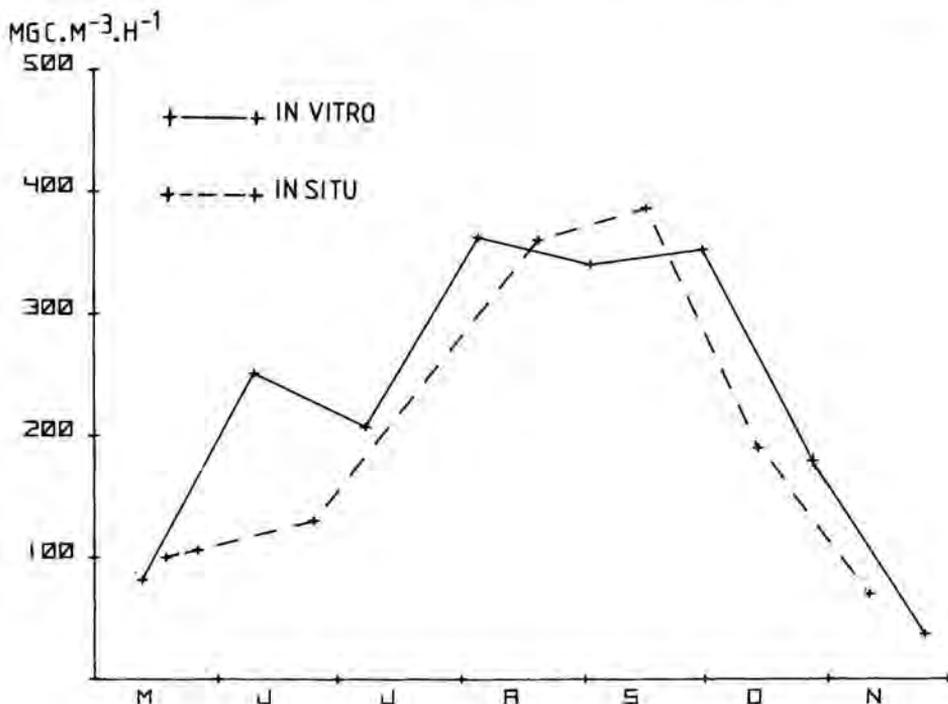


Fig. 27. *In vitro* and *in situ* production rates at station 9 in the Loosdrecht Lakes under comparable light intensities.

in the phytoplankton. Maximum integrated values of seston and epipelton, expressed as chlorophyll-*a* and as dark oxygen uptake rate were  $0.3 \text{ g.m}^{-2}$  and  $0.3 \text{ g.m}^{-2}.\text{h}^{-1}$ , respectively. The contribution of epipelton to these both values was small.

#### 2.4.9. Zooplankton grazing (R.D. Gulati, K. Siewertsen, G. Postema - Workgroup Primary and Secondary Production)

The baseline study on the structure and grazing of zooplankton in the Loosdrecht Lakes and Lake Breukeleveen, started in July 1981, was continued. Up to August, the routine research programme was the same as in the last year. Starting in September station 5 was employed as a representative for the Loosdrecht Lakes but in November also station 9 was included. The routine work at stations 3 and 8 has since been suspended (see Fig. 19 for sampling stations). During September - November besides the routine grazing study four experiments using dilution series were carried out. The food concentrations varied from those *in situ* to well below the incipient limiting level.

#### Structure of zooplankton

Ten species of crustaceans and nineteen of rotifers were recorded in the lakes. Among crustaceans the density of *Eudiaptomus gracilis* did not exceed a few individuals per litre in the main Loosdrecht Lakes, only stray individuals being recorded in Lake Breukeleveen. This species is known to occur less frequently in eutrophic lakes in the Netherlands. *Diaphanosoma* sp. also occurred in low numbers and was absent in Lake Breukeleveen. *Bosmina longirostris* and *B. coregoni* were the most dominant cladocerans in the main Loosdrecht Lakes, the mean annual densities varying between  $109 \pm 136$  and  $198 \pm 369 \text{ ind.l}^{-1}$  at stations 5 and 9, respectively. In Lake Breukeleveen, both *Bosmina* spp. ( $264 \pm 361 \text{ ind.l}^{-1}$ ) and *Chydorus sphaericus* ( $226 \pm 269 \text{ ind.l}^{-1}$ ) were the major filter-feeders.

Among the cyclopoid copepods, *Cyclops* spp. particularly *C. vicinus* was dominant and together with nauplii had an important share in the crustacean zooplankton.

The rotifers were dominated by *Anuraeopsis fissa*, a form typical of eutrophic lakes (Table 11). It formed between 22 and 35 percent of the rotifer populations in the Loosdrecht Lakes and 55 percent of the rotifers in Lake Breukeleveen. In this lake, the mean annual density ( $2387 \pm 3242 \text{ ind.l}^{-1}$ ) is among the highest recorded in temperate lakes. The second most dominant species was *Keratella cochlearis*, followed by *Polyarthra* spp. (*P. vulgaris* and *P. euryptera*). *Filinia longiseta* was sparse in the main Loosdrecht Lakes, but with a mean annual density of  $547 \pm 1293$  quite common in Lake Breukeleveen.

Since simultaneous with the density (N) measurements community biomass (B) was also determined, an attempt is made to calculate the average weight per individual ( $\bar{W} = \bar{B}/\bar{N}$ ). This is confined to the crustacean community, with omission of nauplii that were assumed to weigh  $0.1 \mu\text{g C ind}^{-1}$ . The average weight per individual irrespective of the species was as follows:

Lake	Loosdrecht				Breukeleveen
Station	3	5	8	9	6
Wt. $\mu\text{g C.ind}^{-1}$	1.04	1.07	1.03	1.08	0.71

Strikingly, the average weights per animal at all stations in the main Loosdrecht Lakes were quite similar and that in Lake Breukeleveen significantly lower. These weights in both the lakes will be lower, more so in Lake Breukeleveen, if the mean community biomass is corrected for rotifer biomass. Although a rough estimate, the mean individual weights may provide information about the changes in the course of years in size structure of the community related to those in the

Table 11. Annual means with standard deviation (ind.l<sup>-1</sup>) of zooplankton in the Loosdrecht Lakes (Stations 3, 5, 8, 9 and their grand mean, GM) and in L. Breukeleveen (station 6).

Station (see Fig. 19)		3	5	8	9	GM	6
Total Crustaceans	$\bar{N}$	488	480	622	604	548	939
Filterfeeders*	$\bar{N}$	232	227	194	305	239	530
	SD	256	253	428	428	309	409
<i>Cyclops</i>	$\bar{N}$	75	80	152	105	103	106
	SD	53	54	111	90	35	87
Nauplii	$\bar{N}$	181	173	276	194	206	303
	SD	114	94	165	109	47	145
Total Rotifers	$\bar{N}$	2204	1984	2376	2224	2175	4330
<i>Anuraeopsis fissa</i>	$\bar{N}$	766	658	512	673	652	2387
	SD	1147	1021	754	1056	105	3242
<i>Keratella cochlearis</i>	$\bar{N}$	786	578	802	679	711	481
	SD	989	793	823	722	104	905
Others	$\bar{N}$	652	748	1060	872	812	1462

\* Include all the herbivore Cladocera and *Eudiaptomus* sp. if present, but except nauplii.

trophic level. Undoubtedly, for a conclusive evidence, information on the role of fish predation in structuring zooplankton in these lakes is indispensable.

#### Grazing studies

The mean daily grazing based on annual data (July 1981–August 1982) varied between  $13 \pm 17.4\%$  and  $22 \pm 35\%$  at the four stations in the main Loosdrecht Lakes and was  $23.8 \pm 29\%$  in Lake Breukeleveen. The seasonal variations in the grazing activity were large. This was also true for the assimilation efficiencies (5–67%) and daily rations (25–1600%). These large variations are difficult to explain, though they do reflect the possibilities of sharp changes in the food quality and even accidental ingestion by zooplankton of food particles with abnormally high specific activity. That contamination of the zooplankton samples could be due to the labelled filamentous algae or other large particles was tested by comparing the community uptake with the sum of uptakes of different populations in the community. The latter is likely to suffer much less from contamination with radioactive food since individual animals are sorted one for one, so that chances that active algae will adhere are considerably reduced.

The filtering rates of individual filter-feeder species together with the density of these species provide information about the share of different species in the community grazing and thus the food uptake. *Bosmina* spp. were important in this respect, followed by *Daphnia* sp. Surprisingly, *Cyclops* sp. consumed a substantial amount of seston food in the late summer of 1981 as well as in early spring.

In the experiments using different dilutions of *in situ* food it appears that in short acclimation periods of an hour in the drastically reduced food concentrations the animals could not fully compensate and filtered water at a much slower

rate than would be expected on the basis of the reduced food concentration.

The grazing data collected so far in these and several other lakes were used to construct a general model for lakes varying in their trophic degree, using food concentration (F) and SFR (Y), namely the specific daily filtering rate (SFR) in  $\text{ml} \cdot \text{d}^{-1} \cdot \text{mg}^{-1}$  zooplankton carbon. The annual mean water temperature being more or less similar in all the lakes ( $\pm 13^\circ\text{C}$ ) the relation between SFR and food was found to be significant ( $\text{SFR} = 1333 \cdot \text{F}^{-0.487}$ ;  $P < 0.001$ ;  $N = 26$ ) but limited to a temperature of  $13^\circ\text{C}$ .

The role of temperature in controlling the SFR in the Loosdrecht Lakes was examined further using only the Loosdrecht data from stations 3, 5 and 8. This was done in a separate multiple-regression relationship ( $\text{SFR} = a \cdot X_1^{b_1} \cdot X_2^{b_2}$ ) between SFR (Y), water temperature ( $X_1$ ) and food concentration ( $X_2$ ). The temperature coefficient  $b_1$  of 1.22 thus derived was inserted into the relationship  $\text{SFR} = 1333 \cdot \text{F}^{-0.487}$  to derive a generalized model:  $\text{SFR} = 57.4 \cdot \text{T}^{1.226} \cdot \text{F}^{-0.487}$  specially valid for the Loosdrecht Lakes (Fig. 28).

The observed SFR values at station 9 (Fig. 28) were compared with those calculated from the model based on data from stations 3, 5 and 8. There is a fairly good agreement between the observed and the calculated values, although the model generally overestimates the observed values. In contrast, the linear multiple regression model using data from the Loosdrecht Lakes only (Fig. 28) underestimates SFR in the summer and overestimates it in winter months. This latter is, however, due to horizontal differences in grazing activities which were lower at station 9 than at stations 3, 5 and 8.

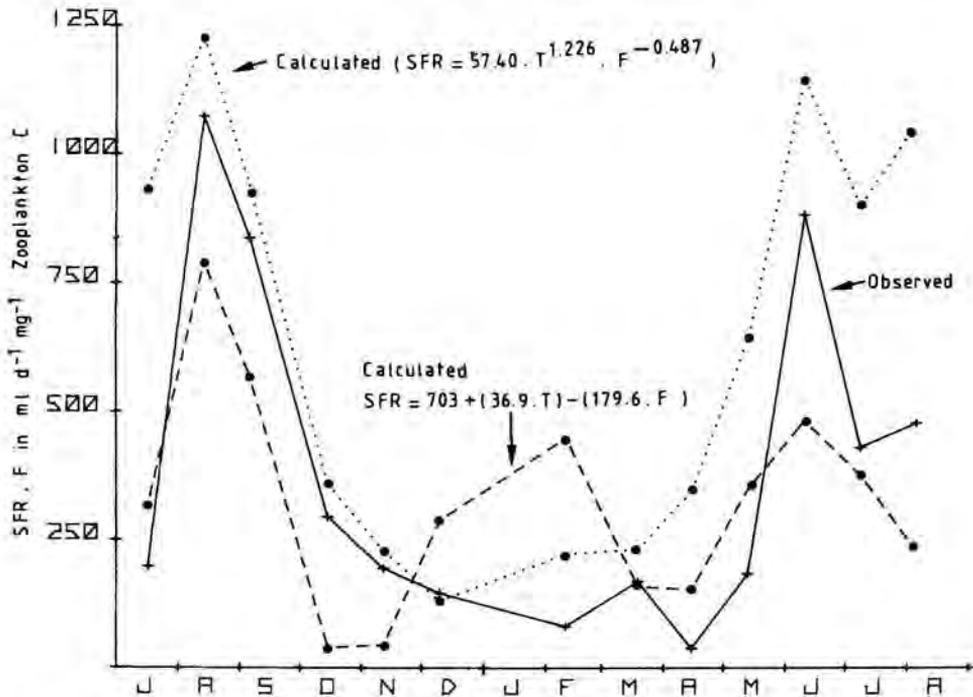


Fig. 28. Comparison of the observed specific filtering rates (SFR) at station 9 in the Loosdrecht Lakes during July 1981 - August 1982 with those calculated via a linear regression model based on stations 3, 5, 8 and 9, and via a generalized power model based on several Dutch lakes varying in their trophic level. For further details see text.

Other important factors such as species composition, size of animals, and quality of food particles were not included in the model, but may be important in determining the grazing activity.

#### 2.5. PROJECT 'POLDER RESEARCH' (R. Veeningen)

The project 'Dynamics of the concentration of dissolved oxygen in polder ditches' is financed by the Ministry of Housing, Physical Planning and Environment (formerly Ministry of Public Health and the Environment), and was started in 1979. The main object is to investigate if and how the dynamics of the concentration of dissolved oxygen (DO) can be used as a criterion for water quality in polder ditches (Fig. 29). The field-work was started in the spring of 1980 in ditches in the polder Groot-Wilnis-Vinkeveen in the vicinity of Nieuwersluis and will be completed in spring 1983.

##### *Temporal and spatial variations of dissolved oxygen*

The study on the temporal and spatial variations of dissolved oxygen concentration in three polder ditches was continued during 1982. Attention was paid specially to the effects of thermal stratification, ice-cover, the cleaning oper-



Fig. 29. Aerial picture of the polder Groot-Wilnis-Vinkeveen, with ditches and meadows. Study site of polder project (Photo Studio Koppelman, Maarssen).

ation and the increased nutrient load of the inlet water on the DO dynamics. The results are summarized (Section 4.2).

#### Environmental variables

The routine work on the environmental variables was continued. It related to the DO dynamics, namely phytoplankton content, macrophytes and nutrients and those concerning the water balance, i.e. chloride and conductivity. The reduction of the water volume during the ice-cover in December 1981 and in January 1982 caused increased concentrations of dissolved substances. Chlorides, bicar-

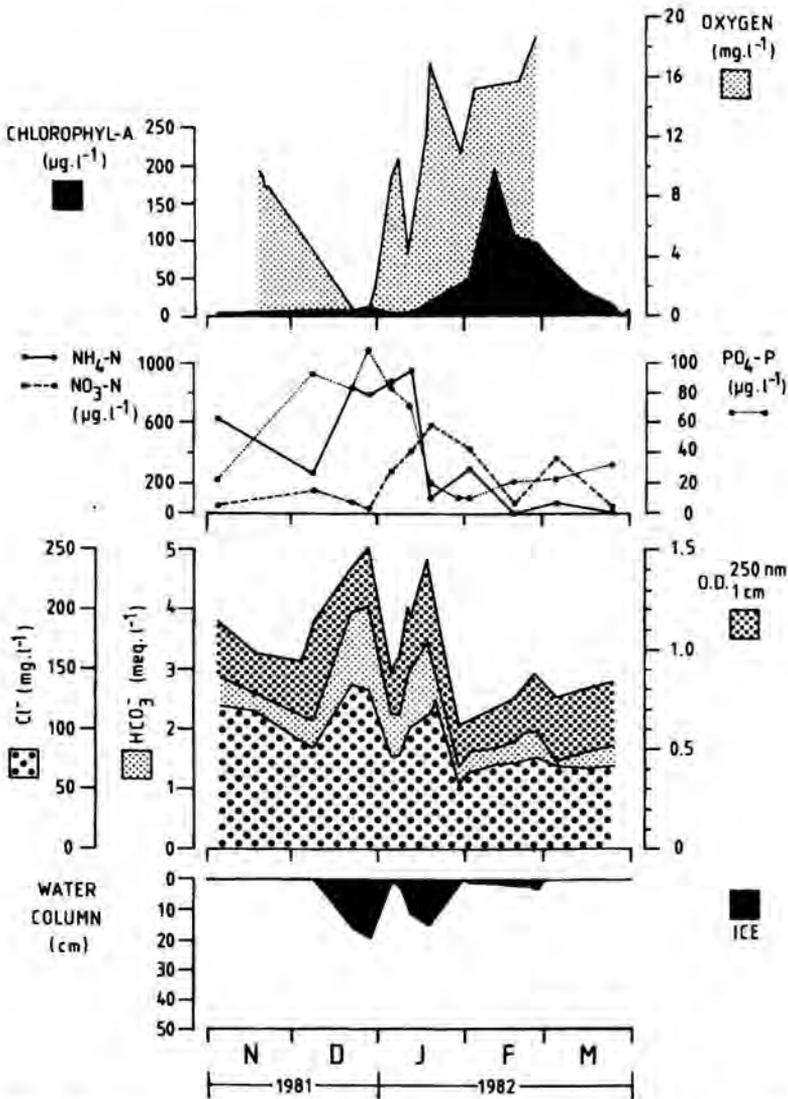


Fig. 30. Concentration patterns of chloride, bicarbonate,  $E_{250}^{1\text{cm}}$ , ammonia, orthophosphates and nitrates, chlorophyll-*a* and dissolved oxygen under ice cover. The thickness of the ice cover is indicated at the bottom of the figure.

bonate and the  $E_{250}^{1.0}$  behaved as conservative constituents, while  $PO_4\text{-P}$ ,  $NH_4\text{-N}$  and  $NO_3\text{-N}$  showed different patterns (Fig. 30). These nutrients appear to have been involved in the algal bloom which gave rise to chlorophyll-*a* concentrations of up to  $200 \mu\text{g.l}^{-1}$  both during and after the ice-cover. Dissolved oxygen concentration was low in December when phytoplankton concentration was low and ice was covered with snow. In January and February the ice was transparent and the phytoplankton bloom caused high concentrations of oxygen.

### 3. Tjeukemeer Laboratory

#### 3.1. GENERAL INTRODUCTION (S. Parma)

The Tjeukemeer is a shallow freshwater lake (area, c.  $21 \text{ km}^2$ ; mean depth c.  $1.5 \text{ m}$ ). It forms a part of the Frisian reservoir system (Fig. 1). The uppermost sediment consists of a mosaic of peat (60%), sand (35%) and mud (5%). In winter the lake receives nutrient-rich and humus-rich water from the surrounding polders. The concentration of 'dissolved' humic acids ( $0.2 \mu\text{m}$ ) varies from  $3.4$  to  $13.7 \text{ mg.l}^{-1}$ . Due to the high  $Ca^{2+}$  (range  $36\text{--}56 \text{ mg.l}^{-1}$ ) and  $HCO_3^-$  (range  $79\text{--}122 \text{ mg.l}^{-1}$ ) concentrations, the pH in this peaty lake is usually  $7.5$  and above. In the summer period, however, water from the IJsselmeer is allowed to enter the lake for agricultural purposes. The humic acid concentration of IJsselmeer water is much lower than that of the polder water but the chloride concentration is much higher. The increase in the chloride concentrations up to  $200 \text{ mg.l}^{-1}$  gives the lake an oligohaline character.

A study of the food chain in the Tjeukemeer was started in 1966 within the framework of the International Biological Programme and finished in 1971. Subsequently, two workgroups were set up.

A large part of the data was published this year in *Developments in Hydrobiology 11*, 1982 'Studies on Lake Vechten and Tjeukemeer, The Netherlands'. (Reprinted from *Hydrobiologia*, vol. 95, 1982.)

#### 3.2. WORKGROUP 'ALGOLOGY'

##### 3.2.1. Introduction (J.R. Moed)

The research programme (1979-1983) is aimed at explaining the algal periodicity. Unicellular centric diatoms, *Melosira* spp., *Diatoma elongatum*, *Oscillatoria redekei*, *O. limnetica*, *O. agardhii*, *Lyngbya* spp. and *Aphanizomenon flos-aquae* show periodicity in the Tjeukemeer since 1972, although in the last years the densities reached by *O. limnetica* and *Lyngbya* spp. were not high. An inverse relationship exists between the maximal density of the diatom *Melosira* spp. and that of the blue-green *O. redekei* occurring a few months later. Its significance in terms of forecasting algal development is recognized. Statistical analysis points to the pH as a possible factor (Project A1).

Density Gradient Centrifugation (DGC) in Percoll was started as a means of characterizing algal species in the Tjeukemeer, besides cell counting. By applying DGC we obtained *Oscillatoria* bands, allowing chemical analysis and information on banding density and gas vacuole resistance (Project A 8.1). This information serves as a reference for laboratory studies to simulate periodicity of *O. redekei* (Project A 8.2). This is a new approach to detect the factors co-regulating algal periodicity, besides bioassays.

Laboratory bioassays presented less evidence for N-limitation in Tjeukemeer water than in the previous years (Project A 7.1). Also the effect of pH regulation was investigated (Project A 7.4) to test the previously applied laboratory conditions. It appears that in a certain period maintaining the lake pH in the cultures resulted in impairment of blue-green algal growth.

The variations in the physicochemical regime of the lake, which are due to the hydrology may influence the availability of P, N, Fe and trace elements to algae. Thus the hydrology may co-regulate the algal periodicity and play a greater role than only transport of nutrients and algae. The use of the new atomic absorption spectrophotometer (AAS) together with the colorimetric method, helped understand the Mn cycle further (Project A 6.6). We can now explain a number of the seasonal changes in terms of oxidation and sorption in dependence of the redox potential and the pH.

Using the AAS with graphite furnace, the Tot-Cu concentrations could be determined without concentrating. The previously noted high average Cu-concentrations in the Tjeukemeer could not be confirmed (Project A 6.4). In contrast, the direct Zn analysis gave results quite comparable with those obtained with the indirect methods (Project A 6.7).

For studying the different physicochemical forms of the nutrients gel filtration, ultrafiltration and ion-exchange chromatography are being used. Ultrafiltration (Project A 6.2) showed that the molecular size of fulvic acids in the Tjeukemeer decreases if the pH is lowered, as observed earlier with Sephadex gel-filtration and dialysis. The nutrient ultrafiltration programme was extended by investigating lake samples at both the natural pH and at pH = 7. This may be considered significant in the role of pH in Mn-specification owing to sorption (Project A 6.6). It appears that almost all the 'dissolved' Fe ( $< 0.2 \mu\text{m}$ ) is located in colloidal particles; this holds good for the organic C and a great deal of the P, too. With increase in pH and humus concentration the magnitude of these colloids increases as well.

The availability of nutrients for algae is being tested in chemostats. The colloidal Fe-fraction from the lake was only one-third as much available as Fe in  $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in an Fe-limited chemostat of *Scenedesmus quadricauda* at pH = 8 and  $2 \text{ mg} \cdot \text{l}^{-1}$  of EDTA (Project A 6.2).

### 3.2.2. Phytoplankton dynamics in the Tjeukemeer (Project A 1; H.L. Hoogveld, J. Voerman, J.R. Moed)

The general pattern of the algal succession in 1982 agrees with the trends since 1972. The maximal densities of the usually abundant species were moderate. This does not contradict the earlier observations (Progress Report 1981) that a high maximal density of *Melosira* spp. coincided with low maximal densities of unicellular centric diatoms and *Oscillatoria redekei* (Fig. 31 a and b). The *O. redekei* maximum since 1971 occurred 1-2 months later than that of *Melosira* spp.; therefore, forecasting the growth of *O. redekei* in the Tjeukemeer is a possibility. The pH may be a factor involved. The density maxima of *Melosira* spp. are reached when the pH is about 8.5. If this pH value occurs relatively late in spring, the density maximum of *O. redekei* is generally high. Relatively lower maxima are observed if the pH value of 8.5 is reached earlier in spring.

### 3.2.3. Nutrient dynamics in the Tjeukemeer (Project A 2)

Determination of nitrogen compounds (Project A 2.1; H.A. Kramer, G. Semplonius, J.R. Moed)

Research on nitrate determination was continued. A column method in which nitrate is reduced to nitrite by zinc powder and platinum was tried. A reduction of 85% has been achieved so far without reduction of nitrite.

Unexpectedly, the zinc-batch method showed a strongly decreased nitrate reduction. This could be counteracted by Cu addition, which suggests that Cu plays a role in the nitrate reduction under the batch conditions.

The work on characterization of the org- $\text{N}_{\text{diss}}$  in the Tjeukemeer was continued. Adsorption to  $\text{Al}_2\text{O}_3$  at pH 6.5, to Dowex 50 W-X8 $\text{Na}^+$  and to a mixed bed of Dowex 50 W-X8 $\text{Na}^+$  and 2X-8 $\text{Cl}^-$  showed a similar trend as in 1981. Adsorption to Dowex 50 W-X8 $\text{Na}^+$  was 7.5% ( $n = 17$ ) but was c. 50% for the other two, in accordance with a supposed negative net charge of the org- $\text{N}_{\text{diss}}$  under the conditions

applied. Fulvic acids, estimated by yellow colour determination, adsorbed qualitatively in a similar manner, namely 6% ( $n = 12$ ) to Dowex 50 W-X8Na<sup>+</sup> and 80-90% to the others ( $n = 12$ ). The labile fraction of the org-N<sub>diss</sub> was determined as the capacity to produce ammonia at 110°C at natural pH and at a pH of 12. At the latter pH the percentage of NH<sub>4</sub>-N in Total-N was 21 ( $n = 13$ ). Correlations were calculated between the org-N<sub>diss</sub>, its fractions obtained after column chromatography, and after ammonia releasing conditions on one hand and algal bio-volume, Tot-N, Mn and Cu concentrations on the other hand. The correlation, particularly that between dissolved Mn ( $< 0.2 \mu\text{m}$ ) and the labile org-N<sub>diss</sub>-fraction, which produced ammonia during heating at 110°C, draws the attention ( $n = 12$ ;  $r = 0.849$ ;  $p > 99.95\%$ ). It suggests either a role for this special org-N<sub>diss</sub>-fraction in dissolving Mn or that the release of ammonia at 110°C is promoted by Mn. Ultrafiltration of Tot-N<sub>diss</sub> was started (Project A 6).

**Nutrient monitoring** (Project A 2.2; T. de Boer, H.A. Kramer, J. Voerman, J.R. Moed)

Monitoring of yellow colour ( $E_{365}$ ), pH, Cl<sup>-</sup>, P, N, Si and Fe was continued. The lake was covered with ice in December 1981 and from 10 January 1982 to the end of the month. The seasonal changes in  $E_{365}$  and Cl<sup>-</sup> indicated that between November 1981 and mid-February 1982 the lake was filled with drainage water from the polders and the higher grounds, and this water was replaced by chloride-rich water from the IJsselmeer from May to the end of July. From August to October the chloride concentration remained constant at c.  $150 \text{ mg.l}^{-1}$ .

The sharp decrease in SiO<sub>2</sub>-Si, namely from 4.5 to c.  $0.1 \text{ mg.l}^{-1}$  during March and early April coincided with diatom growth. The pH which reached 8.5 at the end of March seemed to be important in 'linking' the development of the diatom *Melosira* spp. and the potential growth of the blue-green *Oscillatoria redekei* (Project A 1), and for the extent to which Mn sorption occurred (Project A 6.6).

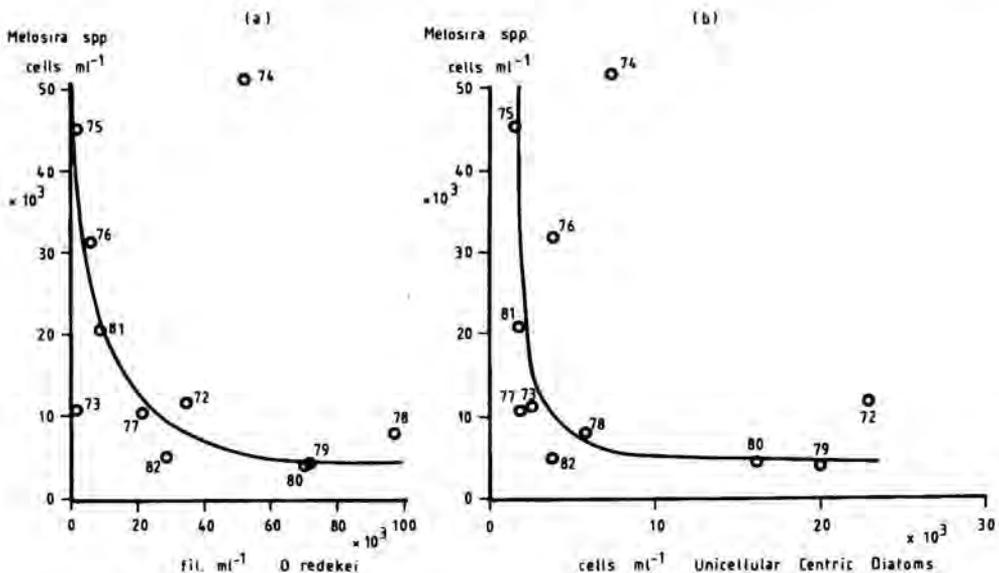


Fig. 31. (a) Relationship between the annual maximal spring densities of *Melosira* spp. and *Oscillatoria redekei* in the Tjeukemeer. The maximum of the latter occurred 1-2 months later; (b) relationship between the maximal spring densities of *Melosira* spp. and unicellular centric diatoms in the Tjeukemeer.

Tot-Fe<sub>diss</sub> slowly decreased from 1.2 to 0.02 mg.l<sup>-1</sup> early May, being absent only in August. The PO<sub>4</sub>-P followed a similar course, decreasing from 0.18 to 0 mg.l<sup>-1</sup> early May. Tot-P<sub>diss</sub>, starting at 0.19 decreased to 0.015 mg.l<sup>-1</sup> at the end of May. The average concentrations of Tot-P<sub>diss</sub> and PO<sub>4</sub>-P between June and November were 0.055 and 0.020 mg.l<sup>-1</sup>, respectively. These values are clearly lower than those in 1981 and are in the range of those in 1977-1980 (Progress Report 1981, p. 47). The initial NH<sub>4</sub>N-concentrations of c. 2 mg.l<sup>-1</sup> are rather high, possibly related to the ice period. NH<sub>4</sub>-N was absent in mid-April and increased significantly only in November. NO<sub>3</sub>-N behaved similarly, except for an increase during the second half of March, possibly originating from NH<sub>4</sub> oxidation, and for reaching zero by the end of May. The org-N<sub>diss</sub> varied from 1 to 2.3 mg.l<sup>-1</sup>.

*Determination of dissolved organic carbon (Project A 2.3; T. de Boer, H. de Haan)*

A fortnightly sampling programme on the DOC-fraction, determined with a TOCsin II analyser (Phase Sep. Ltd, UK), was started. Results of the DOC- and E<sub>250</sub>-measurements are drawn in Fig. 32.

As reported by De Haan *et al.* (1982) there is a significant correlation between the UV light absorbance at 250 nm (E<sub>250</sub>) and the COD of filtered samples  $r = 0.78$ ;  $n = 209$ ;  $P < 0.005$ , viz.

$$\text{mg C l}^{-1} (\text{COD}) = 17.0 E_{250} + 5.1 \quad (1)$$

Both E<sub>250</sub> and COD are indirect measures for the concentration of DOC. Since a carbon analyser measures DOC directly a more distinctly formulated correlation between E<sub>250</sub> and DOC is expected. Therefore the analyses of E<sub>250</sub> and DOC were extended by sampling not only the Tjeukemeer stations 1-10 but also station 11, the Tjonger and a polder ditch near Bantega. In Table 12 the correlation coefficients, slopes, intercepts and data on the linear regressions both for the separate sampling stations and for all stations together are listed. The correlation between E<sub>250</sub> and DOC data based on all sampling stations is highly significant ( $r = 0.93$ ;  $n = 88$ ;  $P < 0.0005$ ).

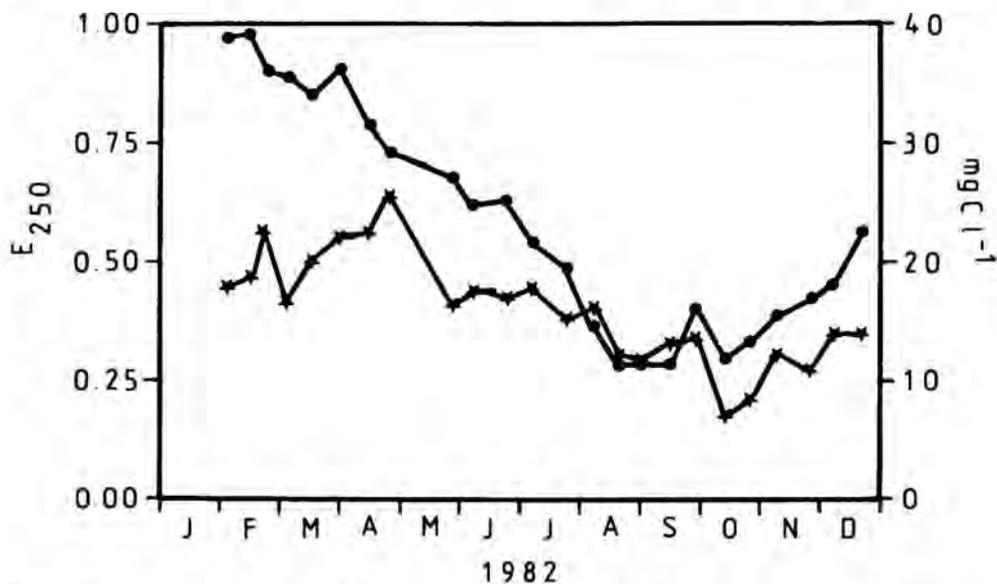


Fig. 32. Concentration of fulvic acids (dots) estimated as optical density at 250 nm (E<sub>250</sub>), and DOC (asterisks) in mg C l<sup>-1</sup> in the Tjeukemeer-east in 1982.

Table 12. Correlation coefficients ( $r$ ), slopes ( $B$ ), intersects on Y axis ( $A$ ) and number of data ( $n$ ) of the linear correlations between  $E_{250}$  and DOC of filtered water from different sampling stations in 1982.

Sampling Station	$r$	$B$	$A$	$n$
Tjeukemeer pt 1 - 10	0.806	13.3	7.7	22
Tjeukemeer pt 11	0.852	16.6	5.0	22
Tjonger	0.679	14.9	6.7	22
Bantega	0.812	16.9	7.6	22
Tjeukemeer pt 1 - 11	0.834	15.5	6.0	44
Tj. meer + Tjonger + Bantega	0.933	18.5	4.7	88

The regression equation is:

$$\text{mg C l}^{-1} (\text{DOC}) = 18.5 E_{250} + 4.7 \quad (2)$$

An almost identical correlation exists between the concentrations of COD and  $E_{250}$  (1) and between DOC and  $E_{250}$  (2) (Fig. 33), suggesting that  $E_{250}$  is a suitable estimate of DOC in humic waters. However, preliminary ultrafiltration experiments show that for the different size fractions the equations differ too. More work is needed to determine the relations between extinction and different size classes of dissolved organic carbon.

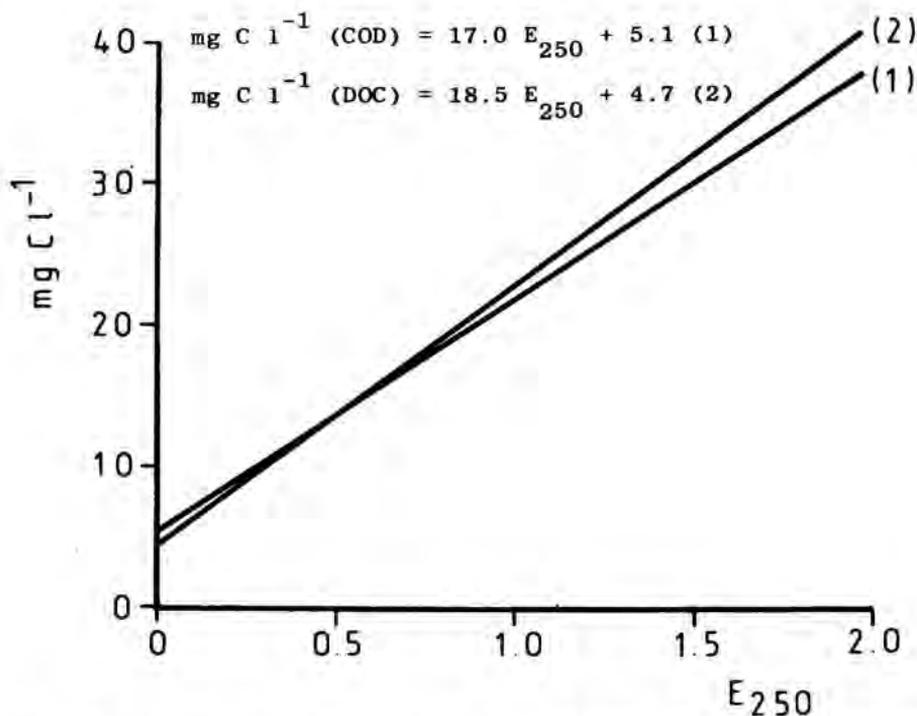


Fig. 33. Correlation between concentration of fulvic acids measured as optical density at 250 nm and DOC as measured respectively as COD between 1970 and 1980 (1) and by direct analysis with TOCsin II (DOC) in 1982 (2) in Tjeukemeer east.

### 3.2.4. Monitoring physical factors (Project A 3; H.L. Hoogveld, J. Bloem, J.R. Moed)

The solar irradiation was registered continuously and pH (Project A 2.2), temperature and turbidity (Secchi-disc) recorded once a week. The pH which was measured incidently during 24 hour periods showed a decrease of about one unit during the night. The importance of the pH in nutrient availability makes a continuous pH registration of the lake necessary.

J. Bloem started work on light conditions. He determined light intensity as a function of wavelength monthly at different depths, and total photosynthetic active radiation fortnightly.

### 3.2.5. Availability of nutrients (Project A 6)

The aim is:

- to test the physicochemical form of the various nutrients and their degree of availability for the abundant algal species; and
- to study the capacity of the algal species to compete for the nutrients Fe, N, P, and trace-elements ( $K_S$  values).

*Isolation of abundant algal species* (Project A 6.1; J.R. Moed, P.J. Timmer, H.L. Hoogveld)

*Oscillatoria redekei* isolates obtained from the Tjeukemeer water are:  $K_1$  and  $K_2$ , both brown-green;  $KR_3$  and  $KR_4$ , both red; and  $K_5$  and  $K_7$ , both green. In addition, isolate  $K_0$  of *O. redekei* and  $K_4$  of *O. limnetica* (identified by J.B.W. Wanders) are available. They are being tested as a representative of the *O. redekei* periodicity in the Tjeukemeer (Project A 8.2).

*Ultrafiltration and availability of iron* (Project A 6.2; H. de Haan, T. de Boer, G. Werlemark, J. Voerman, H. Kramer, J.R. Moed)

The pH-dependent molecular weight and size of fulvic acids (FA), demonstrated by Sephadex gel filtration and dialysis, was confirmed by applying ultrafiltration to lake water at pH 3.0, 5.0, 7.0, and 9.5 using Schleicher & Schüll AC filters. Amicon filters did not give corresponding results probably because of interactions between these filters and fulvic acids. However, Schleicher & Schüll filters also appeared to affect the results depending on the filtration procedure. Using a separate series of filters at each pH value gave the best results. The ratio of the UV light absorbances at 250 and 365 nm ( $E_{250}/E_{365}$ ) of lake water between pH 2.0 and 10.0 predicted the increase of molecular weight and size of FA with increasing pH (De Haan and Werlemark, 1982).

The bio-availability of algal nutrients is connected with their physicochemical speciation. Speciation of nutrients in the oxygenated, alkaline and humus-rich water of the Tjeukemeer is likely to result in different size classes (particulate, colloidal and dissolved). Therefore the speciation of N, P, Fe, Si, Na, K, Mg, and Ca was investigated by ultrafiltration using Schleicher & Schüll filters. Since organic matter forms an important speciation factor organic C was included in this study. Generally speaking, chemical composition and pH of Tjeukemeer show dependent seasonal patterns which may influence the speciation of algal nutrients. From the ultrafiltrations of natural and neutralized lake water samples some general results emerged, published in de Haan (1982). The lake water samples were prefiltered prior to ultrafiltration, to remove particulates  $> 0.2 \mu\text{m}$ , so that the specification was confined to the so-called 'dissolved' nutrient fractions.

Usually, independent of pH, all Na and K was recovered in the smallest ( $50 \text{ \AA}$ ) fraction. However, part of the Ca and Mg occurred frequently in colloidal form ( $50 - 75 \text{ \AA}$ ), especially at natural pH, suggesting a  $\text{Ca}(\text{HCO}_3)_2$  saturation of the lake water.

Si passed each filter and can be regarded as dissolved throughout the year.

More than 80% of the Fe mostly occurred in colloidal sizes exceeding  $150 \text{ \AA}$ . This percentage was not noticeably affected by pH variations (7.1 - 9.2) in the

samples. In winter when the 'dissolved' Fe concentration exceeded  $1 \text{ mg.l}^{-1}$  most Fe was in colloids even larger than  $250 \text{ \AA}$ . If less than 80% of Fe was found to be larger than  $150 \text{ \AA}$ , the Tot-Fe<sub>diss</sub> concentration in the lake was around its detection limit, possibly resulting in inaccurate fractionation data. Only 10 - 20% of Fe and org-C appeared to have the same particle size, indicating that most Fe is not chelated by FA in the humic Tjeukemeer. Therefore, most of the Fe will be of colloidal inorganic nature.

In summer the 'dissolved' P concentrations in the lake were low ( $10 - 50 \text{ \mu m.l}^{-1}$ ), causing inaccurate size fractions. Yet, the results indicate that most P is in the same size range as Fe suggesting that 'dissolved' Fe and P in the Tjeukemeer occur as colloidal Fe(III)-hydroxide-phosphate.

The N ( $< 0.2 \text{ \mu m}$ ), distributed over two size fractions, was season dependent. In winter, when most N occurs in  $\text{NH}_3$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , c. 70% of it was in the expected fraction passing pores of  $50 \text{ \AA}$ . In July c. 50% of the N was larger than  $250 \text{ \AA}$ , indicating the occurrence of proteins, possibly of algal origin. This confirms the earlier work on amino acid analysis of organic matter of different molecular weight as isolated by Sephadex gel filtration. The distribution of N was not affected much by pH. The filtrates containing the smallest size fractions ( $< 50 \text{ \AA}$ ) sometimes gave high controls during N-analysis, a problem not yet resolved.

Org-C was estimated indirectly by light absorbance at 365 and 250 nm, by fluorescence ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ;  $\lambda_{\text{em}} = 470 \text{ nm}$ ) and by direct carbon analysis (TOCsin II) (Project A 2.3). All the three methods indicated that the average particle size of org-C was larger in winter than in summer. Lowering the pH decreased the average particle size of org-C; this pH-dependent particle size of most org-C varied between 75 and  $150 \text{ \AA}$ . The relationship between the size of org-C and pH confirms the pH-dependent size relations obtained by Sephadex gel filtration and dialysis. All these data are in accordance with the idea that the molecular arrangement of 'dissolved' brown organic C, namely the fulvic acids in the Tjeukemeer are arranged as aggregated units that reversibly dissociate with decreasing pH. Since almost all 'dissolved' Fe occurs in larger colloidal particles than those of the org-C it is likely that other metals with higher complexing properties, e.g. Cu or Zn, may play binding roles at the association of the FA units. Research to elucidate the possible role of metals in the steric configuration of FA is in progress.

The solubility and the speciation of Fe in fresh water depends on pH and the presence of complexing agents. The effects of these factors on the availability of Fe in a Fe-limited chemostat of *Scenedesmus quadricauda* were studied, assuming that the availability of Fe was reflected in growth and chlorophyll-a synthesis of the alga. The data obtained indicate (Table 13) that if the pH of the

Table 13. Dry weight (DW) and chlorophyll-a concentration (chl-a) in a Fe-limited chemostat of *Scenedesmus quadricauda* in medium M26 ( $S_R = 58 \text{ \mu g Fe.l}^{-1}$ ) at  $20 \pm 0.5^\circ\text{C}$  as a function of the EDTA concentration with and without a pH-stat.

EDTA $\text{mg.l}^{-1}$	Without pH-stat			With pH-stat (pH = 7.5)	
	DW $\text{mg.l}^{-1}$	chl-a $\text{\mu g.l}^{-1}$	pH	DW $\text{mg.l}^{-1}$	chl-a $\text{\mu g.l}^{-1}$
0.5	29	144	7.8	116	535
1.0	220	1117	8.7	138	655
2.0	221	1135	9.1	-	-
2.5	225	1165	9.7	154	715
3.0	179	805	8.5	135	763

culture is kept constant with a pH-stat, the EDTA concentration had no effect on the Fe-limited growth and chlorophyll-*a* synthesis of the alga. However, if the pH of the culture was not kept constant the EDTA concentration strongly affected the growth and thus the pH of the medium. Apparently, for optimal Fe-availability to Fe-limited growing *S. quadricauda* about 2 mg.l<sup>-1</sup> EDTA is needed in medium M 26. It is not yet known at which pH Fe-limited growth of *S. quadricauda* is optimal.

Preliminary work on Fe-limited chemostats of the blue-green algae (Cyanobacteria) *Oscillatoria agardhii* and *O. limnetica* indicates that the Fe-availability for especially the former species was independent of the EDTA concentration. Cyanobacteria have been demonstrated to release siderochromes that are strong Fe-chelating hydroxamates. If this is true for the *Oscillatoria* species in our Fe-limited chemostats, their Fe-limited growth would have been expected to be independent of the EDTA concentration.

#### Availability of copper (Project A 6.4; H. de Haan, T. de Boer)

The relatively high average Cu-concentrations in the Tjeukemeer and in polderwater as determined by preconcentration followed either by colorimetric analysis or by flame atomic-absorption spectrophotometry could not be confirmed by direct graphite-furnace atomic-absorption spectrophotometry (Table 14). Also the annual differences observed in both water types with the two indirect methods were not found with the direct graphite-furnace atomic-absorption spectrophotometry. As the indirect methods may have suffered from Cu pollution the three methods will be compared with special reference to the influence of clean handling during sampling and concentrating.

#### Availability of nitrogen (Project A 6.5; J.R. Moed, J.F. van Weerden)

The previous laboratory bioassays related specially to possible nitrogen limitation, whereas enhanced ratios of carotenoid and chlorophyll-*a* were measured in Tjeukemeer. It is being investigated, therefore, to what extent pigment analysis can help identify nutrient limitation or depletion. Miss J.F. van Weerden continued her investigations on this subject using chemostats of *Oscillatoria limnetica* (isolate K4). Results are being statistically analysed. The tentative conclusion is that mere pigment analysis can be used to discriminate between two different nutrient limitations in a few cases only (see also Arkesteijn, 1982).

#### Availability of manganese (Project A 6.6; G.J. Schrottenboer, J.R. Moed)

Labile-Mn analysis of Tjeukemeer, polder, and Tjonger water by reaction with leucomalachite green was continued and extended to include Tot-Mn determination by flame atomic-absorption spectrophotometry (FAAS), in filtered as well as in unfiltered samples (Fig. 34). The Tot-Mn values in unfiltered lake water appeared to be much higher than those in the filtered water except in January and

Table 14. Average Tot-Cu concentrations ( $\mu\text{g.l}^{-1}$ ) in Tjeukemeer and polder water in 1980, 1981 and 1982 as determined by preconcentration by freeze-drying either followed by colorimetric analysis (FD-DBC) or flame atomic-absorption spectrophotometry (FD-FAAS) or direct graphite-furnace atomic-absorption spectrophotometry (GFAAS).

Method	Tjeukemeer			Polder water		
	1980	1981	1982	1980	1981	1982
FD-DBC	25	23	9	30	41	11
FD-FAAS	-	33	9	-	50	10
GFAAS	-	3	3	-	2	2

February. Also in the first half of the year the FAAS values are mostly much higher than the colorimetric values. From August onwards they become more or less comparable and relatively low.

The dissolved Mn ( $< 0.2 \mu\text{m}$ ) seems to play a very important role in the algal uptake. The fraction  $< 0.2 \mu\text{m}$  had high initial values which decreased to almost zero within a month, but increased temporarily at the end of March and during May - July, after which they remained low. Although both in March and in May the Tot-Mn and labile-Mn concentrations increased, they did more so in March. Thus there is evidence for the formation of 'stable' and labile Mn compounds. From mid-February to mid-March both the decrease of Mn in the filtrates and the increase of Tot-Mn in the unfiltered samples coincided with an increase in the redox potential ( $E_h$ ), pH being relatively low ( $\text{pH} < 8$ ) and constant (Fig. 34). These changes in the Mn concentrations can be explained, therefore, by Mn oxidation, resulting in fairly large particles of 'stable'  $\text{MnO}_x$ . At the end of March and in May the increase of 'stable' and labile Mn compounds coincided with increase in the pH to 8.5 and almost 10, respectively. At these pH values we expect both the acceleration of oxidation and the occurrence of sorption processes (Progress Report 1981, p. 51). In the latter case Mn may be bound by adsorption and ion-exchange and considered as labile Mn.

This information by the colorimetric and FAAS methods supports the idea that in the Mn cycle of the lake both oxidation and sorption processes are important. In relation to algal periodicity, it cannot be excluded that the pH-dependent sorption of Mn, and of other metal-ions probably as well, is competitive with the algal uptake of these ions.

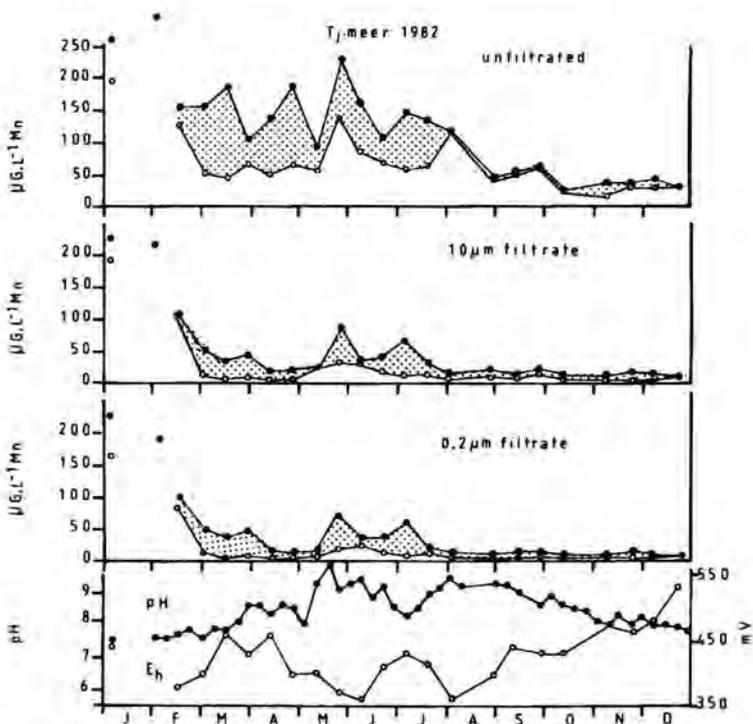


Fig. 34. Seasonal changes in Mn concentrations, pH and  $E_h$  in Tjeukemeer water. Black dots: Tot-Mn; open circles: labile Mn.

### Availability of zinc (Project 6.7; H. de Haan, J. Voerman)

In contrast with the Cu analyses, the direct Zn analysis and the indirect methods (Table 15) gave comparable results. Apparently ammonium pyrrolidine-1-dithiocarboxylate (APDC) is not able to complex all Zn. Since APDC appeared to bind relatively less of the Zn in relatively humus-rich polder water, humic materials might have interfered with the complexation by APDC. Hydrolysis of the samples before direct and indirect Zn analysis did not result in higher concentrations. We therefore consider the direct flame atomic-absorption spectrophotometric analysis to determine Zn concentrations accurately, and thus discontinued the APDC pre-concentration in the Zn analysis.

The relatively high Zn concentration in polder water suggests strongly that in the Tjeukemeer metal originates to a large extent from the surrounding peaty polders. Indeed, the fortnightly Zn concentrations exhibited significant positive correlations ( $P < 0.001$ ;  $r = 0.84$ ) with the brown colour of filtered lake water, indicating the presence of polder water.

Table 15. Average Tot-Zn concentrations ( $\mu\text{g}\cdot\text{l}^{-1}$ ) in Tjeukemeer and polder water in 1982 as determined either by preconcentration by freeze-drying (FD) or complexation by ammonium pyrrolidine-1-dithiocarboxylate (APDC) followed by flame atomic-absorption spectrophotometry (FAAS) or as determined by direct-flame atomic-absorption spectrophotometry.

Method	Tjeukemeer	Polder water
APDC-FAAS	8	8
FD-FAAS	12	16
FAAS	11	16

### 3.2.6. Bioassay experiments (Project A 7)

*Detection of nutrient limitation* (Project A 7.1; J.R. Moed, H.L. Hoogveld, H.A. Kramer, H. de Haan)

Laboratory bioassays following the multiple addition technique were continued. Poor growth was observed in Tjeukemeer water sampled in the period May - November. Medium addition greatly stimulated growth in most cases. Nitrate omission resulted in c. 50% growth reduction until April and from June to September; there was virtually no growth in May and from September to December. In 1981, on the other hand, nitrate omission had resulted in poor growth from May to September.

The Tot-N concentrations in the flasks increased c. 30% on the average. Since no evidence for algal nitrogen fixation by heterocysts was observed, it was assumed to be owing to air pollution caused by fish analysis in the laboratory. Indeed, a significant positive correlation was found between the percent increase in Tot-N values and the number of fish catches per 14 days ( $n = 12$ ;  $r = 0.77$ ;  $P < 0.0005$ ). It follows that the air used for our laboratory experiments needs to be made nitrogenous product free.

*Detection of regulating physical factors* (Project A 7.4; J.R. Moed, H.L. Hoogveld)

In 1981 evidence of a pH-dependent Mn sorption in the lake was obtained (Progress Report 1981, p. 51). We realized that intensive aeration in laboratory bioassays at night would cause a decrease in the pH. This may lead to desorption of metal-ions, thus masking possible limitation or depletion of trace elements.

This hypothesis was tested in Tjeukemeer water enriched with nutrients, but without trace elements. The pH was kept at the value measured in the lake at the start of the bioassay. That a CO<sub>2</sub> co-limitation was induced cannot be excluded. Intensive aeration would have caused a considerable inflow of NaOH to keep the pH within the desired narrow range. The increase in chlorophyll-*a* in the pH-regulated flasks was relatively low compared with that in the non-regulated flasks, in the period from April to mid-June; but only if the pH was > 8.2 was the supposed margin of error ( $\pm 20\%$ ) exceeded.

Pigment absorption spectra showed that particularly the blue-green algal growth was impaired.

### 3.2.7. Laboratory model of algal periodicity in the Tjeukemeer (Project A 8)

This simulation project is being undertaken to identify factors regulating the algal periodicity in the Tjeukemeer.

#### Density gradient centrifugation of Tjeukemeer seston (Project A 8.1; J.R. Moed)

J. Bloem started density gradient centrifugation (DGC) in Percoll for isolation of *Oscillatoria agardhii* and *O. redekei* from Tjeukemeer seston, in order to analyse their pigment composition. DGC proved to be suitable, although isolation of *O. redekei* required pre-settling of diatoms in the period they were abundant. Further checks are needed to confirm that *O. redekei* samples obtained in this way are representative.

Density marker beads, in the range 1.006–1.120 g.ml<sup>-1</sup>, were used to analyse the specific gravities of both the gradient and the algal bands (banding densities). Between 27 April and 9 June, the relative abundance of *O. redekei* in the lake varied from 10 to 50%, but was from 60 to 98% in the single *O. redekei* band. A similar concentration could be obtained with *O. agardhii*. The pattern of this alga was different, showing one band in April, but 2–3 bands during May–December. Filaments in the bands appearing later had almost lost their gas vacuoles owing to centrifugation at 38,000 g. The vacuole resistance to this centrifugal force probably depends on the prevailing turgor and therefore on the balance between photosynthesis and growth. Thus, the occurrence of these *O. agardhii* bands showed that, physiologically speaking, the *O. agardhii* population in the lake was not homogeneous. Pigment composition was determined by means of absorption spectra of algal bands on glass fibre filters. The ratios both between phycocyanine and chlorophyll-*a*, and between carotenoid and chlorophyll-*a*, were fairly constant for *O. redekei*. No phycoerythrin was observed. *O. agardhii* showed more variation in pigment composition, thus the ratio between phycocyanine and chlorophyll-*a* fluctuated more than 30%. Furthermore, two periods could be recognized for the differences in ratio between carotenoid and chlorophyll-*a*. From April to 18 May, the period in which a maximal density of *O. redekei* was counted, the ratios were relatively low and constant. Thereafter, however, the ratio increased by 20–30%; *O. agardhii* dominated and chlorophyll-*a* did not decrease. It is suggested, therefore, that during the disappearance of *O. redekei*, *O. agardhii* responded to the changed environmental conditions with an enhanced carotenoid content.

Concluding, the DGC in Percoll is suitable for isolating *O. redekei* and *O. agardhii* from Tjeukemeer seston. This allows characterization by means of banding density, vacuole resistance, pigment analysis and probably additional chemical parameters. Thus, the field results of single species can be compared with those in the laboratory to judge the validity of simulating the field situation (Project A 8.2). This may help to identify the regulating factors in the lake.

#### Simulation of the *Oscillatoria redekei* growth (Project A 8.2; J.R. Moed, P.J. Timmer, H.L. Hoogveld)

Since 1972 *O. redekei* is one of the most abundant algae in the Tjeukemeer. Its maximal densities coincide generally with the chlorophyll-*a* maxima in May. Thus

the selection of *O. redekei* for the simulation studies. For a successful simulation, the prerequisites are: an *O. redekei* isolate representative for the population of this species in the Tjeukemeer, and field data as reference. To fulfil these requirements, isolates were collected (Project A 6.1) and information gained on the seasonal changes in density (Project A 1) and on cell characteristics (Project A 8.1).

First, the available isolates were compared. As no evidence for the presence of phycoerythrin in *O. redekei* in the Tjeukemeer was obtained (Project A 8.1), only green-coloured isolates were selected. In cells of the isolate *O. limnetica* K<sub>4</sub> small gas vacuoles could be recognized, thus its identification as *O. limnetica* instead of *O. redekei* is questionable. Therefore, we selected this isolate too. It was cultivated in M26 in batch-cultures under light-dark rhythm at 18°C, and at various irradiation intensities. The changes in optical density, dry weight, nitrate, pigments and banding density in Percoll were followed and compared with the reference data. Nitrogen depletion limited the maximal biomass. The ratio between carotenoid and chlorophyll-*a* greatly increased and that between phycoerythrin and chlorophyll-*a* strongly decreased. Also the banding density reached values of  $> 1.12 \text{ g.ml}^{-1}$  which is much higher than those observed in the lake (c.  $1.006 \text{ g.ml}^{-1}$ ).

Preliminary experiments revealed that the isolate *O. redekei* K<sub>7</sub> behaved differently. For instance, a considerable carotenoid loss occurred during preparation to determine pigment spectra. To prevent this, a special procedure was developed. The maximal biomass seemed to be affected by unknown factors. Also, the course of its banding density was different, values becoming lower than  $1.006 \text{ g.ml}^{-1}$ . Preliminary results show that the pigment ratios and banding densities may resemble the field data.

Summarizing, a) the two isolates of *O. redekei*/*O. limnetica* behave differently under similar growth conditions, which justifies the investigation whether the available isolates are representative; and b) some cell characteristics of one of the isolates (*O. redekei*) show a fair resemblance with the field data, which would allow research on identification of the limiting factors during the batch-growth of this isolate.

### 3.3. WORKGROUP 'FOODCHAIN AND PRODUCTION STUDIES'

#### 3.3.1. Introduction

The workgroup is engaged in a study of the relations between the fish population and their food organisms (Fig. 35). Population structure, population density, fecundity, mortality, growth, standing crop biomass, and production are being estimated for both the fish species and the most important fish-food species. The latter include zooplankton (copepods and cladocerans) and macro-fauna elements as chironomids, gammarids and the opossum shrimp, *Neomysis integer*. The work is directed mainly on the ecology of the fish-feeding in nature as well as under experimental conditions in aquaria. The results of these studies will be used in a simulation model in order to describe the subsystem fish and their food organisms and to get more insight into its dynamics.

The Tjeukemeer is the main object for most of these studies. However, aspects like migration of fish larvae, growth of 0<sup>+</sup> fish, feeding ecology of bream, and population dynamics of *Neomysis* are being also studied in other lakes of the Frisian Lake District.

#### 3.3.2. A discrete event-oriented simulation model of the subsystem 'fish and its food organisms' in the Tjeukemeer (Project V 1; J. Vijverberg, A.F. Richter)

The 'INSTAR' model was improved in two ways. Firstly, the model was generalized so that it can now be applied more universally to copepod or cladoceran species without any essential changes in the programme of the model. Secondly,

the model is now much easier to operate. A user's guide describing the improved model is in preparation.

Our modelling experience with *Daphnia* showed clearly that for a satisfactory simulation of the population dynamics of a cladoceran species, more precise information is needed about juvenile instar duration, number of juvenile instars and juvenile growth.

Therefore, using laboratory cultures, we studied in detail the instar durations and growth of five cladoceran species: *Bosmina coregoni*, *B. longirostris*, *Chydorus sphaericus*, *Ceriodaphnia pulchella* and *Leptodora kindtii*. Larger species had higher numbers of juvenile instars, two for the smallest species (*Bosmina coregoni*, *B. longirostris* and *C. sphaericus*), three for the medium-sized *Ceriodaphnia pulchella* and seven for the large species *L. kindtii*. For each species, duration of the different juvenile instars was generally constant and took 60-70% of the duration of the adult instars. *Leptodora* showed a somewhat different pattern; the first juvenile instar developed c. 5 times, and the second and third instar c. 2 times faster than the more advanced juvenile instars.

### 3.3.3. Population densities, population structure and biomass of copepods and herbivorous cladocerans in the Tjeukemeer (Project V 2; J. Vijverberg)

Sampling was regular and more intensive during the growing season. There were 23 sampling dates in total. Especially the small cladoceran species: *C. sphaericus*, *B. coregoni* and *B. longirostris* showed high densities, with a mean annual density of 100 ind. l<sup>-1</sup>. *Daphnia hyalina* densities were low during early summer, but relatively high during mid-summer and autumn. The closely related *D. cucullata* recorded very high densities, those during June-September being c. 7 times higher than the annual maxima recorded from 1968 to 1982.

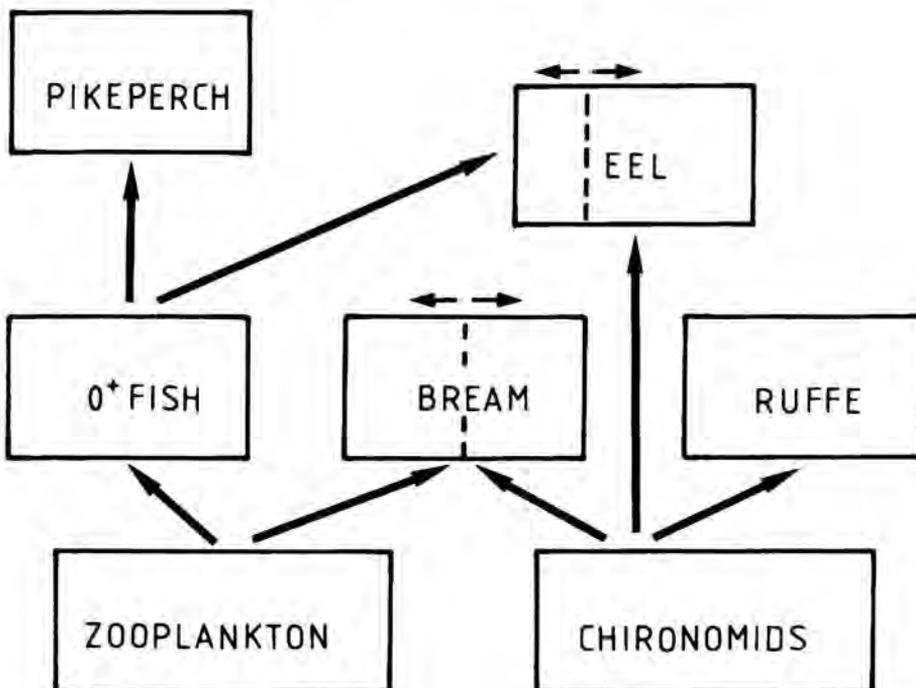


Fig. 35. Research field of the workgroup 'Foodchain and Production Studies'.

3.3.4. *Autecology of Leptodora kindtii in the Tjeukemeer* (Project V 3; J. Vijverberg)

The sampling frequency was less than during 1978-1980. Densities were very high during June ( $1.0-3.7 \text{ ind. l}^{-1}$ ), with a lower peak in August.

3.3.5. *Autecology of Neomysis integer* (Project V 4; J. Vijverberg, P.J. Mac Gillavry)

The sampling frequency in the Tjeukemeer was less than during 1978-1981. During most of the year densities were very low, with a peak density of  $2.1 \text{ ind. m}^{-2}$  only at the end of the year.

For calculating production and population dynamics of *N. integer* data on egg duration time in relation to water temperature are needed, information from the literature being incomplete. Egg development time was studied in the laboratory using females of the overwintering population. These females started carrying eggs in early April. The egg development time was strongly dependent on temperature, namely 36, 24, 16 and 11 days at 10, 12.5, 15 en  $17.5^{\circ}\text{C}$ , respectively.

3.3.6. *Population dynamics and production of chironomid larvae* (Project V 5; J. Vijverberg, N.J. van Benthem, Th.H. Frank)

Samples taken in the Tjeukemeer during 1980 in the reed-beds and in the adjacent zone without macrophytes were analysed. In the sand substrate chironomid densities were lower than outside, but the biomass per  $\text{m}^2$  was c. 50% higher ( $n = 12$ ). This because the densities of larger species like *Glyptotendipes pallens*, *Polypedilum nubeculosum* and *Endochironomus* sp. in the reed-beds were higher. In the mud the densities in the reed-beds were very similar to those outside, but the biomass per  $\text{m}^2$  was about twice as high. This difference was mainly the result of the higher *Chironomus plumosus* densities in the reed-beds.

The routine sampling in the Tjeukemeer was continued. Compared with those in 1979-1981 both the densities and the standing crop biomass were relatively high, the mean annual values for the whole lake amounted to  $600 \text{ ind. m}^{-2}$  and  $400 \text{ mg ashfree dry wt m}^{-2}$ , respectively. *Einfeldia carbonarius* was dominant in the sand; in the mud *Chironomus plumosus* was co-dominant. In the Tjeukemeer as a whole *Einfeldia* sp. represented 75% of the mean densities and 65% of the larval biomass.

In the Langweerder Wielen the chironomid densities were dominated by *Polypedilum nubeculosum* (49%), whereas *Einfeldia carbonarius* (24%) and *Glyptotendipes pallens* (18%) were sub-dominant. Although the larval densities were only half of those in the Tjeukemeer, the mean larval biomass was quite similar.

3.3.7. *Ecology of smelt (Osmerus eperlanus), perch (Perca fluviatilis) and pikeperch (Stizostedion lucioperca) larvae* (Project V 6; W.L.T. van Densen, G.A.A. Schoon, A.G. Frank-Landman)

Smelt (7-22 mm), perch (7-14 mm) and pikeperch (7-12 mm) larvae were studied in the Tjeukemeer and the IJsselmeer. Both fish larvae and their food (copepods) were sampled simultaneously. The copepod samples were concentrated on a  $40 \mu\text{m}$  mesh sieve. Initially, when the larvae were still very small, mainly the smallest copepod instars, the nauplii, were selected. Later, when the larvae had a size of c. 10-15 mm, predominantly the larger copepodite instars were eaten. Size class of copepodites maximally consumed and larval length of pikeperch were correlated positively. In the Tjeukemeer the fish larvae switched at a smaller size from nauplii to copepodites than in the IJsselmeer.

3.3.8. *Ecology of 0+ fish in the Tjeukemeer* (Project V 7; W.L.T. van Densen, A.G. Frank-Landman, P.J. Mac Gillavry)

To study the availability of prey fish for older pikeperch, perch and eel, the

numbers, growth and production of 0<sup>+</sup> fish of all species were recorded. The recruitment of pikeperch larvae was the highest, and this species dominated the 0<sup>+</sup> fish production in the open water zone of the lake during the growing season. Its instantaneous daily mortality rate, however, was high (10% d<sup>-1</sup>) and, therefore, the resultant recruitment to the population of 1<sup>+</sup> and older fish at the end of the season was low. A small part of the 0<sup>+</sup> pikeperch population succeeded in becoming piscivorous in the course of the season, but the conditions for piscivory deteriorated fast to the end of the season. The 0<sup>+</sup> pikeperch mainly acquired its food by consuming *Leptodora kindtii*. Although *Leptodora* sp. is the largest zooplankton species present in the lake, it contributes less than 3% to the annual zooplankton production, implying a very selective use of the secondary production for channelling energy to the top-predators in the lake ecosystem.

### 3.3.9. Ecology of 1<sup>+</sup> and older bream (*Abramis brama*) in the Tjeukemeer (Project V 8; E.H.R.R. Lammens)

Bream were sampled at least once a month with a small-meshed trawl. Gut contents of different length classes of the fish (9.5–10.5, 14.5–15.5 and 34.5–35.5 cm forklength) were collected. The relations between length and weight and those between weight and gonad weight were determined. Scales and fin rays were collected in spring and autumn for determination of growth.

As a consequence of bad feeding conditions in 1980 and 1981 the condition and gonad development were poor even before the spawning period of 1982. Many mature females failed to develop gonads, or produced only very small eggs. During summer the feeding conditions improved. The bream smaller than 20 cm increased c. 6 cm in length, that between 20 and 30 cm increased 1–4 cm, and that larger than 30 cm hardly grew, but their conditions improved greatly. Gonad development was much better than in 1980 and 1981.

The food of the bream was generally characterized by *Daphnia* and *Bosmina* species, but from mid-July to mid-August *Cladotanytarsus* and *Einfeldia* spp. dominated the food of larger bream (> 20 cm), and the benthic cladocerans (*Alona* and *Leydigia* sp.) that of the smaller fish.

### 3.3.10. Ecology of 1<sup>+</sup> and older bream (*Abramis brama*) in the Langweerder Wielen (Project V 9; E.H.R.R. Lammens)

Sampling was less frequent than in the Tjeukemeer i.e. once in two months. The growth, condition and gonad development as in the preceding year were poor because the zooplankton population was over-exploited by very large numbers of young bream, and the chironomid population by a very large amount of large bream. Only the smaller bream (< 25 cm) increased in length, on a diet predominated by benthic cladocera (*Alona* and *Leydigia* spp.). The larger bream could not restore its condition, even an increased mortality for this size class was found. The diet of the larger bream was composed of chironomids (*Poly-pedilum* and *Chironomus* spp.) and a very large amount of detritus.

### 3.3.11. Ecology of 1<sup>+</sup> and older white bream (*Blicca björkna*) and roach (*Rutilus rutilus*) in the Tjeukemeer (Project V 10; E.H.R.R. Lammens)

White bream were sampled at least once a month during the summer using a small meshed trawl. Gut contents, scales and fin-rays were collected. The diet resembled strongly that of bream, species of *Daphnia*, *Bosmina* and *Leptodora* and chironomids (*Cladotanytarsus* spp. and *Einfeldia* sp.) being the most important food organisms. For the same size classes the size of the food organisms of white bream was larger than for bream: the average size of *Daphnia* was larger and the share of small chironomids was smaller. Benthic cladocera, which are often important for small bream, were virtually absent.

Growth of white bream was very moderate with only fish smaller than 22 cm increasing in length.

Table 16. Estimation of consumption by the eel population during the growing season in the open water zone of the Tjeukemeer.

year	mg fresh weight.m <sup>-2</sup> d <sup>-1</sup>				Total	g fr.w.m <sup>-2</sup> eel biomass
	molluscs	<i>G. tigrinus</i>	chiro- nomids	fish		
1979	5.2	0.15	17	3	27	2.4
1980	3.2	7.1	25	31	68	3.5
1981	0.18	3.5	17	34	55	2.8

3.3.12. Ecology of the eel (*Anguilla anguilla*) in the Tjeukemeer. (Project V 11; H.W. de Nie)

The practical work carried out during 1979-1982 was completed during autumn this year.

From 1979 to 1981, 7884 eels were caught in the Tjeukemeer, measured and weighed. Out of the 1800 fish stomachs removed 1080 were analysed both quantitatively and qualitatively. The chironomids *Chironomus plumosus*, *Einfeldia carbonarius* and *Glyptotendipes pallens* and the amphipod *Gammarus tigrinus* occurred most frequently in the stomachs. The chironomids, fishes, *G. tigrinus* and the molluscs *Anodonta* sp. and *Dreissena polymorpha* had a high biomass share in the diet, together representing 85-96% of the consumed biomass (Table 16).

Chironomids are relatively scarce in the lake benthos. They are heavily predated by the very abundant bream but form also the main food of eel < 250 mm. In 1979 the larger eels fed mainly on molluscs, while in 1980 and 1981 they fed chiefly on young fish. If the eel is an optimal forager, the energy cost of eel > 250 mm for predated on young fish is high. The feeding behaviour of the eel in aquaria revealed that, because of a longer search-handling time for tube dwelling *Chironomus* larvae, the fish preferred pupae to larvae. Pupae are more immobile and if offered under similar conditions as larvae, the satiated eel preferred larvae.

The standing stock of eel in the open water zone of the Tjeukemeer is 24-35 kg fresh weight ha<sup>-1</sup>. In 1979 the length classes 200-250 mm and 250-300 mm dominated the population, in 1980 and 1981 the length class 150-200 mm became more abundant in August and September. The percentage of females became higher, and their Fulton index (weight x length<sup>-3</sup>) lower in 1981 (Table 17).

Table 17. Sex ratio and Fulton index of eel larger than 280 mm in the Tjeukemeer.

year	Females				Males			
	% in number		Fulton index kg.m <sup>-3</sup>		% in number		Fulton index kg.m <sup>-3</sup>	
	280 - 339 mm	340 - 399 mm	280 - 339 mm	340 - 399 mm	280 - 339 mm	340 - 399 mm	280 - 339 mm	340 - 399 mm
1979	7.0	28.3	1.73	1.71	93.0	71.7	1.80	1.80
1980	7.9	27.4	1.69*	1.75*	92.1	72.6	1.79*	1.96*
1981	17.6	36.4	1.58	1.75*	82.4	63.6	1.65	1.90*

\* significant difference between females and males (p < 0.05).

**3.3.13. Population structure, growth and feeding of I<sup>+</sup> and older pikeperch (*Stizostedion lucioperca*) and perch (*Perca fluviatilis*) in the Tjeukemeer (Project V 12; W.T.L. van Densen)**

The stock of pikeperch further expanded because the adults were only lightly exploited by sport fishermen, and the subadults in the strong year-classes 1980 and 1981 grew considerably. The I<sup>+</sup> fish was highly available during spring and early summer, but was overexploited as was 0<sup>+</sup> pikeperch at the end of the summer (Section 3.3.8).

The somatic condition of the pikeperch was high in June and July, but no significant mid-summer development of the ovaria, as in 1980, was observed. Although condition should play an important role in the mid-summer gonad development and the related manifestation of a second spawning time, this is apparently not the only factor involved. Therefore, research will be directed to the storage and use of energy reserves (lipids) in relation to the availability of preyfish and the dynamics of the gonad cycle.

**3.3.14. Experimental feeding of eel (*Anguilla anguilla*) and bream (*Abramis brama*) (Project V 13; E.H.R.R. Lammens, H.W. de Nie)**

Factors influencing the efficiency of feeding on chironomids (the amount of chironomids eaten per time unit) by eel and bream were determined. The effects of variations in chironomid density and distribution and those of composition of the substrate and light intensity were studied. Density had, within certain limits only, a great influence on the feeding efficiency; outside these limits feeding efficiency tended to be constant. For a bream of 600 g the upper limit was approximately 30 g.fr wt m<sup>-2</sup>, the lower limit 0.5 g.fr wt m<sup>-2</sup>. The efficiency increased 2-3 times when the chironomids were clumped in 1/6 th of the original surface area.

For two size classes of bream an inverse relationship between feeding efficiencies and particle size of substrate was found. Besides, feeding efficiency and depth at which the chironomids were present in the substrate were related inversely. A relation between feeding efficiency and light intensity could not be found.

**3.3.15. Ecology of 0<sup>+</sup> fish in the Frisian lakes (Project V 14; W.L.T. van Densen)**

The project is now completed and the results have been published (van Densen & Vijverberg, 1982).

**3.3.16. Effects of the Bergum Power Station on zooplankton and 0<sup>+</sup> fish in the Bergumermeer (Project V 15; W.L.T. van Densen, H.W. de Nie)**

The project is now completed and the results have been published (de Nie, 1982; van Densen & Hadderlingh, 1982).

**3.3.17. The effects of experimental removal of bream in the Morra (Project V 16; W.L.T. van Densen, E.H.R.R. Lammens, H.W. de Nie, J. Vijverberg)**

The feeding behaviour of bream, eel and ruffe and the possible competitive relationships with each other and with 0<sup>+</sup> fish were studied in the Morra, a lake with an area of 234 ha in the SW-part of the Frisian Lake District.

The fish populations were sampled with a trawl thrice during the growing season (June, July, September), simultaneously with the sampling of zooplankton, chironomids and *Neomysis integer*. The abundance of 0<sup>+</sup> fish at the end of the season was high compared with that in the Tjeukemeer. This was also indicated by a smaller size of *Daphnia* compared with that in the Tjeukemeer. This smaller size reduced the availability of zooplankton for the larger bream which, therefore, is forced to feed on rather low chironomid densities (500-2000 ind. m<sup>-2</sup>) and thus shows poor condition during the whole summer. Because *Chirono-*

*mus plumosus* - a very large species - dominated the standing stock the chironomid biomass was c. 10 times higher than in the Tjeukemeer. This stock was also exploited by eel and ruffe, most energy being derived from *Chironomus* as was the case for the bream. Larger eel supplemented its diet with 0<sup>+</sup> fish, but ruffe consumed only chironomids. The higher standing stock of chironomid biomass in the Morra than in the Tjeukemeer did not result in a better growth or condition for eel or ruffe.

### 3.3.18. Distribution of cyprinids in the Tjeukemeer (Project V 17; E.H.R.R. Lammens)

Gill-nets with mesh-sizes of 35, 50 and 60 mm were placed parallel along the shore-line at 10, 100 and 1000 m. For each distance a length-frequency distribution of each species (bream, white bream, roach and ide) was made. Gut contents were collected and sexes ascertained. Simultaneously the density of chironomids was determined (Section 3.3.6). Biomass of the bream caught at 1000 m was approximately 3 times higher than at 10 and 100 m; numbers were c. 5 times higher. Bream smaller than 33 cm forklength were most abundant at 1000 m; bream larger than 33 cm were most abundant at 10 m. So there existed an opposite gradient of frequency of occurrence for small and large bream from the shore to the middle of the lake.

Most chironomids were found near the shore, which corresponded with the gut contents. In spring there was also a differentiation in the distribution of sexes. At 10 m the males outnumbered the females and at 1000 m the opposite was true. This differentiation already started in late winter.

The distribution of white bream was more even but the same gradient as found for bream was noticed: the large individuals close to the shore-line, the small ones more in the middle of the lake.

Roach and ide frequented the shore more than the open water, but their numbers were too small to differentiate for the size classes.

## 4. Publications

### 4.1. PAPERS PUBLISHED IN 1982

Beattie, D.M. - Distribution and production of the larval chironomid populations in Tjeukemeer. *Hydrobiologia* 95, 287-306\*

Best, E.P.H. - Growth modelling in aquatic macrophytes. In: Studies on aquatic vascular plants; Eds. J.J. Symoens, S.S. Hooper and P. Compère. Brussels, Royal Bot. Soc. of Belgium; 102-111.

Best, E.P.H. - Hormonal interactions in *Ceratophyllum demersum*. *Aquat. Bot.* 13, 87-95

Best, E.P.H. - The aquatic macrophytes of Lake Vechten. Species composition, spatial distribution and production, *Hydrobiologia* 95, 65-77\*

Best, E.P.H., M. Zippin and J.H.A. Dassen - Studies on decomposition of *Phragmites australis* leaves under laboratory conditions. *Hydrobiol. Bull.* 16, 21-33

Blaauboer, M.C.I. - The phytoplankton species composition and the seasonal periodicity in Lake Vechten from 1956 to 1979. *Hydrobiologia* 95, 25-36\*

Blaauboer, M.C.I., R. van Keulen and Th.E. Cappenberg - Extracellular release of photosynthetic products by freshwater phytoplankton populations, with special reference to the algal species involved. *Freshwat. Biol.* 12, 559-572

Bremer, P. and J. Vijverberg - Production, population biology and diet of *Neomysis integer* (Leach) in a shallow Frisian Lake (The Netherlands). *Hydrobiologia* 93, 41-51. - Also in: Ecology of Mysidacea; Ed. M.D. Morgan. The Hague etc., Junk, 1982; *Developments in Hydrobiology* 10; 41-51

- Cappenberg, Th.E., K.A. Hordijk, G.J. Jonkheer and J.P.M. Lauwen - Carbon flow across the sediment-water interface in Lake Vechten, The Netherlands. *Hydrobiologia* 91/92, 161-168. - Also in: Sediment/freshwater interaction; Proceedings of the Second International Symposium held in Kingston, Ontario, 15-18 June 1981; Ed. P.G. Sly. The Hague etc., Junk, 1982; *Developments in Hydrobiology* 9, 161-168
- Cappenberg, Th.E. and H. Verdouw - Sedimentation and breakdown kinetics of organic matter in the anaerobic zone of Lake Vechten. *Hydrobiologia* 95, 165-179\*
- Densen, W.L.T. van - Sampling with purse seines (p. 61-66); Sampling with perch traps (p. 77-81); Sampling with fykes (p. 83-90); Sampling with gill nets (p. 91-101); Estimation of the population size by intensive fishing (Leslie method) (p. 137-142) (in Dutch). In: *Methoden ter bemonstering van visbestanden* (Methods for sampling of fish populations). Nieuwegein, Organisatie ter Verbetering van de Binnenvisserij, 1982
- Densen, W.L.T. van - The eel and bream problem in Dutch inland waters (in Dutch). *Onze Zoetwaterviss.* 75(5), 1-6
- Densen, W.L.T. van and R.H. Hadderingh - Effects of entrapment and cooling water discharge by the Bergum Power Station on 0<sup>+</sup> fish in the Bergumermeer. *Hydrobiologia* 95, 351-368\*
- Densen, W.L.T. van and J. Vijverberg - The relations between 0<sup>+</sup> fish density, zooplankton size and the vulnerability of pikeperch, *Stizostedion lucioperca*, to angling in the Frisian lakes. *Hydrobiologia* 95, 321-336\*
- Dvořák, J. and E.P.H. Best - Macro-invertebrate communities associated with the macrophytes of Lake Vechten: structural and functional relationships. *Hydrobiologia* 95, 115-126\*
- Gons, H.J. - Structural and functional characteristics of epiphyton and epipelton in relation to their distribution in Lake Vechten. *Hydrobiologia* 95, 79-114\*
- Gulati, R.D., K. Siewertsen and G. Postema - The zooplankton: its community structure, food and feeding, and role in the ecosystem of Lake Vechten. *Hydrobiologia* 95, 127-163\*
- Haan, H. de - Physico-chemical environment in Tjeukemeer with special reference to speciation of algal nutrients. *Hydrobiologia* 95, 205-221\*
- Haan, H. de, T. de Boer, H.A. Kramer and J. Voerman - Applicability of light absorbance as a measure of organic carbon in humic lake water. *Wat. Res.* 16, 1047-1050
- Haan, H. de, J.B.W. Wanders and J.R. Moed - Multiple addition bioassay of Tjeukemeer water. *Hydrobiologia* 88, 233-244
- Hogeweg, P. and A.F. Richter - INSTAR, a discrete event model for simulating zooplankton population dynamics. *Hydrobiologia* 95, 275-285\*
- Kloet, W.A. de - The primary production of phytoplankton in Lake Vechten. *Hydrobiologia* 95, 37-57\*
- Lammens, E.H.H.R. - Growth, condition and gonad development of bream (*Abramis brama* L.) in relation to its feeding conditions in Tjeukemeer. *Hydrobiologia* 95, 311-320\*
- Leenen, J.D. - Hydrology of Tjeukemeer. *Hydrobiologia* 95, 199-203\*
- Moed, J.R. and H.L. Hoogveld - The algal periodicity in Tjeukemeer during 1968-1978. *Hydrobiologia* 95, 223-234\*
- Nie, H.W. de - Effects of thermal effluents from the Bergum Power Station on the zooplankton in the Bergumermeer. *Hydrobiologia* 95, 337-349\*

Nie, H.W. de - A note on the significance of larger bivalve molluscs (*Anodonta* spp. and *Dreissena* sp.) in the food of the eel (*Anguilla anguilla*) in Tjeukemeer. *Hydrobiologia* 95, 307-310\*

Olie, J.J. and Th.E. Cappenberg - Aspects of aerobic mineralization during spring in Lake Vechten with special reference to the <sup>14</sup>C-labelling technique. *Hydrobiologia* 95, 181-190\*

Olie, J.J., M.C.I. Blaauboer, R. van Keulen and Th.E. Cappenberg - Aerobic mineralization of organic matter in the open-water region of Lake Vechten. *Hydrobiol. Bull.* 16, 69-70

Parma, S. - The twenty-fifth anniversary of the Limnological Institute, The Netherlands (1957-1982). *Hydrobiologia* 95, 1-9\*

Progress Report 1981, eds. S. Parma and R.D. Gulati. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Reeks 2*, 79, 66 p.

Reins, A. - The effect of food concentration and water temperature on the filtering rate of zooplankton. *Neth. J. Zool.* 31, 613-614. (1981)●

Steenbergen, C.L.M. - Contribution of photosynthetic sulphur bacteria to primary production in Lake Vechten. *Hydrobiologia* 95, 59-64\*

Steenbergen, C.L.M. and H. Verdouw - Lake Vechten: aspects of its morphometry, climate, hydrology and physico-chemical characteristics. *Hydrobiologia* 95, 11-23\*

Steenbergen, C.L.M. and H.J. Korthals - Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of lake Vechten (The Netherlands). Pigment analysis and role in primary production. *Limnol. Oceanogr.* 27, 883-895

Studies on Lake Vechten and Tjeukemeer, The Netherlands; 25th anniversary of the Limnological Institute of the Royal Netherlands Academy of Arts and Sciences; Eds. R.D. Gulati and S. Parma. The Hague etc., Junk, 1982; *Developments in Hydrobiology* 11, VIII, 383 p. - Also as: *Hydrobiologia* 95

Veeningen, R. - Temporal and spatial variations of dissolved oxygen concentrations in some Dutch polder ditches. *Hydrobiologia* 95, 369-383\*

Verdouw, H. and E.M.J. Dekkers - Nitrogen cycle of Lake Vechten: concentration patterns and internal mass-balance. *Hydrobiologia* 95, 191-197\*

Verdouw, H. and E.M.J. Dekkers - Nitrogen cycle of Lake Vechten (The Netherlands); role of sedimentation. *Arch. Hydrobiol.* 94, 251-263

Vijverberg, J. and A.F. Richter - Population dynamics and production of *Daphnia hyalina* Leydig and *Daphnia cucullata* Sars in Tjeukemeer. *Hydrobiologia* 95, 235-259\*

Vijverberg, J. and A.F. Richter - Population dynamics and production of *Acanthocyclops robustus* (Sars) and *Mesocyclops leuckarti* (Claus) in Tjeukemeer. *Hydrobiologia* 95, 261-274\*

Vries, D.A. de - A comparison of five sampling techniques for zooplankton. *Neth. J. Zool.* 32, 273-274●

\* Also in:

Studies on Lake Vechten and Tjeukemeer, The Netherlands; 25th anniversary of the Limnological Institute of the Netherlands Academy of Arts and Sciences; Eds. R.D. Gulati and S. Parma. The Hague etc., Junk, 1982; *Developments in Hydrobiology* 11 (same pages).

● Abstract only.

#### 4.2. PAPERS IN PRESS

- Best, E.P.H. - Effects of water pollution on freshwater submerged macrophytes. *Wat. Pollut. Manag. Rev.* 1982, 27-56 (1983)
- Best, E.P.H. and K. Kersting - Oxygen production and consumption in a *Ceratophyllum demersum* vegetation; measurements in enclosed and "open" communities. In: Proceedings 1st European Workshop on aquatic macrophytes
- Cappenberg, Th.E. - Microbial break-down processes of organic matter in anaerobic freshwater ecosystems. *Advan. Microb. Ecol.*
- Cappenberg, Th.E., L. van Breemen and J. Kaper - Microbial interactions in anaerobic mineralization: a case of interspecies hydrogen transfer. *Microb. Ecol.*
- Densen, W.L.T. van - Fish ecological studies in Tjeukemeer and fishery management. *Hydrobiol. Bull.* 17, 59-65 (1983)
- Densen, W.L.T. van and J. Vijverberg - De rol van de vis in het voedselweb van het Tjeukemeer. In: Oecologie van meren en plassen; Symposium Biologische Raad. Wageningen, PUDOC
- Duncan, A. and R.D. Gulati - A diurnal study of the planktonic rotifer populations in Parakrama Samudra Reservoir, Sri Lanka. In: Limnology of Parakrama Samudra - Sri Lanka; Ed. F. Schiemer. The Hague etc., Junk, 1983; *Developments in Hydrobiology* 12, 95-106
- Duncan, A. and R.D. Gulati - Feeding studies with natural food particles on tropical species of planktonic rotifers. In: Limnology of Parakrama Samudra - Sri Lanka; Ed. F. Schiemer. The Hague etc., Junk, 1983; *Developments in Hydrobiology* 12, 117-125
- Flik, B.J.G. and W.A. de Kloet - Measurements of freshwater phytoplankton; p. 35-36 in: The measurement of primary production: problems and recommendations; Eds. F. Colijn, W.W.C. Gieskes and W. Zevenboom. *Hydrobiol. Bull.* 17, 29-51 (1983)
- Gons, H.J. - De koolstofkringloop in drie Nederlandse meren. In: Oecologie van meren en plassen; Symposium Biologische Raad. Wageningen, PUDOC
- Gons, H.J. and R. van Keulen - Seasonal changes in organic matter and dark oxygen uptake of epiphyton and epipelon in relation to seston deposition in Lake Vechten (The Netherlands). *Developments in Hydrobiology*
- Gulati, R.D. - Zoöplankton en de grazing ervan als indicatoren voor de trofiegraad. In: Ecologische indicatoren voor kwaliteitsbeoordeling van lucht, water, bodem en ecosystemen; Eds. P.H. Best and J. Haeck. Wageningen, PUDOC
- Gulati, R.D. - Zooplankton and its grazing as indicators of trophic status in Dutch lakes. *Environm. Monitor Assessm.* 3
- Haan, H. de - Use of ultraviolet spectroscopy, gel filtration, pyrolysis/mass spectrometry and numbers of benzoate-metabolizing bacteria in the study of humification and degradation of aquatic organic matter. In: Aquatic and terrestrial humic materials; Eds. R.F. Christman and E.T. Gjessing. Ann Arbor, Ann Arbor Science, 1983; 165-182
- Hordijk, C.A. and Th.E. Cappenberg - Quantitative High-Pressure Liquid Chromatography-fluorescence determination of some important lower fatty acids in lake sediments. *Appl. Environm. Microbiol.* 46, 361-369
- Olie, J.J., C.L.M. Steenbergen, H. Verdouw and Th.E. Cappenberg - Mineralization of organic matter in a stratifying lake-ecosystem. *Antonie van Leeuwenhoek*
- Parma, S. - Aquatisch-oecosysteemonderzoek in wetenschappelijk en maatschappelijk kader. In: Oecologie van meren en plassen; Symposium Biologische Raad. Wageningen, PUDOC

Veeningen, R. - The dynamics of dissolved oxygen concentration for water quality monitoring and assessment in polder ditches. Environm. Monitor. Assessm. 3

Veeningen, R. - De zuurstofhuishouding als ecologische indicator voor de kwaliteitsbeoordeling van poldersloten. In: Ecologische indicatoren voor kwaliteitsbeoordeling van lucht, water, bodem en ecosystemen; Eds. P.H. Best and J. Haeck. Wageningen, PUDOC

#### 4.3. INTERNAL REPORTS

Arkesteijn-Dijksman, L. - The effect of limitation by some nutrients on the cell characteristics of *Oscillatoria limnetica* in continuous culture (in Dutch). Internal Report 1982-1. 52 p.

Siewertsen, K. - Excretion of nitrogen and phosphorus by zooplankton in Lake Vechten (in Dutch). Internal Report 1982-4. 22 p.

Steenbergen, C.L.M. - Photosynthetic pigment analysis as a tool to characterize the structure of phytoplankton communities (in Dutch). Note 1982-6. 13 p.

Werlemark, G. and H. de Haan - Particle size of fulvic acids from polder water as a function of pH, using gel filtration, dialysis, and ultrafiltration (in English). Internal Report 1982-5. 10 p.

Wisselo, A.G. - A study of the exchangeable phosphates and total phosphates at the mud-water interface in the Loosdrecht Lakes (in Dutch). Internal Report 1982-2, 11 p.

#### 4.4. STUDENT AND TRAINEE REPORTS

Bakker, H. - Age composition and growth of the pikeperch (*Stizostedion lucioperca*) in the Tjeukemeer in 1980 (in Dutch). Student Report 1982-10. 41 p.

Bakker, J. - Bioassay experiments with Tjeukemeer water, 1982 (in Dutch). Student Report 1982-8, 33 p.

Bijlard, A.F. - Comparison of the food intake of the bream (*Abramis brama*) and the eel (*Anguilla anguilla*) in the Tjeukemeer (in Dutch). Student Report 1982-9. 50 p.

Bos, J.J. - The water balance of Lake Vechten (in Dutch). Student Report 1981-14. 54 p.

Eijgenraam, A. - Physicochemical aspects of manganese in the Tjeukemeer (in Dutch). Student Report 1982-7. 45 p.

Jagtman, E. - The effects of oil pollution on the macrophytes of aquatic ecosystems (in English). Student Report 1982-11. 52 p.

Kroon, A. - Growth and changes in density of 0<sup>+</sup> fish, especially the pikeperch (*Stizostedion lucioperca*), the perch (*Perca fluviatilis*) and the smelt (*Osmerus eperlanus*) in some Frisian lakes (in Dutch). Student Report 1982-12. 96 p.

Loenhout, R.C.J. van - Population structure, condition, reproductive cycle and food uptake of pikeperch (*Stizostedion lucioperca*) older than one year in the Tjeukemeer in 1981 (in Dutch). Student Report 1982-14. 41 p.

Mathijssen, J.T.G. - Turnover and diffusion rates of methane and lower fatty acids in Lake Vechten (in Dutch). Student Report 1982-2. 81 p.

Pot, R. - The role of bacteria as a food source for herbivorous zooplankton in Lake Vechten (in Dutch). Student Report 1982-13. 67 p.

Toet, W.A. - Food and food selection of pikeperch larvae in the Tjeukemeer, 1981 (in Dutch). Student Report 1982-3. 24 p.

Verbrugge, R. - Recruitment of 0<sup>+</sup> pikeperch in the Tjeukemeer in 1981 (in Dutch). Student Report 1982-4. 20 p.

Vlieger, C. de - Phytoplankton studies in the Loosdrecht Lakes area using photosynthetic pigment analysis (in Dutch). Student Report 1982-5. 31 p.

Werkhoven, J.A. - Growth and photosynthesis of the pennate diatom *Nitzschia palea* (in Dutch). Student Report 1982-6. 94 p.

Westhoff, M.J. - Composition of the photosynthetic pigments of phytoplankton in the Loosdrecht Lakes area (in Dutch). Student Report 1982-1. 22 p.

## 5. Acknowledgements

We thank Miss M.C.G. Röling for typing the manuscript, Mr. E.M. Mariën for photographic work and Drs. B.Z. Salomé for the arrangement of chapter 4 and for proof-reading.

# **Delta Institute for Hydrobiological Research**

---

## **Progress Report 1982**

**Editing team: E.K. Duursma, J. Coosen, E.A.M.J. Daemen,  
A.B.J. Sepers, A.H.L. Huiskes, E.S. Nieuwenhuize (convener),  
M.J. Van Leerdam (word-processing), A.A. Bolsius (design)  
and R.H.G. Kleingeld (photographs)**



Publication  $\Delta$ -263 of the Delta  
Institute for Hydrobiological  
Research, Vierstraat 28,  
4401 EA Yerseke, The Netherlands

## **CONTENTS**

- I. HISTORY AND ORGANIZATION OF THE INSTITUTE 5
- II. INTRODUCTION (E.K. Duursma) 7
- III. CONTRACT RESEARCH AND COOPERATION 10
- IV. GENERAL ECOLOGICAL CONDITIONS IN 1982 (R. Peelen) 12
- V. WORKING GROUP: ELEMENTS CYCLING AND FOOD CHAINS (CODE G + K) 15
- V.1. Introduction (P.H. Nienhuis) 15
- V.2. Identification of faunal shifts in the Keeten-Krammer-Volkerak area using numerical classification (A2) (J. Coosen and A. Van den Dool) 15
- V.3. Some remarks on the occurrence of waders (oystercatcher, curlew) and macrozoobenthos before, during and after the attempt of closing the western Markiezaatsdam (A2) (B. Van Dessel and J. Coosen) 19
- V.4. Influence of production and mineralization on nutrient balances in Lake Grevelingen (G1, 2, 3, 5) (I. De Vries and M.F. Veul - Delft Hydraulics Laboratory, WABASIM project -) 20
- V.5. A simulation curve of the phosphate concentration in Lake Grevelingen (G 2) (P. Kelderman) 23
- V.6. Carbon budget of the eelgrass (*Zostera marina*) community in Lake Grevelingen (G6) (P.H. Nienhuis) 25
- V.7. The continuing saga of an introduced brown alga (*Sargassum muticum*) and its establishment within the s.w. Netherlands (G 6, K 5) (A. Critchley - Royal Society European Exchange Fellowship; present address: University of Natal, Dept. of Botany, Pietermaritzburg 3200, Natal, South Africa - ) 27
- V.8. A survey of the shore crab (*Carcinus maenas* L.) in Lake Grevelingen (G7) (R.H.D. Lambeck and E.G.J. Wessel) 29
- V.9. Population dynamics and migration of the netted dogwhelk (*Nassarius reticulatus*) in Lake Grevelingen (G7) (E.J.H. Sterenborg and R.H.D. Lambeck) 31
- V.10. A tentative population model of the mussel *Mytilus edulis* in Lake Grevelingen (G7) (J.H.G. Verhagen) 33
- V.11. Role of the meiofauna in the breakdown of organic matter in Lake Grevelingen (G8) (M.J. Orban) 35
- V.12. Fast growth of 0-group plaice (*Pleuronectes platessa* L.) in Lake Grevelingen (G9) (G. Doornbos, R.H. Bogaards and P. De Koeijer) 37
- V.13. Effect of water temperature on gear efficiency (G 9) (G. Doornbos and F. Twisk) 40
- V.14. Oxygen, sulphide, redoxpotential and pH microgradients in Lake Grevelingen sediment (G 10) (H.J. Lindeboom, A.J.J. Sandee and H.A.J. De Klerk) 41

- V.15. Transport of organic matter in the Oosterschelde (K 1) (J.H.B.W. Elgershuizen) 43
- V.16. Seston analysis (K1, K3) (E.T. Van Ierland and L. Peperzak) 45
- V.17. Phytoplankton production measurements in the Oosterschelde (K3) (F. Vegter) 46
- V.18. The importance of seston dependant variables during tidal cycles for daily primary production calculations (K 3) (P.R.M. De Visscher) 48
- V.19. Coulter countings of fresh and preserved seston samples (K3) (M.L.M. Tackx and J.W. Francke) 49
- V.20. Contribution and nature of  $\mu$ -cell aggregates in the seston of the Oosterschelde (K3) (C. Bakker, J.C.M. Rijk, M.L.M. Tackx) 50
- V.21. Seston, zooplankton biomass (I) and - grazing (II) during a 24-hour measurement in the Oosterschelde (K4) (C. Bakker, J.C.M. Rijk and P. Van Rijswijk - DIHO; M.H. Daro, O. Cromboom and R. Van den Wijngaert - VU, Brussels) 52
- V.22. Zooplankton biomass, succession and interrelationships in the Oosterschelde (K4) (C. Bakker and P. Van Rijswijk) 54
- V.23. A comparison of grazing measurements on two types of particulate matter-distributions (K4) (M.L.M. Tackx and J.W. Francke) 55
- V.24. Biomass measurements of the microphytobenthos in the Oosterschelde (K5) (E.A.M.J. Daemen and M.T.T. De Leeuw) 57
- VI. WORKING GROUP: BRACKISH WATERS (Code B) 58
- VI.1. Introduction (A.B.J. Sepers) 58
- VI.2. Aquatic and semi-aquatic Hemiptera in the inland waters of the s.w.-Netherlands (B3) (R. Luyendijk and B.P.M. Krebs) 59
- VI.3. The activity of heterotrophic bacteria at variable environmental conditions (B8) (A.B.J. Sepers and F.W. Melissen) 61
- VI.4. The role of the salinity in the selection of brackish water phytoplankton (B10) (J.W. Rijstenbil) 63
- VI.5. The impacts of the storm-surge barrier on the macrozoobenthos of the Oosterschelde (B17) (H. Hummel) 65
- VII. RESEARCH GROUP: SALT-MARSH ECOSYSTEMS (Code S) 65
- VII.1. Introduction (A.H.L. Huiskes) 65
- VII.2. Structure and production of salt-marsh vegetation (S1) (G.J.C. Buth and H. Fuchs) 66
- VII.3. Distribution and ecology of mycorrhizae in salt marshes (S1) (S. Mastenbroek and W.G. Beeftink) 68
- VII.4. Geohydrology of salt marshes in relation to the tides (S1) (W.G. Beeftink, M.C. Daane and R.J. Kolpa - Technical University Delft) 68

- VII.5. Transport of seeds and seedlings in the salt marsh east of Krabbendijke (S1) (B.P. Koutstaal, M.M. Markusse and W. De Munck) 69
- VII.6. A scanner for the recording of infrequently fluctuating environmental parameters (S1) (A.H.L. Huiskes and R. Middel) 69
- VII.7. The demography of *Aster tripolium* in various vegetation zones of the salt marsh near Ellewoutsdijk (S2) (A.H.L. Huiskes and J. Van Soelen) 70
- VII.8. Survival of seedlings of *Aster tripolium* in various vegetation zones in the salt marsh near Bergen op Zoom (S2) (A.H.L. Huiskes and M.M. Markusse) 71
- VII.9. Germination of *Aster tripolium* seeds collected at different locations (S2) (A.H.L. Huiskes, J. Van Soelen and M.M. Markusse) 72
- VII.10. The nitrate content and the growth of 2 ecotypes of *Aster tripolium* (L.) (S2) (A.W. Stienstra) 73
- VII.11. Population ecology of *Atriplex littoralis* and *A. hastata* (S2) (B.P. Koutstaal) 75
- VII.12. The role of *Orchestia gammarella* on the decomposition rate of dead salt marsh plants (S3) (M. Lambert and A.M. Groenendijk) 75
- VII.13. Decomposition of some halophytes (S3) (G.J.C. Buth and R. Voeselek) 76
- VII.14. Accumulation of heavy metals in the salt marsh (S4) (W.G. Beeftink and J. Nieuwenhuize) 77
- VII.15. Influence of tidal management on salt-marsh angiosperms (S5) (A.M. Groenendijk and M.A. Lievaart) 77
- VIII. A-SUBJECTS (ANTHROPOGENIC SUBSTANCES AND OTHER INTERWORKING GROUP PROJECTS) 78
- VIII.1. Artificial radionuclides in Rijn-Maas-Schelde delta (A12) (A. Thomas, J.M. Martin - Lab. Géol. Paris -, E.K. Duursma, J. Nieuwenhuize - DHO - and R. Penners, M.J. Frissel - ITAL, Wageningen-) (30% funding by CEC, Brussels) 78
- VIII.2. Wet deposition of toxic metals from the atmosphere in the Oosterschelde region (V.D. Nguyen - Institute of Applied Physical Chemistry, Nuclear Research Centre (KFA) Jülich, FRG - and A.G.A. Merks) 80
- VIII.3. Trace metal sorption experiments at elevated temperatures (L.A. Van Geldermalsen) 81
- IX. ARTICLES SUBMITTED FOR PUBLICATION OR IN PRESS 83
- X. PUBLISHED ARTICLES AND REPORTS IN 1982 86
- X.1. Working group 'Elements cycling and food chains' 86
- X.2. Working group 'Brackish waters' 88
- X.3. Working group 'Salt-marsh ecosystems' 89
- X.4. A-Subjects (Anthropogenic substances and other interworking group projects) 89

## 1. HISTORY AND ORGANIZATION OF THE INSTITUTE

In 1957 the Division of Natural Sciences of the Royal Netherlands Academy of Arts and Sciences, reacting on an initiative of the Commission for Ecology, created an institute to be established in the delta area of the south-west Netherlands, with the aim of studying the environmental changes to be expected as result of the closing of the various river mouths and sea arms in this area.

When the Zuiderzee, in the centre of The Netherlands was closed by a dam and converted into the freshwater Ysselake in 1932, extensive biological research was carried out by a group of fishery biologists, members of botanical and zoological societies and academic staff. The results obtained during this study, warranted the expectation that in the more diversified delta area of the rivers Rijn, Maas and Schelde, even more results could be achieved, especially so when one institute located in the area was given the task to make a coordinated effort to study the problems from various angles. After an exploratory phase, in which a distribution of biota was studied from an ecological point of view, research was initiated to elucidate the causal background of the changes observed, and to develop concepts on ecosystem functioning and management.



Photograph 1.  
Presentation of the first copy of the book "The Dutch Delta, a compromise between environment and technology in the struggle against the sea" to Her Royal Highness Princess Juliana, 20 October 1982. This joint jubilee book (see Progress Report of last year) is for sale with Natuur and Techniek, P.O. Box 415, 6200 AK Maastricht NL. An extra edition with a 48 page English summary and captions is available. (Photograph Jaap Wolterbeek).

The institute is located at Yerseke on the Oosterschelde, the sea arm to be semi-closed in the last stage of the s.c. 'Delta Plan'. The exploitation of the institute and management is financed by means of funds allotted to the Academy, by the Ministry of Education and Science.

The institute had in 1981 a permanent staff of personnel of 55 including 13 scientists. Additionally 12 short-term contract scientists with assistants, and students and trainees took part in the programme of the institute (Table 1).

Table 1. Scheme of personnel (1 Dec. 1982)

WORKING GROUPS		
Elements cycling and food chains (G, K)	Salt-marsh ecosystems (S)	Brackish waters (B)
<u>Botany:</u> Dr. P.H. Nienhuis (w.g. leader) B.H.H. De Bree J.M. Verschuure	<u>Communities:</u> Dr. Ir. W.G. Beeftink <sup>■</sup> (w.g. leader) M.C. Daane B.P. Koutstaal W. De Munck	<u>Microbiology:</u> Dr. A.B.J. Sepers (w.g. leader) F.W. Melissen  <u>Plankton biology:</u> Ir. J.W. Rijstenbil L. De Wolf
<u>Zoology:</u> Drs. R.H.D. Lambeck E.G.J. Wessel A.J.J. Sandee <u>Meiofauna</u> Drs. M.J. Orban (temporary)	<u>Populations:</u> Dr. A.H.L. Huiskes Drs. A.W. Stienstra M.M. Markusse J. Van Soelen	<u>Entomology:</u> B.P.M. Krebs
<u>Plankton:</u> Drs. C. Bakker P. Van Rijswijk J.C.M. Rijk	<u>Biomass budget</u> Drs. G.J.C. Buth (BION project)	<u>Zoology:</u> Drs. C.H. Borghouts R.H. Bogaards <sup>■</sup> J.W. Francke <sup>■</sup>
<u>Primary production:</u> Drs. F. Vegter P.R.M. De Visscher		
SHORT-TERM PROJECTS		
<u>BALANS-R.W.S. (K)</u> <u>Research Food balance</u> <u>Oosterschelde</u>	<u>Effects storm-surge barrier</u> <u>salt marsh (VEGIN R.W.S.): (S)</u> Drs. A.M. Groenendijk M.A. Vink	<u>MISCELLANEOUS</u> Dr. E.K. Duursma <sup>■</sup> (coordinator)
<u>Organic matter transport (K)</u> Drs. J.H.B.W. Elgershuizen A. Nijse D.J. Van Hekken	<u>Diversity research- R.W.S. (B)</u> Drs. H. Hummel	<u>Projectgroup ANTHROS:</u> <u>(Anthropogenic substances)</u> Dr. E.K. Duursma <sup>■</sup> Dr. Ir. W.G. Beeftink <sup>■</sup> Dr. A.H.L. Huiskes <sup>■</sup> A.G.A. Merks <sup>■</sup> J. Nieuwenhuize <sup>■</sup>
<u>Qualification and quantification</u> <u>of the microphytobenthos (K)</u> Drs. E.A.M.J. Daemen M.T.T. De Leeuw	<u>ZOWEC-R.W.S. (G)</u> saline water ecology	<u>Litoral-water exchange:</u> Drs. L.A. Van Geldermaisen
<u>Consumption and assimilation by</u> <u>zooplankton: (K)</u> Drs. M.L.M. Tackx <sup>■</sup> J.W. Francke <sup>■</sup>	<u>Fishery: (G)</u> Drs. G. Doornbos F. Twisk R.H. Bogaards <sup>■</sup>	<u>Ecological research</u> <u>zoobenthos R.W.S. (ZACHTSUB)</u> Drs. J. Coosen A. Van den Dool
<u>Decomposition of organic matter</u> <u>by bacteria (G, K)</u> Drs. J.G.C.M. Goossens Dr. H.J. Lindeboom H.A.J. De Klerk J.C. Verplanke	<u>Seston research: (G)</u> Drs. E.T. Van Ierland	

## GENERAL DEPARTMENTS

### Science information:

(book, courses, excursions):

Dr. E.K. Duursma<sup>■</sup>  
Drs. R. Peelen  
R.H.G. Kleingeld<sup>■</sup>  
P.J. Van Boven

### Library:

M.A. Pronk  
E.S. Nieuwenhuize<sup>■</sup>

### Photography:

R.H.G. Kleingeld<sup>■</sup>

### Design and off-sets:

J.A. Van den Ende  
A.A. Bolsius

### Research vessels:

W.J.L. Robër  
C.M. De Rooy  
J.A. Van Sprundel  
P. De Koeljer

### Biomathematics:

Dr. A.G. Vlasblom  
J.J. Guerand

### Sedimentology:

J. Nieuwenhuize<sup>■</sup>  
J.M. Van Liere  
C.H. Vos

### Chemistry:

A.G.A. Merks<sup>■</sup>  
J.J. Sinke  
J.O. Van de Zande

### Aquarium:

P.J. Van Boven<sup>■</sup>

### Administration:

L.J. Goud<sup>■</sup>  
M.A. Manneke  
J.C. Ruissen  
M.E.W. Van Veen (temporary)

### Reception/typing:

M.J. Van Leerdam

### Technical Service:

C. Almekinders  
J.P. Hoekman  
Electronics  
R. Middel

### Household Service:

K.C. Zweedijk  
J.J. Braam  
J.A. Goedhart

Director: Dr. E.K. Duursma<sup>■</sup>  
Managers: L.J. Goud<sup>■</sup>  
Secretary: E.S. Nieuwenhuize<sup>■</sup>

<sup>■</sup> = double mentioned

### Students and Trainees (1982)

B. Van Dessel, H. Fuchs, A. Kroon, S.J. Lemmens, A. Paarlberg, E. Sterenborg, B. Van Tussenbroek,  
R. Voeselek, R.M. Wolfs.  
H.A.J.M. De Bie, R. Brand, E.J. Dingemanse, J.W.C. Oostdijk, A.J. Pouwer, R.L. Willems.

## II. INTRODUCTION (E.K. Duursma)

By 1987 the so-called Delta Plan will attain its final achievement. Four former estuaries of the s.w. Netherlands will be protected against storm floods by barriers, while a fifth one will remain in its original state, surrounded only by enforced dikes. Two barriers are large dams, while the other two have complex sluices to allow release to or exchange with the North Sea. Additional secondary dams were and are built, dividing the estuaries up in a river head (Haringvliet), a saline lake (Grevelingen), a brackish lake (Lake Veere), and a saline tidal sea arm (Oosterschelde) (Fig. 1A).

Drastic changes have occurred and still will occur in the aquatic and semi-terrestrial ecosystems of the former estuaries (Fig. 1B), from which parts have great nature-reserve values and are used for fisheries and aquaculture. Surrounded by the highly populated industrial area of Rotterdam, Antwerp and Ghent there is a potential anthropogenic stress from shipping, recreation and pollution, although the Oosterschelde and the Lake Grevelingen are still considered as well-developed, rather undisturbed ecological systems. In particular the Oosterschelde has a great value for development of young marine species and for winter refuge of migrating European and Northwestasian birds.

The hydrobiological studies of the institute have been started in 1957 with

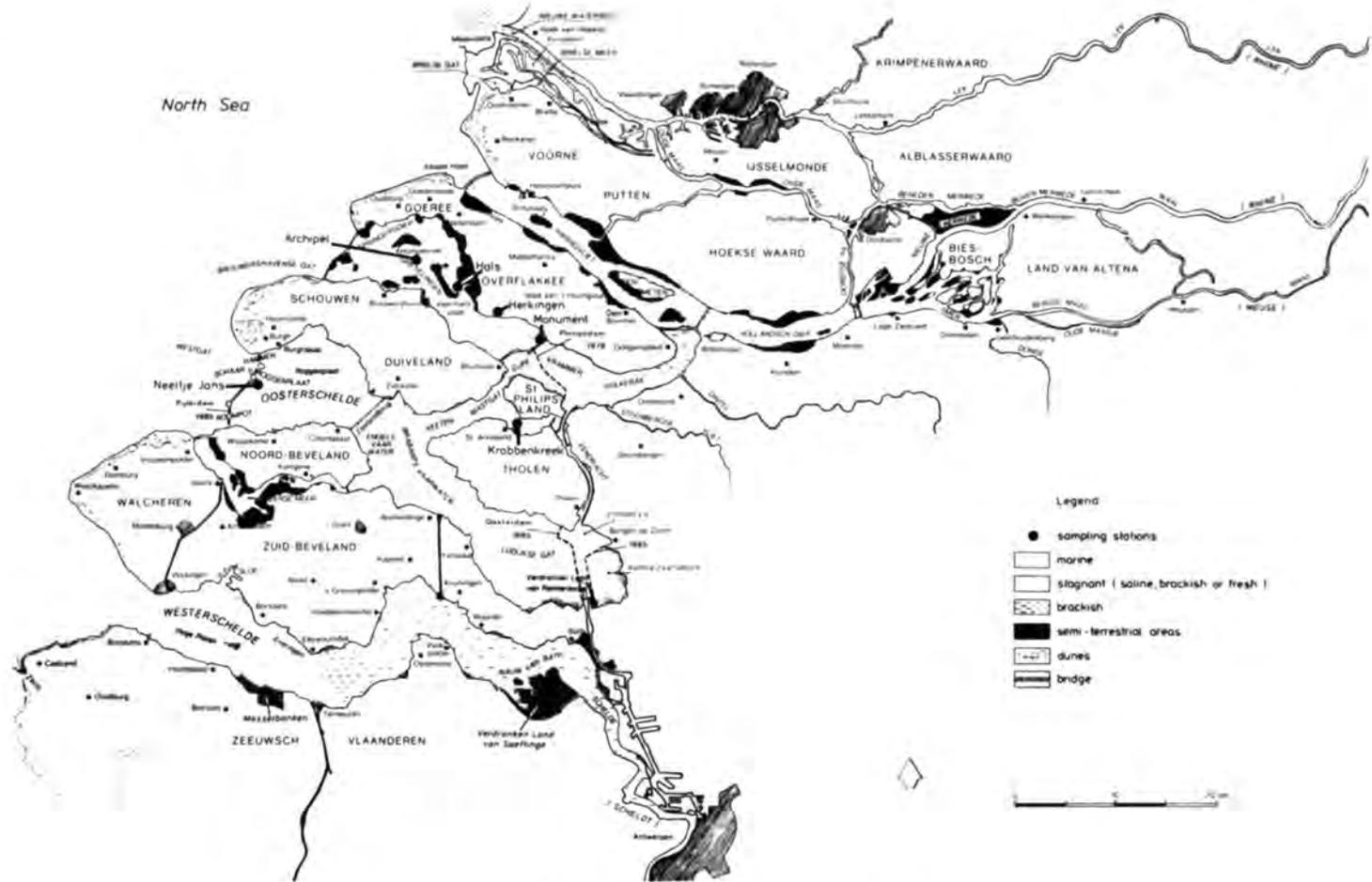


Fig. 1A. Delta of the rivers Rijn, Maas and Schelde.

detailed surveys of flora and fauna and their reactions on the changes caused by the successive closing of the sea arms with dams. These changes have created a number of water systems of different character at short distance of each other, which were bound to be in an ecologically unbalanced position for certain periods of time. It is thus possible to carry out ecosystem studies under a variety of environmental conditions.

The last years and also in 1981 these studies have been focussed on the functioning of estuarine and lagoon systems in general and those of the south-west Netherlands in particular. The results are considered essential for the environmental agencies in charge of the management policy of these waters.

The research was concentrated around a number of central themes on which the investigations were carried out by working groups (see also Table I). The working-group themes are (i) the element cycling and food chains in the Grevelingen and Oosterschelde, (ii) structure, functioning and dynamics of ecosystems in brackish waters and (iii) ecosystems studies on salt marshes.

Parallel to the working groups a project group was created on anthropogenic influences, caused by pollutants, on the aquatic and semi-terrestrial ecosystems. The group, consisting of members of all working groups on salt-marsh ecosystems, the two chemical laboratories and Drs. J. Al, DDMI-Delta Department of Rijkswaterstaat and Drs. M. Smies, Shell Internationale Petroleum Mij., is intending to coordinate various activities on contamination investigations in the Delta region. The topics concern metal, organochlorine and radionuclide contamination, and particular attention will be given to the Westerschelde and the Zoommeer (Fig. 1A) during and after its conversion from an estuary into a stagnant freshwater system, flushed with Rhine-Meuse water.

For all these working-group and project-group studies, it is estimated that towards 2010 the particular Delta Plan related topics will be gradually replaced by topics which should be studied in the diverse freshwater, brackish and saline systems of the Delta region. Primarily it is the institute's intention to remain focussed on semi-applied problems of stress on ecosystems and the production of scientific background data which are required for the management of these ecosystems or are required by legislation on the so-called environment-impact-assessment.

The work of the institute is grouped into projects, each of them bearing a code number. G and K are codes for the energy flow studies, B for the brackish-

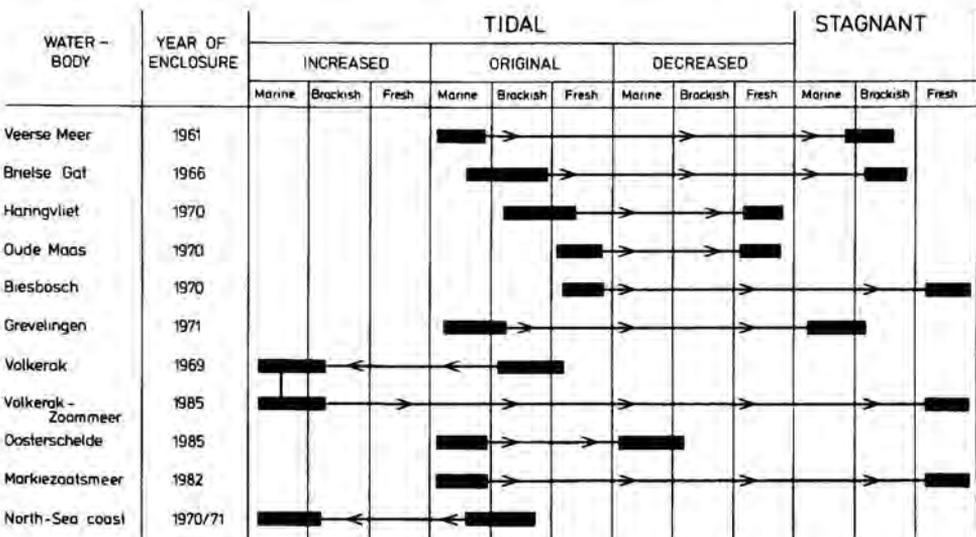


Fig. 1B. Hydrological changes due to the 'Delta Plan'.

water investigations, S for the salt-marsh studies while A denotes miscellaneous subjects, among which are the pollution studies.

Since the studies of the Delta Institute for Hydrobiological Research are of a long-range character, the research planning is usually made for both the working-group projects and the more individually carried-out projects for a long period of years. Reference is made to the working plan 1980-1990 of the institute (DIHO, 1980).

*Reference*

DIHO - 1980. Werkplan 1980-1990. Delta Institute for Hydrobiological Research. Yerseke, WP-04, 74 pp.

### III. CONTRACT RESEARCH AND COOPERATION

The extensive pressure on the Delta Institute to produce data and results that can be applied for technical and environmental management purposes, has resulted



Photograph 2.

A stainless steel sculpture of the Dutch Delta by Bob Klaassen, Middelburg. Donated to the Delta Institute at the occasion of its 25 years anniversary, by the municipality of Middelburg on the basis of the so-called BKR regulation. (Photograph René Kleingeld).



Photograph 3.

A bronze sculpture of two courting great crested grebes by Peter de Jong, Middelburg. Also donated by the municipality of Middelburg on the same basis and at the same occasion as mentioned with photograph 2. (Photograph René Kleingeld).

in a number of short-term contracts between our institute and other organizations. The major part concerns 3-years research projects funded by the Delta Department of Rijkswaterstaat for which the programmes were determined by mutual agreement between the Environmental Division (DDMI) of this Delta Department and our institute. These projects are: ZOWEC (salt-water ecology) in which 5 scientists and 5 technical assistants are engaged. This project will be reported in the Working group "Elements cycling and food chains" and will expire in 1982-1983; ZACHTSUB (soft-bottom benthos), which involves 1 scientist and 1 technical assistant for the investigation of benthic organisms in the Keeten-Krammer-Volkerak; VEGIN (vegetation-inventory), being an experimental study on the prognosis of the effects from the construction of the storm-surge barrier in the Oosterschelde on the salt-marsh vegetation, involving 1 scientist and 1 technical assistant; BALANS (budget of organic matter in the Oosterschelde), which is a project starting in 1980, involving 5 scientists and 5 technical assistants. Cooperative studies with the Delta Department to be mentioned are WABASIM (water-basin modelling) with the Hydraulics Laboratory in Delft and the section DDMI of the Delta Department in Middelburg, as well as the project ZOUVER (salt-water refreshment, Grevelingen).

A smaller contract, financed for 30% by the Commission of European Communities, section Biology, concerns the analysis of plutonium in the Delta waters. The first contract of 1979-1980 is extended to 1984. The analyses are carried out by the ITAL at Wageningen and the Laboratoire Géologique at Paris (F). The Delta Institute carried out the sampling and the nonradioactive analyses.

A joint cooperative investigation with closed budgets, and initiated through the Scientific Markets organized in Yerseke in 1977 and 1979, has been carried out with the Chemical Institute of the KFA at Jülich (FRG) on the heavy metal distribution in water, plants and organisms of the Delta area.

Based on the available know-how in the institute on adsorption and diffusion of radionuclides in marine sediments, an additional 1½ year contract was granted by the Commission of European Communities and the Dutch Ministry of Economics for 1981-1982, to be financed for 40% by the Commission and for 60% by the Ministry. The topic concerns heat effects on the processes of adsorption and diffusion for which investigations one scientist was seconded to the institute and one to the Technical University "Twente" at Enschede.

#### **IV. GENERAL ECOLOGICAL CONDITIONS IN 1982 (R. Peelen)**

##### *Temperature*

January was a little colder than normal, as was April, but February and March were a little warmer. The months May till September were much warmer than normal as like as November. October and December were nearly normal (Table 2).

##### *Solar radiation*

De Bilt is the representative station of our country and Vlissingen that of the Delta region for solar radiation. In the first half of the year the radiation was higher than normal especially in April and May. From July the measurements were lower, except in September, till the end of the year. Altogether were 386 183 Joules cm<sup>-2</sup> measured in Vlissingen, that is 4 440 Joules cm<sup>-2</sup> more than normal or + 1.15% (Fig. 2).

##### *Rainfall*

Dryer than normal were the months January, February, April, July, August, September and November. Wetter than normal were the months March, May, June, October and December. The yearly rainfall was 698.7 mm, that is 5.4% less than the mean at Vlissingen.

##### *Windspeed*

The windspeed is recorded in  $\frac{1}{2}$  m s<sup>-1</sup>. The months January, February and September

were close to the mean. More wind than normal was recorded in the months March, April, June, October and December. Very much wind was recorded in the months August and November. Less was measured in the months May and July.

#### River discharges and North Sea water exchanges

The average normal discharge of the three rivers Rijn, Maas and Schelde are respectively 2200 at Lobith, 330 at Lith and  $100 \text{ m}^3 \text{ s}^{-1}$  at Schelle. The relative daily discharges of Rijn and Maas are given in Table 2. The Rijn is a combined rainfed and glacier river, therefore the discharge is better buffered than that of the Maas and the Schelde, which are rainfed rivers. The discharge of the Rijn fluctuated from  $2/3$  till  $3\frac{1}{2}$  times the normal discharge, which was higher during the whole year than the normal discharge. The river Maas had discharges from  $1/10$  till 4 times the normal discharge and the river Schelde had the same tendency: the precipitation areas border each other.

#### Technical works by Rijkswaterstaat in the Delta area

Oosterschelde works at the storm-surge barrier.

1. The compacting work of the vessel "Mytilus" within the range of the Oosterscheldedam was completed in December 1982.
2. The barge "Cardium" and "Jan Heymans" the floating asphalt plant which are to lay the foundation mattresses together, were tested. The special lifting vessel "Ostrea" lifted a pier with success.
3. The construction of the gates for the Oosterscheldedam was started in May. Accessories for the operating mechanisms are in production.
4. The operating house of the Oosterscheldedam was officially named "Ir. J.W. Topshuis" on the 28th October.
5. The Roompot sluice is approaching to completion.
6. One underwater inspection equipment proved to be disappointing in running water and was subsequently removed so that the inspection work will now have to be carried out by human divers.
7. The mattress factory commenced production in De Hammen in November. The first mattress was damaged and the cause has been discovered.

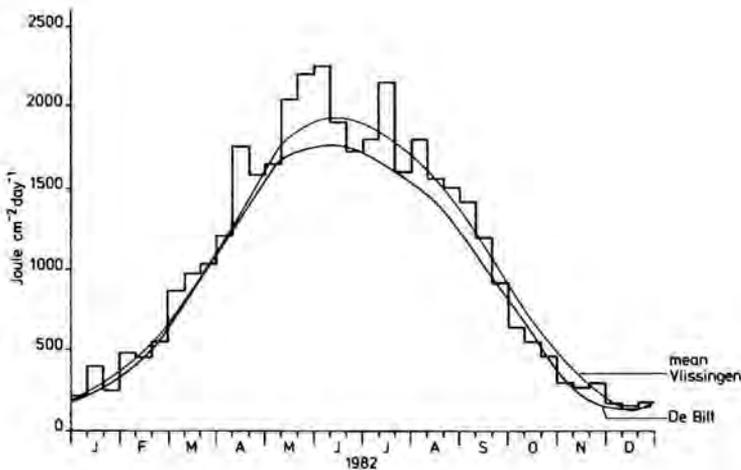


Fig. 2. Solar radiation at station Vlissingen in decades (block diagramme) for 1982 as compared to normal radiation at Vlissingen (upper line) and De Bilt (lower line).

Oosterschelde compartmentation works

1. The work of the sluices in the Philipsdam is proceeding successfully. The concrete work of the Flakkee siphon in the Grevelingendam continues to make good progress.
2. Preparations are in progress for the construction of a sluice for small ships (size 30 x 5.5 m) in the Oesterdam.

Table 2. Climate and river discharge conditions in 1982

	Temperature °C			Rainfall mm month <sup>-1</sup>			
	measur.	normal	deviation	measur.	normal	deviation	
January	1.9	3.1	-1.2	54.7	60.8	- 6.1	
February	3.6	3.1	0.5	11.9	49.4	-37.5	
March	5.6	5.2	0.4	45.8	45.6	0.2	
April	7.8	8.0	-0.2	25.5	44.1	-18.6	
May	12.7	11.9	0.8	47.5	44.2	3.3	
June	16.5	14.9	1.6	95.7	58.4	37.3	
July	18.4	16.7	1.7	50.4	71.4	-21.0	
August	17.7	17.0	0.7	51.0	77.4	-26.4	
September	16.8	15.2	1.6	33.4	67.2	-33.8	
October	11.8	11.7	0.1	148.0	72.9	75.1	
November	9.1	7.2	1.9	63.3	76.8	-13.5	
December	4.6	4.5	0.1	71.5	70.3	1.2	
				total:	698.7	738.5	-39.8 (5.4%)

	Windspeed $\frac{1}{2}$ m s <sup>-1</sup>			River discharge x normal	
	measur.	normal	deviation	Rijn	Maas
January	13	13	0	1½-3½	1½-4
February	12	12	0	1-3½	1-3
March	14	12	2	1-1½	1-2
April	12	11	1	1-1½	½-2
May	10	11	-1	1	½-1½
June	11	10	1	1-1½	½-1
July	10	11	-1	1	1/5- ¾
August	13	10	3	1	1/5- 1/3
September	11	11	0	2/3	1/10- ½
October	13	11	2	2/3-1½	1/10-2
November	17	13	4	2/3-1	1/3- ¾
December	15	13	2	2/3-3½	3/4-3

3. On the 11th March the Markiezaat dyke was broken. The opening was enlarged to protect the navigation of ships in the canal.
4. The weir of Bath has been demensioned for 6 outlets. Building of the siphon and the weir has been started.
5. A decision has been taken to close the Philipsdam with sand. During this work the Oosterscheldedam will be closed temporarily.
6. A further work has been started near Hansweert for a new shipping sluices in the Kanaal door Zuid-Beveland.

## **V. WORKING GROUP: ELEMENTS CYCLING AND FOOD CHAINS (CODE G + K)**

### **V.1. Introduction (P.H. Nienhuis)**

The working group 'Carbon cycle in the Grevelingen' for the greater part terminated its integrated ecosystem studies in saline Lake Grevelingen in 1982, and turned its attention to the Oosterschelde estuary. This estuary is ment to be closed by a storm-surge barrier in 1987, allowing the tides to enter in a diminished way. A new research plan has been developed, dealing with production, transformation and mineralization of organic matter and based upon the results of the Grevelingen study. As a result, the working group has two main projects under study, viz. (a) Carbon cycle in Lake Grevelingen, and (b) Food chains, production and mineralization of organic matter in the Oosterschelde estuary. The central question to be answered in both projects is: in which way and to which extent will estuarine elements cycles and food chains be influenced by an extinction (Grevelingen) or reduction (Oosterschelde) of the tidal movements.

The Grevelingen project is in its final integrative phase. During community-metabolism measurements the micro-electrode technique has been successfully applied *in situ*, estimating oxygen microgradients and fluxes through the sediment-water boundary layer. The role of larger carnivores (fish, birds, crabs) in the lake got much attention. Although these animals are unimportant in terms of organic carbon, their position in the structure and functioning of the ecosystem is obvious. Large efforts were paid to mathematical modelling of complex data sets (macrozoobenthos, nutrient dynamics, phyto and zooplankton) in cooperation with the Delta Department of Rijkswaterstaat and the Delft Hydraulics Laboratory. The contract-research project ZOWEC (5 scientists and 5 technical assistants for 3 years), financed by Rijkswaterstaat, came to an end, except for the fish project. This enterprise has been succeeded by BALANS, a 3 to 6 years project of the same size as ZOWEC and also paid by Rijkswaterstaat.

The Oosterschelde project asked increasing attention of the investigators. The complicated pattern of transport of organic material in the tidal system has been analyzed. Seston analyses were done in relation to a series of environmental factors. Both phytoplankton and zooplankton structural and functional parameters were measured, including grazing by zooplankton. Biomass measurements of micro-phytobenthos have been continued and revealed a complicated pattern for the entire estuary. Biomass distributions of benthic macrophytes and macro-zoobenthos have been established. The Oosterschelde is a large and complex estuary and concentration of research on a number of selected sampling sites is in progress.

The geographical names used in this report can be found in Fig. 3.

### **V.2. Identification of faunal shifts in the Keeten-Krammer-Volkerak area using numerical classification (A2) (J. Coosen and A. Van den Dool)**

During winter 1978-1979 the chlorinity in the Keeten-Krammer-Volkerak area was considerably lowered, due to an experimental doubling of the freshwater discharge at the Volkeraksluices. On average, interstitial chlorinity dropped 2-3 ‰ during the experiment (Coosen and Van den Dool, 1981; Leewis *et al.*, 1981). To evaluate the possible impact of such large scale hydrotechnical manipulations on the benthic

macrofauna in the area, samples have been taken before (Oct. 1978; March, Oct. 1979) and after (March, Oct. 1980, March 1981) the experiment, at 35 stations along 6 transects (Fig. 4). For use in the numerical classification the samples of 20 stations from the original 35 were paired, summing up the data from adjacent stations, hence creating 10 new "stations" (surface area:  $0.09 \text{ m}^2$  each - no. 1 and 2 in transect I; 3 and 4 in II; 5 and 6 in III; 7 in IV; 8 in V; 9 and 10 in VI -). The samples of March 1981 were not treated in this way, because of their larger sampling surface ( $0.095 \text{ m}^2$ ). Numerical classification was used to find out whether: 1) the

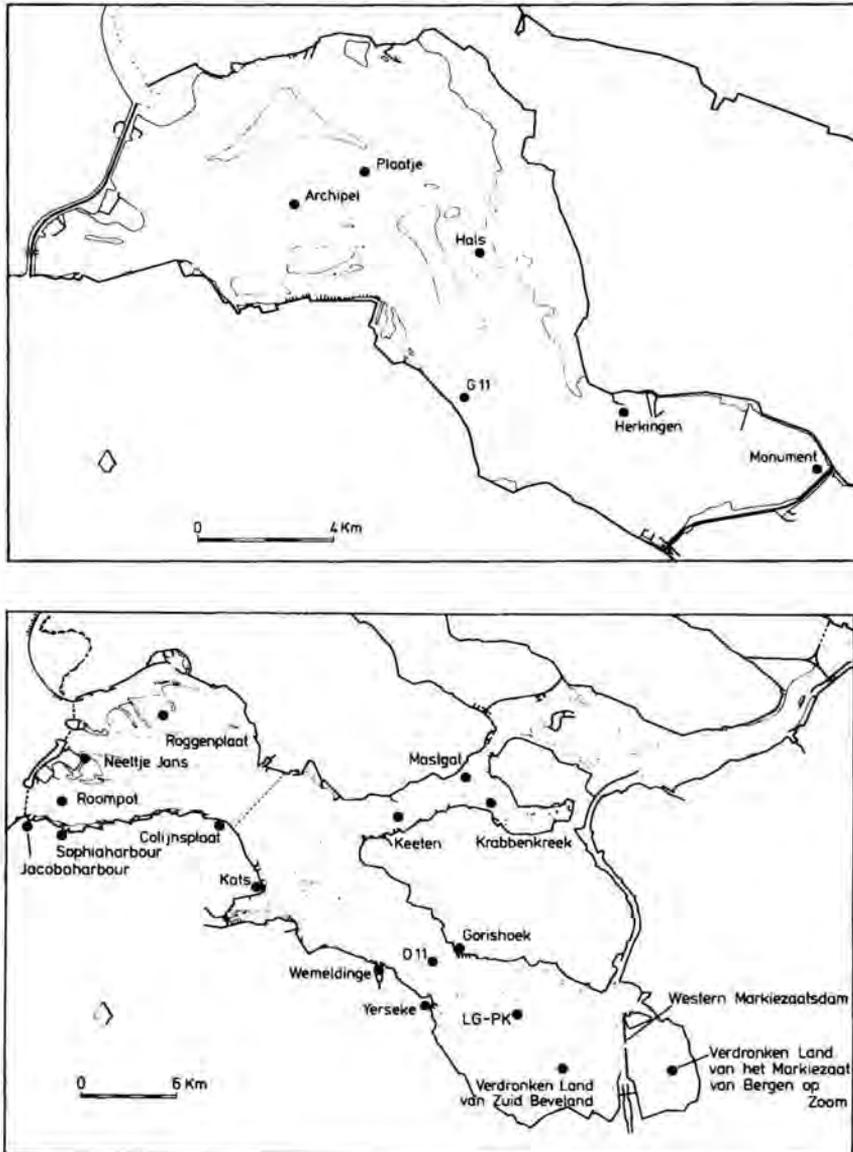


Fig. 3. Grevelingen (above) and Oosterschelde (below) localities as mentioned by the working group "Elements cycling and food chains".

pre-experiment-samples showed more similarity to each other than to the post-experiment-samples, and 2) whether there were any other ecological meaningful sample groups to be recognized.

The similarity between pre- and post-experiment-samples was analyzed by cluster analysis using the Bray-Curtis dissimilarity index and group-average sorting strategy on double rooted ( $\mathcal{W}$ ) species counts. Calculations were performed at the Rijksuniversiteit Gent with the aid of Mr. A. De Kimpe. A detailed explanation of the technique applied is given in Clifford and Stephenson (1975).

A clustering of the pooled samples of 10 new "stations" over all seasons resulted in the sample dendrogram presented in Fig. 5. In the lower part of the figure the numbers of the stations are set on different seasonlines. The dissimilarity coefficient of fusion is rather low, so stations throughout the area contain a comparable fauna within the time studied. Nevertheless, by analyzing the 6 main clusters, a difference between March and October values can be recognized. The clusters 1, 2 and 3 mainly contain the October samples from the stations in transects I, II, III and IV and include the March 1981 samples from 6 stations in I, II, III and IV. The October samples from transects V and VI are mainly grouped together in cluster 4, again including the March 1981 samples. The other March-samples (of 1979 and 1980 together) can be found in cluster 5 (containing one station in transect IV and all stations in V and VI) and in cluster 6, distinctly separated from all other samples (containing the other station in transect IV and all stations in I, II and III). In other words, no faunal shifts caused by the experiment could be observed. Separation of stations with low salinity (transects V and VI) and high salinity (I and II) is shown. Differences in median grain size may also contribute to this separation.

A more detailed and longer study in the Oosterschelde has to be done in order to find out whether the seasonal differences in macrozoobenthos composition are related to a regular faunal shift.

Faunal shifts related to differences in chlorinity, occurring in true estuarine habitats, often coincide with the seasons: the oligochaete-fauna of the Fraser estuary (Canada) changed considerably after high river discharge in summer (Chapman

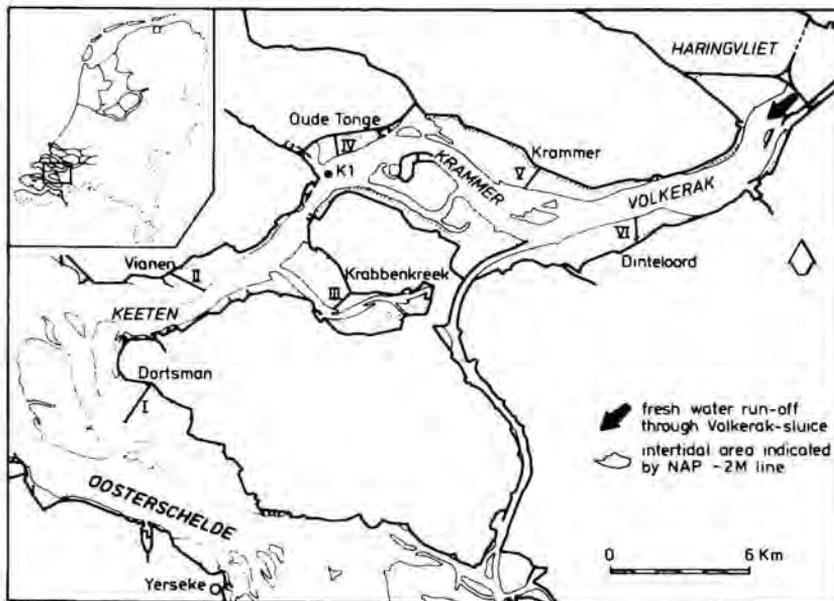


Fig. 4. Map of the Keeten-Volkerak area. The positions of the transects (I-VI) are indicated by solid lines.

and Brinkhurst, 1981). Tenore (1972) noted that in the Pamlico River estuary (USA), species with low salinity tolerance fluctuated in abundance, coinciding with fresh-water discharge. Large shifts in the macrobenthos fauna of Bramble Bay (Australia) after a river flood have been recorded by Stephenson *et al.* (1977). In all these cases salinity differences have been much larger than during the Volkerak experiment.

**References**

Chapman, P.M. and R.O. Brinkhurst - 1981. Seasonal changes in interstitial salinities and seasonal movements of subtidal benthic invertebrates in the Fraser River estuary, B.C. Est. Coast. Shelf Sci. 12, 49-66.  
 Clifford, H.T. and W. Stephenson - 1975. An introduction to numerical classification. Academic Press, New York, 1-229 pp.  
 Coosen, J. and A. Van den Dool - 1981. De gevolgen van het experimenteel lozingsprogramma Volkeraksluizen op het macrozoobenthos. Yerseke/Middelburg. DIHO-DDMI Interimrapportage ZACHTSUB.  
 Leewis, R.J., A.J.M. Meijering, P.B.M. Stortelder and J.P.G. Van de Kamer - 1981. Zoutgehaltewisselingen en mosselconditie: Milieukundige begeleiding experimenteel lozingsprogramma Volkeraksluizen. Middelburg. RWS-DDMI. Nota DDMI-81.14, 32 pp.  
 Stephenson, W., S.D. Cook and U.I. Raphael - 1977. The effect of major flood on the macrobenthos of Bramble Bay, Australia. Mem. Queensl. Mus. 18 (1), 95-119.

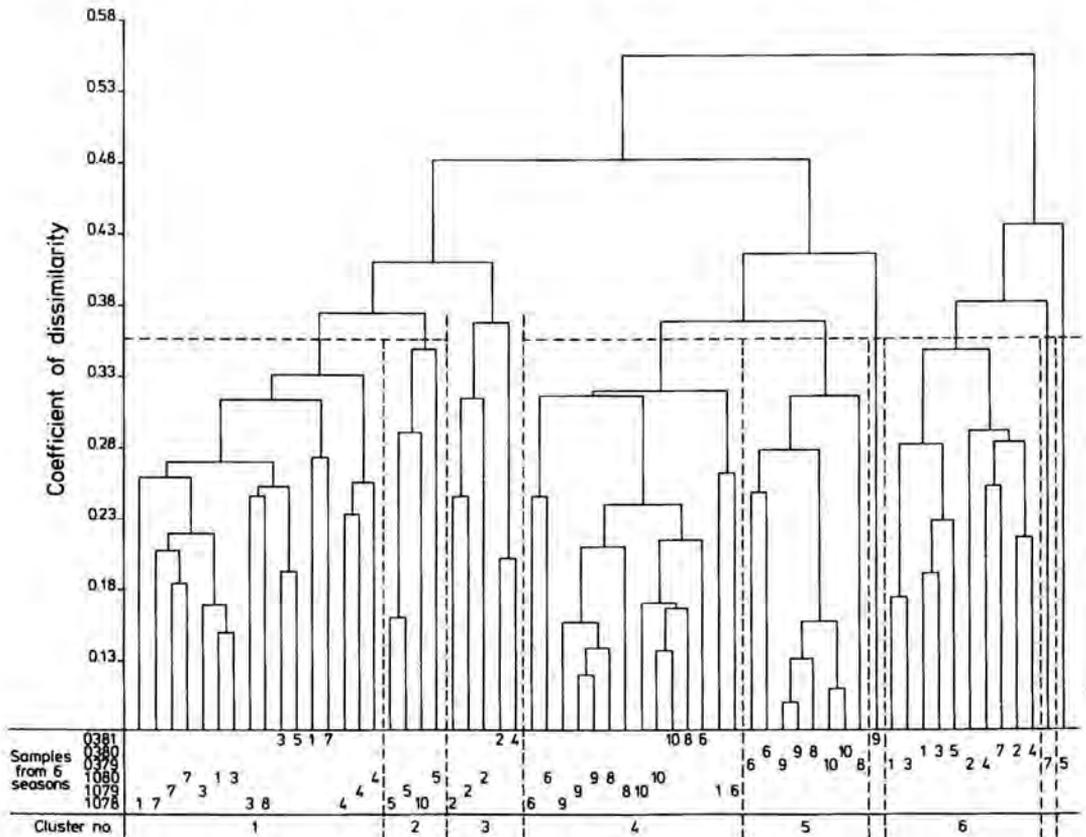


Fig. 5. Dendrogram of 60 samples taken at 10 stations over 6 seasons in the Keeten-Volkerak area.

**V.3. Some remarks on the occurrence of waders (oystercatcher, curlew) and macrozoobenthos before, during and after the attempt of closing the western Markiezaatsdam (A2) (B. Van Dessel and J. Coosen)**

At the start of this study (Aug. '81) the Verdrongen Land van Het Markiezaat van Bergen op Zoom was an intertidal area with extended mudflats and salt-marshes (1660 ha). Due to the closure of the western Markiezaatsdam, this part of the Oosterschelde, would become a tideless basin. The closure of the dam would cause a, temporary decrease of the tidal range. In the future after the closure a stagnant fresh water lake is ment to arise (Markiezaatsmeer). Before, during and shortly after this closure, bird counts were carried out, in a transect of 9 permanent quadrats (PQ's), from -1.25 to +1.00 m NAP. Biweekly the numbers of curlew (*Numenius arquata*) and oystercatcher (*Haematopus ostralegus*) present during one tidal cycle, were counted every half hour in all PQ's (Fig. 6). Foraging intensity was also determined. Macrozoobenthos samples were taken in September and December 1981. Highest biomass figures (550-700 g ADW m<sup>-2</sup>) were found on the natural mussel beds (*Mytilus edulis*), where foraging oystercatcher and curlew were concentrated (Fig. 7).

Numerical classification, using several diversity-indices and Spearman-Rank correlation analysis were carried out at the Rijksuniversiteit Gent (thanks to Mr. P. Meire). This analysis confirmed the importance of the musselbeds as an excellent foraging area for both oystercatcher and curlew. Generally, the other PQ's were visited only when the musselbeds were inundated and could not be reached. Observations on the tidal migration of the oystercatcher were continued during the closure of the dam and the cold period afterwards, when ice covered the whole area.

The closure caused a rapid decrease of the intertidal foraging area of the waders. The musselbeds, situated low in the intertidal area, became inaccessible.

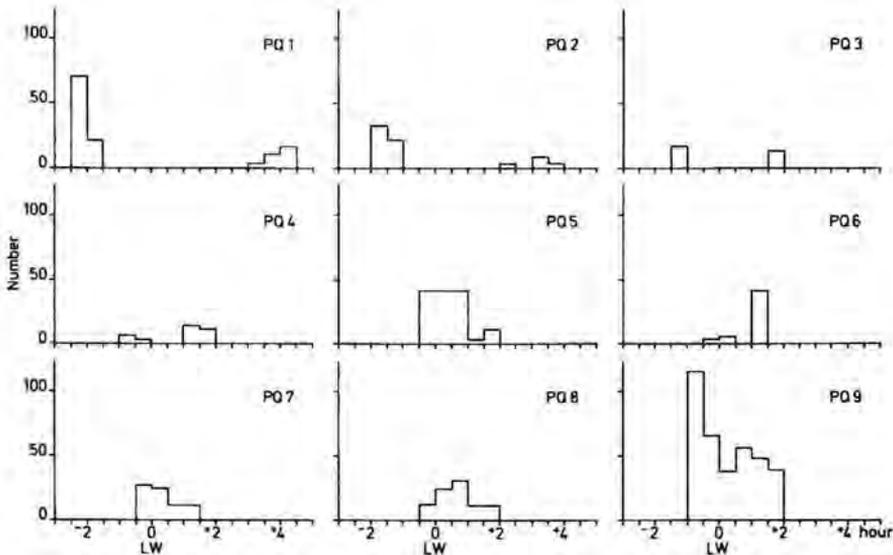


Fig. 6. Number of oystercatchers present in 9 PQ's, in relation to time of low water (LW). PQ-8 and -9 are musselbeds. Observations on 16-10-'82.

The oystercatcher still showed the same tidal migration pattern, following the waterline in high densities.

When ice covered the whole area, the tidal migration came to an end, but it lasted a week before the oystercatchers fled in great numbers to the west. By that time already hundreds of birds were frozen to death. Curlew and other bird species left the area sooner.

In conclusion, the loss of rich foraging grounds like musselbeds, due to a rise of the mean low water level, will have drastic consequences for the wading birds in the area. Furthermore, the absence of tidal water movements during a severe winter when ice covers the area might cause a substantial loss in the oystercatcher population.

On March 11, 1982 a storm destroyed part of the almost finished dam, allowing the tidal movements to enter the "lake" for at least another year.

#### V.4. Influence of production and mineralization on nutrient balances in Lake Grevelingen (G1, 2, 3, 5) (I. De Vries and M.F. Veul - Delft Hydraulics Laboratory, WABASIM project -)

Concentrations of dissolved nutrients in Lake Grevelingen show pronounced fluctuations. The inorganic dissolved fractions of nitrogen (N) and silicon (Si) behave similar. Winter concentrations of these nutrients are rather high, and during summer the inorganic dissolved fractions are almost zero. Inorganic dissolved phosphate (orthophosphate) behaves different. The concentration decrease of orthophosphate begins during winter, instead of at the beginning of the growing season. The subsequent concentration increase already starts early summer, whereas the other two nutrients remain at low levels for still some months. So nitrogen and silicon seem to be limiting for primary production in the waterphase during summer, in contrast to orthophosphate.



Fig. 7. Tidal migration movements of the oystercatcher in the study-area, before closure of the dam. HVP = High Water Roost.

However, the amount of nutrients in the suspended particulate material during summer is far less than the quantity that disappears out of the dissolved pools. Fig. 8 shows this phenomenon for nitrogen. The difference between winter and summer concentration of inorganic dissolved nitrogen is about  $0.6 \text{ g N m}^{-3}$ , (measurements of DIHO at G11). The particulate nitrogen concentration is maximal  $0.2 \text{ g N m}^{-3}$  (averaged measurements at 7 stations of DDMI), i.e. only 25% of the quantity that disappears out of the dissolved pool.

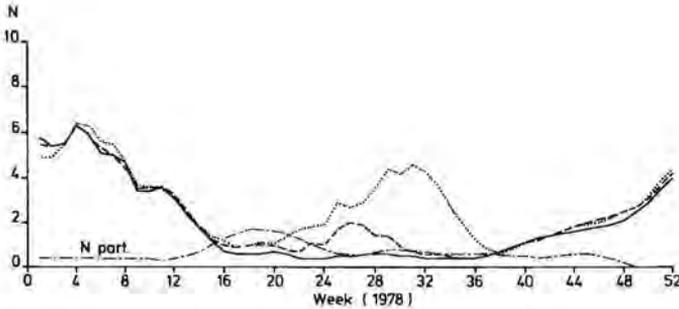


Fig. 8. Dissolved inorganic nitrogen (— 0-5 m; --- > 5-15 m; ..... > 15-22 m) and suspended particulate nitrogen (—·— 0-5 m) in  $\text{g Nm}^{-3}$  in 1978 in Lake Grevelingen.

Another comparison concerns the potential amount of algal biomass that can be formed from the available nutrients. From the amount of dissolved silicon, available during winter, more than  $50 \mu\text{g Chl-a l}^{-1}$  of diatoms can be formed and from the amount of inorganic dissolved nitrogen, more than  $100 \mu\text{g Chl l}^{-1}$  of algal biomass can be formed. Measured phytoplankton concentrations are lower than  $10 \mu\text{g Chl-a l}^{-1}$  i.e. less than 10% of the maximal possible amount. To explain this difference of one order to magnitude, nutrient balance calculations are carried out.

The crux of the balance calculations consists of a dynamic calculation of detritus pools in a water and bottom compartment. The formation of detritus is deduced from the mortality of microphytobenthos and phytoplankton, which is calculated from available biomass and primary production measurements (data from De Bree, Bakker, Vegter, De Visscher and Lindeboom). Decay of detritus is simulated by calculation of the mineralization of carbon, nitrogen and phosphorus and dissolution of silicon, as function of temperature and substrate concentration.

The balances for 1978 of silicon, nitrogen and phosphorus are given in the Figures 9A, 9B and 9C. The nutrient pools are obtained by: - measurements of cell-volumes, resp. bottomchlorophyll and conversion to nutrient via minimum stoichiometry (pool 1 and 2); - calculation of detritus pools (3, 4). Pool 3 is derived from phytoplankton. Pool 4 is derived from the calculated mortality of microphytobenthos and sedimented phytoplankton detritus taking into account the mineralization of detritus; - measuring the amount of nutrient present in inorganic dissolved form (pool 5).

The total amount of nutrients (pool 1-5) is subtracted from the cumulative load in the course of the year, resulting in the net load (■—■). For phosphorus the net load, results in net export to the North Sea, and is presented on top of the cumulative pools.

From these balances it appears that 80-90% of the amount of silicon and nitrogen that is present in a dissolved form during winter, is 'stored' in the bottom detritus pool during summer. So not the absolute amount of nitrogen and silicon is limiting for primary production, but the rate by which it is mineralized resp. dissolved during summer.

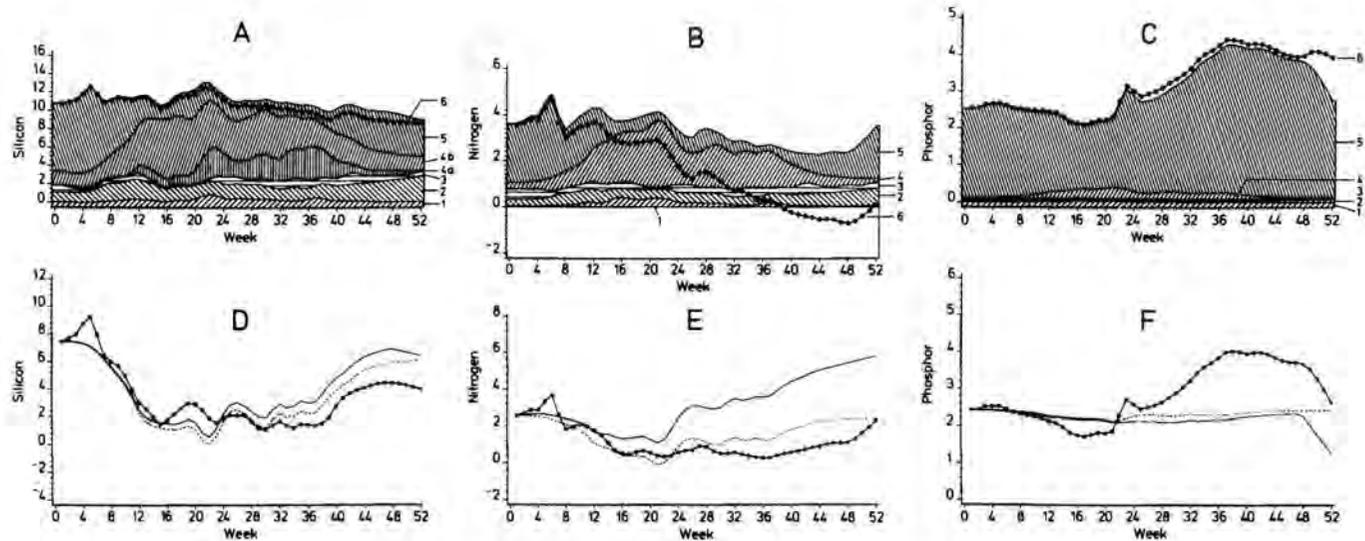


Fig. 9. A, B, C. Inorganic nutrient balances in Lake Grevelingen in 1978 (in  $\text{g m}^{-2}$ ). Amounts of nutrient present in: 1 phytoplankton; 2 microphytobenthos; 3 suspended detritus; 4 a sedimented phytoplankton; b bottom detritus; 5 the water phase; 6 net load.

Fig. 9. D, E, F. Calculations of the inorganic dissolved nutrient pools in Lake Grevelingen in 1978, combined with the measured concentrations (in  $\text{g m}^{-2}$ ). Legend: : measured concentration; : calculated concentration; : calculated concentration without load.

The nitrogen balance is complicated by the load (mainly due to rainfall and polderwater discharge) which is larger than the total amount of nitrogen incorporated in the biological cycle. Apparently redox processes like denitrification remove approximately the same amount of nitrogen out of the system, that is imported into the system by loadings. These redox processes are not incorporated in the nitrogen balance calculations.

The phosphorus balance confirms the fact that phosphorus is not limiting for primary production in Lake Grevelingen. Only 10% of the dynamics of orthophosphate is caused by uptake by primary producers and subsequent release resulting from mineralization of detritus.

In the Figures 9D, 9E and 9F calculations of the inorganic dissolved nutrient pools for 1978 are shown, combined with the measured concentrations. These figures present the calculated magnitude of the dissolved nutrient pools, when these pools are only influenced by the processes that are taken into account in the balances. As a starting value of the dissolved pool, the measured nutrient concentration at the beginning of the year is given. The following processes are taken into account: Uptake of nutrients resulting from the net primary production of phytoplankton and microphytobenthos; Release of nutrients by mineralization and dissolution of detritus assuming that all the nutrients are released into the water compartment; Net load on or net export from the lake.

The course of the silicon concentration (Fig. 9D) indicates that the above mentioned processes explain nearly all the dynamics of the dissolved pool. This is in agreement with the results of the silicon balance calculations.

The figure on nitrogen (Fig. 9E) again indicates the importance of loadings, and the importance of denitrification (not incorporated in the calculations).

When only production and mineralization should influence the orthophosphate concentration, it would hardly change at all during the whole year (Fig. 9F), which means that, whenever eutrophication becomes a problem in a salt lake Grevelingen, it will be caused by nitrogen.

It will only be possible to solve such problems by means of reducing nitrogen loads, and not by phosphorus removal.

#### **V.5. A simulation curve of the phosphate concentration in Lake Grevelingen (G 2) (P. Kelderman)**

Laboratory experiments were carried out, dealing with the dependence of phosphate sediment-water exchange on water temperature and phosphate concentration in the overlying water. For detailed results, see Kelderman (1983). Sediment cores (30 cm depth, 11 cm diameter) were taken at four stations in the lake, with sediment types ranging from medium to muddy sand. The (aerobic) cores with overlying water were placed in the dark and were slowly adapted to one of the fixed experimental temperatures, i.e. 5°C, 10°C, 15°C or 20°C. Next, a  $\text{PO}_4\text{-P}$  loading of ca. 1.5 mg  $\text{P l}^{-1}$  was added to the overlying water.

The phosphate sediment-water exchange was investigated by monitoring the overlying water phosphate concentration daily.

After a P accumulation by the sediment, an equilibrium P concentration in the overlying water was usually reached within a few weeks. The equilibrium P concentrations were distinctly higher for the high temperatures, compared to the low temperatures. During the experiment, a set of ca. 20 overlying water phosphate concentrations with corresponding P sediment-water exchange figures was obtained for each sediment core. A linear correlation may be expected between these two factors, according to Fick's first law of diffusion (Kelderman, 1983). For the 40 sediment cores used, significance levels for linear correlation have been found of resp.: 20x  $p < 0.001$ , 11x  $p < 0.01$  and 9x n.s.

Considering the results for the four temperatures, a relationship could be computed between: a) water temperature, b) overlying water P concentration and c) P sediment-water exchange.

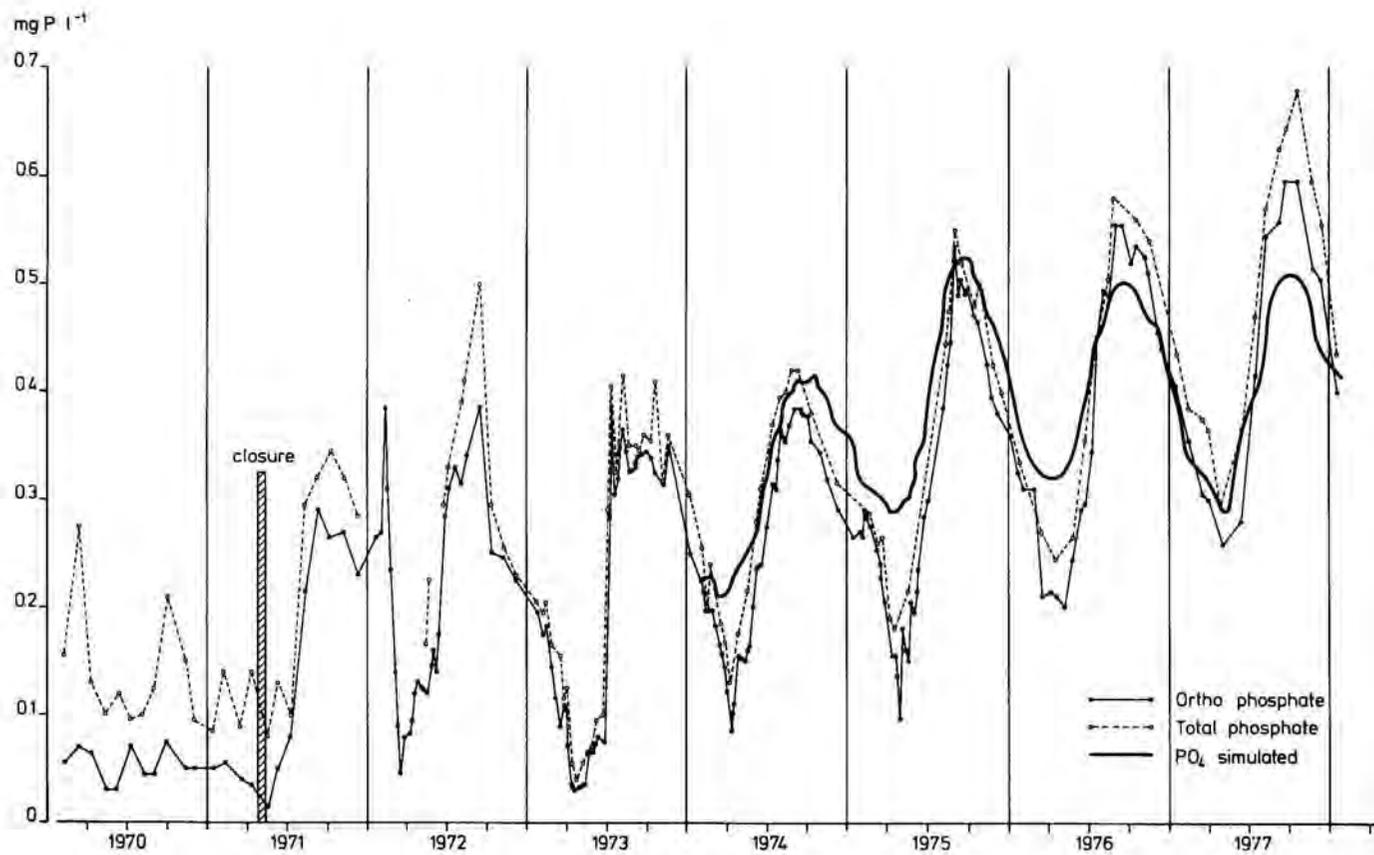


Fig. 10.  $\text{PO}_4$ -P simulation curve for Lake Grevelingen.

This relationship has been used in a phosphate simulation model in Lake Grevelingen over the years 1974-1977. A minor term in the phosphate simulation was the external P loading into the lake (e.g. through polder and waste water, rain, etc.) (Kelderman, 1980).

The resulting phosphate simulation curve (Fig. 10) shows a good resemblance with the actual phosphate course in the lake. The typical sinoidal shape of the simulation curve coincides exactly with the actual P course. Shortcomings in the simulation model - basically deviations of the P maxima and minima - can be ascribed to the different "field" and "laboratory" conditions (Kelderman, 1983).

#### References

- Kelderman, P. - 1980. Phosphate budget and sediment-water exchange in Lake Grevelingen (S.W. Netherlands). *Neth. J. Sea Res.* 14, 229-236.  
 Kelderman, P. - 1983. Sediment-water exchange characteristics in Lake Grevelingen under different environmental conditions. *Neth. J. Sea Res.* 17 (in press).

#### V.6. Carbon budget of the eelgrass (*Zostera marina*) community in Lake Grevelingen (G6) (P.H. Nienhuis)

In the course of 1980, 1981 and 1982 community metabolism of an eelgrass stand in Lake Grevelingen (water depth 1-1.5 m) has been investigated several times, both with the carbon-14 method and with the oxygen method. Team leader of these field experiments was H.L. Lindeboom.

Applying the  $^{14}\text{C}$ -method a sample containing either eelgrass, phytoplankton, macro- or microphytobenthos, has been incubated *in situ* in overlapping experiments running 4 hours each, over a period of 28 hours. After the incubation period the labeled organic compounds are caught - occasionally after combustion-in a counting fluid. The radioactivity of the sample is measured in a scintillation counter.

Applying the oxygen method a number of perspex bell jars, provided with an oxygen electrode a thermo couple and a stirrer have been placed in an eelgrass bed on the bottom of the lake. The bell jars have been placed over the most substantial

Carbon budget eelgrass community Grevelingen 1978 - 1979

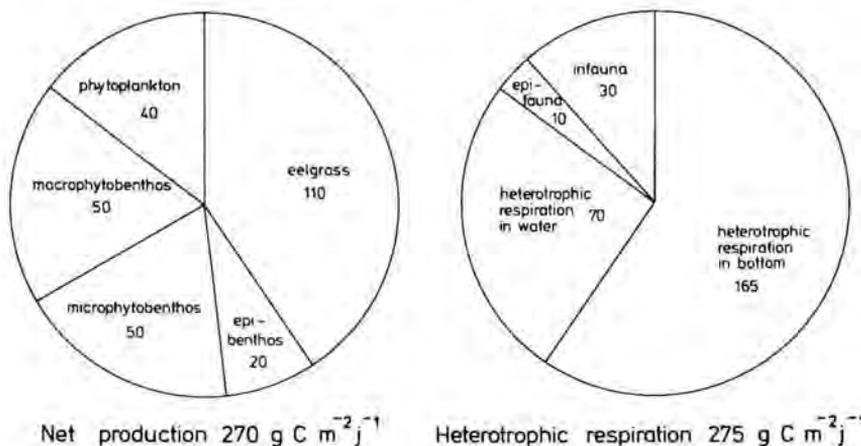


Fig. 11. Organic carbon budget in an eelgrass bed in Lake Grevelingen at a locality with 1-1.5 m waterdepth, average over 1978 and 1979. The data in  $\text{g C m}^{-2}\text{yr}^{-1}$  have been based on measurements, estimates and extrapolations; for further explanation see text.

parts of the community viz. eelgrass, macroalgae, microphytobenthos, filterfeeding benthic animals, in all cases including bacteria, in order to measure oxygen production and consumption. The use of darkened bell jars excluded primary production, so production and consumption have been measured independently.

During a period of 30 hours, divided into experiments of 2-4 hours each, oxygen concentrations in the domes have been measured and recorded continuously aboard a research vessel. Oxygen production and consumption per time unit have been calculated (Lindeboom and De Bree 1982; Lindeboom *et al.*, 1982). After the experiment the biomass of the most conspicuous parts of the community underneath the bell jars has been estimated in order to calculate production per unit biomass.

A summarizing carbon budget over the years 1978-1979 is shown in Fig. 11. Net primary production is compared in this budget with oxygen consumption, considered as mineralization by heterotrophic bacteria and respiration by micro and meiofauna (= heterotrophic respiration) and respiration by larger animals (epifauna and infauna). Considering the production circle, the eelgrass data are most reliable (Nienhuis and De Bree, 1980), whereas the other values, a.o. because of methodological problems, are less reliable. The data in the consumption circle have been estimated exclusively with the oxygen method in 1980-1982 and extrapolated to the situation in 1978-1979.

At the production side of the budget eelgrass is conspicuous, contributing  $110 \text{ g C m}^{-2} \text{ yr}^{-1}$ . However, the macro and micro-algae together account for 60% of the total production, which means that the equilibrium between *Zostera* and the other plants is unstable. Owing to eutrophication with anorganic nutrients the algal production might be favoured, compared to that of eelgrass. On a world scale a considerable number of communities of aquatic phanerogams have vanished in this way (Phillips *et al.*, 1978).

At the consumption side of the budget the activities of small biota in the sediments (viz. bacteria, micro and meiofauna) predominate ( $165 \text{ g C m}^{-2} \text{ yr}^{-1}$ ). Especially the share of heterotrophic bacteria will be large. Lindeboom *et al.* (1983) estimated the bacterial and microfaunal share at a level of  $135 \text{ g C m}^{-2} \text{ yr}^{-1}$ . The remainder of the heterotrophic respiration is of minor importance. In the water column the respiration values shall be mainly caused by bacteria. The infauna data include mainly respiration of worms, and to a lesser extent of molluscs. Although isopods, mysids *etc.* play an important role in the structure of the community, the significance of the epifauna in community metabolism is but small. Zooplankton and carnivores (especially fish) have been omitted: their share in the overall respiration data is far below  $10 \text{ g C m}^{-2} \text{ yr}^{-1}$ .

It is hypothesized that high mineralization rates in the bottom sediments indicate a high loading with organic material. The relations between the various metabolic activities of the groups of biota are unstable at the consumption side of the budget, just as at the production side. The soil in eelgrass beds contains approximately 0.5-2% particulate organic carbon. The aerobic toplayer of the sediment is only 2-3 mm thick, the underlying sediment is almost anaerobic, except in the immediate surroundings of the rhizomes and roots. Together with an increasing loading of the sediment with organic material - up to a level of 3% and higher - the bottom sediments stand an increasing chance of becoming anaerobic.

Notwithstanding its extensive area of approximately 3000 ha, the eelgrass community in Lake Grevelingen appears to be a vulnerable sub-system. At the production side increasing eutrophication and consequent excessive blooming of macroalgae or phytoplankton is a continuous potential threat. At the consumption side a slight increase of organic matter loading of the sediment may cause anaerobic conditions and die-back of rhizomes and roots of eelgrass.

### References

Lindeboom, H.J. and B.H.H. De Bree - 1982. Daily production and consumption in an eelgrass (*Zostera marina*) community in saline Lake Grevelingen: discrepancies between the  $\text{O}_2$  and  $^{14}\text{C}$  method. *Neth. J. Sea Res.* 16, 362-379.

Lindeboom, H.J., H.A.J. De Klerk-Van den Driessche and A.J.J. Sandee - 1982. Production and decomposition of eelgrass (*Zostera marina*) in saline Lake Grevelingen. *Hydrobiol. Bull.* 16, 93-102.

Lindeboom, H.J., H.A.J. Van den Driessche and A.J.J. Sandee - 1983. Mineralization of organic carbon on and in the sediment of Lake Grevelingen. *Neth. J. Sea Res* (in press).

Nienhuis, P.H. and B.H.H. De Bree, 1980. Production and growth dynamics of eelgrass (*Zostera marina*) in brackish Lake Grevelingen (The Netherlands). *Neth. J. Sea Res.* 14, 102-118.

Phillips, G.L., D. Eminson and B. Moss - 1978. A mechanism to account for macrophyte decline in progressively eutrophicated fresh waters. *Aquat. Bot.* 4, 103-126.

**V.7. The continuing saga of an introduced brown alga (*Sargassum muticum*) and its establishment within the s.w. Netherlands (G 6, K 5) (A. Critchley - Royal Society European Exchange Fellowship; present address: University of Natal, Dept. of Botany, Pietermaritzburg 3200, Natal, South Africa - )**

Few new localities of attached *Sargassum muticum* were recorded in the s.w. Netherlands during 1982, but a marked consolidation and expansion of already established populations was noted, since the 1981 Progress Report.

Figure 12A illustrates sites of drift material and attached *Sargassum* populations as recorded for the entire Netherlands during 1982. A precondition for the successful establishment of an attached *S. muticum* population is that a suitable habitat of hard substratum, covered at all states of the tide, must be present. Hence, large areas of dunes and mudflats will never support attached *Sargassum* populations.

Suitable habitats are found in the Oosterschelde. *Sargassum* colonizes these areas of dyke wall and building material which were not previously utilized by indigenous macroalgae. Therefore the presence of the introduced alga is unlikely to affect the growth and success of other seaweeds. Expansion and minor changes in distribution of *Sargassum* populations were noted along the coast of Noord-Beveland

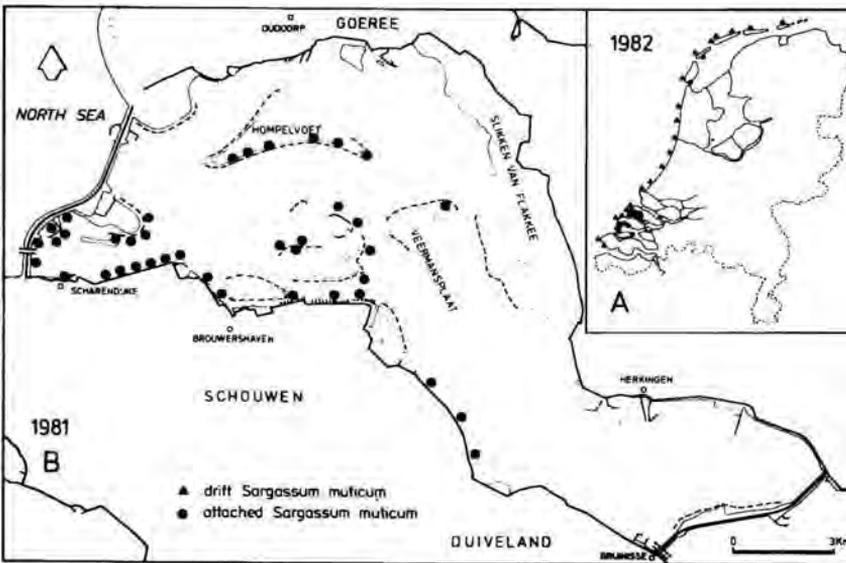


Fig. 12A. Distribution of *Sargassum muticum* in The Netherlands in 1982 and in B. Lake Grevelingen in 1981.

and Schouwen. Some development of populations has been noted on the work island of Neeltje Jans (in the mouth of the estuary), although construction and recontouring of the island has destroyed some of the former *Sargassum* habitats. Once the island is completed, as part of the storm-surge barrier, an extensive area will be presented to colonization by *S. muticum*. Any effect the algal may have on the operation of the barrier remains to be seen when the construction is completed.

Within the non-tidal, saline, Lake Grevelingen the *Sargassum* population is confined to points of attachment around the dyke perimeter and rock walls (rip-rap dams), placed to protect the internal islands. The available substrata range from mussel beds to dyke building material. Development of the attached *Sargassum* population within Lake Grevelingen has been spectacular. Figure 12B illustrates the distribution of attached plants for 1981 (Bom, 1982). Figure 13 shows the increase of attached plants over a single growing season. By 1982 virtually all available substrata were colonized to some extent.

The rapid spread of *Sargassum* is thought to have been enhanced by the favourable environment, with a plentiful supply of hard substrata, where drifting fertile material could be easily disseminated by winds to establish new colonies. *S. muticum* is expected to continue to reinforce its position upon the dyke walls and rip-rap dams. However, most of the available substrata are now colonized; areas such as Slikken van Flakkee will remain free due to the lack of substrata. The absence (so far) of *S. muticum* from the shallow oyster beds of Lake Grevelingen is surprising, those beds of c. 4-5 m depth are expected to be colonized, in some form, in the near future.

The success of *Sargassum muticum* within Lake Grevelingen is such that by early summer (May-June) of 1982, extensive beds of upto 10 m wide could be seen forming an almost continuous "carpet" in the areas of Scharendijke and Brouwershaven (Fig. 13). This 'carpet' was formed by the buoyant fronds (up to 3 m in length) standing upright in the shallow water and forming a canopy at the surface. During early summer the canopy was extensive and particularly dense, having a significant effect on the penetration of light. Ephemeral, sub-canopy algae, such as *Dumontia incrassata*, *Enteromorpha* spp., *Petalonia fascia*, *Scytosiphon lomentaria* and *Ulva* spp. did appear to be affected in size and density beneath the *Sargassum* canopy

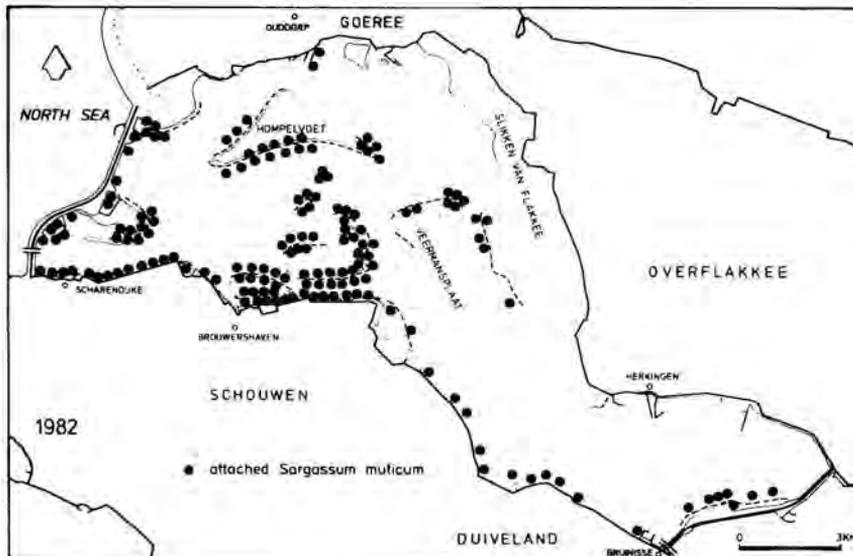


Fig. 13. Distribution of *Sargassum muticum* in Lake Grevelingen in 1982.

during summer 1982. Loss of fronds due to senescence and wind action led to the canopy brake-up from mid-June. However, in the shallow waters, due to the present density of the *Sargassum* stand, the canopy was seen to reform as early as November, i.e. plants of 50 cm length in water of 40-50 cm depth, come to the surface and create a cover which will persist through to mid-June of the following year. This factor should be investigated further as a permanent shading regime, in a non-tidal situation, for up to 8 months must have a significant effect upon the composition and growth of sub-canopy algae (see Ambrose and Nelson, 1982).

The oyster industry of the s.w. Netherlands is currently in a state of recession due to the oyster sickness (*Bonamia ostreae*) which has affected stocks of *Ostrea edulis* throughout Europe. There is, at present, no relay in or fishing of *O. edulis* from the Yerseke Bank fishery. When the area is declared disease free, the beds will be restocked with resistant *O. edulis* from lake Grevelingen. Unfortunately, once the oysters are re-established in these open pans, they will provide a very suitable substratum for the attachment of *S. muticum*, and will undoubtedly become colonized by the weed eventually; if only from fertile drift material originating at the mouth of the Oosterschelde. Once this happens stock losses will be inevitable from oysters attached to buoyant *Sargassum* plants being carried off in sea currents.

*Sargassum muticum* in the s.w. Netherlands does have a current nuisance value. Dense stands of the alga can cause problems to small outboard engines by entangling in the propeller and blocking cooling water intakes, also making areas unpleasant for sailboarders and sub-aqua divers. Similarly areas of *Sargassum* canopy do form a barrier and must have a detrimental effect on the recreational use of Lake Grevelingen, with swimmers reluctant to wade out through a bed of weed. To date, no amenity areas in the Oosterschelde are affected by the attached alga, probably due to the tidal regime and lack of suitable substrata for development of extensive populations. However, if large quantities of drifting weed are washed onto beaches and left to rot, may cause offence to tourists.

*Sargassum muticum* is now a permanent, well-established component of the Dutch marine flora. Only some five years after its introduction the alga is seen to form extensive populations in the s.w. Netherlands. In certain areas, some selective control of *S. muticum* may be necessary, in order to maintain amenity usage. A further commercial consideration will be the protection of oysters from the attachment of the weed.

#### References

- Ambrose, R.F. and B.V. Nelson - 1982. Inhibition of giant kelp recruitment by an introduced brown alga. *Bot. Mar.* 25, 265-267.  
Bom. H. - 1982. Inventarisatie van en (auto)ecologisch onderzoek aan *Sargassum muticum* (Yendo) Fensholt in Z.W. Nederland. Rijksherbarium, Leiden. Doct. onderwerp. 101 pp.

#### V.3. A survey of the shore crab (*Carcinus maenas* L.) in Lake Grevelingen (G7) (R.H.D. Lambeck and E.G.J. Wessel)

Macrozoobenthic sampling programmes with Van Veen grabs and bottom corers are inappropriate for the large mobile epifaunal species occurring in low densities. Owing to their high individual weight, average biomass per m<sup>2</sup> may not be negligible. Especially in the case of predators large species can have an important impact on the energy flow in the ecosystem.

From fishery research it was already known that shore crabs (*Carcinus maenas*) occurred relatively widespread in Lake Grevelingen. To get at least an idea about their importance a survey has been carried out in late September 1981 (in close cooperation with the fishery group of the Delta Institute). Sampling stations were randomly chosen within five depth strata, viz. 0-0.6 m, 0.6-2 m, 2.5-6 m, 6.5-13 m and 13.5-22 m. The three deepest strata were fished with a 3 m beam trawl (6 x 6 mm mesh size in the tail), each haul covering 1000 m<sup>2</sup>. In the two shallow strata

a 2 m beam trawl was used (5 x 5 mm mesh size), towed by a small motor flat in the 0.6-2 m zone and pulled by hand (with thanks to the students V. Erenst and P.J.F. De Graaf) in the 0-0.6 m zone. In the latter cases hauls covered 285 m<sup>2</sup>.

Animals have been sexed and carapace width has been measured. Biomass has been calculated on basis of a carapace width- ash-free dry weight (ADW) regression-function, determined for both sexes.

Densities in the two most shallow strata, only 4 individuals . 1000 m<sup>-2</sup>, were significantly ( $p < 0.001$ ) lower than in the deeper ones (Fig. 14). Biomass showed a comparable pattern. Most of the crabs were found between 2.5 and 13 m depth. The decrease in the deepest stratum was not significant. Numbers of hauls were relatively small, however (Fig. 14).

Considering the surface areas represented by the strata, a weighted average density of 28.0 shore crabs , 1000 m<sup>-2</sup> Grevelingen bottom (95% confidence interval 20.6-38.0) could be calculated with a concomittant biomass value of 71.8 g ADW.1000 m<sup>-2</sup> (37.0-139.4 g). These values should be regarded as absolute minima, because the net efficiency for crabs of the two trawls is unknown. For e.g. flatfish this value is in summer about 20% (Doornbos *et al.* 1982).

Surprisingly few small (< 20 mm) animals were found. According to Klein Breteler (1976) juveniles can grow to a maximum size of 20 mm in autumn at the tidal Balgzand (Dutch Wadden Sea). Very small juveniles may escape through the net. Perhaps juveniles live more burrowed than adults or try to escape the approaching trawl by burrowing instead of running away. It can neither be excluded that there was a near recruitment failure in 1981.

In their second year Balgzand crabs can reach a size of 50 mm (Klein Breteler, 1976). Maximum size found during this survey amounted to 69 mm for an exceptionally large female and to 68 mm for some males. If growth conditions in Lake Grevelingen are comparable with Balgzand ones, this suggest the presence of a third season

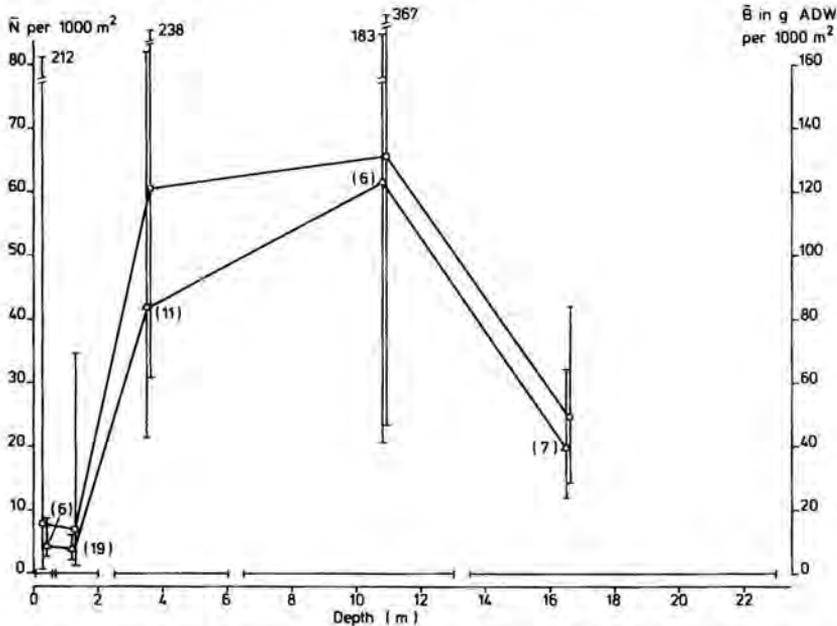


Fig. 14. The relation between average number of shore crabs caught per 1000 m<sup>2</sup> ( $\Delta$ ), average biomass in g ADW.1000 m<sup>-2</sup> (o) and water depth; 95% confidence intervals (after  $\log(x + 0.375)$  transformation) and depth strata indicated. (Numbers of hauls between brackets).

year class. A bimodal size frequency distribution was lacking, however, suggesting a wide variation in individual growth rates.

In shallow water ( $\leq 2$  m) only about 5% of the crabs was infected by the parasitic barnacle *Sacculina carcini*, but below 2.5 m the infected overall percentage amounted to 14% in males ( $N_t = 557$ ) and 31% in females ( $N_t = 355$ ). It was striking that nearly all infected animals were caught in the western part of the lake, hence local values were even much higher. Rasmussen (1973) found in the Danish Isefjord only a few percent of the crabs affected by *Sacculina*.

The infected males were on average considerably smaller than the non-infected ones. There was no difference within the generally smaller sized females (Table 3). Especially in unaffected males there is a size decrease towards deeper water, the difference of about 10% between the 2-6 and 6.5-13 m strata is significant ( $p < 0.001$ ).

#### References

- Doornbos, G., F. Twisk and R.H. Bogaards - 1982. Kwantificering van vissen. 2nd. Interimrapport ZOWEC Projekt III. Yerseke/Middelburg. DIHO/RWS DDMI. Nota Z 82 III 4.
- Klein Breteler, W.C.M. - 1976. Migration of the shore crab, *Carcinus maenas* in the Dutch Wadden Sea. *Neth. J. Sea Res.* 10, 338-353.
- Rasmussen, E. - 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia* 11, 1-507.

#### V.9. Population dynamics and migration of the netted dogwhelk (*Nassarius reticulatus*) in Lake Grevelingen (G7) (E.J.H. Sterenborg and R.H.D. Lambeck)

In 1976 a mass colonization by the prosobranch snail *Nassarius reticulatus* (L.) took place in Lake Grevelingen (Lambeck, 1982). It was tried to study the population dynamics of this new cohort in 1976 and 1977 by sampling frequently four permanent stations, situated at different sediment types and depths. However, a seasonal migration pattern prevented the estimation of a reliable mortality rate.

To overcome this problem a one year study into the dynamics of the whole lake population was started in April 1982. In water deeper than 1.8 m (hence accessible for our research vessel) every six weeks, 150 (0.093 m<sup>2</sup>) Van Veen grab samples were taken randomly within three depth strata, viz. 1.8-6.0 m, 6.5-13.5 m and deeper than 14 m.

Such approach took too much time in the extensive shallow ( $< 1.8$  m) areas, covering about one third of the lake surface. Four transects were chosen in this

Table 3. Average carapace width (in mm) in male and female shore crabs (*Carcinus maenas*) in relation to water depth and infection by *Sacculina carcini*. Animals smaller than 20 mm excluded. Sample sizes between brackets

	Depth stratum				
	0-0.6 m	0.6-2.0 m	2.5-6.0 m	6.5-13.0 m	13.5-22 m
Males without <i>Sacculina</i>	47.2 (13)	46.6 (38)	45.7 (240)	41.0 (168)	43.3 (72)
Males with <i>Sacculina</i>	33 (2)	40 (1)	33.8 (33)	30.9 (31)	33.6 (13)
Females without <i>Sacculina</i>	36 (5)	33.6 (16)	37.4 (99)	33.0 (109)	37.9 (38)
Females with <i>Sacculina</i>	-	29 (1)	34.7 (39)	34.7 (59)	34.9 (11)

area, representing the most important shallow "flats". Along each transect 0.1 m<sup>2</sup> has been sampled with a 0.02 m<sup>2</sup> flushing sampler (Van Arkel and Mulder, 1975) and a 0.018 m<sup>2</sup> corer at seven fixed depths between 0.1 and 1.6 m.

All bottom material was washed through a 1.0 mm sieve in the field. Residues were preserved by deep-freezing. In the laboratory snails were sorted out by the naked eye and stereomicroscopy. Numbers per sample, shell heights of the individuals and ash-free dry weights were determined in order to allow production calculations.

Based on the mean densities in the four strata, a weighted average density per m<sup>2</sup> Grevelingen bottom can be calculated for each date. Numbers of adult *Nassarius* declined gradually from 71 in early April to 54 m<sup>-2</sup> in mid August. *Nassarius* produces only one juvenile cohort a year (Tallmark, 1980; Lambeck, 1982). The first juveniles were caught in mid August, the average density amounted to 12 m<sup>-2</sup>, hence hardly compensating for the adult losses in the previous months.

Densities vary considerably with water-depth. At three of the sampling dates maxima were found at 3-3.5 m depth (Fig. 15). Numbers at 1.8-2.5 m were only 5-10% smaller. The *Nassarius* density found along the transects in the most shallow parts of the lake was relatively low. Towards deeper water there was a gradual decline. By far the smallest numbers were found in the deep channels (14-30 m).

Only the June distribution differed somewhat with a pronounced peak at 4-4.5 m and also an above average density at 5-6 m depth. The June 1982 data are in accordance with a dip in numbers at two 3 m stations in June 1977, followed by an increase later on in the season (Lambeck, 1983).

These seasonal variations might be related with reproduction behaviour, but in 1977 the population was dominated by sexually immature animals, so other factors may also be involved.

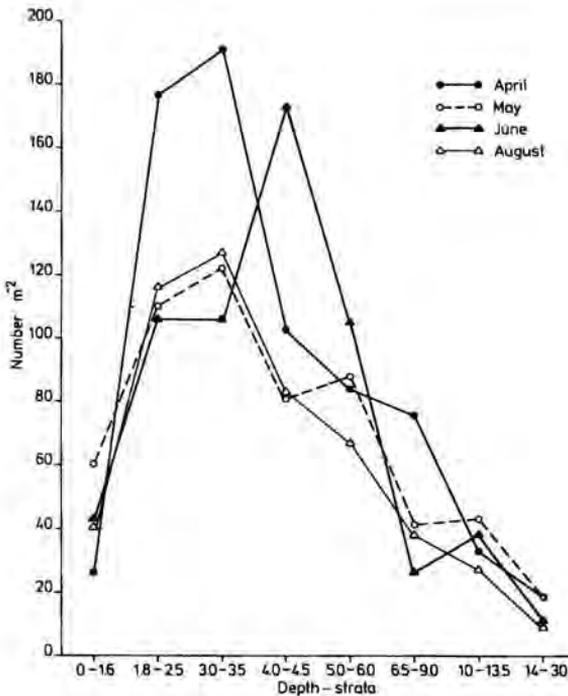


Fig. 15. Densities of *Nassarius reticulatus* during 1982 in the various depth strata in Lake Grevelingen at 4 sampling dates in 1982.

A more elaborate analysis of changes in depth distribution and hence of migration patterns will take place after completion of the field work.

#### References

- Lambeck, R.H.D. - 1982. Colonization and distribution of *Nassarius reticulatus* (Mollusca: Prosobranchia) in the newly created saline Lake Grevelingen (S.W. Netherlands). *Neth. J. Sea Res.* 16, 67-79.
- Lambeck, R.H.D. - 1983. Dynamics, migration and growth of *Nassarius reticulatus* (Mollusca, Prosobranchia) colonizing saline Lake Grevelingen (S.W. Netherlands). *Neth. J. Sea Res.* 17 (in press).
- Tallmark, B. - 1980. Population dynamics of *Nassarius reticulatus* (Gastropoda, Prosobranchia) in Gullmar Fjord, Sweden. *Mar. Ecol. Progr. Ser.* 3, 51-62.
- Van Arkel, M.A. and M. Mulder - 1975. A device for quantitative sampling of benthic organisms in shallow water by means of a flushing technique. *Neth. J. Sea Res.* 9, 365-370.

#### V.10. A tentative population model of the mussel *Mytilus edulis* in Lake Grevelingen (G7) (J.H.G. Verhagen)

In addition to the general C-budget approach, dynamical models for the main components in the aquatic system are constructed. The model development for the eelgrass component has been finished for the time being (Verhagen and Nienhuis, 1983). Also a preliminary version of a model for the macro-zoobenthos component has been developed (Verhagen, 1982).

The model is based on the assumption that the benthic filter feeders are food-limited. In that case the benthic filter feeders will utilize the available food in the water column as efficiently as possible. In fact it is shown that the musselbeds are located at places where the transport of food to the bottom is maximal. This transport is controlled by the pattern of prevailing wind generated currents, the food level in those currents and the vertical component of the velocity towards the bottom.

The wind driven currents in Lake Grevelingen as calculated by the Delft Hydraulics Laboratory for a westerly wind are shown in Fig. 16A.

From the calculated velocity field the pattern of wind driven circulation can also be reproduced as shown in Fig. 16B. It can be observed that several circuits exist. The exchange of water between those circulation "cells" will be less than within one "cell". Optimum food utilization by benthic filter feeders implies that

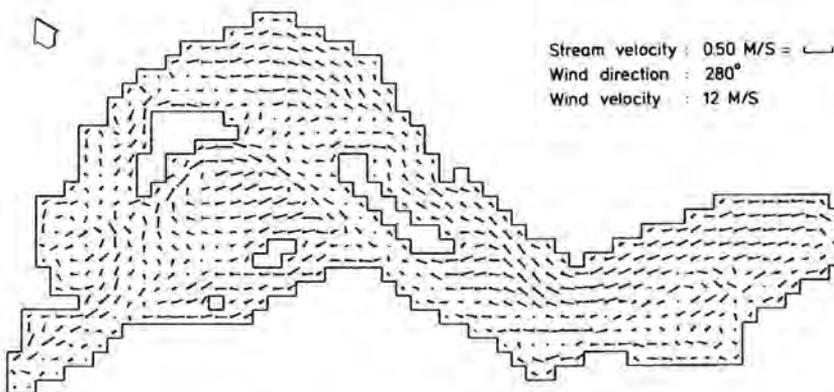


Fig. 16A. Calculated circulation pattern in Lake Grevelingen. Dashes originating from the spots are pointing in the direction of the flow. The length of the dashes corresponds to the strength of the flow velocity averaged over the waterdepth.

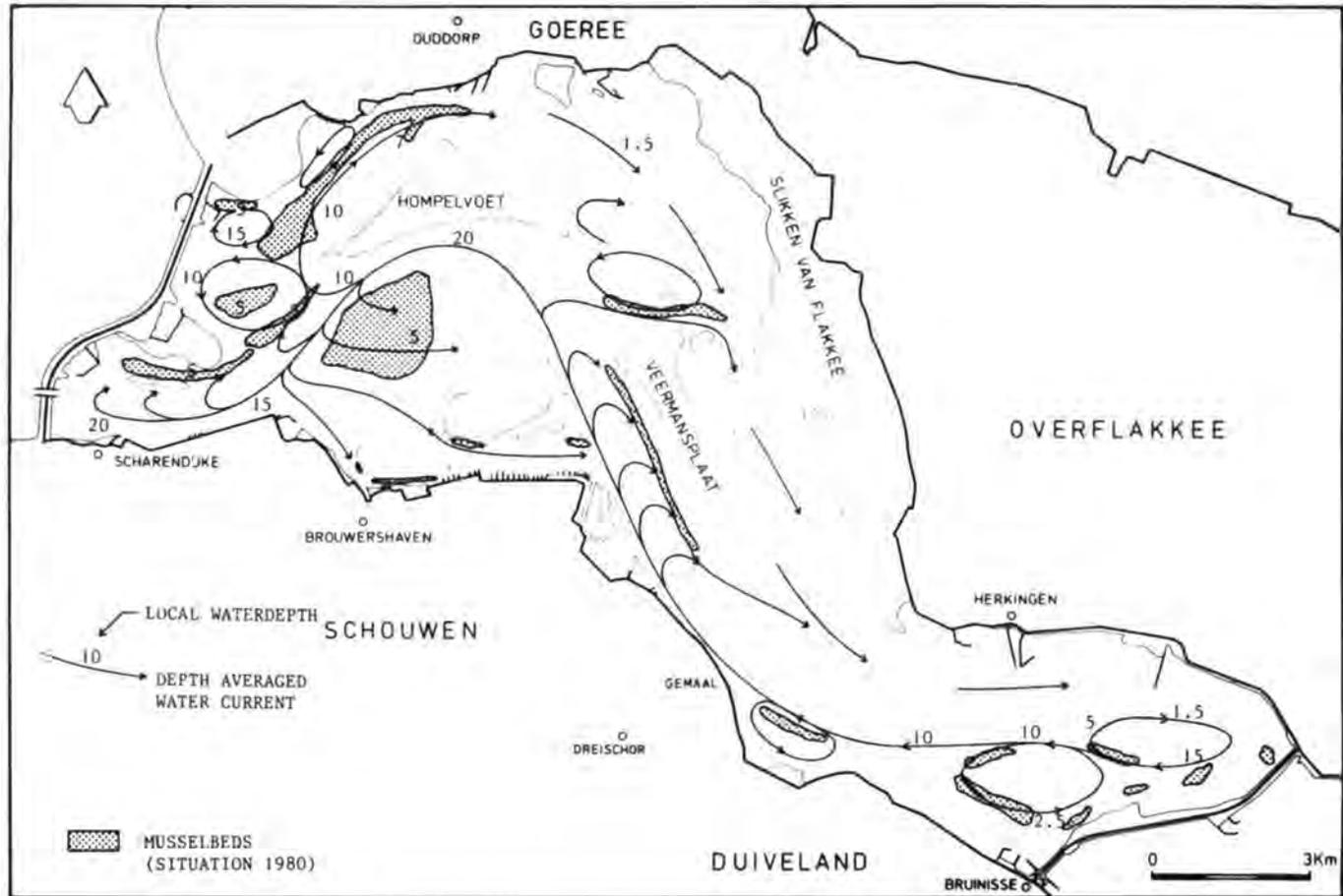


Fig. 16B. Distribution of mussel beds as surveyed by the Institute for Fishery Research in relation to the wind driven circulation pattern. The numbers along the streamlines indicate local waterdepth.

every circulation cell should at least contain one musselbed. Moreover the musselbeds will be located where the vertical component of the velocity directed towards the bottom is maximal, hence they will be located on gently sloping bottoms in converging flows. The distribution of musselbeds as surveyed by the Institute for Fishery Research is shown also in Fig. 16B. The agreement between measured distribution and expected distribution on the basis of calculated circulation cells and bottom slopes is surprisingly good.

Next to the distribution model a population model has been developed mainly based on data from Bayne *et al.* (1976, 1983). Growth, mortality, reproduction and recruitment of *Mytilus edulis* have been described as functions of external variables as temperature, salinity, season, suspended solids, food concentration, flow rate and vertical mixing in the water column reaching the musselbed.

A workshop aiming to improve that tentative model version has been organized in September 1982 at Middelburg. Several invited investigators on mussels from various research institutes in The Netherlands, Belgium and England contributed to the discussion on the formulation of the reproductive cycle as given in the model. The work on this submodel will be continued.

### References

- Bayne, B.L. (ed.) - 1976. Marine mussels: their ecology and physiology. Cambridge. Cambridge Univ. Press. International Biological Programme 10, pp. 1-506.
- Bayne, B.L. - 1983. The physiological ecology of marine molluscan larvae. In: The Biology of Molluscs: Reproduction and Development. Ed. N.H. Verdonk, A. Tourpa and J.A.M. Van den Biggelaar, Academic. Press (to be published).
- Verhagen, J.H.G. - 1982. A distribution and population model of the mussel *Mytilus edulis* in Lake Grevelingen. Paper presented at the Third Internat. Conf. on State-of-the-Art in Ecological Modelling, Colorado State University, USA, May 24-28, 1982. Delft, Publication no. 279 Delft Hydraulics Laboratory, 11 pp.
- Verhagen, J.H.G. and P.H. Nienhuis - 1983. A simulation model of production, seasonal changes in biomass and distribution of eelgrass (*Zostera marina*) in Lake Grevelingen. Mar. Ecol. Prog. Ser. 10, 187-195.

### V.11. Role of the meiofauna in the breakdown of organic matter in Lake Grevelingen (G8) (M.J. Orban)

Meiozoobenthos (animals passing through a one mm sieve) might play a substantial role in the "small foodweb" (Kuipers *et al.*, 1981). Their role in the production and mineralization processes in Lake Grevelingen has been studied in a one-year project. Cultivation experiments with different media were carried out to obtain animals for the mineralization measurements.

Monocultures were created by introducing several adult males and females of one species into fresh agar plates. In this way monocultures of *Theristus pertenus* and *Chromadora nudicapitata* were obtained. Because of methodological problems the number of animals in the monocultures were too low to use them for mineralization experiments. For this reason another species, *Monhystera microphthalmia* (successfully cultivated in Gent, Belgium) was brought onto the sediment cores used for the experiments. It was found out experimentally that *M. microphthalmia* could live and reproduce on Lake Grevelingen sediment (from station Archipel). Moreover, for each nematode species an optimal agar concentration was determined.

Oxygen gradients were determined in cores that were placed in four different tanks with a constant circulation of filtered Grevelingen water. Before the experiments the sediment from the cores has been frozen and thawed twice to kill all the macro- and meiofauna. On the cores a spontaneous growth of micro-organisms arose. Four different tanks were used in the experiment:

1. The cores of tank I served as control. Only the micro-organisms developed.
2. On the cores of tank II, nematodes of a natural source were introduced after a stabilization period of about 1 month.

3. The number of nematodes in tank II increased too slowly, for that reason *Monhystera microphthalmia* was introduced on the cores of tank III.

4. Ten weeks after the incubation of the cores from tank I, II and III, cores from Lake Grevelingen were placed in tank IV.

Experiments were carried out at 18°C. The light climate was adapted to the natural situation. Oxygen consumption measurements were carried out in the dark to prevent the production of oxygen. Micro-electrodes were used as described by Revsbech *et al.* (1980). This method is suitable for these measurements because:

1. direct readings of the O<sub>2</sub>-concentration are possible;
2. disturbance of the sample is small, so more measurements can be made on one sample;
3. differences in concentration over small distances (up to 0.25 mm) can be recorded, allowing the registration of fairly accurate gradients.

Fig. 17 shows the results of a measurement; several cores of each tank were examined.

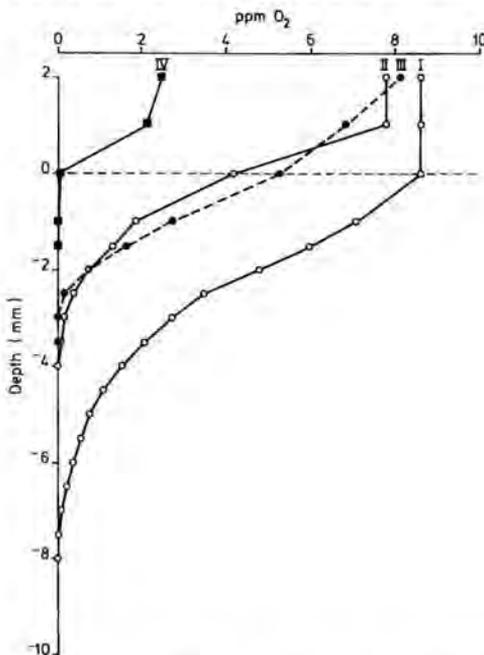


Fig. 17. Oxygen profiles in four tanks. Average curves of several cores for each tank.

The average oxygen gradients for the cores of each tank are given in this figure. After the measurements, 2 cores were taken from each tank. Cores were sliced in 0.5 cm layers, and the number of nematodes was counted (Table 4). In tank IV the oxygen consumption is high because of the presence of other larger organisms than nematodes (*Polydora spec.*, harpacticoids and harpacticoid larvae). There appeared to be a relation between the oxygen gradient in the sediment and the number of nematodes present in the cores. In other words: the more meiofauna, the more oxygen will be consumed, implying more mineralization of organic matter.

#### References

Kuipers, B.R., P.A.W.J. De Wilde and F. Creutzberg - 1981. Energy flow in a tidal flat ecosystem. *Mar. Ecol. Progr. Ser.* 5, 215-218.

Table 4. Number of meiofauna organisms present in 4 experimental tanks

Tank/Organisms	Nematodes	Others
I	41	-
II	2224	-
III	2019	-
IV	1096	145

Revsbech, N.P., J. Sørensen, T.H. Blackburn and J.P. Lomholt - 1980. Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.* 25, 403-411.

**V.12. Fast growth of 0-group plaice (*Pleuronectes platessa* L.) in Lake Grevelingen (G9) (G. Doornbos, R.H. Bogaards and P. De Koeijer)**

From an economical point of view the growth rate of commercial fish species is very important.

In The Netherlands the statutory minimum size of catchable plaice *Pleuronectes platessa* is 25 cm, reached in the North Sea in 3-4 years. Factors as e.g. density, food supply, diseases, water temperature etc. can influence growth rate, which may explain the remarkable differences in average length reached by juvenile plaice at the end of their first growing season ( $L_1$ ) in the various nursery areas in n.w. Europe (Table 5).

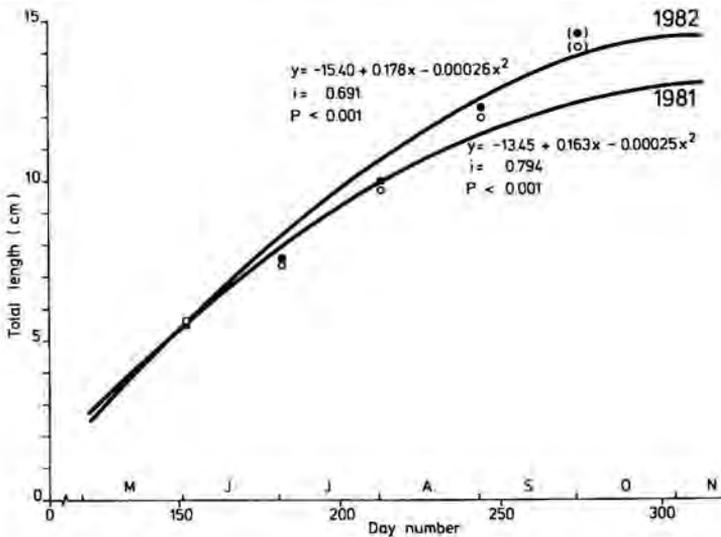


Fig. 18. Growth of 0-group plaice, year classes 1981 and 1982, in Lake Grevelingen compared to the simulated increase in mean length according to Fonds (1979) (O = 1981; ● = 1982).

Table 5. Differences in mean total body length ( $L_1$ ) of juvenile plaice at the end of their first growing season in various nursery areas and years

Area	Year	Mean $L_1$ (in cm)	Reference
Red Wharf Bay	1963	8	Macer, 1967
" " "	1964	8	" "
" " "	1965	6	" "
Loch Ewe	1965	7	Steele and Edwards, 1970
" " "	1966	7	" " " "
" " "	1967	6	" " " "
" " "	1968	7	" " " "
Balgzand	1972	8	Kuipers, 1977
" "	1973	10	" "
" "	1974	10	" "
" "	1975	11	" "
Belgian coast	1976	12	De Clerck, 1978
" " "	1977	9	De Clerck, 1980
" " "	1978	9	" " "
" " "	1979	12	De Clerck, 1981

On the Balgzand (western Waddenzee) Zijlstra *et al.* (1982) observed a negative correlation between the abundance of post-larvae during settling (mainly in April and May) and the length of the fish on June 1st. However, after that date, monthly growth ( $\Delta L$ ) of juvenile plaice in the field could be described as a linear function of body length ( $L$  in cm) and ambient water temperature ( $T$  in  $^{\circ}\text{C}$ ), according to the empirical model of Fonds (1979):  $\Delta L = 0.12 T + 0.05 L - 0.4$ . So during summer period growth was neither density-dependent nor limited by feeding conditions.

The  $L_1$  values in Lake Grevelingen for the year classes 1981 and 1982, respectively 13.0 cm (range 7-17 cm) and 14.5 cm (range 8-20 cm), appeared to be the highest known for natural waters in n.w. Europe (Table 5). Only under artificial growing conditions, e.g. in sea water mixed with warm effluent of the Hunterston nuclear power plant (Scotland), the  $L_1$  of plaice became somewhat larger, namely 15.3 cm (Bardach *et al.*, 1972).

Over the period May to November growth of 0-group Lake Grevelingen plaice could be described better by a second degree polynome than by the von Bertalanffy expression (Fig. 18). From June through September the linear function of Fonds fits almost just as well. This implies that the fast growth of juvenile plaice is primarily a result of the high water temperatures in the lake during summer time (up to  $20^{\circ}\text{C}$ ), which are a consequence of the enclosing of the former Grevelingen estuary in 1971.

The increase in weight is more spectacular than the length growth, because of the length-weight relationship  $W = a L^b$ , in which the exponent  $b$  has a value close to 3. At the end of their first growing season 0-group Lake Grevelingen plaice of year classes 1981 and 1982 had reached a mean fresh weight of 27.4 and 37.3 g, respectively. Compared to the Wadden Sea the Grevelingen plaice gained weight approximately three times as fast.

### References

- Bardach, J.E., J.H. Ryther and W.O. McLarney - 1972. Aquaculture. The farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York, 868 pp.
- De Clerck, R. - 1978. Growth of juvenile soles, plaice, and dab off the Belgian coast. *Ann. Biol., Copenh.* 33, 161-163.
- De Clerck, R. - 1980. Growth of juvenile sole, plaice and dab off the Belgian coast in 1977 and 1978. *Ann. Biol., Copenh.* 35, 225-229.
- De Clerck, R. - 1981. Growth of 0-group sole, plaice, and dab off the Belgian coast in 1979. *Ann. Biol., Copenh.* 36, 175-176.
- Fonds, M. - 1979. A seasonal fluctuation in growth rate of young plaice (*Pleuronectes platessa*) and sole (*Solea solea*) in the laboratory at constant temperatures and a natural daylight cycle. In: E. Naylor and R.G. Hartnoll (eds.). Cyclic phenomena in marine plants and animals. Proc. 13<sup>th</sup> Eur. Mar. Biol. Symp., Isle of Man, Great-Britain. Pergamon Press, Oxford, pp. 151-156.
- Kuipers, B.R. - 1977. On the ecology of juvenile plaice on a tidal flat in the Wadden Sea. *Neth. J. Sea Res.* 11, 1, 56-91.
- Macer, C.T. - 1967. The food web in Red Wharf Bay (North Wales) with particular reference to young plaice (*Pleuronectes platessa*). *Helgoländer wiss. Meeresunters.* 15, 1/4, 560-573.
- Steele, J.H. and R.C. Edwards - 1970. The ecology of 0-group plaice and common dabs in Loch Ewe. IV. Dynamics of the plaice and dab population. *J. Exp. Mar. Biol. Ecol.* 4, 174-187.
- Zijlstra, J.J., R. Dapper and J.Y. Witte - 1982. Settlement, growth and mortality of post-larval plaice (*Pleuronectes platessa*) in the western Wadden Sea. *Neth. J. Sea Res.* 15, 2, 250-272.



Photograph 4.

This young female Common or Harbour Porpoise *Phocoena phocoena* (TL = 128 cm) drowned in a weir at the Oosterschelde on 6-12-1982. After World War II the common porpoise became rare in the Dutch coastal waters. Pollution, *f.i.* by toxic chemicals, may be one of the main causes of this decline. During 1970-1980 annually 7-26 (mean 17) animals, most of them dead, were washed ashore in The Netherlands. (Photograph Fred Twisk).

**V.13. Effect of water temperature on gear efficiency (G 9) (G. Doornbos and F. Twisk)**

To assess the size of a fish population by means of active fishing it is necessary to know the efficiency of the gear used. A figure for the latter can be obtained experimentally. In practise gear efficiency is usually assumed to be a more or less constant factor, although strictly speaking this assumption will only be valid for a particular species or size-group and only under the prevailing conditions during the experiment.

Indeed, results from a fish study in lake Grevelingen indicated a seasonal dependency of the gear efficiency. In the lake the number of plaice *Pleuronectes platessa* (excluding 0-group), caught with a 3 metre beam trawl, was low in spring and autumn and high in summer, although no migration could occur (Doornbos et al., in press). This might indicate a seasonal pattern in gear efficiency. Escape underneath the ground rope of the trawl is one of the factors that will influence the gear efficiency. To reveal the seasonal differences in relation to this factor three experiments with plaice and sand goby *Pomatoschistus minutus* were carried out according to Kuipers (1975).

An experiment consisted of a number of hauls, each of four minutes and covering about 1000 m<sup>2</sup>, with a varying number of tickler chains in front of the ground rope. Fishing was done 6-11 times with each chain combination. During fishing the chains drive the hiding fish off the bottom and prevent them to escape underneath the trawl. It is assumed that in the case of the highest mean catch of a series the underneath escape is zero and efficiency is 100%. Results pointed out that in the case of plaice there is a relationship between the water temperature and the number of chains needed to obtain maximum efficiency (Fig. 19A). When water temperatures are above 10°C the best results are obtained with one tickler chain. Then efficiency with respect to underneath escape will be approximately 100%. With more than two chains disturbance probably decreases the efficiency. The too early activated fish may escape by swimming away from the mouth of the trawl. Besides, in that case the increasing amount of by-catch (shells, seaweed etc.) makes fishing more difficult. To obtain a comparable high efficiency at low temperatures up to five chains are needed.

Data for the sand goby showed the same pattern (Fig. 19B). No relation could be detected between the length of the fish and the efficiency with respect

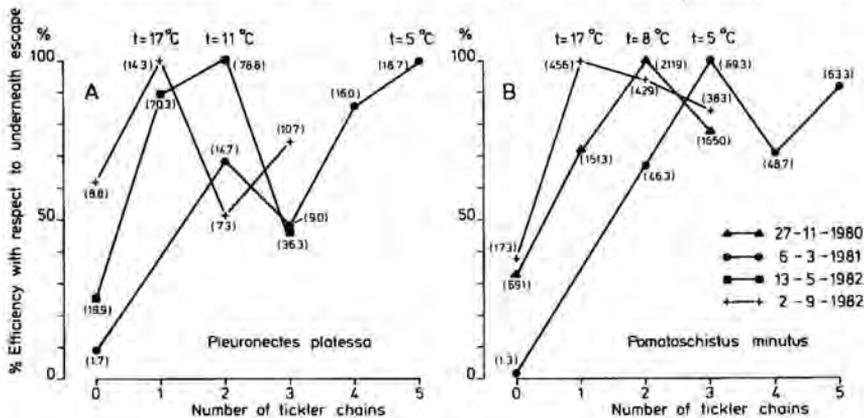


Fig. 19. The effect of tickler chains on the mean number (in brackets) of plaice (A) and sand goby (B) caught per 1000 m<sup>2</sup> with a 3 m beam trawl at different water temperatures (t). The catch has been related to the highest mean number in a test series (= 100% Efficiency with respect to underneath escape). All experiments have been done in Lake Grevelingen, except the 13 May one (Oosterschelde).

Table 6. Mean, minimum and maximum length of plaice and sand goby caught with a 3 m beam trawl during the experiments.

	Date	Length (in cm)		
		mean	min.	max.
<i>Pleuronectes platessa</i>	06-03-1981	19.8	11.8	30.5
	13-05-1982	16.0	3.7	32.4
	02-09-1982	17.6	6.3	38.0
<i>Pomatoschistus minutus</i>	27-11-1980	4.5	3.1	7.7
	06-03-1981	4.3	3.1	8.0
	02-09-1982	4.8	3.1	8.0

to underneath escape. In addition the length of the fish caught during the experiments is given (Table 6).

As a consequence of low water temperatures during winter time, plaice and sand goby are less active and probably "burrow" deeper in the substrate than during summer time. This behaviour of the fish might affect the efficiency of the trawl. It may be concluded that the catches of the beam trawl, normally equipped with one tickler chain, are well comparable during the period the water temperature is above 10°C. In Lake Grevelingen this period lasts from about April to October. During winter time the efficiency is much lower and catch data may not directly be compared to the summer ones.

In total, for gear efficiency not only underneath escape is important, but also lateral escape and mesh size selection (Kuipers, 1975). As normally a fine meshed (6 x 6 mm in the cod end) shrimpnet is used mesh size selection is only relevant to the small individuals of sand goby. There are indications that in the case of plaice the lateral escape and hence the efficiency is also influenced by water temperature. More experiments are needed to test this hypothesis.

#### References

- Doornbos, G., R.H. Bogaards and F. Twisk - . Density, growth and annual food consumption of plaice (*Pleuronectes platessa* L.) and flounder (*Platichthys flesus* (L.)) in Lake Grevelingen, The Netherlands. Neth. J. Sea Res. (in press).  
 Kuipers, B.R. - 1975. On the efficiency of a two-metre beam trawl for juvenile plaice (*Pleuronectes platessa*). Neth. J. Sea Res. 9, 1, 69-85.

#### V.14. Oxygen, sulphide, redoxpotential and pH microgradients in Lake Grevelingen sediment (G 10) (H.J. Lindeboom, A.J.J. Sandee and H.A.J. De Klerk)

With the introduction of microelectrodes for ecological research Revsbech *et al.* (1980) opened a new frontier in the study of biological processes in sediments. With these electrodes it is now possible to measure *in situ* concentrations without disturbing the sediment. Knowledge about these concentration gradients gives insight into the mineralization processes taking place in the sediment and into the way in which different groups of organisms influence these processes.

In 1982 we started a sampling programme in Lake Grevelingen. Bimonthly sediment cores were collected by SCUBA-divers at four different sampling stations. Within 5 minutes of retrieval the oxygen, sulphide and redoxpotential gradients

were measured in the laboratory on board the research vessel. In December 1982 we also succeeded in measuring the pH gradient with electrodes built according to the technique developed by the Physiology Department of the University of Gent (Belgium).

Fig. 20 shows the profiles measured in the sediment at A) the Monument sampling station (depth 8.0 m) on June 4, 1982 and at B) the Hals sampling station (depth 7.0 m) on December 17, 1982. The sediments at these sampling sites are characterized by their muddy structure and high organic carbon loading (Lindeboom and De Klerk, 1983).

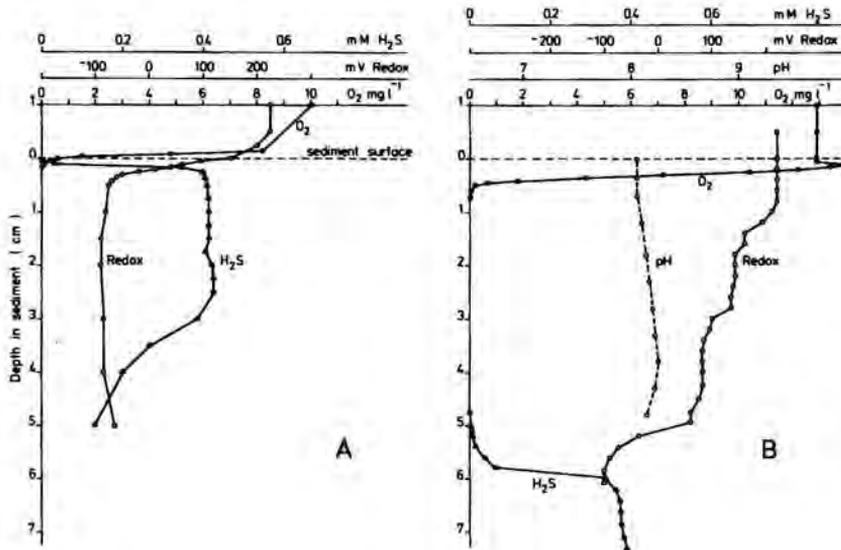


Fig. 20. Distribution of hydrogen sulphide, redox potential, oxygen, and pH in the sediment of sampling site Monument on June 4 (A) and in the sediment of station Hals on December 17, 1982 (B).

In the summertime (Fig. 20A), the high load with organic carbon and the high mineralization rates have caused a depletion of oxygen to almost the sediment-water interphase while a steep oxygen gradient is found just above the bottom. Hydrogen sulphide shows a steep gradient just below the sediment surface. This gradient is typical for the summer situation. On sunny windless days, part of the overlying water may become anaerobic allowing sulphide to diffuse into the water-phase. A milky coloration in the water caused by molecular sulphur, and many dead organisms on the bottom was noticed on one of these days.

In winter (Fig. 20B), the sediment is more aerobic due to considerably lower mineralization rates as well as to autumn and winter storms when large waves and current velocities stir up the sediment. The profiles shown in Fig. 20B were recorded just after a heavy storm with galewinds up to  $26 \text{ m s}^{-1}$ . Apparently all sulphide in the upper 5 cm was removed or oxidized. This gradient pattern is typical for the winter situation. The layer between free oxygen and free hydrogen sulphide probably contains oxidized nitrogen, iron and manganese compounds (Jones, 1982). These compounds are reduced in the spring when the microbial mineralization rates increase due to the rising temperature.

Our future research is focussed on the sediment of the Oosterschelde, where we will study both aerobic and anaerobic mineralization. With the help of large

bell jars and microelectrodes we hope to obtain further insight into mineralization processes and into the way in which different groups of organisms influence these processes, either via production, respiration or bioturbation.

### References

- Jones, J.G. - 1982. Activities of aerobic and anaerobic bacteria in lake sediments and their effect on the watercolumn. In: D.B. Nedwell and C.M. Brown (eds.), *Sediment Microbiology*. Academic Press, London, pp. 107-145.
- Lindeboom, H.J. and H.A.J. De Klerk - 1983. C-mineralisatie op en in de bodem van de Grevelingen. Eindrapport ZOWEC II. Yerseke/Middelburg. DIHO/RWS DDMI. Nota Z 83 II 5 (in press).
- Revsbech, N.P., J. Sørensen, T.H. Blackburn and J.P. Lomholt - 1980. Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.* 25 (3), 403-411.

### V.15. Transport of organic matter in the Oosterschelde (K 1) (J.H.B.W. Elgershuizen)

The year programme of 1982 included:

- a. Twelve flux-measurements of POC, seston, chlorophyll a and total pigments by use of three ships (one scanner and the other ones at a fixed anchor station) in the Roompot during a complete tidal cycle;
- b. A monthly monitoring of the above-mentioned parameters and chlorinity, DOC, DON and total carbohydrates at twelve stations in the Oosterschelde by ship within two days; a monthly monitoring on sixty-six stations in the Dutch coastal waters between Hoek van Holland and the Belgium frontier up to sixty kilometers off shore by helicopter within three hours;
- c. Five flux-measurements at different trajects in the Oosterschelde during a complete tidal cycle.

The information derived from these measurements was put into existing data files. Depth-time profiles with isopleths of concentration or current velocity of the anchor station measurements, were presented. These profiles will be analyzed by a method called "singular value decomposition" (Nash and Lefkovitch, 1976), which implicates data description by one depth function and two time functions per profile. Longer time series of POC and seston data at a reference point might be useful to link up the different flux-measurements and elucidate the effects of spring and neap tide, storms, plankton blooms etc. Measurements at Sophia harbour, Jacoba harbour and Roompot showed that the fluctuations of particulate parameters in the harbours differed widely from those in the main channel Roompot. So none of the harbours can serve as a reference point. A pontoon with monitoring equipment situated in one of the main channels is needed to get good reference values. Research into the use of a turbidity meter for estimating POC concentrations revealed that a Monitek 160/131 can be applied. By use of a daily standard graphic, POC can be estimated at an accuracy of  $0.15 \text{ g m}^{-3}$ . At present, horizontal distribution of e.g. POC is established by scanning the water mass using a water sucking up system and a throughflow turbidity meter.

In an estuary like the Oosterschelde the horizontal distribution of POC and seston changes with the tide. It was hypothesized that sources and sinks could be derived from these changes. This could be done faster, more easily and synoptically by remote sensing technics. For ground truth a scanning by ship is needed. The remote sensing pictures taken by aircraft and satellite (LANDSAT) showed a very regular turbidity pattern in the Oosterschelde and the coastal waters directly in front of it, when weather conditions were favourable i.e. not too windy. In order to analyse these turbidity patterns, charts of the grain size, mud and POC-distribution were compiled (Elgershuizen, 1982) by use of data from different authors. Actually, the data on POC-distribution are rather inaccurate because of the many ecological processes in the bottom (primary production by microphytobenthos, mineralization and consumption by epi and infauna etc.).

In collaboration with the Hydraulics Laboratory two different related studies are carried out. One is directed to characterize sources of POC in suspension by application of stable isotopic geochemics ( $C^{12}/C^{13}$  ratio) and is done by Dr. A. Salomons (WL-BI, Groningen). The other one is a follow up of earlier studies on settling velocity of POC and seston in the Oosterschelde and is guided by Ir. W. Van Leussen (WL, Delft). Research into the effects of turbulence of the water on aggregation of particles in suspension was a prerequisite for further studies on this subject. A tube (height: 4 m) was constructed at the Delft Hydraulics Laboratory. In this apparatus turbulence can be generated by an oscillating gate construction.

This year, two students joined the project. M. Van Steen (TH-Twente) analyzed the total flux-measurement carried out at the transect Wemeldinge-Tholen on April 20 1979. He concluded that the relative accuracy of the net transport of POC during that measurement was very close to 25%, which corresponds with earlier estimates (Elgershuizen and Stortelder, 1981). R. Wortelboer (RU Utrecht) confirmed the hypothesis of the apparent conservative behaviour of DOC in the estuary. The parameter itself is too rough to conclude that no fluctuations occur at all due to primary production etc. Actually, the large fluctuations of DOC that are observed are caused mainly by the local sugar beet processing industry.

At the end of 1982 an initial interim report appeared (Elgershuizen, 1982). An overall insight in the study of the transport balance of POC and mud in the

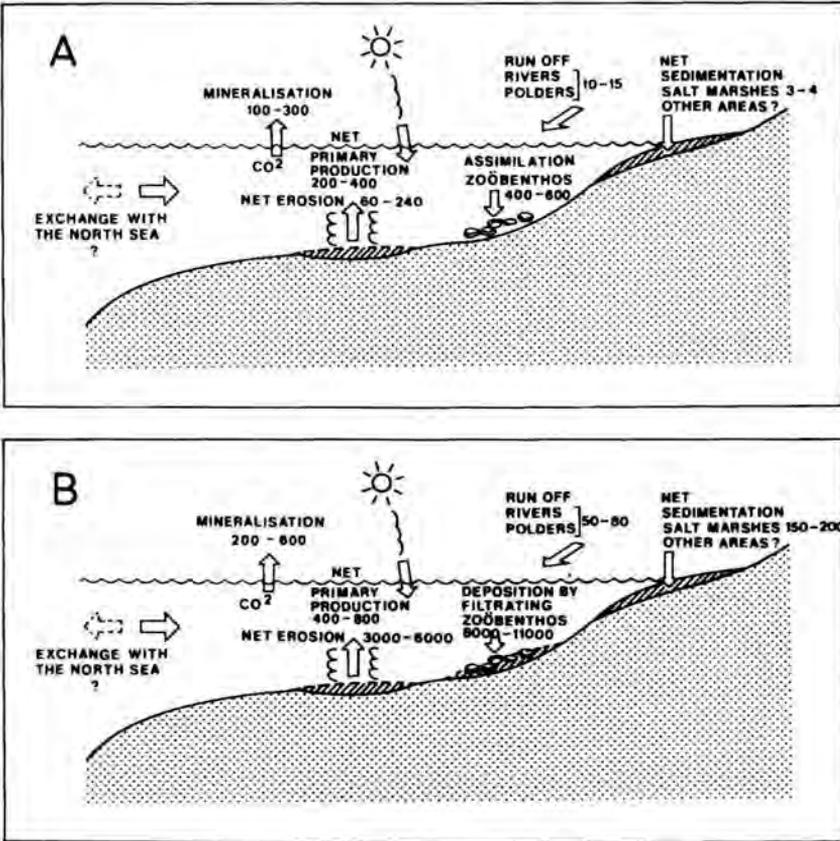


Fig. 21. Current view of average seston-flows (A) and POC-flows (B) in tons per day in the Oosterschelde. (From Dronkers, 1982).

Oosterschelde is given by Dronkers (1982). The present knowledge of the balance itself is summarized in Fig. 21A and B.

#### References

- Dronkers, J. - 1982. Slibtransport in de Oosterschelde. Driemaand. Ber. Deltawerken 101, 39-48.
- Elgershuizen, J.H.B.W. - 1981. Some environmental impacts of a storm surge barrier. Mar. Poll. Bull. 12 (8): 265-271.
- Elgershuizen, J.H.B.W. - 1982. Eerste interimrapport van het deelproject BALANS-transport: bijdragen van J.H.B.W. Elgershuizen. DIHO Yerseke/DDMI Middelburg, VN 218, 113 pp.
- Elgershuizen, J.H.B.W. and P.B.M. Stortelder - 1981. A direct measurement of the transport of organic matter in the Eastern Scheldt (S.W.-Netherlands). application of a trend surface method. Proc. Symp. Dynamics of Turbid Coastal Environments, Halifax.
- Nash, J.C. and L.P. Lefkovitch - 1976. Principal components and regression by singular value decomposition on a small computer. Appl. Statist. 25, 3, 210-216.

#### V.16. Seston analysis (K1, K3) (E.T. Van Ierland and L. Peperzak)

An attempt was made to separate the constituents of marine seston samples (inorganic material, detritus, phytoplankton and zooplankton) from each other without affecting the physiological state of the living material. The zooplankton was lured from the sample with the aid of light. For that purpose the efficiencies of four different light traps were compared. A black painted erlenmeyer, height 9 cm, opening  $\phi$  1.4 cm, filled with the sample, placed in a 1 l beaker containing filtered sea water, was most satisfactory. Approximately 65% of the zooplankton came out of the sample. The standard deviation was approximately 20%. The method is useful for grazing experiments and chemical analysis of zooplankton.

The other components were subjected to density gradient centrifugation in a relatively inert gradient, based on Percoll (Pharmacia Chemicals). In most of the experiments no separation was achieved: the densities of detritus and algae as well as the densities of the different algal species overlapped each other. Of the 100 samples processed in the course of the year 10 showed a reasonable separation, which means that one or more fractions consisted mainly of cells of one species. The succes of the separation procedure depends on the composition of the sample.

The separation experiments also yielded information about the densities of different algal species. Although not all the cells of one species had exactly the same density, representative densities could be determined for most of the species and differences between species were obvious (Table 7). No seasonal trend in these densities was found.

Table 7. Densities of planktonic species

<i>Biddulphia aurita</i>	1.18 - 1.23	$\text{g cm}^{-3}$
<i>Biddulphia sinensis</i>	1.03 - 1.08	"
<i>Cerataulina bergonii</i>	1.03 - 1.06	"
<i>Ditylum brightwellii</i>	1.07 - 1.13	"
<i>Rhizosolenia delicatula</i>	1.04 - 1.09	"
<i>Skeletonema costatum</i>	1.12 - 1.17	"
<i>Streptotheca thamensis</i>	1.04 - 1.10	"
<i>Thalassiosira rotula</i>	1.05 - 1.10	"

The possibilities of the FACS II Flow Cytometer (Reppo TNO, Rijswijk) in relation to marine field samples were examined. The apparatus is able to recognize and count cells of different diatom species. Chain-forming species present difficulties however. Separation is possible only with cells smaller than 40  $\mu\text{m}$ .

### V.17. Phytoplankton production measurements in the Oosterschelde (K3) (F. Vegter)

Phytoplankton production measurements in the Oosterschelde started in 1981 (at buoy 0-11) and were continued in 1982. Daily primary production is calculated from simulation experiments in an incubator with the  $^{14}\text{C}$ -method. By means of these experiments photosynthesis-light curves were obtained. From these curves phytoplankton photosynthetic efficiencies (initial slope of the curve expressed as  $\text{mg C (mg chlor-a)}^{-1}\text{joule}^{-1}\text{h}^{-1}$ ) and  $P(\text{opt})$  were determined. Daily primary production has been calculated from the photosynthesis-light curve and the daily light climate in the water column. The latter has been estimated from daily irradiance and extinction coefficients.

$P(\text{opt})$  was significantly correlated with chlorophyll concentrations as it was in 1981. About the same relation was obtained

$$P(\text{opt}) = 11.05 (\text{chlor-a}) - 12.5 \quad P < 0.001$$

$$n = 29$$

$$r = 0.91$$

Primary production data are presented in Fig. 22A. From this figure it may be concluded that phytoplankton activity during 1982 started in June. However since measurement frequencies have been chosen lower than once a week, phytoplankton blooms could have been missed easily. Obviously this is the case with a *Phaeocystis* bloom at the end of April (Bakker, this volume).

By integration of the data, presented in Fig. 22A a primary production of  $264 \text{ g C m}^{-2}$  was calculated for 1982.

During most incubations, the photosynthesis-light curve showed no light inhibition. In winter and spring a few cases of light inhibition were demonstrated, but since the main part of primary production occurred in summer, light inhibition was not important for the overall annual primary production as is shown by Fig. 22B.

The main species which contributed to the primary production in June and

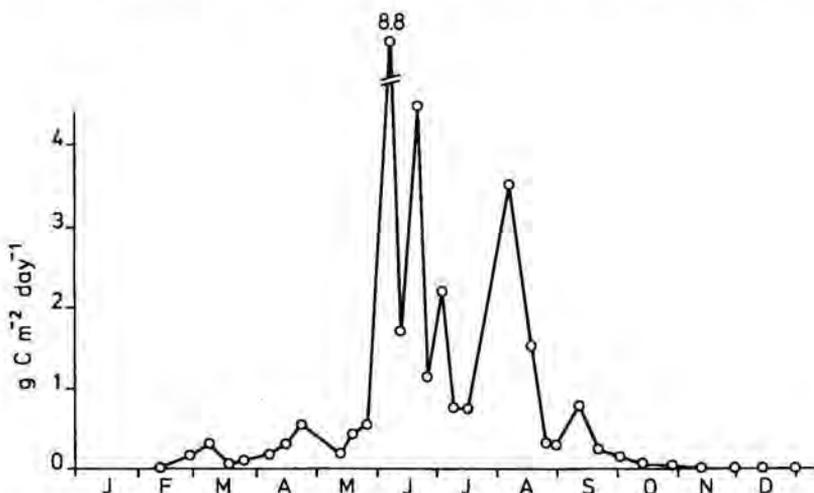


Fig. 22A. Phytoplankton primary production in 1982.

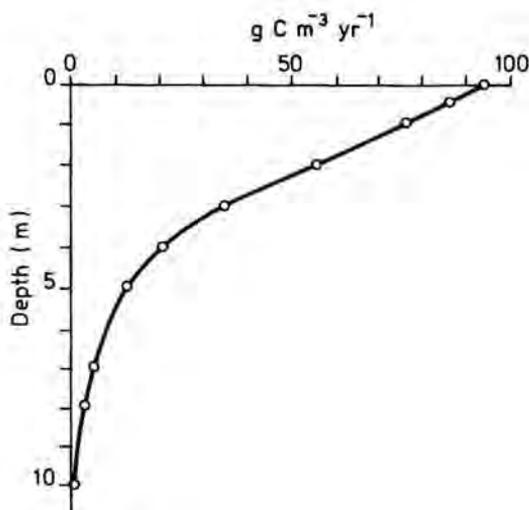


Fig. 22B. The annual production-depth curve.

July was *Cryptomonas* spec.; in August *Rhizosolenia* spec. was also important. However, the numerically not dominating part of the phytoplankton community may also be significant for the phytoplankton production. Fig. 22C represents the photosynthetic efficiency; this figure shows no distinct pattern during the year. Maximum efficiencies correspond with maximum daily primary productivity, but photosynthetic efficiencies are not significantly correlated with  $P(\text{opt})$  or with the daily irradiance, nor are they correlated with the daily irradiance of the days preceding to the day of measurement. We explain the large fluctuations of photosynthetic efficiencies by rapid changes in species diversity and abundance.

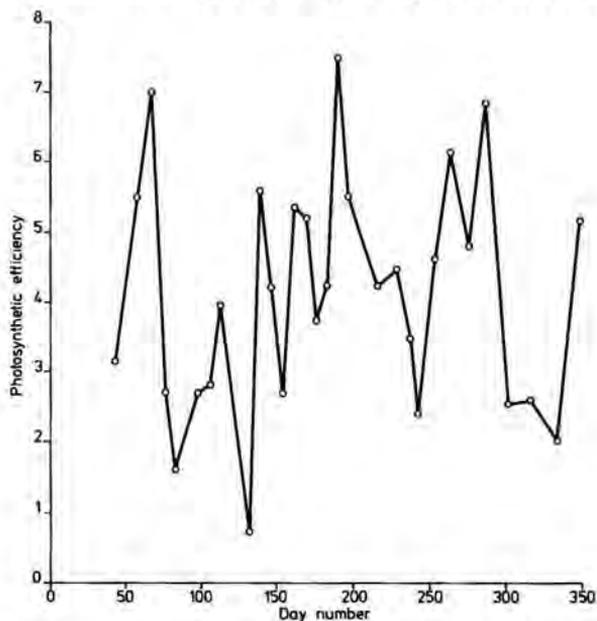


Fig. 22C. Average of hourly variation in ratio  $P_2/P_1$ .

**V.18. The importance of seston dependant variables during tidal cycles for daily primary production calculations (K 3) (P.R.M. De Visscher)**

Since the tidal movement in the Oosterschelde moderates some parameters that influence the extinction coefficient, several 13 hr measurements were carried out in 1982 to examine the parameters that depend on current velocity and seston content (extinction-coefficient itself, secchi depth, chlorophyll a content, POC content). The examined tidal cycles were all covering a day's light period.

The result of 6 measurements carried out in spring and early summer (the most productive period for the Oosterschelde), are illustrated in this paper. Even without statistical analysis (forthcoming results) the consistent relations between seston and respectively current velocity, POC, chlorophyll and extinction coefficient/secchi depth are distinct. Primary production was measured using the  $^{14}\text{C}$ -method (see Vegter, this volume). To test the sensitivity of the primary production calculations, two methods of calculation were compared:

the first method includes the input of changing chlorophyll and extinction figures into the common daily production calculation: biomass and light climate can be adapted over short periods (one hour) thus obtaining integral daily column production (reference production P1); the second method includes the input of average values over the 13 hr measurement period of chlorophyll and extinction coefficient in the calculation, thus obtaining P2. The ratio of P2/P1 should approximate 1 (optimal ratio) + 0.1.

In Fig. 23 the hourly ratios of P2/P1 (the averages of all 6 measurements) are given. In Table 8 the integral production and the ratios are presented. The average value for all ratios is 1.073. The high value for LG-PK (1.236) might be caused by the morphometrical situation: LG-PK is situated nearby a large shallow area which makes the extinction coefficient more dependent on seston variations than the chlorophyll content does.

From Fig. 23 an optimal sampling/measuring time can be derived. This conclusion is not merely visual but has also a theoretical basis in terms of expectance:

Averaged P2/P1

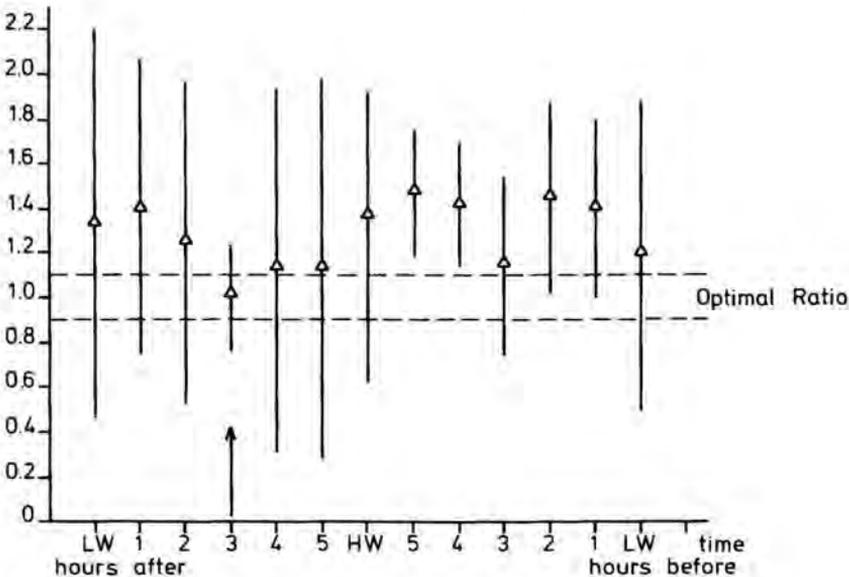


Fig. 23. Average of hourly variation in ratio P2/P1.

during the largest part of the tidal cycle (about 70%) the current velocities are high enough (more than  $30 \text{ cm s}^{-1}$ ) to maintain an almost constant seston content.

Fig. 23 shows that the optimal sampling time is 3 hr before high water and that the use of high or low water data will lead to large errors: during this period particulate material is sedimenting which causes a decrease in biomass as well as in extinction figures.

The overall daily production calculation with flood half-tide data is presented in Table 8 as P3. The average ratio P3/P1 (1.155) is higher than the average ratio P2/P1 (1.073) and differs more than 10% from the optimal ratio. The P3/P1 ratios show more variation than the P2/P1 ratios.

Table 8. Calculated productions and production ratios for 6 stations in the Oosterschelde

Date	Sampling station	P1	P2	P3	P2/P1	P3/P1
23-03-82	LG5	153.7	150.2	147.4	0.977	0.959
19-04-82	O11	773.8	744.7	783.0	0.962	1.012
28-05-82	LG-PK	978.0	1208.9	1126.6	1.236	1.152
03-06-82	LG14	5285.8	5808.3	4369.4	1.099	0.827
11-06-82	BV20	1189.3	1282.1	1912.5	1.078	1.61
14-07-82	LG-PK	886.2	962.3	1214.0	1.086	1.37
Average					1.073	1.155
St. dev.					0.099	0.290

Production calculations on the basis of all hourly variations will give the best results, but are very time-consuming. Optimalization cannot be achieved by using averages, because in that case all parameters have to be measured too. The use of flood half-tide data in the production calculations is more efficient, although errors up to 60% (BV20) can occur.

To give a rough estimate of the yearly primary carbon production for the whole Oosterschelde (or part of it) the use of flood half-tide data will be satisfying.

#### V.19. Coulter countings of fresh and preserved seston samples (K3) (M.L.M. Tackx and J.W. Francke)

Electronic counters provide a quick and accurate way of analyzing seston particle concentrations. The high mechanical and electrical stability required by these instruments often makes use in the field impossible. The question arises whether seston samples will change substantially in concentration and size class distribution during the time of transport to the laboratory. We have investigated whether the

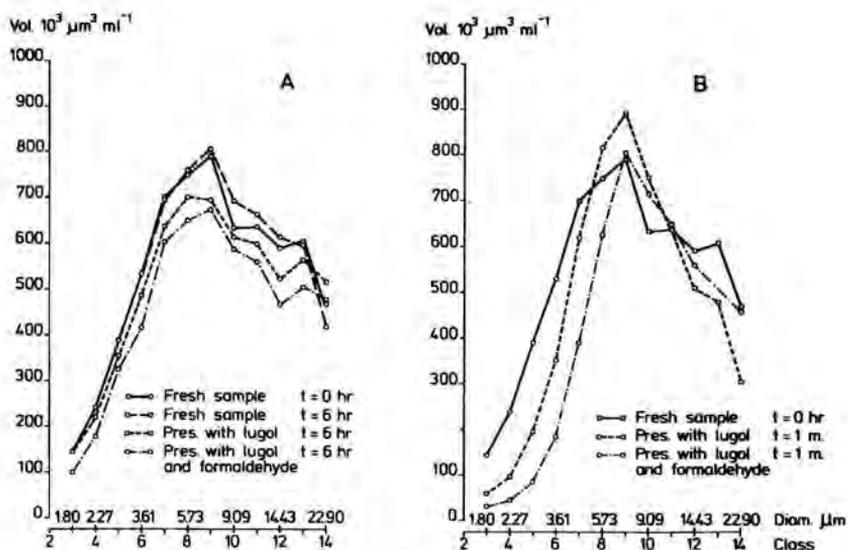


Fig. 24A and B. Coulter-size class distribution of Oosterschelde samples treated in different ways.

classical preservation methods used for microscopical plankton counting are also appropriate in relation to a coulter analysis of Oosterschelde samples.

Fig. 24A gives the mean counts of 5 replicates treated in 3 different ways: A: Fresh samples stored in a cooling box at approximately *in situ* temperature (*i.e.* filled with sample water).

B: Samples preserved with 0.5% lugol's solution and stored in the dark at room temperature.

C: Samples preserved first with 0.5% lugol's solution and afterwards with 4% formaline; stored as B.

All counts were corrected for background controls using the appropriate electrolyte.

From Fig. 24A it can be seen that addition of one or both preservatives creates larger concentration changes than those occurring in the non-preserved samples after 6 hours. However, neither preserved nor fresh samples show an important deviation of the distribution after a short period. After 1 month storage (Fig. 24B), both the lugol and the lugol + formaline preserved samples show an important decrease in the concentration of both small and large particles (classes 1-6 and 12-14), so the shape of the size class distribution narrows considerably.

In other words it seems preferable to use no preservatives when coulter analysis can be performed within a few hours after sampling.

## V.20. Contribution and nature of $\mu$ -cell aggregates in the seston of the Oosterschelde (K3) (C. Bakker, J.C.M. Rijk, M.L.M. Tackx)

Seston samples were collected at the depths of 2.5, 12.5 and 22.5 m at four localities in the western, central and eastern part of the Oosterschelde. The samples were preserved with Lugol's solution and studied after sedimentation by means of an inverted microscope (Reichert Universal Kamera Mikr. MeFII).

The sedimented seston was divided in the following categories: phytoplankton (mainly diatoms and flagellates); microzooplankton (mainly ciliates);  $\mu$ -cells (separate

unidentified coccoid cells with  $\phi$  1-5  $\mu$ m) and aggregates of the same  $\mu$ -cells (divided in the fractions:  $\phi$  5-20  $\mu$ m;  $\phi$  20-50  $\mu$ m and  $\phi$  > 50  $\mu$ m); detritus particles (same size fractions as the aggregates); sand (*idem*).

The species composition of the plankton was determined and the abundance of dominant species was estimated. Volumes of all counted species were calculated on the basis of regularly measured dimensions. Volumes of aggregates and detritus particles were estimated too. This seston analysis was accompanied by determination of POC and chlorophyll *a*.

Oosterschelde seston is characterized by a large content (70-90%) of  $\mu$ -cell aggregates and detritus (see also Prins, 1983). To reveal the nature and ecological significance of these  $\mu$ -cell aggregates a linear regression analysis of  $\mu$ -cell data with both chlorophyll and POC-data was performed. No correlation was found between the aggregates and chlorophyll (Fig. 25A), indicating that the hypothesis that  $\mu$ -cells are of algal nature, is improbable. However, the aggregates contributed indeed significantly ( $p < 0.001$ ;  $r^2 = 0.56$ ) to POC content (Fig. 25B) especially the smaller size fractions. (Contribution of phytoplankton to POC was only small and not significant). The preliminary conclusion is that the aggregates can be considered very important detritus components of the Oosterschelde seston.

Further,  $\mu$ -cell aggregates demonstrated a significant ( $p < 0.001$ ;  $r^2 = 0.62$ ) negative correlation with temperature. This correlation, however, may be considered an indirect one. A rise of temperature will probably play a stimulating role in one or more processes involving a decrease in the quantity of the aggregates. To be mentioned are: 1. increasing mineralizing activity of bacteria; 2. increasing abundance of zooplankton grazing on phytoplankton as well as on aggregates, especially the 5-20  $\mu$ m fraction.

Further studies will be directed especially to zooplankton grazing influences (*cf.* Tackx and Francke, 1983, this volume).

#### References

Prins, Th. - 1983. Een mikroskopische en coulter counter analyse van samenstelling en hoeveelheid van het seston in Oosterschelde en Grevelingen. DIHO Stud. Rep. D2-1983, 84 pp.

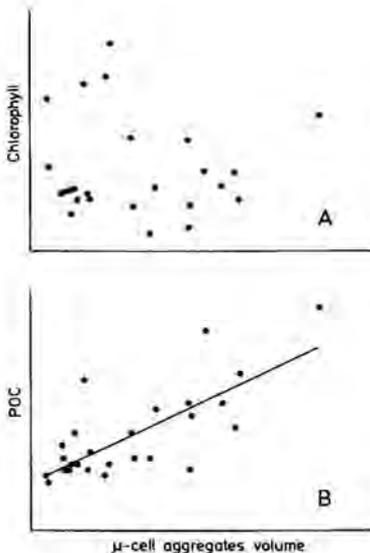


Fig. 25A. Volume of  $\mu$ -cell aggregates (all size fractions) versus chlorophyll in the eastern shallow part of the Oosterschelde. B. *Idem* versus particulate organic carbon.

**V.21. Seston, zooplankton biomass (I) and - grazing (II) during a 24-hour measurement in the Oosterschelde (K4) (C. Bakker, J.C.M. Rijk and P. Van Rijswijk - DIHO; M.H. Daro, O. Cromboom and R. Van den Wijngaert - VU, Brussels)**

In the marine coastal environment of the Oosterschelde, tidal rhythms interfere with day-night rhythms. During a 24-hour measurement in the shallow eastern part of the estuary (July 1981), seston and zooplankton standing stocks were estimated (every 2 hours) as well as zooplankton grazing (every 4 hours). The sampling depths were: 2.5 and 12.5 m. Preliminary results are presented in this paper.

I. Chlorophyll demonstrated peak values at low tide and minimum values at high tide. Analysis of the phytoplankton composition revealed high numbers of cryptomonad flagellates during low tide. POC values corresponded well with detritus content of the seston, including  $\mu$ -cell aggregates.

Phytoplankton analysis resulted in a complete spectrum of contributing species, and demonstrated clear difference in species composition between high and low tide periods. Total biomass values were maximal at low as well as at high tides, especially at the 12.5 m level. Percentages of detritus particles including aggregates of  $\mu$ -cells were very high, sometimes approaching 90% of total seston content (microscopically determined, cf. Bakker, Rijk and Tackx, 1983, this volume).

In the phytoplankton a number of species groups could be distinguished according to their behaviour in relation to the current pattern. Some diatoms (*Streptotheca*, *Lithodesmium*) and flagellates (*Cryptomonas*, *Eutreptiella*) demonstrated evident maxima around low tide. These species may partly prefer the shallow water covering the extensive mud flats (*Streptotheca*), or partly live at the mud interface (*Lithodesmium*, the flagellates). Some small diatoms, the *Asterionella* species - *kariana* and - *glaciag*, were always most abundant at high current velocities suggesting resuspension after the tides set in as well as rapid sinking when current velocity decreases. The diatoms *Rhizosolenia imbricata* var. *shrubsolaei* and *Chaetoceros* spp. were present in large numbers at high tide, decreasing during the ebb current and at low tide. Still other species did not demonstrate any consistency in relation to their distribution during the tidal cycle.

Total zooplankton biomass consisted for 50-60% of copepods. Zooplankton biomass was related to current velocity. All minimum values of 2.5 m depth were found at high and low tide. During the ebb tide, when current velocity was stronger than during flood, a larger zooplankton biomass was transported at a depth of 12.5

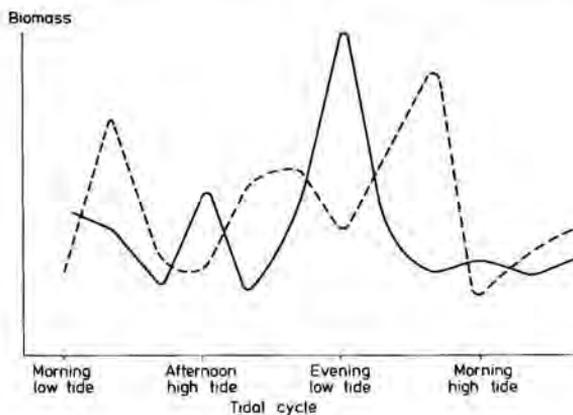


Fig. 26. Scheme of phytoplankton (detritus-included) (—) versus zooplankton (---) biomass during a 24-hour measurements in the eastern shallow part of the Oosterschelde estuary (July 1981).

m than at 2.5 m. Thus, during flood the transport of zooplankton in the surface layers prevailed, during ebb the transport in deeper water. Sinking of zooplankton at 12.5 m depth lagged strongly behind the curve of 2.5 m. *Acartia tonsa* and *Balanus nauplii* dominated around low tide (during last ebb as well as during beginning flood), other copepod species and *Oikopleura dioica* prevailed at high tide.

In conclusion: the most interesting result of the combined seston- and zooplankton biomass measurements is the different tidal periodicity of the phytoplankton (including all forms of small detritus) and the zooplankton, resulting in maximum and minimum biomass for respectively phytoplankton and zooplankton around low and high tide (Fig. 26).

II. Grazing measurements were carried out using a radiocarbon method (Daro, 1978), so that only ingestion of living or active phytoplankton could be measured. Fractioning was applied in order to realize measurements on 3 different fractions: particles smaller than 25  $\mu\text{m}$ , between 25 and 100  $\mu\text{m}$ , and larger than 100  $\mu\text{m}$ . Incubation time was four hours.

It was demonstrated that a large part of the 2 dominant groups (*Acartia* adults and copepodites on one hand, and nauplii of *Balanus* on the other hand), did not feed on the 100  $\mu\text{m}$  fraction, at least in the range of concentrations of this fraction occurring during the measuring period (0.5-1 mg chlorophyll a per  $\text{m}^3$ ).

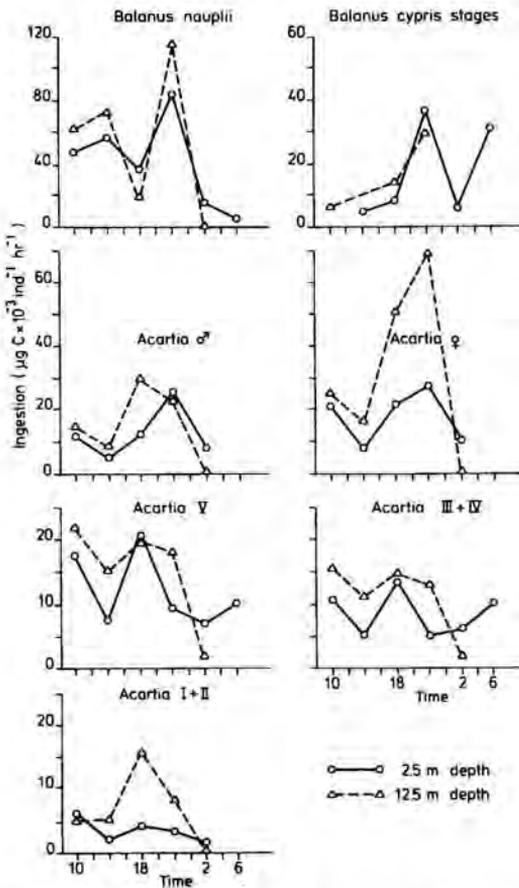


Fig. 27. Total ingestion rates of dominant zooplankton groups and developmental stages in the Oosterschelde (July 1981).

No preference for the two other fractions (< 25  $\mu\text{m}$ , 25-100  $\mu\text{m}$ ) could be demonstrated. Total ingestion per animal per hour as a function of time is given in Fig. 27. Following features can be mentioned: 1) the values of the depth of 12.5 m generally exceed those of 2.5 m, for all developmental stages of *Acartia*. 2) a day-night feeding rhythm occurs with peaks at 17.30-21.30 hr. 3) the maximum values coincided with the high total seston peak of low tide (Fig. 27), especially with the *Cryptomonas*-maximum. Consequently, a more or less selective grazing on this flagellate cannot be excluded. Both features 2) and 3) point in the same direction: increase of edible food superponed by day-night rhythms led to enhanced grazing activity.

Grazing figures, expressed as carbon values, show that if zooplankton would graze on phytoplankton only, the daily ingestion might reach maximum values of 77% of the own biomass at 2.5 m depth and 97% at 12.5 m.

However, it does not seem realistic to assume that the Oosterschelde zooplankton should graze on phytoplankton only, since this living fraction represents only a small part of total particulate matter.

In 1982 the coulter counter method as well as microscopical analysis have been applied for grazing measurements in the Oosterschelde (cf. Tackx and Francke, 1983, this volume).

#### Reference

Daro, M.H. - 1978. A simplified  $^{14}\text{C}$ -method for grazing measurements on natural planktonic populations. *Helgoländer wiss. Meeresunters.* 31, 241-248.

#### V.22. Zooplankton biomass, -succession and -interrelationships in the Oosterschelde (K4) (C. Bakker and P. Van Rijswijk)

In 1982 the shallow eastern part as well as the central and mouth areas of the estuary were sampled, weekly and biweekly respectively, allowing to determine longitudinal gradients in zooplankton distribution and biomass.

The zooplankton was sampled 1) by pumping 100-200 litres of water, subsequently filtered through 63  $\mu\text{m}$  mesh gauze, 2) by using a high speed sampler (Nackthai, Hydrobios) provided with a net of 300  $\mu\text{m}$  mesh width. Pump samples were taken from the depths of 2.5, 7.5, 12.5, 17.5 and 22.5 m and pooled. High speed sampling was performed in the water layers of ca. 10 and 20 m depth.

The abundance of all dominant species was estimated, with special emphasis on the copepods. In the shallow eastern part *Acartia* spp. dominated nearly continuously, except in June, when *Temora longicornis* was most numerous, immediately followed by *Centropages hamatus*. Also *Pseudocalanus minutus elongatus* demonstrated a (moderate) peak during that period (Fig. 28). Biomass of the adult copepods was determined by dry weight measurements using a Cahn electrobalance. Length and weight of the animals decreased in the course of spring and summer and the preliminary data suggest differences in this respect between the western and eastern section.

Comparison of the densities of the rotifer genus *Synchaeta* in Oosterschelde and Lake Grevelingen revealed that peak densities of the Oosterschelde populations were nearly an order of magnitude (5-10x) smaller than in Lake Grevelingen. Maximum densities of the subitaneous eggs, on the other hand, were rather similar in both areas. This indicates higher egg production of rotifers in the tidal Oosterschelde than in the stagnant Lake Grevelingen, compensating for the increased mortality in the tidal basin.

During spring naupliar stages of *Balanus* were far more numerous in the shallow eastern area than in the central and western regions of the sea arm. This coincided with higher abundance of *Pleurobrachia* in the mouth during that period, indicating high predation pressure on the *Balanus* nauplii. When *Pleurobrachia* reached its peak, *Beroe* began to develop strongly, eliminating *Pleurobrachia* within two weeks. After the disappearance of *Pleurobrachia* in summer, abundances of *Balanus* nauplii in the 3 Oosterschelde sections were more comparable. All copepods, species and stages together, demonstrated maximum abundance in the last decade of June when *Pleurobrachia* strongly decreased in numbers and especially in biomass.

A remarkable phenomenon was the abrupt disappearance of *Oikopleura dioica* in the samples of July 7 along the entire longitudinal axis of the estuary. Also most copepod species and stages decreased strongly in numbers. Both decreases coincided with a strong development of the Hydromedusa *Eucheilota maculata*.

One of the major aims of the study was to discover the structural differences between the assemblages of the shallow eastern area and the mouth region. When in 1987 the storm surge barrier is operational, the largest changes of structural patterns may be expected in the shallow eastern area. Therefore we have to study the present situation in detail in order to be able to evaluate the future differences.

### V.23. A comparison of grazing measurements on two types of particulate matter-distributions (K4) (M.L.M. Tackx and J.W. Francke)

Grazing measurements were performed on two types of particulate matter distributions: A: Oosterschelde samples, with a high particle concentration distributed quite evenly over a broad size range of 4 to 30  $\mu\text{m}$  particle-diameter. B: A culture of *Chlamydomonas* sp. with an equally high particle concentration (expressed in volume), situated in a narrow size range of 2 to 10  $\mu\text{m}$  diameter.

A. For the Oosterschelde samples, the following experimental procedure was used: Water samples (20 l) were collected at the station LG-PK and transported to the laboratory together with zooplankton collected at the same station. Both were stored overnight. The next day, 8 one-l bottles were filled with the collected sample. To each of 4 bottles 50 or 25 adult copepods (*Acartia* sp., *Temora longicornis*) were added. The remaining 4 bottles served as controls. The bottles were rotated at 2 rpm in the dark. Both storage and incubation took place at temperature close to the *in situ* temperature on the day of collection. From the remaining 12 l Oosterschelde water four 100 ml-samples were taken at the beginning of the experiment. At several incubation times, 100 ml samples were taken from each bottle. All samples were preserved with Lugol's solution and counted afterwards with a Coulter Counter.

B. For the experiments with *Chlamydomonas* sp. the same procedure was used except that the animals (*Acartia tonsa*) were collected at the sluice-dock in Ostend (Belgium) and that the samples were counted immediately without preservation.

For each size class of the coulter distribution, filtering and ingestion rates were calculated according to Frost (1972).

Total ingestion rates were calculated by summing up the ingestion rates for all size classes, each multiplied by the corresponding specific volume.

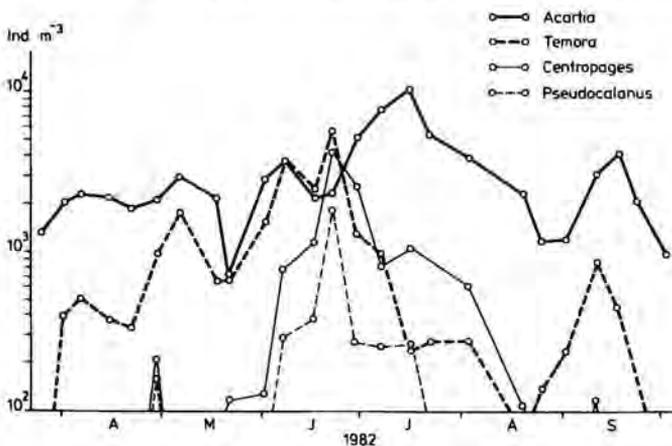


Fig. 28. Seasonal abundance of copepodite and adult stages of 4 copepod species in the eastern shallow part of the Oosterschelde in 1982.

Table 9 gives these total ingestion rates, expressed as percentage of the available particulate matter removed during the experiment.

Comparison of experiment A1 with B1 and A2 with B2 shows that comparable percentages removal are measured in cases with approximately equal particle concentration. In other words, quantitatively the grazing measurements are equal. In series A however, the coefficients of variation (CV) between the 4 replicate measurements are higher than in series B.

The results of experiments A3, A4 and A5 show that this high variability of grazing measurements is common for the Oosterschelde experiments (except for experiment 4, 4 hours incubation time).

A control experiment with Oosterschelde water showed a reduction of the CV from 93 to 48% when fresh samples were used instead of preserved ones. This CV of 48% is still higher than the CV's of series B.

The Oosterschelde samples used in these experiments show a particle distribution of the type found throughout the year over the entire estuary (Prins, 1983).

Table 9. Grazing measurements: A. Oosterschelde seston size range 4-30  $\mu\text{m}$   
B. *Chlamydomonas* sp. size range 2-10  $\mu\text{m}$

exp. A	P $\mu\text{m}^3\text{l}^{-1}$	t h	I %	CV	exp. B	P $\mu\text{m}^3\text{l}^{-1}$	t h	I %	CV
1	2007	6	15	113	1	1903	4	11	22
		18	14	32			16	15	22
		24	8	52			24	21	20
2	5664	4	4	51	2	4867	4	5	26
		8	3	67			16	10	33
							24	13	40
3	6666	4	7	120					
		8	6	110					
4	6884	4	11	7					
		20	1	61					
		24	3	69					
5	12715	4	25	105					
		8	14	98					
		16	7	88					

P: Concentration of particulate matter at the beginning of the experiment

t: Incubation time

I: Total amount of particulate matter removed during the experiment expressed as % of P

= Ingestion rate ( $\mu\text{m}^3 \cdot \text{ind.}^{-1} \cdot \text{hr}^{-1}$ ), nr of animals in experimental bottle.  
incubation time . 100/P . 1000

CV: Coefficient of variation of the 4 replicate grazing measurements (in %)

The tendency of higher variability in the measured ingestion rates, in samples that cover a more extended size range than narrow peaked samples, may be of biological significance. When abundant food that covers a broad size range is offered, the copepods may not be obliged to feed on the total range. Numerous combinations of particles present in the 4-30  $\mu\text{m}$  size range can be used as food. So the ingestion rate may also vary between the experimental bottles.

This hypothesis will be checked experimentally with fractionated Oosterschelde samples, appropriate statistical tests on the original coulter data and extra control experiments on the influence of preservation.

#### References

- Frost, B.W. - 1972. Effects of size and concentration of food particles on the behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* 17, 805-815.
- Prins, Th. - 1983. Een mikroskopische en coulter counter analyse van samenstelling en hoeveelheid van het seston in Oosterschelde en Grevelingen. *DIHO Stud. Rep.* D2-1983, 84 pp.

#### V.24. Biomass measurements of the microphytobenthos in the Oosterschelde (K5) (E.A.M.J. Daemen and M.T.T. De Leeuw)

As part of the BALANS-project the biomass of the microphytobenthos, an assumed substantial primary producer in the Oosterschelde ecosystem, was determined. Biomass was estimated by measuring spectrophotometrically the chlorophyll-a content of the top cm of the sediment.

In April 1981 a sampling programme at 72 stations, distributed over the Oosterschelde, has been started. The sampling was finished in November 1982, completing a one-year cycle with large overlaps at both ends. Stations were situated in five sections of the Oosterschelde, 4 of which have been sampled about monthly. The 16 stations in the 5th section (on the tidal flats of the Verdrongen Land van Zuid-Beveland) were sampled biweekly.

Preliminary estimates of the chlorophyll-a content show a mean value of about 200  $\text{mg m}^{-2}$  over the year, for the 16 stations of the Verdrongen Land van Zuid-Beveland. In the other sections, in which subtidal stations are represented too, the mean value is 100-120  $\text{mg m}^{-2}$ . No clear differences existed between mean chlorophyll contents of these sections, although the differences between individual sampling stations of a section may be considerable. Peak values were always highest in the central part of the Oosterschelde. In 1982 all sections showed a clear seasonal pattern with a more or less distinct peak in May-June and a very distinct minimum in July (20-40  $\text{mg m}^{-2}$ ). In August chlorophyll content increased again and on the last sampling date (mid-November), mean value was about 120  $\text{mg m}^{-2}$ . Chlorophyll values of the Verdrongen Land van Zuid-Beveland are roughly twice as high as those of the other sections. During winter large areas of the tidal flats were covered with ice and snow, nevertheless sediment samples showed considerable amounts of chlorophyll and after thaw, even a peak was found. This agrees with findings from Asmus (1982) for the northern Waddenzee. The fast raise of temperature in spring and early summer might be, directly or indirectly, the cause for the decline of biomass in July because grazing by zoobenthos and mineralization processes will increase at higher temperature (Van den Hoek *et al.*, 1979, Hargrave *et al.*, 1983). Oosterschelde microphytobenthos consists mainly of diatoms, but in summer blue-green algae (mainly *Merismopedia glauca*) may be important too, especially on the higher parts of the tidal flats. This phenomenon is also mentioned by Asmus (1982).

At 2 tidal stations the chlorophyll content was determined frequently to find out whether the sampling frequency used in the 5 distinguished sections of the Oosterschelde was sufficient to get a good insight into the seasonal fluctuations. Fig. 29 shows the determined chlorophyll values for one station. As can be seen

short-time fluctuations are considerable, so one might expect that a monthly sampling will not give a satisfying impression of the variation over the year. By sampling about monthly a more smoothed picture of this variation is obtained (Fig. 29). But, comparing the mean chlorophyll content over the year based on weekly sampling with that based on monthly sampling, the difference appears to be small over the year. For the two stations these mean values were 152 and 166 mg chl m<sup>-2</sup> for the weekly samplings (n=38) and resp. 153 and 164 mg chl m<sup>-2</sup> (n=10) if samples were taken once a month. Obviously, in this case a monthly sampling is sufficient to get an idea about the mean chlorophyll value over the year.

#### References

- Asmus, R. - 1982. Field measurements on seasonal variation of the activity of primary producers on a sandy tidal flat in the northern Wadden Sea. *Neth. J. Sea Res.* 16, 389-402.
- Hargrave, B.T., N.J. Prouse, G.A. Phillips and P.A. Neame - 1983. Primary production and respiration in pelagic and benthic communities at two intertidal sites in the upper Bay of Fundy Com. *J. Fish. Aquat. Sci.*, 40 (Suppl. 1), 229-243.
- Van den Hoek, C., W. Admiraal, F. Colijn and V.N. De Jonge - 1979. The role of algae and seagrasses in the ecosystem of the Wadden Sea: A review. Page 55. In: *Flora and Vegetation of the Waddensea*, W.J. Wolff, ed. Leiden, St. Veth tot Steun aan Waddenonderzoek. Report 3 Wadden Sea Working Group. 206 pp.

## VI. WORKING GROUP: BRACKISH WATERS (Code B)

### VI.1. Introduction (A.B.J. Sepers)

In the Delta area of The Netherlands there is a great number of large and small waterbodies with very diverse characteristics. Beside the great differences in size there are also considerable differences regarding the influence of the bottom and

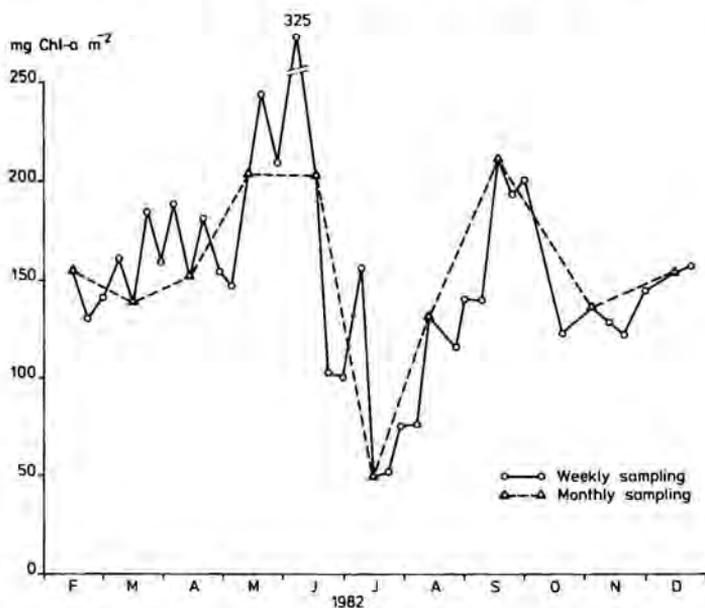


Fig. 29. Comparison of the seasonal variation in Chl-a content of a tidal station based on weekly (○—○) and monthly (Δ---Δ) samplings.

the littoral zone on the metabolism of the aquatic system as a whole. The mean chlorinity varies from near zero to about 18 ‰ Cl<sup>-</sup>. The variation in the value of the abiotic environmental parameters (stability in the meaning of constancy of the environmental parameters) shows great differences, resulting in a wide range of mean nutrient concentrations. The presence of this great variety of aquatic environments offers the opportunity to perform experiments in very different environments, so as to determine the most relevant parameters for the considered process. Moreover, it is possible to test hypotheses originating from laboratory experiments, in the natural environment.

In natural waters the abiotic environmental parameters vary continuously. The range of these fluctuations determines the stability. A comparison of a stable environment with an environment with a great variation in the abiotic parameters indicates that the community in the stable environment is characterized by a higher diversity. There occur more species and there exist more interrelations between the species than in less stable environments. Instable environments are generally characterized by communities with a relatively low complexity. Comparative research with organisms which are characteristic for stable and instable environments yields information about the fulfilment of a particular function within the aquatic system and indicates what the determinant parameters are.

In addition to this research dealing with the functioning of an ecosystem, it is the aim of the working group to set up investigations related to the structural aspects of an ecosystem. In this context the structure will be defined as the distribution of organisms in time and space, by which an instable environment will show a structure with a low level of complexity, and a stable environment a more complex structure. It is the ultimate goal of this research to yield better definitions of conceptions which are in general use in order to characterize ecosystems, like for instance the diversity concept.

The development of communities in waters with strong fluctuations will be interrupted repeatedly with the resultant effect, that these communities remain in the pioneer stage of development. Investigations into the community structure during this phase of development and into the functioning of the organisms yield information about the phenomena which govern the development of ecosystems during the initial phase.

## **VI.2. Aquatic and semi-aquatic Hemiptera in the inland waters of the s.w.-Netherlands (B3) (R. Luyendijk and B.P.M. Krebs)**

During a period of 25 years, aquatic and semi-aquatic Hemiptera were collected from the Delta-region in the south-western part of The Netherlands. Sampling over 1200 localities, a total of 42 species including 11 semi-aquatic Hemiptera were found. This number is slightly higher than that reported for other areas near the Delta-region. Thus Verstraete (1978) listed 37 species for an area south of the Delta region. Almost equal numbers were presented by Cuppen (1977), Van Tol and Van Nieuwerkerken (1978) and Worrel-Schets (1978) for the northern and eastern areas near the Delta region.

The majority of the collected aquatic and semi-aquatic Hemiptera were insects with an immense geographic distribution. Many species have been found all over Europe and in Asia, other species inhabit the entire Palearctic region (Nieser, 1978). Only a few species obtained during this study can be considered as characteristic for the region: *Corixa affinis* (Leach), *Sigara stagnalis* (Leach), *Sigara selecta* (Fieber) and *Micronecta meridionalis* (Costa). Some remarkable findings have to be mentioned: *Hesperocorixa moesta* (Fieber), *Micronecta minutissima* (L.) and *Microvelia umbricola* (Wroblewski). Although the latter mentioned species is not rare, *M. umbricola* is not as common as suggested by Nieser (1974); up till now this species was found at a dozen localities in The Netherlands, whereas no recordings are known from Belgium (Dethiers and Bosmans, 1979).

The dominant species were: *Gerris thoracicus* (Shum.), *Sigara lateralis* (Leach),

*Sigara striata* (L.), *Sigara stagnalis* (Leach), *Corixa affinis*, *Corixa punctata* (Ill.), *Callicorixa concinna* (Fieber) and *Notonecta viridis* (Delc.). A number of species known from areas near the Delta region were not found. Most of these species prefer nutrient-poor or lotic waters, which waters are virtually absent in the s.w.-Netherlands (e.g. *Gerris gibbifer* (Schummel), *Corixa dentipes* (Thomson), *Glaenocorisa propinqua* (Fieber)).

As an example the distribution pattern of the three related species *Sigara falleni* (Fieber), *S. striata* and *S. stagnalis* is presented in Fig. 30A, B, C. The distribution map of *S. falleni* shows a limnetic species with a weak tolerance for  $\beta$ -oligohaline waters. Limnetic waters occur in the northern and southern part of the Delta region. In the brackish central region the species is rare. *Sigara striata* is also a limnetic species, but has a greater tolerance for brackish waters than *S. falleni*; consequently this species shows a uniform distribution pattern over the whole Delta region. Whereas *S. falleni* is restricted to the greater limnetic waters, *S. striata* can also be found

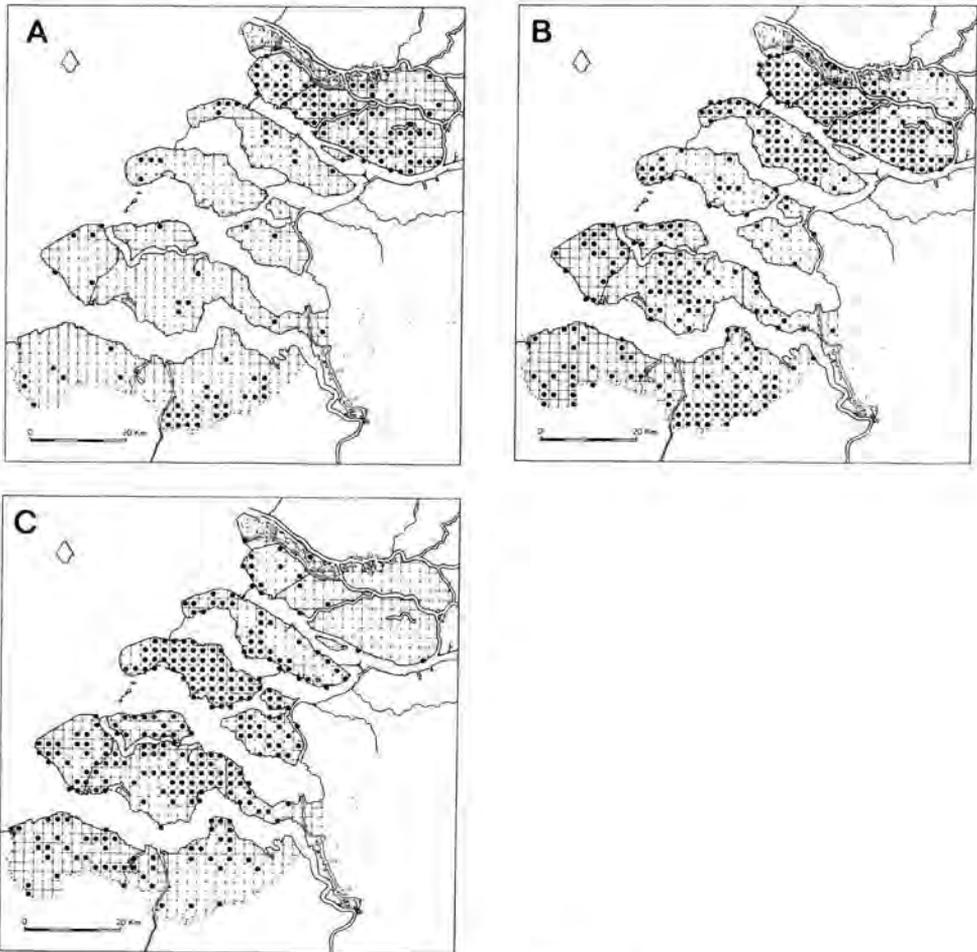


Fig. 30A. Distribution pattern of *Sigara falleni* (Fieber).

Fig. 30B. Distribution pattern of *Sigara striata* (L.)

Fig 30C. Distribution pattern of *Sigara stagnalis* (Leach).

in small waters like cattle drinking pools. The distribution pattern of *S. stagnalis* shows that this organism is a typical brackish water species. In the limnetic northern and southern part of the Delta area this species is less abundant than in the brackish central part.

#### References

- Cuppen, H.P.J.J. - 1977. Een hydrobiologisch onderzoek naar de macrofauna en de hogere waterplanten van een aantal wateren in Noord-Limburg. Versl. Lab. Aquat. Oecologie, Katholieke Universiteit Nijmegen, 53 pp.
- Dethier, M. and R. Bosmans - 1979. Les Hétéroptères aquatiques de Belgique. Bull. Ann. Soc. Roy. Belge Ent. 115, 272-303.
- Nieser, N. - 1974. De Nederlandse Water- en Oppervlaktewantsen (*Heteroptera aquatica* et *semiaquatica*). Hoogwoud, Wetensch. Meded. KNNV No. 77, 56 pp.
- Nieser, N. - 1978. Heteroptera. In: J. Illies (Ed.), Limnofauna Europaea, Fischer Verlag, Stuttgart etc., p. 280-285.
- Van Tol, J. and E.J. Van Nieukerken - 1978. Fauna van wateren in Meijndel. II. Lijst van water- en oppervlaktewantsen van Meijndel (Heteroptera). Zool. Bijdr. 23, 70-91.
- Verstraete, A.M. - 1978. De verspreiding van de water- en oppervlaktewantsen in het noordwesten van de provincie Oost-Vlaanderen. Rapp. Rijks Universiteit Gent, 141 pp.
- Worrel-Schets, M. - 1978. De verspreiding van water- en oppervlaktewantsen (*Heteroptera aquatica* en *semi-aquatica*) in een deel van de provincie Utrecht. Rapp. Rijks Universiteit Utrecht, 32 pp.

### VI.3. The activity of heterotrophic bacteria at variable environmental conditions (B8) (A.B.J. Sepers and F.W. Melissen)

In natural aquatic environments the growth of heterotrophic bacteria is normally limited by organic carbon. In a chemostat culture bacteria can be grown at very low, growth limiting nutrient levels. In this respect the chemostat culture can be regarded as a model of the natural environment. The growth of bacteria in a chemostat is represented by the Michaelis-Menten equation.

In nature the environmental parameters change continuously. These continuous variations will have their impact on the growth rate of bacteria and on the composition of the bacterial community. Very recently in our laboratory a study was initiated into the effect of these fluctuations on the ecophysiology of heterotrophic bacteria. For that purpose a continuous culture system with a microprocessor controlled pump was used. In the applied experimental set up the dilution rate was varied, implying a similar variation of the addition of the growth limiting nutrient to the culture.

The experiments were done with the heterotrophic bacterial strain HIS 53 (Sepers, 1981). The maximum specific growth rate ( $\mu_{max}$ ) of HIS 53 for the growth on aspartate is  $0.36 \text{ h}^{-1}$ . Samples taken from the continuous culture were used to determine the respiratory capacity on aspartate. The respiratory capacity is defined in this paper as the maximum uptake rate of the substrate, used for respiratory purposes. This maximum uptake rate was determined by measurement of the oxygen consumption rate at saturating substrate concentration. The measured oxygen consumption rate was converted to an uptake rate for aspartate assuming a full respiration of the substrate. Before the measurement of the oxygen consumption, the samples were filtered so as to remove the residual aspartate.

The dilution rate was varied from  $0.08$  to  $0.17 \text{ h}^{-1}$  on a sinusoidal curve with 10 hours for a complete sinus. In a second culture the dilution rate was varied stepwise from  $0.08$  tot  $0.18 \text{ h}^{-1}$  (and the other way round), with intervals of 5 hours. A third culture was a chemostat culture with a constant dilution rate of  $0.12 \text{ h}^{-1}$ , which corresponds with the mean dilution rate of the other two cultures.

The data on the respiratory capacity of HIS 53 for aspartate are summarized

Table 10. The respiratory capacity of HIS 53 for aspartate at different regimes of the dilution rate.

Culture	Mean respiratory capacity ( $\mu\text{mol asp l}^{-1} \text{h}^{-1}$ )	Mean protein level ( $\text{mg l}^{-1}$ )	Mean specific respiratory capacity ( $\mu\text{mol asp mg protein}^{-1} \text{h}^{-1}$ )
Chemostat	54.5	14.2	3.84
Sinusoidal variation of the dilution rate	45.3	12.8	3.54
Stepwise variation of the dilution rate	46.3	3.3	14.3

in Table 10. The mean respiratory capacity in the continuous cultures with a variation of the dilution rate was roughly comparable with the respiratory capacity measured in the chemostat culture. The mean protein content of the culture with the sinusoidal variation of the dilution rate was comparable with the protein level of the chemostat culture, whereas the mean protein content of the culture with the stepwise variation of the dilution rate was about three times lower. This indicates that the percentage of respired substrate depends on the variation of the dilution rate.

The mean specific respiratory capacity of the culture with the sinusoidal variation of the dilution rate and of the chemostat culture were quite comparable (Table 10), whereas the mean specific respiratory capacity of the culture with the stepwise variation of the dilution rate was about three times higher.

The gathered data demonstrate that the character of the variation in the nutrient supply has an important effect on the physiology of the organisms. Yoon *et al.* (1977) designed a model for the growth of micro-organisms and showed that the diversity of the nutrient sources contributes significantly to the diversity of the heterotrophic bacterial population. In view of the diverse characteristics of bacteria in relation to the continuously changing environment, such as the chemical and physical parameters, these environmental variations will have their impact on the composition of the bacterial population. In the natural aquatic environment the organic compounds, supporting bacterial growth, will be supplied with a variable rate, which will be related to the activity and condition of the phytoplankton population. It seems reasonable to assume that bacteria differ with respect to the regulation of their metabolic activity in a changing environment. The recently started research with the continuous culture system with a microprocessor controlled pump is expected to yield more information about the contribution of changing environmental parameters on the activity and diversity of the heterotrophic bacterial population.

#### References

- Sepers, A.B.J. - 1981. Diversity of ammonifying bacteria. *Hydrobiologia* 83, 343-350.
- Yoon, H., G. Klinzing and H.W. Blanch - 1977. Competition for mixed substrates by microbial populations. *Biotechnol. Bioeng.* 19, 1193-1210.

#### VI.4. The role of the salinity in the selection of brackish water phytoplankton (B10) (J.W. Rijstenbil)

The influence of the salinity on the phytoplankton species distribution was investigated in several water types, both in lakes and in estuarine waters (Braarud, 1951; Paasche, 1975). In some studied mesohaline environments in the s.w.-Netherlands euryhaline species like *Skeletonema costatum* and *Actinocyclus ehrenbergii* were normally present. These species are well adapted to salinity fluctuations (Qasim *et al.*, 1972). Important species of the polyhaline environments were *Detonula confervacea*, *Ditylum brightwellii* and *Rhizosolenia delicatula*.

More information about the response of natural phytoplankton assemblages to salinity changes was obtained in a chemostat at a dilution rate of  $0.02 \text{ h}^{-1}$ ; the phytoplankton was grown at an ammonia limitation and at a light level of about  $90 \mu\text{Ein m}^{-2}\text{s}^{-1}$ . Four salinity patterns were applied to these natural phytoplankton populations during a one week period. In the first case the salinity was gradually lowered from  $19.5 \text{ ‰}$  to  $0.8 \text{ ‰}$ . In this chemostat *S. costatum* started to develop, but was in the oligohaline medium replaced by the small diatom *Chaetoceros muellerii*; the latter species however, was with respect to its biomass, not important in the studied water types. In the second case the salinity was lowered from  $19.5 \text{ ‰}$  to  $9.0 \text{ ‰}$ . *S. costatum* appeared the most successful competitor. The same development was observed during a decrease of the salinity from  $19.5 \text{ ‰}$  to  $7.8 \text{ ‰}$ , followed by an increase to  $17.6 \text{ ‰}$  (case 3). Only if the salinity was stable at  $18 \text{ ‰}$ , as in the fourth case, *S. costatum* and *D. brightwellii* coexisted.

In order to obtain a better insight into the behaviour of the dominant phyto-

plankton species in environments with changing salinities, similar experiments were performed with a mixed inoculum of laboratory cultures of *Biddulphia sinensis*, *D. brightwellii*, *R. delicatula* and *S. costatum*. Both *B. sinensis* and *R. delicatula* were washed out very rapidly from the chemostats. The developments in the chemostats were followed during 5 weeks (Fig. 31). In the cultures where the salinity was lowered either to a constant level of 9.0 ‰ S (A), or with fluctuations down to about 4.0 ‰ S (B), *S. costatum* outcompeted *D. brightwellii* after 3 weeks. In experiments where the salinity varied between 16.0 and 2.5 ‰ S, *D. brightwellii* initially outcompeted *S. costatum*; however, after 5 fluctuations *S. costatum* gradually superseded *D. brightwellii* (C). At a constant level of 18 ‰ S *D. brightwellii* dominated (D), but one week after lowering the salinity to about 14 ‰ S, the number of *S. costatum* increased significantly.

The minimum salinity endured by *S. costatum* and *D. brightwellii* was 4 and 11.5 ‰ S respectively (Paasche, 1975). The latter species developed slower in a medium with a varying salinity, than in a medium with a constant salinity (Fig. 31C and D). In competition experiments between *D. brightwellii* and *S. costatum*, *S. costatum* had better chances in media with low or varying salinities. The experiments will be continued with monocultures of both species in chemostats with variable salinities.

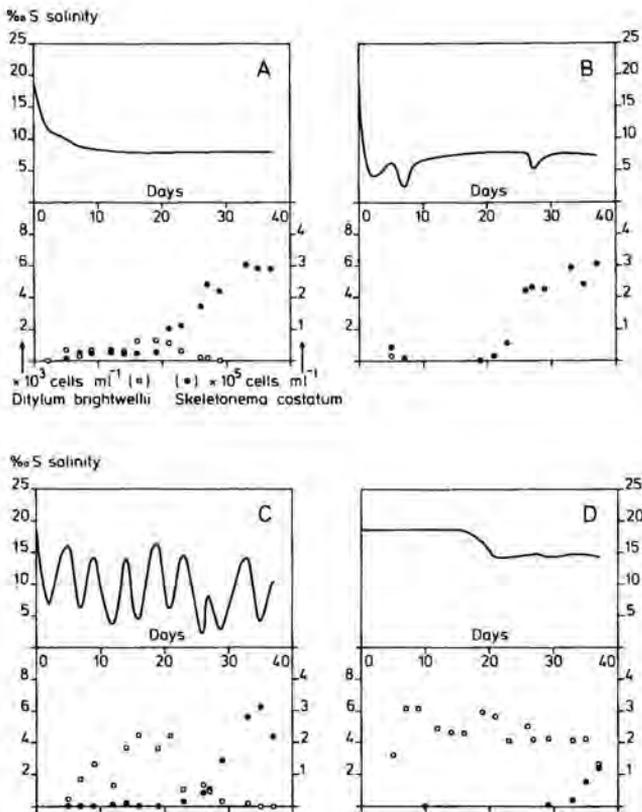


Fig. 31. The influence of salinity on the competition between *Skeletonema costatum* and *Ditylum brightwellii* in continuous cultures.

**References**

Braarud, T. - 1951. Salinity as an ecological factor in marine phytoplankton. *Physiol. Plant.* 4, 28-34.  
 Paasche, E. - 1975. The influence of salinity on the growth of some plankton diatoms from brackish water. *Norw. J. Bot.* 22, 209-215.  
 Qasim, S.Z., P.M.A. Bhattahiri and V.P. Devassy - 1972. The influence of salinity on the rate of photosynthesis and abundance of some tropical phytoplankton. *Mar. Biol.* 12, 200-206.

**VI.5. The impacts of the storm-surge barrier on the macrozoobenthos of the Oosterschelde (B17) (H. Hummel)**

To protect the area around the Oosterschelde from disastrous floodings a storm-surge barrier is under construction. Temporary closure of the barrier will influence

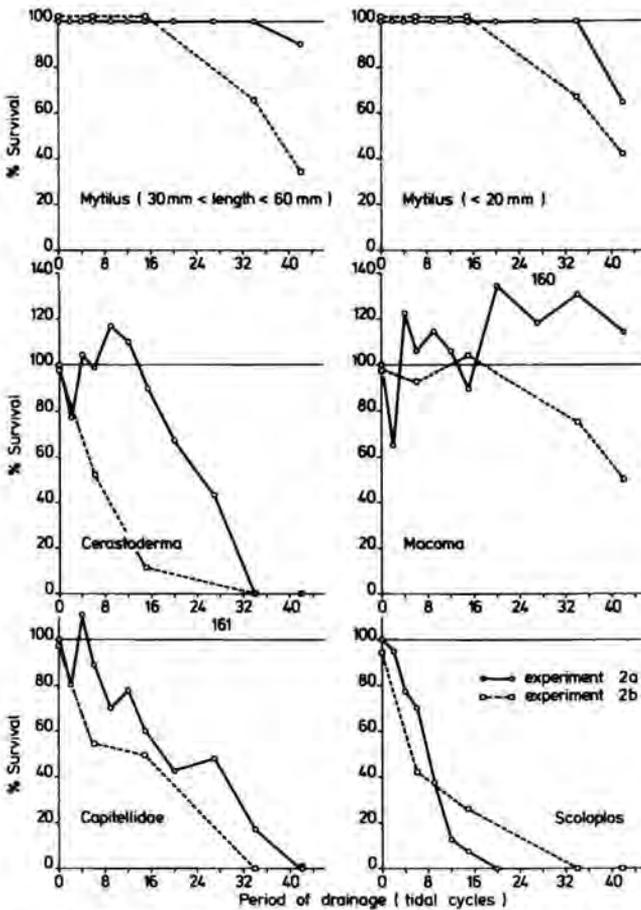


Fig. 32. The survival of some intertidal macrozoobenthos species after different periods of desiccation. The survival is described here as the number of living organisms expressed as a percentage of the number present at the start of the experiment. The survival was determined directly after exposure to desiccation (experiment a) and after a month of reacclimatization to normal tidal conditions (experiment b).

the tidal amplitude and tidal currents. The most important effects of a closure on the benthic animals are the resultant temperature extremes and desiccation. During the winter of 1981-1982 some preliminary experiments were done to investigate the influence of different desiccation periods on the intertidal bottom fauna. Benthic animals like *Cerastoderma edule* and *Scoloplos armiger* suffered heavily from desiccation reaching a 50% mortality within a few days, whereas *Mytilus edulis* and *Macoma balthica* survived several weeks of desiccation (Fig. 32). Restoring the normal tidal conditions most of the animals showed a continuing mortality. Thus in studies dealing with environmental impacts on benthic animals these after-effects have to be taken into account.

#### References

Hummel, H. - 1982. De invloed van verschillende tijden van droogliggen op bodemdieren. DIHO Rapp. en Versl. 1982-2, 22 pp.

## VII. RESEARCH GROUP: SALT-MARSH ECOSYSTEMS (Code S)

### VII.1. Introduction (A.H.L. Huiskes)

The research group salt-marsh ecosystems studies the structure and functioning of saline and brackish tidal and non-tidal salt marshes in the south-west of The Netherlands.

The research activities are divided over five projects; each of these projects is in turn subdivided in a number of research topics.

Project S1 contains research on the community level. Research topics are: The description of the structure of saline vegetation under influence of the changes brought about by the Delta Project. The processes of production and decomposition as related to the structure of various types of salt-marsh vegetation (see VII.2.), this research is funded by BION. The occurrence of mycorrhiza in salt marshes (see VII.3.). The movements of the ground water and the fluctuation of the ground-water table in tidal salt marshes and in embanked salt marshes and tidal flats (see VII.4.). The influence of the tidal movements on the dispersion of diaspores (see VII.5.). The measurement of a number of abiotic- mainly meteorological-parameters (see VII.6.).

Project S2 combines in it all studies on the population level. Research topics are the population ecology and autecology of *Salicornia* spp., of *Aster tripolium* (VII.7., VII.8., VII.10.), of *Atriplex* spp. (VII.11.) and of *Halimione portulacoides*.

Project S3: "Production, decomposition and accumulation of organic matter" overlaps in part with the research funded by BION, mentioned under project S1 (see VII.12.).

Project S4: "Ecotoxic effects of pollutants" has two research topics: a monitoring programme in cooperation with the KFA, Jülich (GFR) (see VII.13.) and experimental research on uptake of heavy metals by plants under different conditions in cooperation with WES, Vicksburg, Mississippi, USA.

Project S5: "Ecological effects of tidal management" is a project funded by the Delta Department (Environmental division) of the Rijkswaterstaat under the code name VEGIN (see VII.15.).

### VII.2. Structure and production of salt-marsh vegetation (S1) (G.J.C. Buth and H. Fuchs)

Vegetation structure is defined as the horizontal and vertical arrangement (the architecture) of the different morphological elements of a phytocoenosis (Barkman, 1979). Also the temporal arrangement of these elements is part of the vegetation structure. To a certain extent the structure of a concrete vegetation stand can be attributed to local environmental conditions. By investigating structural attributes

such as phytomass, leaf size or leaf area index and environmental factors such as soil parameters and microclimate, a functional interpretation of relationships between structure, function and environment will be possible. For three years, from October 1981 onwards, structure of salt-marsh vegetation will be studied. Primary production and decomposition are processes related to structure and aspects of these processes are studied too.

In April 1982 plots of  $0.25 \text{ m}^2$  were marked out in three different vegetation types in a tidal salt marsh in the Oosterschelde (Stroodorpepolder) and in two vegetation types in a non-tidal inland saline marsh (inlaag) near Zierikzee. In the tidal marsh plots were marked out in a *Spartinetum*-, a *Halimometum*-and a *Puccinellietum*-vegetation, in the non-tidal marsh in a *Puccinellietum*-and an *Armerion*-vegetation. Structure and production during the growing season were followed by triplicate harvesting of randomly chosen plots at six weeks intervals. Vegetation samples were collected and transported to the lab without disturbing the vertical structure.



Photograph 5.

Integrated measurements of primary production of algae and higher plants, mineralization, respiration of zoobenthos, movements of organic and inorganic substances by tidal currents and the climate on macro- and micro scale are performed on the salt marshes and tidal flats of the Oosterschelde. Towers of various construction and size are used to keep measuring equipment and personnel dry at high tide. (Photograph René Kleingeld).

The vertical arrangement of phytomass and structural parameters was analyzed by dividing the samples into strata; the samples were cut at intervals of 5- or 10 cm. The phytomass of each plant species in each stratum was divided into a leaf-, shoot- and flower-, c.q. fruit fraction, dried and weighed. At every sampling date 20-30 representative plants of each species were collected in the neighbourhood of the plots. From these plants the mean leaf area and the mean leaf fresh and dry weight were determined. With all these data of every sampling date structural parameters like phytomass, reproductive effort (R.E.) leaf area index (L.A.I.) and leaf area ratio (L.A.D) can be calculated for each species, each stratum and each vegetation type. In 1983 these calculations will be processed with the aid of a computer.

Underground biomass dynamics were followed by collecting at six weeks intervals five soil cores from every vegetation type. At the lab the cores, 60 cm in length, were divided into 15 cm layers, dried and ground. Subsamples were weighed, ignited at 550°C and weighed again.

After every harvest sediment samples were obtained from the clipped plots for determination of the following parameters: silt, carbonate, humidity, salinity, nitrogen and pH. Vegetation structure has a modifying influence upon the microclimate (Woodward and Sheehy, 1983). During some bright summer days in the plant communities of the tidal marsh light-, temperature- and atmospheric humidity profiles were recorded. Other recorded environmental factors were immersion frequency and ground-water table.

#### References

- Barkman, J.J. - 1979. The investigation of vegetation texture and structure. In: M.J.A. Werger (ed.). The study of vegetation. Junk, Den Haag. pp. 123-160.  
Woodward, F.I. and J.E. Sheehy - 1983. Principles and measurements in environmental biology. Butterworths, London. 263 pp.

### VII.3. Distribution and ecology of mycorrhizae in salt marshes (S1) (S. Mastenbroek and W.G. Beeftink)

The main results from a pilot study on the distribution and ecology of mycorrhizae (VAM) in salt marshes were obtained from field work. Different plant species were sampled in various salt marshes in the south-western part of The Netherlands. *Aster tripolium* and *Spartina anglica* were collected along gradients running from a high point in the marsh to a low one. Both these species contained mycorrhiza (varying from 0 to 22% infection on a frequency basis), but no correspondence with their situation along the gradient was found. *Plantago maritima* and *P. coronopus* showed much higher percentages of infection (maximal 55%). Among the species found without mycorrhiza infection were *Salicornia europaea*, *Limonium vulgare* and *Armeria maritima*.

Soil samples were collected together with root sampling. The redox-potential showed a high correlation with the marsh level. Calculations on the percentage of VAM infection and soil analyses suggest correlations between the percentage of infection and soil moisture, P<sub>2</sub>O<sub>5</sub>, nitrogen and NaCl contents. The results of infection experiments with plants under drained and waterlogged conditions are still to be worked out.

### VII.4. Geohydrology of salt marshes in relation to the tides (S1) (W.G. Beeftink, M.C. Daane and R.J. Kolpa - Technical University Delft)

During many years M.C. Daane studied the influence of tides and rainfall on ground-water level and salinity of the ground water in two salt marshes and three beach plains. From this research it appeared that the tidal difference governs both ground-water relations and salinity to a great extent. After barraging the waterbody in which the marsh area is situated both these soil factors changed thoroughly in a

way dependent upon the permeability and geomorphology of the marsh. These measurements give us the opportunity (a) to investigate (1) the relation between tidal and ground-water movements, and (2) the relation between ground-water level and movements and the vegetation composition; and (b) to predict the changes in ground-water level and vegetation composition occurring in the Oosterschelde marshes after the storm-surge barrier will be constructed in 1986.

The third author carried out first examinations on this subject using the dataset of the Spieringschor salt marsh for a geohydrological modelling study. It appeared that the large variation in geomorphological patterns and the many soil factors obstruct finding these relationships. Four models simplifying the geomorphological patterns and hypothesizing a homogeneous soil profile were used. With these models either the soil factor  $\alpha^2 = kD/\mu$  (whereby  $kD$  = transmissivity in  $m^2 day^{-1}$ , and  $\mu$  = storage coefficient) can be calculated from the water levels in creek and soil, or the ground-water levels can be calculated from known  $\alpha^2$ 's and water levels in the creek. For further research the propositions on which this analytical method was based, can be tested in laboratory experiments. Besides that, other, viz. numerical and statistical approaches are recommended.

#### References

Kolpa, R.J. - 1982. De geohydrologie van de Spieringschor (Noord-Beveland). DIHO Stud. Rep. D8-1982, 70 pp.

#### VII.5. Transport of seeds and seedlings in the salt marsh east of Krabbendijke (S1) (B.P. Koutstaal, M.M. Markusse and W. De Munck)

The investigations into the transport of diaspores by the tidal movement from and towards the salt marsh has been continued from February until May, 1982, in the Krabbenkreek, south-east of St. Philipsland. It was decided to terminate these measurements because of the presence of bimodality in the flood curve (Dutch: agger) in this semi-closed estuarine arm causing irregularities in the direction of the tidal stream. From September, 1982 on, this study has been taken up in the Oosterschelde, viz. in the Stroodorpepolder salt marsh and, later on, in the marsh east of Krabbendijke (Rattekaai). There, the measurements were extended with floating nets moving up and down with tides, for trapping floating diaspores.

In these floating nets large numbers of *Spartina anglica* seeds were trapped. The percentage viability in those seeds was sometimes markedly high in the winter season (9-20%, against usually less than 2% in the Bergen op Zoom salt marsh in former years). In January large numbers of *Salicornia dolichostachya* seedlings were caught in these nets. As a consequence of the mild climate at that time many seeds germinated prematurely (at January 27, up to 82% of all *Salicornia* diaspores were seedlings).

The difference in sampling efficiency between floating and standing nets was studied in a comparative experiment, using 3 floating and 12 standing nets. The floating nets trapped 23 *Salicornia* seedlings per 1000  $cm^2$  net mouth, and the standing nets 25 seedlings.

#### VII.6. A scanner for the recording of infrequently fluctuating environmental parameters (S1) (A.H.L. Huiskes and R. Middel)

Measurements of very gradually fluctuating environmental factors, over periods of 24 hours or longer result in a large amount of data of which only a small portion is actually used. Especially a number of soil parameters belongs to this category. With the recording devices used in this research programme (a datalogger or a recorder) each parameter, however, occupies one of a limited number of channels. Combination of a number of these parameters on one channel would result in a more economic use of the recording channels. A scanner was developed for this purpose.

The scanner was designed to scan ten parameters or less. In case less parameters are scanned, the number of channels not in use are skipped. The number of channels to be scanned is to be set by a ten-step switch. The scanning time is equal for all channels but can be set between 1 and 10 minutes. The scanner operates on 9V DC but 12V DC batteries may be used also. Fig. 33 shows a block-diagramme of the channel scanner.

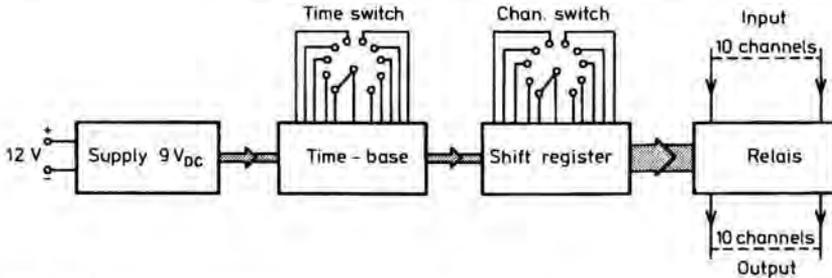


Fig. 33. Block diagramme of a channel scanner.

#### VII.7. The demography of *Aster tripolium* in various vegetation zones of the salt marsh near Ellewoutsdijk (S2) (A.H.L. Huiskes and J. Van Soelen)

*Aster tripolium* is a very variable species of saline and brackish habitats. In the Dutch flora (Van Oostroom 1977) two varieties are described: var. *discoldeus* with capitula without ray florets and var. *tripolium* with ray florets. Gray (1971) describes three types of *A. tripolium* on the tidal salt marshes of Great Britain. To study possible differences in *A. tripolium* on the salt marsh at various heights above Dutch Ordnance Level (NAP) a total of eight plots of 5 m<sup>2</sup> were marked out in four vegetation types in the salt marsh near Ellewoutsdijk situated on the Westerschelde just opposite Terneuzen (Fig. 1A).

These zones are: (1) a zone dominated by *Spartina anglica* C.E. Hubbard and *A. tripolium*; this zone is flooded with almost every high tide; (2) a zone dominated by *Puccinellia maritima* and *A. tripolium*; (3) a zone with a vegetation of *Triglochin maritima*, *P. maritima*, *A. tripolium*, *Limonium vulgare* and some other halophytes; (4) a vegetation dominated by *Halimione portulacoides*: this is not a real zone but *Halimione* covers large areas in this salt marsh.

Figure 34 shows the fate of the marked adult plants in the plots in the various vegetation zones in the salt marsh near Ellewoutsdijk. The graphs show the mean number of marked plants in the two plots in each vegetation zone. Clearly can be seen that the life expectancy of adult plants is much longer in the *Spartina*-zone than in the other zones. The mean estimated life expectancy calculated following Barclay (1958) is for the plants in the *Spartina*-zone (I) 32 months, for the *Puccinellia*-zone (II) 19 months, for the *Triglochin*-zone (III) 16 months, and in the *Halimione*-vegetation (IV) 26 months.

The results from the plots on the salt marsh near Ellewoutsdijk indicate that two kinds of individuals of *A. tripolium* exist. One in the *Spartina*-zone of the marsh with a relatively long mean life expectancy, they have also a relatively high number of shoots per individual, both vegetative and generative, but with average fewer flower heads per flowering shoot than in the other vegetation zones. In the *Puccinellia*-zone, the *Triglochin*-zone and the *Halimione*-vegetation *A. tripolium* has a shorter mean life expectancy, especially in the *Triglochin*-zone, a lower number of shoots per individual plant, both vegetative and generative, but on average a higher number of flowering heads per generative shoot.

These findings are in agreement with the conclusions of Gray (1971) and the two kinds of individuals found in the marsh near Ellewoutsdijk could be labeled

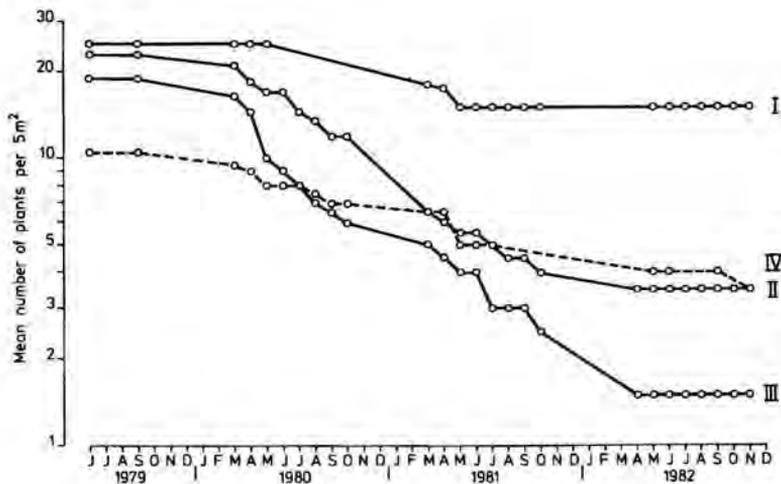


Fig. 34. Survivorship curves of adult *A. tripolium* plants in various vegetation types in the salt marsh near Ellewoutsdijk. I: *Spartina*-zone, II: *Puccinellia*-zone, III: *Triglochin*-zone, IV: *Halimione*-vegetation (for a description of the vegetation types see text). The graphs show the mean number of the two plots in the same vegetation type.

Low Marsh type and Mid Marsh type. Gray's High marsh type occurs in vegetation types not found in the salt marsh near Ellewoutsdijk.

#### References

- Barclay, G.W. 1958. *Techniques of Population Analysis*, Wiley, New York, 311 pp.  
 Gray, A.J. - 1971. *Variation in Aster tripolium L., with particular reference to some british populations*. Thesis, University of Keele, XVI + 381 pp.  
 Van Ooststroom, S.J. - 1977. *Heukels-Van Ooststroom, Flora van Nederland*, 19th ed., Wolters-Noordhoff, Groningen, 925 pp.

#### VII.8. Survival of seedlings of *Aster tripolium* in various vegetation zones in the salt marsh near Bergen op Zoom (S2) (A.H.L. Huiskes and M.M. Markusse)

To study seedling survival of *Aster tripolium* in various vegetation types at different heights above Dutch Ordnance Level, six quadrats of 5 m<sup>2</sup> were marked out in the salt marsh near Bergen op Zoom in the same vegetation zones as mentioned in VII.7. No plots were laid out in the *Halimione* vegetation as this is not covering large patches of the marsh.

The study on seedling survival in the salt marsh near Bergen op Zoom (Fig. 35) over a period of two growing seasons showed that although seedlings were not absent in the *Spartina*-zone of the marsh, their mean life expectancy was shorter than in other zones. A crude estimate gave a mean life expectancy of 16 months in the *Spartina*-zone, 30 months in the *Puccinellia*-zone and 20 months in the *Triglochin*-zone.

The seedlings show a higher death risk in the *Spartina*-zone and the *Triglochin*-zone as compared with the *Puccinellia*-zone. Miedema and Ham (1980) found also none to very little seedlings in the *Spartina*-zone. This zone is flooded with almost every high tide and it could well be that the flooding causes a relatively higher death risk for seedlings and young plants. In the *Triglochin*-zone both young and adult plants have a relatively high death risk, this might be ascribed to competition by other plant species, but this has not been tested as yet.

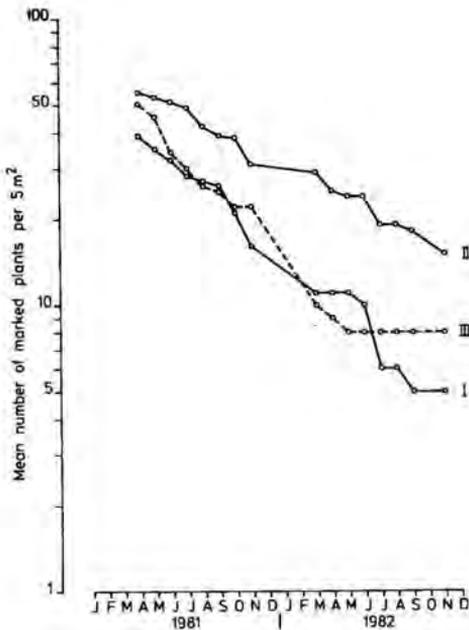


Fig. 35. Survivorship curves of plants of *A. tripolium* marked as seedlings in 1981 in the salt marsh near Bergen op Zoom. The graphs show the average numbers of the two plots in each vegetation type. For the roman figures see caption of Fig. 34.

### References

Miedema, E. and M. Ham - 1980. Demografie van *Aster tripolium* L. DIHO Stud. Rep. D12-1980, 80 pp.

### VII.9. Germination of *Aster tripolium* seeds collected at different locations (S2) (A.H.L. Huiskes, J. Van Soelen and M.M. Markusse)

As Wijnands (1969a, b) suggested in his reports a seed polymorphism in *Aster tripolium* exists, which rectified a study on the germination of seeds collected from different populations of *A. tripolium* growing in different habitats. The seeds were collected in October 1980 at the various sites, dried in a cool dry place and stored in the dark at +5°C. After a few months a germination test was done on the seeds. A 100 seeds of each batch were placed in a petri dish on filter paper which was moistened and kept moist with demineralized water. The dishes were placed in an incubator at 20°C. The seeds received light only when handled. Germinated seeds were counted daily and removed from the trays. The duration of the experiment was 21 days.

Table 11 shows the total germination percentage of the various seed batches. Although the seeds were collected and treated the same way there is a great difference in germination between the seed batches of the various populations. Also was observed that seeds from plants on tidal salt marshes had larger seeds than those collected from other populations.

Further research in germination behaviour of seeds from different populations of *A. tripolium* was continued in 1982 and will continue in 1983.

Table 11. Total germination percentages of seeds collected from populations of *Aster tripolium* growing in different habitats

Locality	Habitat	% germination
Stroodorpepolder	low tidal salt marsh	82
Bergen op Zoom	do.	74
Hinkelenoord	high tidal brackish marsh	58
Waarde	do.	59
Ellewoutsdijk	do.	57
Ritthem	high tidal salt marsh	34
Kakkersweel	non-tidal salt marsh	48
Dijkwater	do.	53
Vlietepolder	do.	40
Westerschouwense Inlaag	do.	22
Heerenpolder	do.	35
Inlaag 1887	do.	45
Fort de Ruyter	brackish ditch	32
Boschplaat Terschelling	high salt meadow	25
Middelplaten	former sand flats	21
Slikken van Flakkee	do.	16

#### References

- Wijnands, D.O. - 1969a. Een onderzoek naar de variabiliteit van *Aster tripolium* in Nederland I. Beschrijvend onderzoek. Report doctoral study Hugo de Vries-laboratory, Amsterdam, 49 pp.+ annexes.  
 Wijnands, D.O. - 1969b. Een onderzoek naar de variabiliteit van *Aster tripolium* in Nederland II. Experimenteel onderzoek. Report doctoral study Hugo de Vries-laboratory, Amsterdam, 35 pp. + annex.

#### VII.10. The nitrate content and the growth of 2 ecotypes of *Aster tripolium* (L.) (S2) (A.W. Stienstra)

*Aster tripolium* is a halophyte, which occurs along the Dutch coast in different ecotypes in "tidal" and "non-tidal" marshes.

It is a succulent plant with a more or less obligatory capacity for the accumulation of inorganic and organic solutions; under non-saline conditions the species accumulates high levels of potassium and nitrate (Stewart *et al.*, 1979). With two ecotypes, a "tidal marsh" (T<sub>1</sub>) and a "non-tidal marsh" (T<sub>2</sub>) type, experiments were done in 1981 and subsequently in 1982 with an interrupted nitrate supply. In the course of a week the plants stood 4-8-24-48-168-hours on a nitrate-rich In Hoagland nutrient solution (11.9 mmol NO<sub>3</sub><sup>-</sup>/l); during the remaining hours, the plants grew on a In Hoagland nutrient solution without nitrate. The NO<sub>3</sub><sup>-</sup>-ions were replaced by Cl<sup>-</sup> or SO<sub>4</sub><sup>2-</sup>-ions respectively. After + 9 weeks the plants were harvested; fresh- and dry weight were determined as well as the mineral compounds.

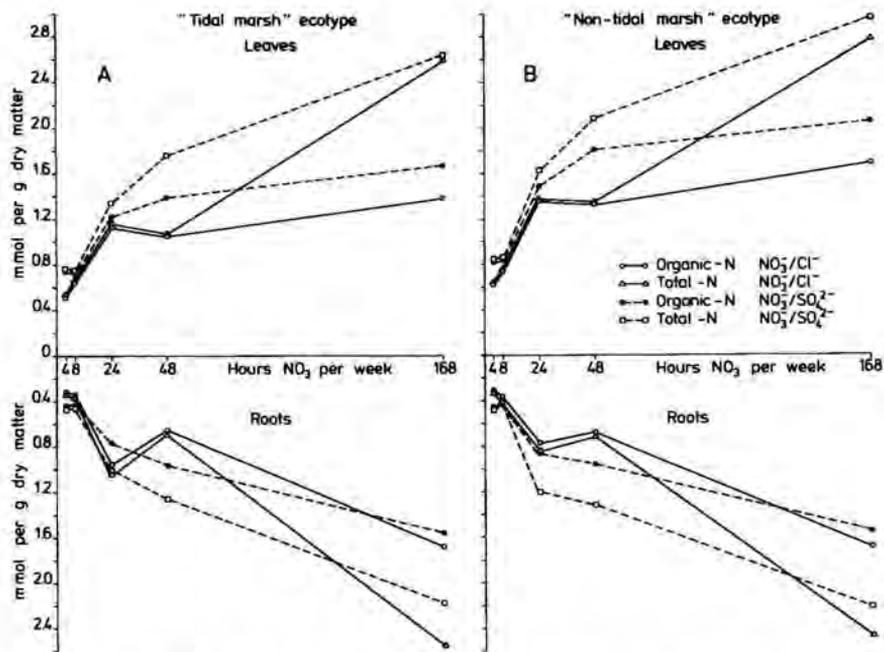


Fig. 36. The nitrogen content (total and organic-N) of leaves and roots of two *Aster tripolium* types. A: The tidal marsh ecotype, B: The non-tidal marsh ecotype.

These experiments were carried out to investigate if the accumulated ions  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  mutually can be exchanged partly without a retarded growth. The results show quite clearly the accumulating character of *Aster tripolium* (Fig. 36A and B). The total nitrogen content in the plant increases with the plant staying more hours on the nitrate-rich nutrient solution.

The course of the organic-N and total-N level during an increasing nitrate supply is for both types the same. However the exact values of the nitrogen content in the leaves differ; the "non-tidal" marsh type has a higher nitrogen percentage than the "tidal" marsh type. The roots of both types contain more or less the same N-percentage. In the  $\text{NO}_3^-/\text{SO}_4^{2-}$  interrupting experiment the leaves of both types have a higher percentage of total-N than in the  $\text{NO}_3^-/\text{Cl}^-$  interrupting experiment.

It is remarkable that, at a supply of 4-8-24-48 hours nitrate, and using  $\text{Cl}^-$  as the replaceable ion, none or hardly any  $\text{NO}_3^-$  can be detected in the leaves. On the other hand in the experiment with the replaceable ion  $\text{SO}_4^{2-}$ , already at a nitrate supply of 24 and 48 hours, more  $\text{NO}_3^-$  is taken up than can be assimilated to organic-N. This suggests a possible restrictive and/or a competitive influence of  $\text{Cl}^-$  on the uptake of  $\text{NO}_3^-$ .

When plants grow up during the whole week (168 hours) on a nitrogen rich nutrient solution, the C-A (cation-anion) content for both ecotypes is the same. If the nitrate supply is partly replaced by a  $\text{Cl}^-$  or  $\text{SO}_4^{2-}$  supply, the C-A content seems to be quite higher in the last treatment. The two-valent  $\text{SO}_4^{2-}$ -ion is taken up less easier than the monovalent  $\text{Cl}^-$  and  $\text{NO}_3^-$ -ions.

By nitrogen deficiency at a low nitrate supply, the growth is much retarded. The dry weight percentage is then about 10.5%. When the plants get more nitrate, the nitrogen deficiency becomes less; the growth increases very much and the dry weight percentage decreases till 6%. An excess of  $\text{NO}_3^-$  (168 hours) causes for both types a retarded root development, and at T<sub>2</sub> even a slight retarded leaf growth.

Comparing 48 with 168 hours a small part of the  $\text{NO}_3^-$ -ions, absorbed in excess, is assimilated to N-organic compounds. The remaining absorbed nitrate is stored in the vacuoles as  $\text{NO}_3^-$ -ions. A partly replacement of  $\text{NO}_3^-$  by  $\text{Cl}^-$  or  $\text{SO}_4^{2-}$  has hardly any or even a small positive influence on the growth of this salt-tolerant plant. It also means, regarding the small decline of weight, that *Aster tripolium* in non-saline environment can accumulate  $\text{NO}_3^-$  without being influenced very much in its growth. So  $\text{NO}_3^-$  could function here as a replaceable osmotic.

#### References

Stewart, G.R., F. Larher, I. Ahmad and J.A. Lee - 1979. Nitrogen metabolism and salt-tolerance in higher plant halophytes. In: Jefferies R.L. and A.J. Davy (eds.), Ecological processes in coastal environments. Blackwell, Oxford. pp. 211-227.

#### VII.11. Population ecology of *Atriplex littoralis* and *A. hastata* (S2) (B.P. Koutstaal)

In the central part of the transect laid out in 1980 in the Stroodorpepolder salt marsh *Suaeda maritima* increased considerably last year. *Salicornia dolichostachya* also germinated for the first time in most of these plots. In all plots of the central part *Atriplex hastata* decreased, and *Atriplex littoralis* disappeared in most of them. Also *Elytrigia pungens* individuals established in 1981 in these plots disappeared. These population changes point to unusual fluctuations in environmental factors, such as water relations and salinity, in relation to the foregoing years. The same tendency can be concluded from the lower part of the transect. There *Puccinellia maritima* increased considerably. In the upper part of the transect *Elytrigia* decreased, probably as a consequence of covering with *Spartina* debris washed ashore onto this zone.

#### VII.12. The role of *Orchestia gammarella* on the decomposition rate of dead salt-marsh plants (S3) (M. Lambert and A.M. Groenendijk)

Each year after the growing season considerable amounts of dead plant parts are deposited along the dikes of the Oosterschelde. These deposits are quickly colonized by several invertebrates of which the terrestrial amphipod *Orchestia gammarella* is one of the most abundant and certainly the largest. The role of this macro invertebrate has been studied previously (Van Koppen, 1981). The project was continued by the first author, who studied the mortality caused by different substrates and the increase of fragmentation at the substrate caused by the activities of *Orchestia*.

*Orchestia* showed lowest mortality when fed on natural debris. On *Elytrigia* and *Spartina* debris there was some moderate mortality. On *Halimione*, however, the extremely high mortality made it impossible to continue with the experiment. High mortality coincides with low caloric content of the material. This suggests not only assimilation of the incorporated microbial biomass, but also the use of plant material itself.

*Orchestia* had a marked influence on the fragmentation spectrum of the debris. Especially the two size classes between 1.0 and 0.075 mm showed a distinct increase as a result of the incubation with *Orchestia*. The fragmentation increases with the duration of the experiment (Fig. 37).

#### References

Van Koppen, K. - 1981. Aspecten van de invloed van *Orchestia gammarella* op de decompositie van vloedmerk. DIHO Stud. Rep. D6-1981, 61 pp.

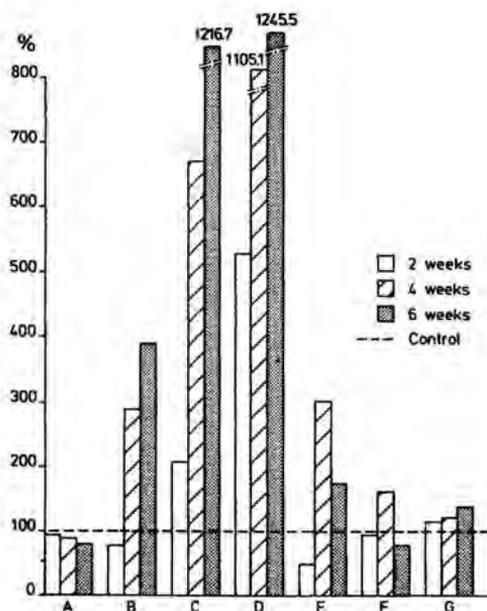


Fig. 37. Relative changes of the fragmentation spectrum after 2, 4 and 6 weeks incubation with (8) *Orchestia* individuals. Fragmentation A -  $x > 2$  mm, B -  $2 \text{ mm} > x > 1$  mm, C -  $1 \text{ mm} > x > 0.6$  mm, D -  $0.6 \text{ mm} > x > 0.075$  mm, E -  $0.075 \text{ mm} > x > 0.45$  mm, F -  $x > 0.45$  mm, G - assimilated.

### VII.13. Decomposition of some halophytes (S3) (G.J.C. Buth and R. Voeselek)

A part of the research-project 'structure of salt-marsh vegetation' is formed by decomposition investigations (see VII.2.). In 1980 and 1981 decomposition of three dominant halophytes was followed from early spring onwards (Buth et al., 1982). In 1983 decomposition was followed from September-November onwards. Above-ground material of dying *Triglochin maritima*, *Limonium vulgare*, *Halimione portulacoides* and *Spartina anglica* was collected from an Oosterschelde salt marsh (Stroordorpolder), divided into a leaf- and a shoot fraction, pre-dried at  $40^{\circ}\text{C}$  and put into 1 mm mesh bags. Some *Spartina*-samples were not pre-dried to test the effect of this treatment. On the marsh the bags were placed in the growing habitats of the plant species; pinned to the ground or hanged on a wire in the vegetation. In this way decomposition of standing and lying dead material was simulated. Sets of bags were retrieved from the marsh regularly. The decomposing material was dried, weighed, ground and analyzed for some chemical parameters. Some subsamples were taken for measurements of oxygen consumption as an index of microbial metabolism.

*Triglochin*-leaves decomposed very fast; in December, after two months, the mean loss of dry weight was 80-90%. Initial decomposition of *Limonium*- and *Spartina*-leaves did not differ so much. The effect of pre-drying only seems to cause difference during the first weeks; initial loss of dry weight of the pre-dried *Spartina*-leaves was faster. This difference had become very small after about six weeks. In most cases decomposition rates of lying and hanging bags differed. However, these differences were very small for the shoot fractions. For all species decomposition during the first 1.5-3 months was significantly slower for the shoot fraction.

### Reference

Buth, G.J.C., P.F.M. Verdonchot and L. De Wolf - 1982. Decomposition of three halophytes in different habitats of an Eastern Scheldt salt marsh. *Hydrobiol. Bull.* 16, 1, 103-112.

### VII.14. Accumulation of heavy metals in the salt marsh (S4) (W.G. Beeftink and J. Nieuwenhuize)

In this year the first project has been concluded as a result of the Scientific Markets, organized in 1977 and 1979 by the Delta Institute and the Directorate-General for Research, Sciences and Education of the Commission of the European Communities at Brussels (Beeftink *et al.*, 1982). A second project was started aiming to study the allocation of heavy metals and nutrients in different parts (roots, stems, leaves, inflorescences) of the plants. Sample plots were chosen at different conditions of soil fertility and contamination with heavy metals. Four species were selected varying in heavy-metal uptake. For this purpose the laboratory equipment was extended with an atomic adsorption spectrophotometer what has been made operational for current analyzing.

### References

Beeftink, W.G., J. Nieuwenhuize, M. Stoepler and C. Mohl - 1982. Heavy metal contamination in salt marshes from the Western and Eastern Scheldt. *Sci. Total Environm.* 25, 199-223.

### VII.15. Influence of tidal management on salt-marsh angiosperms (S5) (A.M. Groenendijk and M.A. Lievaart)

Investigations into the inundation resilience of salt-marsh angiosperms have been carried on (Groenendijk and Lievaart, 1982). This year more attention was given to the tolerance of the generative stages of the plant against immersion. *Salicornia dolichostachya*, *Aster tripolium* and *Plantago maritima* plants with inflorescences were immersed for 2, 4 and 8 days just before, during and just after flowering. In general the immersion of 4 and 8 days had a severe effect on the inflorescences for *Aster* and *Plantago*, species from middle-marsh sites. *Salicornia*, a species from lower sites, was less susceptible to prolonged immersion.

For *Plantago* ripened seeds showed a good tolerance against immersion. However, the germination of these seeds demonstrated a decrease which correlated with the prolongation of the immersion time. This was the case for seeds which ripened on individuals that had been fully immersed, as well as for seeds which had ripened on individuals that had been subjected to waterlogging of the soil (Fig. 38). Prolonged inundation caused a significant reduction of average seed weight for *Aster*, while *Salicornia* seed weight acted indifferent.

The students M. Bouts and H. Geltink finished their work on the effects of temperature of the inundation water on the growth and the die back of salt-marsh plants. Seedlings from several species from the low, middle and high marsh *viz.* *Triglochin maritima*, *Limonium vulgare*, *Plantago maritima*, *Festuca rubra* ssp. *litoralis* and *Juncus gerardii* have been immersed for 2, 4 and 8 days with immersion water of 12, 24 and 36°C.

In general, immersion for 4 and 8 days at 24 and 36°C caused reduction of growth. A 2 day immersion at 24°C was followed by a (slight) increase of growth for most of the species (Bouts, 1982 and Geltink, 1983).

Highest die back was caused by an 8 day immersion at 36°C for all species involved except *Limonium vulgare*, which species seemed to have a good resilience against immersion for all temperatures tested.

The student H. Spieksma finished the first draft of his report on the competition between *Spartina anglica*, *Puccinellia maritima* and *Aster tripolium* under different

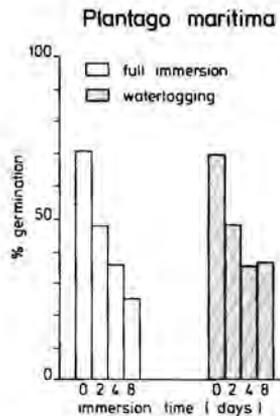


Fig. 38. The reduction of germination of *Plantago maritima* seeds of which the parent plants were subjected to various periods of immersion or waterlogging of the soil. The percentages show the germination after 4 weeks of incubation.

immersion regimes. The results confirm the features of *Spartina* as a good competitor with a wide ecological range. Therefore, it is not likely that species of higher reaches will be able to outcompete *Spartina* after the tidal reduction in the Oosterschelde, after 1986. It is also expected that if *Spartina* succeeds to establish on the by that time less frequently immersed mudflat, the new vegetation developments are for the greater part ruled by this species.

#### References

- Bouts, M., 1982 - Effecten van de temperatuur van het inundatie water op de reactie van de schorvegetatie bij verlengde inundatie. VEGIN-Stud. Rep. Yerseke-Middelburg, 31 pp.
- Geltink, H. - 1983. Effecten van de temperatuur van het inundatiewater op de reactie van de schorvegetatie bij verlengde inundatie. VEGIN-Stud. Rep. Yerseke-Middelburg, 31 pp.
- Groenendijk, A.M. and M.A. Lievaart - 1982. Effecten van verlengde inundatie op de groei en de ontwikkeling van een aantal schorreplanten. Interim Rep. VEGIN. Yerseke-Middelburg, DIHO/RWS, 37 pp.

### VIII. A-SUBJECTS (ANTHROPOGENIC SUBSTANCES AND OTHER INTER-WORKING GROUP PROJECTS)

A number of research projects are not covered by the programmes of the working groups but are inter-working group projects of short or long duration. Those reported concern the ANTHROS project, which concerns pollution studies on organochlorine compounds, heavy metals and radionuclides.

#### VIII.1. Artificial radionuclides in Rijn-Maas-Schelde delta (A12) (A. Thomas, J.M. Martin - Lab. Géol. Paris -, E.K. Duursma, J. Nieuwenhuize - DIHO - and R. Pennders, M.J. Frissel - ITAL, Wageningen-) (30% funding by CEC, Brussels)

The studies on the distribution of plutonium isotopes and various gamma emitters in the Rijn-Maas-Schelde delta of the south-west Netherlands were continued. Taking into account the limitation caused by the restricted number of plutonium analyses, due to long counting times, only selected samples were analyzed. These

are chosen on the basis of earlier results and derived hypothesis of distribution patterns.

In former Euratom progress reports, various results were presented and discussed on the distribution of radionuclides in the region and related to the type of sample: sediment, suspended matter, mussels, plants and lichens. (Euratom, 1979, 1980, 1981). Here the additional achievements will be presented for each studied radioisotope, and well for Cs-137, Co-60, Pu-238, 239, Sb-125, Ru-106 and Ce-144.

In general the behaviour of the detected radionuclides has been investigated by relating their ratios to stable potassium, with the environmental circumstances. Potassium (determined by K-40) is indicative for the clay fractions and most radionuclides are proportionally present in these fractions. Instead of potassium also aluminium can be taken, which is equally related to the clay fractions.

Cs-137/K and Cs-134/Cs-137: The two available isotopes of cesium, Cs-134 ( $t_{1/2}=2.2$  yr) and Cs-137 ( $t_{1/2}=30$  yr) have been investigated. Cs-134 does not exist in fallout, thus the ratio Cs-134/Cs-137 is an indication of local contamination by nuclear installations. The results for suspended matter and top-layer sediment from Rijn, Maas, Delta region, Schelde and southern North Sea towards Boulogne, showed Cs-137 levels of 0.1 - 0.5 pCi/g per % K. The Cs-134/Cs-137 ratios in % were about 5-10%.

In general there could not be distinguished a clear pattern for the Delta region, since the levels are relatively constant, while little variation was observed from river to sea. No clear increase was detectable close to the nuclear power stations Doel and Borssele.

Co-60: The concentrations of Co-60 ( $t_{1/2}=5.24$  yr) ranged, equally for toplayer sediments and suspended matter, from 6 to 430 fCi/g per % K. The highest values were detected for the Channel (Calais) and Schelde, while the lowest concentrations were found in the Grevelingen. Thus Co-60 is a clear indicator for inputs from nuclear installations like Doel (Schelde), and Gravelines (Channel) and those of the English coast.

Pu-238, 239 (+240): The suspended matter and sediment values for Pu-239 ( $t_{1/2}=2.4 \times 10^4$  yr) mixed with Pu-240 ( $t_{1/2}=6.6 \times 10^3$  yr) ranged in the Delta region from 0.9 to 43 fCi Pu-239, 240/g per % K, 240 fCi Pu-239, 240/g per % K, with the highest values in the southern North Sea (26-43 fCi/g/%K) with North Sea water. No influences of Doel and Borssele could be detected, probably the surplus Pu-239, 240 above fallout is explained from releases of the Windscale and/or La Hague reprocessing plants.

The Pu-238/Pu-239,240 ratios (Pu-238,  $t_{1/2}=86$  yr) ranged from 4 to 54%, with again the highest values (19-43%) in the southern North Sea, but also in the upper Schelde (29-54%). The explanation is difficult to give for the Schelde values, since the average source ratios are 35% for La Hague and 5% for fallout. Abnormal high ratios also have been found in sediments of French rivers downstream of nuclear facilities (Loire, Thomas, pers. comm.) but also in rivers only exposed to fallout (Var-river, Ballestra, 1980 and Gironde, Jeandel *et al.*, 1981).

Sb-125: Antimony-125 ( $t_{1/2}=2.8$  yr) attached to sedimentary material is mainly from marine origin and in fact from the La Hague effluents. Values in the Channel of 330-475 fCi Sb-125/g/%K were found, descending to 15 fCi/g/%K in the upper Schelde and 36-37 fCi/g/%K in the Rijn and Maas.

Ru-106: An identical picture as Sb-125 was shown by Ru-106 ( $t_{1/2}=1.0$  yr). High values in the Channel were found for both suspended matter and toplayer sediment of 1300-5700 fCi Ru-106/g/%K. In the mouth of the Westerschelde these values were reduced to about 1000-1100, while the river concentrations of Rijn, Maas and Schelde were between 60 and 230 fCi/g/%K.

Ce-144: The Ce-144 ( $t_{1/2}=0.78$  yr) data, determined in a similar way as the other gamma emitters, are not very reliable. The values are too close to the detection limit. Approximately 50 fCi/g/%K in the rivers and 100-2500 fCi/g/%K in the Channel. Probably the source is equally the La Hague reprocessing plant.

In conclusion: The observed patterns of the various radionuclide distribution

in the Dutch Delta Region become more clear, in particular for radionuclides attached to suspended matter and top-layer sediment. In particular the presentation of the radioactivity per % K gives the opportunity to understand the transport of these radionuclides in the environment as related to their sources.

Additional measurements are proceeding on mussels, as filter-feeders of suspended matter. For this investigation the sampling area will be extended from the Channel and the Delta region to the Dutch North Sea coast northwards.

The lichen studies are completed, while the salt-marsh plant analysis revealed too low concentrations at the level so the detection limits (Euratom, 1980, 1981).

Some core samples are still under investigation in order to get a better view on the fallout maximum of the years 1958-1963.

#### References

- Ballestra, S. - 1980. Radioactivité artificielle et environnement marin. Etude relative aux transuraniens en Méditerranée. Thèse Univ. Nice. 220 pp.  
 Euratom - 1979, 1980, 1981. Plutonium in Rhine-Meuse-Scheldt delta. Progress Reports CEC Progr. Radiat. Prot., Eur, 6766, 211-218, Eur. 7169, 285-290, Eur. 7800, 211-216.  
 Jeandel, C., J.M. Martin and A.J. Thomas - 1981. Les radionucléides artificielles dans les estuaires français. IAEA Vienne SM/284-123, 15-32.

### VIII.2. Wet deposition of toxic metals from the atmosphere in the Oosterschelde region (V.D. Nguyen - Institute of Applied Physical Chemistry, Nuclear Research Centre (KFA) Jülich, FRG - and A.G.A. Merks)

For some metals, inclusive the a priori toxic metals Cd and Pb, the wet deposition with rain and snow represents the major part of their input onto the terrestrial and aquatic ecosystems from the atmosphere. Metals that are adsorbed at the small particles of flying ash are dissolved or washed out in rain and snow. The input of toxic metals dissolved in rain water constitutes a particular hazard, because it provides the metals in a form which is most favourable for their uptake by the vegetation blanket and inland waters.

In collaboration with the Institute of Applied Physical Chemistry of the KFA, FRG, the monitoring of the wet deposition of toxic metals from the atmosphere in the Oosterschelde region was started in 1980 and continued up to now.

The investigations are imbedded in the programme created to clarify the

Table 12. Average daily wet deposition of toxic metals and of the acid in the Oosterschelde region (Delta Institute in Yerseke)

Metal or acid	Daily wet deposition ( $\mu\text{g m}^{-2}\text{d}^{-1}$ )		Average properties		
	1980	1981	1980	1981	
Cadmium	0.38	0.30	pH	4.30	4.17
Lead	23.2	20.2	conductivity	48.9 $\mu\text{S cm}^{-1}$	89.4 $\mu\text{S cm}^{-1}$
Zinc	24.4	27.7	Sulphate	6.05 $\text{mg l}^{-1}$	5.85 $\text{mg l}^{-1}$
Copper	5.1	13.5	Ammonia	0.79 "	0.61 "
Selenium	0.05	0.04	Nitrate	0.49 "	0.60 "
H <sup>+</sup>	103	159	DOC	1.74 "	3.82 "

situation of the environmental burden produced via the atmospheric pollution in the Federal Republic of Germany (Nürnberg *et al.*, 1981). To realize it the KFA has set up a network of automated samplers which monitors since 1980 the wet deposition in various types of ecosystems (coastal zones, rural regions, urban agglomerations and areas of heavy and metallurgical industry).

In the rain water or snow samples at first the pH is determined electrometrically which characterizes the acidity of the rain. Then the concentration of toxic trace metals Cd, Pb, Zn, Cu and Se is determined by differential pulse voltammetry. The wet deposition of toxic metals and of the acid and the pH value of the rain in 1980 and 1981 in the Oosterschelde region are summarized in Table 12. Compared with the results obtained in various regions of the FRG, the Oosterschelde region shows, for all metals investigated, typical coastal values without special pollution.

The relative high value of the conductivity is caused by the sea spray. Compared to Luyten (1982) the data of sulphate, ammonia, nitrate and dissolved organic carbon (DOC) of Yerseke seem to be rather high. At this moment no explanation can be given for this.

#### References

- Luyten, J.A. - 1982. Drie jaren meetnet regenwater kwaliteit. Voorlopig overzicht van de neerslagmetingen en de analyses van de neerslagmonsters. Leidschendam. Rijksinstituut v. Drinkwatervoorziening, 1981.
- Nürnberg, H.W., P. Valenta, and V.D. Nguyen - 1981. Deposition of toxic metals from the atmosphere in the Federal Republic of Germany. In: H.W. Georgii and J. Pankrath (eds.), Proc. Int. Symp. "Deposition of atmospheric Pollutants", Oberursel 1981, (Reidel, Publ. Comp. Dordrecht). pp. 143-157.

### VIII.3. Trace metal sorption experiments at elevated temperatures (L.A. Van Geldermalsen)

This research is part of a CEC project which deals with the mathematical simulation of migration of radionuclides in Atlantic Ocean sediment due to point heat sources. Sorption plays an important role in the migration of trace metals and radionuclides through sediments.  $K_d$  values describe the partition of trace metal in solution and adsorbed to sediment and are used in mathematical studies. These values might change at elevated temperatures.

The well established batch-suspension technique to measure  $K_d$ 's, as used and described by Duursma and Bosch (1970) and Sanchez *et al.* (1981), was adjusted to use at higher temperatures. The technique was tested in the Delta Institute with cadmium and several sediments before applying it to the radionuclides in the laboratory of the ECN, Petten.

The batch technique includes the incubation of the trace metal or radionuclide in sea water together with an amount of sediment until equilibrium of the distribution of trace metal or radionuclide between solvent and sediment is reached. Experiments with low tracer to sediment ratio's allow us to use the simple formula

$$K_d = \frac{\text{amount of element/gram sediment}}{\text{amount of element/ml medium}}$$

$K_d$  is not dimensionless. For application to diffusion calculations, as applied by Duursma and Hoede (1967), this  $K_d$  needs to be dimensionless, determined by all amounts of element per ml (for sediment only the dry material), which requires transformation with a factor equal to the density of the sediment. Thus this  $K = K_d : 1.6$  for sediment with + 40% porosity. Premises are that  $K_d$  is determined at equilibrium and is constant in the concentration range of interest. Equally the adsorption-desorption is supposed to be a reversible process, at least in the time scale migration in the sediment proceeds. Polypropylene centrifuge tubes of 16 ml volume with screw on tops were used in  $Cd^{2+}$  experiments. Various amounts of sediment were incubated together with a range of concentrations of  $Cd^{2+}$  in

sea water at several temperatures. All incubations were always performed in triplo. In order to avoid adsorption of tracer material to naturally occurring particles in sea water, a filtered standard sea water was used.

After incubation the tubes are centrifuged for 10 min. at 3000 rpm and the supernatant was used to determine  $C_t$ .  $K_d$  is calculated with the formula

$$K_d = \frac{(C_o - C_t) (g l^{-1})}{C_t (g l^{-1})} \times \frac{\text{Volume of medium (ml)}}{\text{Mass of sediment (g)}}$$

in which  $C_o$  is the starting concentration of trace metal and  $C_t$  the concentration at time  $t$ , when the adsorption/desorption processes are expected to be in equilibrium.  $Cd^{2+}$  was determined according to a standard procedure on a Perkin Elmer AAS. The capacities of this instrument restricted the concentration range in which we could work. We aimed at a  $C_o/C_t$  ratio of about 50 to avoid large errors in calculation, so the lowest start concentrations are about  $10 \text{ mg } Cd^{2+} l^{-1}$ . However, this depends also on the adsorption capacity of the sediment itself, since a high  $K_d$  forces the use of a higher starting concentration. Oosterschelde sediment was obtained from the intertidal zone in the Oosterschelde and the Atlantic sediment was from the Western Madeira Abyssal Plain. For further information on the sediments see Duursma *et al.* (1983).

Oosterschelde and Atlantic sediments were incubated with a range of concentrations at several temperatures. The results for the Oosterschelde sediment showed no indication for a constant  $K_d$  over the applied concentration range, probably due to the fact that the tracer to ligand ratio (determined by the surface area and/or the cation-exchange capacity of this particular sediment) is too high. The  $K_d$  ranged from 5 to 60 ( $\text{mg } l^{-1}$ ). The Atlantic seabed sediment showed a better constancy of the  $K_d$ 's at the lower  $C_t$  concentration range, probably due to a better tracer to ligand ratio, the  $K_d$ 's ranged from 90 to 1000, see Figure 39.

A general feature arises: a) In the high  $C_t$  concentration range, the amount of available ligands on the mineral surface (or cation exchange capacity) is the limiting factor for  $K_d$  and temperature plays no significant role. b) At the lower  $C_t$  concentration ranges, the temperature plays a significant role at least for the cadmium-mineral interaction. This can be explained when the cadmium-mineral interaction is considered to be a reaction in dilute solution. For this type of reactions,

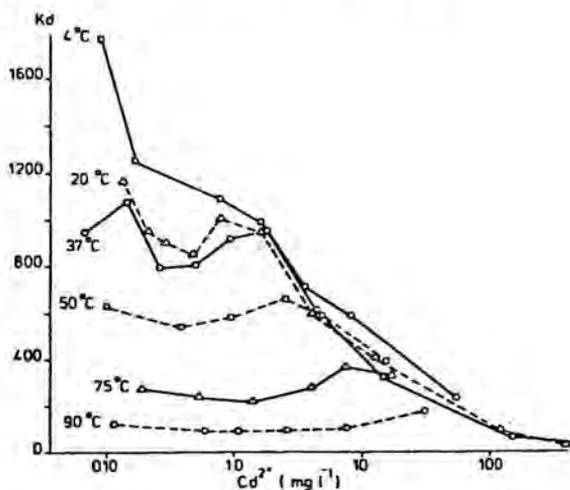


Fig. 39. Sorption constants ( $K_d$ ) of  $Cd^{2+}$  with Atlantic seabed sediment in relation to various  $Cd^{2+}$  concentrations for six temperatures.

the change of equilibrium constant with temperature is described by the Law of Le Chatelier and Van 't Hoff that states

$$\frac{d \ln K}{dT} = -\frac{\Delta H}{RT^2},$$

in which K is the equilibrium constant of the reaction  $Me + Li \rightarrow Me-Li$ ; in this case  $K = K_d$ ,  $\Delta H$  is the reaction enthalpy, R is the gas constant and T the absolute temperature.

By dividing the variables we get

$$d \ln K = -\frac{\Delta H}{RT^2} dT,$$

integrating yields:  $\ln \frac{K_1}{K_2} = -\frac{\Delta H}{R} \times \frac{T_2 - T_1}{T_2 T_1}$ ,

which is a constant factor with temperature.  $\Delta H$  is calculated for temperature intervals 37°, 50° and 75°C. Mean  $\Delta H$  is  $-42 \pm 11 \text{ kJ.mol}^{-1}$ , when  $K_d$  values at 20° and 90° are taken into account,  $\Delta H$  becomes  $-50 \pm 18 \text{ kJ.mol}^{-1}$ . Theoretically  $\Delta H$  ought to be constant. It is not remarkable that this was not found, since the sediments consist of several clay minerals and a large portion  $\text{CaCO}_3$ .

c) In the intermediate  $C_d$ -concentration range of 0.1 to 100  $\text{mg l}^{-1}$  of  $\text{Cd}^{2+}$ , trace element and its adsorption sites on the mineral surface are probably of the same order of magnitude. The lower the temperature, the more trace metal reacts with the mineral surface and vice versa, in accordance with the mechanism described in b. However, there is a limited amount of ligands (sites) available, so not all trace metal can react and  $K_d$ 's are not constant over the concentration range, in accordance with the mechanism described in a. Sorption experiments with radionuclides Pu, Am and Np are now being executed at the ECN laboratory to investigate whether these nuclides show the same  $K_d$  characteristics in relation to temperature as  $\text{Cd}^{2+}$  does.

### References

- Duursma, E.K., L.A. Van Geldermalsen and W.J. Wegereef, 1983. Migration processes in marine sediments caused by heat sources; simulation experiments related to deep sea disposal of high-level radioactive wastes. Rep. HEEG-83-1, TW 83/Ta-Z to CEC Brussels and Min. E.A. The Hague. 61 pp.
- Duursma, E.K. and C. Hoede - 1967. Theoretical, experimental and field studies concerning molecular diffusion of radioisotopes in sediments and suspended solid particles of the sea. Part A: Theories and mathematical calculations. *Neth. J. Sea Res.* 3, 423-457.
- Duursma, E.K. and C. Bosch - 1970. Theoretical, experimental and field studies of radioisotopes concerning diffusion in sediments and suspended particles in the sea. Part B: Methods and experiments. *Neth. J. Sea Res.* 4, 395-469.
- Sanchez, A.L., W.R. Schnell and T.H. Sibley - 1981. Distribution coefficients for radionuclides in aquatic environments. *NUREG/CR - 1852, 5*, 64 pp.

## IX. ARTICLES SUBMITTED FOR PUBLICATION OR IN PRESS

### (Δ-231) publication number of the Delta Institute

Beeftink, W.G. Geography of European halophytes.

Beeftink, W.G. and P. Paalvast. Demographic and ecological studies in *Salicornia europaea* agg. in the South-West of the Netherlands. III. Intra- en interspecific relationships under tidal conditions. *New Phytologist*.

Beukema J.J. and P.H. Nienhuis - Processen in oecosystemen. Hoofdstuk 10 Leerboek Inleiding in de oecologie. Bohn, Scheltema & Hoekema, Utrecht.

Buth, G.J.C. and M.G.M. Jansen - 1983. Historie en vegetatie van de Schouwse polderdijken. *Natura* 80/2 (905), 119-126.

Coosen, J. and R.J. Leewis - 1983. Response of macrozoobenthos to a temperal lowering of chlorinity: a large scale field experiment in the Dutch Delta Area. *Wat. Sci. Techn.* 16, Rotterdam, pp. 141-150 ( $\Delta$  - 251).

Critchley, A.T., W.F. Farnham and S.L. Morell. A chronology of new European sites of attachment for the invasive brown alga, *Sargassum muticum* (Yendo) Fensholt, 1973-1981. *J. mar. biol. Assoc. U.K.* ( $\Delta$  -254).

Critchley, A.T. A taxonomic history and world-wide distribution of the Japanese seaweed, *Sargassum muticum*. *J. mar. biol. Assoc. U.K.* ( $\Delta$  - 253).

Critchley, A.T. *Sargassum muticum* (Yendo) Fensholt: a description of European material. *J. mar. biol. Assoc. U.K.* ( $\Delta$  - 255).

Critchley, A.T. Ecological studies on European population of *Sargassum muticum* (Yendo) Fensholt. II. Experimental observations on variability of leaf and air vesicle shape. *J. mar. biol. Assoc. U.K.* ( $\Delta$  - 256).

Critchley, A.T. The establishment and increase of *Sargassum muticum* (Yendo) Fensholt populations within the Solent area of southern Britain. I. An investigation of the increase in number of population individuals. *Bot. mar.*

Critchley, A.T. The establishment and increase of *Sargassum muticum* (Yendo) Fensholt populations within the solent area of southern Britain. II. An investigation of the increase in canopy cover of the alga at low water. *Bot. mar.*

Critchley, A.T. A consideration of the spread of an immigrant brown alga, *Sargassum muticum*, as an introduced species. *Journal of Biogeography*.

Daemen, E.A.M.J. and M.T.T. Vereecken - 1983. Kwalificering en kwantificering van het microfytobenthos in de Oosterschelde, Yerseke/Middelburg. 's-Gravenhage. DIHO/RWS Deltadienst. DDMI Hoofdafd. Waterloopkunde. Interimrapport BALANS 1983-1, 28 pp.

Doornbos, G., R.H. Bogaards and F. Twisk - Density, growth and annual food consumption of plaice (*Pleuronectes platessa* L.) and flounder (*Platichthys flesus* (L.)) in lake Grevelingen, The Netherlands. *Neth. J. Sea Res.*

Duursma, E.K. The biogeochemical cycle of lead in the Oceans, GESAMP Working group (1978).

Duursma, E.K. Estuarine management and science problems. *Thalassia Jugosl.*

Duursma, E.K. Aspects of residence time in estuaries. *Bull. des Oceanogr. de France.*

Duursma, E.K., J. Nieuwenhuize and J.M. Van Liere - 1983. Organochlorine contamination of the Dutch Delta Region as "watched" by mussels. *Wat. Sci. Techn.* 16, Rotterdam, 619-622.

Duursma, E.K. Problems of sediment sampling and conservation for Radionuclide accumulation studies. *TECDOC ... I.A.E.A., Vienna.*

Duursma, E.K., J.W. Wegereef and L.A. Van Geldermalsen. Migration processes in marine sediments caused by heat sources; simulation experiments related to deep sea disposal of high-level radioactive wastes. Rep. HEEG-83-1 TW 83/TA-Z to CEC Brussels and Min. E.A. The Hague, 64 pp.

Duursma, E.K., J.M. Martin and M.J. Frissel - 1983. Differential migration of plutonium in the delta estuaries of Rhine, Meuse and Scheldt. Artificial radionuclides in Rhine-Meuse-Scheldt Delta, CEC-contract BIO-B-326-81-NL. Ann. Rep. 1982, 2 pp.

Groenendijk, A.M. - 1983. Tidal management: consequences for the salt-marsh vegetation. Water, Science and Technology.

Huiskes, A.H.L., H. Schat and P.F.M. Elenbaas. Demographic and ecological studies on *Salicornia europaea* agg. in the South-West of the Netherlands. I. Cytotaxonomic status and morphological characteristics of the populations. New Phytologist.

Huiskes, A.H.L., A.W. Stienstra and B.P. Koutstaal. Demographic and ecological studies on *Salicornia europaea* agg. in the South-West of the Netherlands. II. Germination ecology. New Phytologist.

Kelderman, P. - 1983. Sediment-water exchange characteristics in Lake Grevelingen under different environmental conditions. Neth. J. Sea Res.

Kelderman, P., J. Nieuwenhuize, A.M. Meerman-Van de Repe and J.M. Van Liere - 1983. The sediment map of Lake Grevelingen, a closed-off estuary in the S.W. Netherlands. Neth. J. Sea Res.

Krebs, B.P.M. Aquatische macrofauna van buitendijkse wateren in het Deltagebied. Deel II. Zeeuws-Vlaanderen. DIHO Rapp. en Versl. 1983 -.

Lambeck, R.H.D. - Dynamics, migration and growth of *Nassarius reticulatus* (Mollusca: Prosobranchia) colonizing saline Lake Grevelingen (SW Netherlands). Neth. J. Sea Res. 17: (Δ - 252).

Lindeboom, H.J. and H.A.J. De Klerk-v.d. Driessche - 1983. C-mineralisatie op en in de bodem van de Grevelingen. Eindrapport ZOWEC II, Yerseke/Middelburg. DIHO/RWS. Nota Z 83 II.

Lindeboom, H.J. and A.G.A. Merks. Annual changes in nutrients, DOC and POC concentrations and their relationship with chemical and biological processes in a closed estuary. Mitt. Geol. Sonderheft 52. Paläent. Inst. Univ. Hamburg. SCOPE/UNEP.

Lindeboom, H.J. and A.J.J. Sandee - 1983. The effect of coastal engineering projects on microgradients and mineralization reactions in sediments. Wat. Sci. Techn. 16, Rotterdam, 87-94. (Δ - 248).

Luyendijk, R. and B.P.M. Krebs. Aquatic and semi-aquatic Hemiptera in the estuarine area of the south-west Netherlands. Hydrobiol. Bull. 17.

Merks, A.G.A. and A.G. Vlasblom - Preservation and storage of samples for the determination of dissolved organic carbon (in press).

Nienhuis, P.H. - Temporal and spatial patterns of eelgrass (*Zostera marina* L.) in a former estuary in The Netherlands, dominated by human activities. Mar. Techn. Soc. J.

Nienhuis P.H. - Zeegrasgemeenschap in het Grevelingenmeer: In: Symposium Aquatische Oecologie, PUDDOC, Wageningen.

Nienhuis, P.H. and B.H.H. De Bree - Carbon fixation, including primary production, pigment and organic carbon dynamics in bottom sediments of brackish Lake Grevelingen, The Netherlands. *Neth. J. Sea Res.*

Nienhuis, P.H. and J. Huis In 't Veld - Grevelingen: from an estuary to a saline lake. *Wat. Sci. Techn.* 16, Rotterdam, pp. 27-50. (Δ - 250).

Sepers, A.B.J. and P.R.M. De Visscher. A preliminary investigation into the primary production of the mineralization in saline Lake Grevelingen. *Neth. J. Sea Res.*

Van Soelen, J. and M.M. Markusse - 1983. Notes on the distribution of some insect species living in the stems of *Aster tripolium* L. (Compositae). *Entomol. Ber. (Amst.)*.

Vegter, F. and P.R.M. De Visscher. Primary Production in Lake Grevelingen from 1976 up to 1981. *Neth. J. Sea Res.*

Vegter, F. and P.R.M. De Visscher. Excretion of phytoplankton during photosynthesis in lake Grevelingen. *Neth. J. Sea Res.*

#### **X. PUBLISHED ARTICLES AND REPORTS IN 1982**

(Δ - 231 = publication number of the Delta Institute)

##### **X.1. Working group 'Elements cycling and food chains'**

Bakker, C. - 1982. Het plankton van de brakwaterzone van de Westerschelde. *Zeeuws Nieuws Natuur, Landschap en Milieu* 8 (2), 29-33.

Bakker, C. - 1982. De kruiskwal *Gonionemus vertens* A. Agassiz in de zeegrasvelden van het Grevelingenmeer. *Vita Marina/Zeebiol. documentatie/holtdieren.* 27-34.

Bouwer, S. Th. - 1982. Ontwikkeling van het meiobenthos in het voorjaar op en om zeegrasplanten (*Zostera marina* L.) in het Grevelingenmeer DIHO. *Stud. Rep. D2-1982.* 33 pp.

Daemen, E.A.M.J., H. Pankow and P.H. Nienhuis - 1982. The benthic diatomflora of saline Lake Grevelingen (S.W. Netherlands). *Acta Bot. Neerl.* 31 (3), 153-167. (Δ - 231).

Doornbos, G., F. Twisk and R.H. Bogaards - 1982. Kwantificering van vissen. 2<sup>e</sup> Interimrapport. Yerseke/Middelburg. DIHO/RWS DDMI. *Nota Z 82 III 4*, 43 pp.

Doornbos, G. - 1982. Changes in the fish fauna of the former Grevelingen estuary, before and after the closure in 1971. *Hydrobiol. Bull.* 16 (2/3): 279-283. (Δ - 242).

Elgershuizen, J.H.B.W. - 1982. Eerste Interimrapport van het deelproject BALANS-TRANSPORT. Yerseke/Middelburg. DIHO/RWS DDMI. *Nota BALANS 1983-2*, 46 pp.

Glazenburg, A.K. - 1982. Interspecifieke concurrentie als mogelijke verklaring voor aantalsfluctuaties van twee grondelsoorten in de Grevelingen (*Pomatochistus minutus* en *P. microps*). DIHO. *Stud. Rep. D6-1982*, 36 pp.

Goossens, J.G.C.M. - 1982. C-mineralisatie in het water van de Grevelingen (ZOWEC I), 2<sup>e</sup> interimrapport. *Nota Z 82 I 4* Yerseke/Middelburg. DIHO/RWS DDMI, 11 pp.

- Kelderman, P. and A.M. Van der Repe - 1982. Temperature dependence of sediment-water exchange in Lake Grevelingen. S.W. Netherlands. *Hydrobiologia* 92, 489-490.
- Kelderman, P. - 1982. Bodem/wateruitwisseling in de Grevelingen. 2<sup>e</sup> interimrapport Yerseke/Middelburg. DIHO/RWS DDMI. Nota Z 82 VI, 46 pp.
- Lambeek, R.H.D. - 1982. Colonization and distribution of *Nassarius reticulatus* (Mollusca: Prosobranchia) in the newly created saline Lake Grevelingen (SW Netherlands). *Neth. J. Sea Res.* 16: 67-79. (Δ - 215).
- Lindeboom, H.J. and B.H.H. De Bree - 1982. Daily production and consumption in an eelgrass (*Zostera marina*) community in saline Lake Grevelingen: discrepancies between the O<sub>2</sub> and 14C method. *Neth. J. Sea Res.* 16, 362-379. (Δ - 240).
- Lindeboom, H.J. and H.A.J. De Klerk-v.d. Driessche - 1982. C-mineralisatie op en in de bodem van de Grevelingen (ZOWEC II), 2<sup>e</sup> interimrapport Yerseke/Middelburg. DIHO/RWS DDMI. Nota Z 82 II 4, 72 pp.
- Lindeboom H.J., A.H.L. De Klerk-v.d. Driessche and A.J.J. Sandee - 1982. Production and decomposition of eelgrass (*Zostera marina* L.) in Lake Grevelingen. *Hydrobiol. Bull.* 16: 93-102. (Δ - 236).
- Nienhuis, P.H. - 1982. De oecologische consequenties van de Deltawerken. (The ecological consequences of the Deltaplan). In: W.J. Wolff e.a. Wadden, duinen, delta. Wageningen/'s Graveland, Pudoc/Ver. tot Behoud van Natuurmonumenten in Nederland. Biologische Raad Reeks, pp. 101-132. (Δ - 234).
- Nienhuis, P.H. - 1982. 25 jaar DIHO: een kijkje in verleden, heden en toekomst van het milieuonderzoek in het Deltagebied. (25 years Delta Institute: a look in the past, to-day and future of the environmental research in the Delta area). *Zeeuws Tijdschrift* 32 (5): 145-151. (Δ - 247).
- Nienhuis, P.H. - 1982. De schorren. *Zeeuws Nieuws Natuur, Landschap en Milieu* 8, 40-43.
- Nienhuis, P.H. - 1982. Attached *Sargassum muticum* found in the S.W. Netherlands. *Aquat. Bot.* 12, 189-195. (Δ - 213).
- Nienhuis, P.H. - 1982. A simulation model of production, seasonal changes in biomass and distribution of eelgrass (*Zostera marina*) in Lake Grevelingen. *Hydrobiol. Bull.* 16, 286.
- Pellikaan, G.C. - 1982. Evaluatie van de methodieken voor het meten van de primaire produktie van het microfytobenthos, zoals toegepast op het DIHO. *DIHO Rapp. en Versl.* 1982-3. 56 pp.
- Pellikaan, G.C. - 1982. Decomposition of eelgrass, *Zostera marina* L. *Hydrobiol. Bull.* 16, 83-92. (Δ - 235).
- Prud'homme van Reine, W.F. and P.H. Nienhuis - 1982. Occurrence of the Brown Alga *Sargassum muticum* (Yendo) Fensholt in The Netherlands. *Bot. Mar.* 25, 37-39. (Δ - 227).
- Tackx, M. and P. Polk - 1982. Feeding of *Acartia tonsa* Dana (Copepoda, Calanoida): predation on Nauplii of *Canuella perplexa* T. et A. Scott (Copepoda, Harpacticoida) in the sluicdock at Ostend. *Hydrobiologia* 94: 131-133.

Van Ierland, E.T. - 1981. Geautomatiseerde sestonanalyse (ZOWEC V), 2<sup>e</sup> interim-rapport. Yerseke/Middelburg. DIHO/RWS DDMI. Nota Z 81 V 4, 12 pp.

Verhagen, J.H.G. and P.H. Nienhuis - 1982. A simulation model of production, seasonal changes in biomass and distribution of eelgrass (*Zostera marina*) in Lake Grevelingen. Mar. Ecol. Progr. 10 (2), 187-195. (Δ - 238).

## X.2. Working group 'Brackish waters'

Beniers, A.J.M.C. - 1982. De invloed van grazing op de ontwikkeling van *Skeletonema costatum* in een brakwatermeer. DIHO, Stud. Rep. D4-1982, 101 pp.

De Nieuwe, R. - 1982. De opnamecapaciteit van heterotrofe bacteriën voor organische stof. DIHO. Stageverslag, 22 pp.

Dingemanse, E.J. - 1982. DNA als biomassaparameter voor bacteriën. DIHO, Stageverslag.

Hummel, H. - 1982. De invloed van verschillende tijden op droogliggen op bodemdieren. DIHO Rapp. en Versl. 1982-2, 22 pp.

Krebs, B.P.M. - 1982. Nota on the distribution of *Sigara selecta* (Fieber) in the brackish inland waters of the South-West Netherlands. Hydrobiol. Bull. 16, 159-164.

Krebs, B.P.M. - 1982. Note on the distribution of *Dixella autumnalis* (Meigen) in the South-West Netherlands (Diptera: Dixidae). Entomol. Ber. (A'dam) 42, 62-64.

Krebs, B.P.M. - 1982. Chironomid research in the Netherlands. Chironomus 2, 17-19.

Krebs, P.B.M. - 1982. Chironomid communities of brackish inland waters. Chironomus 2, 19-23.

Luteyn, A. - 1982. De opnamecapaciteit van heterotrofe bacteriën voor organische stof (II). DIHO, Stageverslag, 21 pp.

Sepers, A.B.J. - 1982. The enumeration of heterotrophic bacteria in aquatic environments on simple and complex media. Int. Revue ges. Hydrobiol. 67, 567-573.

Sepers, A.B.J. - 1982. The uptake of organic compounds by heterotrophic bacteria in relation to growth rate. Publ. CNEXO (Actes Colloq.) no. 13, 45 and 86-90.

Sepers, A.B.J., G. Cahet and H. Goossens - 1982. Comparison between the carbon-14 and oxygen consumption method for the determination of the activity of heterotrophic bacterial populations. Mar. Biol. 66, 237-242.

Van der Boog, H.M. - 1982. Vergelijkend onderzoek naar de aquatische makrofauna en makroflora van enkele wateren op Noord- en Zuid-Beveland in relatie tot milieufactoren. DIHO Stud. Rep. D5-1982, 70 pp.

Van Duyl, Ch. - 1982. Fytoplankton en fyto bentos en de zuurstofhuishouding van een brakke sloot met een zoutgradiënt. DIHO, Stud. Rep. D3-1982, 96 pp.

Verdonschot, P.F.M., M. Smies and A.B.J. Sepers - 1982. The distribution of aquatic oligochaetes in brackish inland waters in the SW Netherlands. Hydrobiologia 89, 29-38.

### **X.3. Working group 'Salt-marsh ecosystems'**

Beeftink, W.G., J. Nieuwenhuize, M. Stoeppler and C. Mohl - 1982. Heavy-metal accumulation in salt marshes from the Western and Eastern Scheldt. *Sci. Tot. Environ.* 25, 199-223.

Bouts, M. 1982. Onderzoek naar het voorkomen van enkele macrozoobenthosoorten over het intergetijdegebied, in vergelijking met het voorkomen in een aantal vaste stations. Stage Rapp. DIHO-DDMI, 22 pp.

Buth, G.J.C. and A.M. Groenendijk - 1982. Comparative investigations of vegetation and soil of former mud- and sandflats in relation to the time of embankment. *Acta Bot. Neerl.* 31, 1/2, 142.

Buth, G.J.C., P.F.M. Verdonshot and L. De Wolf - 1982. Decomposition of three halophytes in different habitats of an Eastern Scheldt salt marsh. *Hydrobiol. Bull.* 16, 1, 103-112.

Buth, G.J.C. - 1982. Schouwen-Duiveland, één groot recreatie-park? *Zeeuws Nieuws Natuur, Landschap en Milieu.* 3, 73-75.

De Loos, F. - 1982. Onderzoek naar de gevolgen van langdurige inundatie op de grassen *Festuca rubra rubra f. litoralis*, *Elytrigia pungens* en *Puccinellia maritima*. *VEGIN Stud. Rep.* 1-1982, 59 pp.

Drok, W.J. - 1982. Is *Spartina anglica* Hubbard wel een goede soort? Taxonomie en oecologie van *Spartina* spp. (slijkgrassen) in zuidwest Nederland. *DIHO, Stud. Rep.* D1-1982, 88 pp.

Groenendijk, A.M. en M.A. Lievaart - 1982. Effecten van verlengde inundatie op de groei en de ontwikkeling van een aantal schorreplanten. Interimrapport *VEGIN, Yerseke-Middelburg*, 37 pp.

Kolpa, R.J. - 1982. De geohydrologie van de Spieringschor (Noord-Beveland). *DIHO Stud. Rep.* D8-1982, 70 pp.

Luytjes, W. and P.J. Waeijen - 1982. Vegetatiekundig en bodemkundig onderzoek aan een zestal inlagen rond de Oosterschelde. *DIHO Stud. Rep.* D7-1982, 142 pp.

Mol, I. - 1982. Een analyse van ruimtelijke en tijdelijke patronen in een schorrevegetatie. *Stud. Rep.* D9-1982, 79 pp.

Van Geldermalsen, L.A. and A.M. Groenendijk - 1982. Decomposition experiments with *Spartina anglica* leaves. *Neth. J. Sea Res.* 15 (3/4), 340-348. ( $\Delta$  - 257).

Van Steen, A. - 1982. Bepaling van de invloed van getijreductie op een aantal abiotische factoren en op de groei van een aantal schorreplanten. *VEGIN Stud. Rep.* 2-1982, 35 pp.

### **X.4. A-Subjects (Anthropogenic substances and other interworking group projects)**

Duursma, E.K. - 1982. Techniques and methods of interfacial transfer and transport processes in the sediment water layer CEC - IAEA Joint Techn. Rep., Ispra/Vienna, 219-227.

Duursma, E.K. and M. Smies - 1982. Sediments and transfer at and in the bottom-interfacial layer. In: 'Pollutant Transfer and Transport in the Sea'. G. Kullenberg (ed.) C.R.C. Press Inc. West Palm Beach U.S.A., Vol. II, 101-139.

Duursma, E.K. (ed.) - 1982. Progress Report 1981. Delta Institute for Hydrobiological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Nat., 2e reeks, 79, 1-98 ( $\Delta$  - 237).

Duursma, E.K., H. Engel en Th.J.M. Martens (eds.). De Nederlandse Delta. Een compromis tussen milieu en techniek in de strijd tegen het water (The Dutch Delta. A compromise between environment and technology in the struggle against the sea). Secr. ed. board: R. Peelen. Authors of the Delta Institute: C. Bakker, W.G. Beeftink, R.H. Bogaards, C.H. Borghouts, G. Doornbos, E.K. Duursma, J.W. Francke, B.P.M. Krebs, R.H.D. Lambeck, P.H. Nienhuis, R. Peelen, A.B.J. Sepers, P.J. Van Boven and F. Vegter. Natuur en Techniek, Maastricht, 511 pp (in Dutch + 48 pp English summary + 'Zelandia Descriptio' ).

Huiskes, A.H. en M. Smies - 1982. Oosterschelde 1987: stormvloedbeheer en wat nog meer? Natuur en Milieu 6, 9, 4-9 ( $\Delta$  - 239).

Merks, A.G.A., J.J. Sinke and J.O. Van de Zande - 1982. Enkele in het Deltagebied voorkomende parameters en hun onderlinge samenhang. DIHO Rapp. en Versl. 1982-4, 24 pp.

Nieuwenhuize, J. en J.M. Van Liere - 1982. De calibratie van totaal kwik in sediment, plantaardig materiaal en mosselen door middel van vlamloze atomaire adsorptie. DIHO Rapp. en Versl. 1982-1, 17 pp.

Pronk, M.A. - 1982. Bibliografie over natuur, milieu en landschap in het Deltagebied. Suppl. II 1 juli 1980 - 1 aug. 1982, DIHO Rapp. en Versl. 1982-5, 69 pp.

Smies, M. - 1982. Biological redistribution of environmental contaminants in estuarine sediments. In: European Communities Commission/Communautés européennes Commission. EUR 7549 - Principles for the interpretation of the results of testing procedures in ecotoxicology. EUR 7549 - Principes à appliquer pour l'interprétation des résultats d'essais en écotoxicologie. Luxembourg, Office for Official Publications of the European Communities. Environment and quality of life series, pp. 545-549 ( $\Delta$  - 205).

Valenta, P., H.W. Nürnberg, P. Klahre, A.G.A. Merks, a.o. - 1982. Comparative study of toxic trace metals in the estuaries of the Eastern and Western Scheldt and of the Sierra Leone River. Paper presented at the Seminar on Estuaries - Their Physics, Chemistry, Biology, Geology and Engineering Aspects. 7-11 December 1981. National Institute of Oceanography, Dona Paula, Goa, India, 14 pp.