

Institutes of the Royal Netherlands Academy of Arts and Sciences

Progress Report 1979

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Netherlands Institute for Brain Research, Amsterdam

Hubrecht Laboratory, International Embryological
Institute, Utrecht

Centraalbureau voor Schimmelcultures, Baarn/Delft

Delta Institute for Hydrobiological Research, Yerseke

Institute for Ecological Research,
Arnhem/Oostvoorne

Limnological Institute, Nieuwersluis/Oosterzee

Netherlands Institute for Brain Research

Progress Report 1979

Edited by D.F. Swaab

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Groups and participants

Director

Prof. D.F. Swaab (NIBR and University of Amsterdam)

Manager

K.E. de Roos

I. Adaptability of the nervous system of adult organisms (including histological technicum)

Dr. H.B.M. Uylings, biologist (leader)
Drs. J. Blaauw, biologist (TAP)
Dr. R.W.H. Verwer, biologist (from 1-3-79)
Dr. P. McConnell, neuroanatomist
Drs. W.A.M. Veltman, physicist
S. Maslam-Suharti (from 1-9-79)
B.M. Przybylski-Zweesaardt
S.W.G. van Kan (from 1-9-79)

II. Interaction of nerve cells and behaviour during maturation (including department of electron microscopy)

Dr. M.A. Corner, biologist (leader) (NIBR and University of Amsterdam)
Dr. R.E. Baker, biologist
Drs. H.L.M.G. Bour, biologist (prom.)
Drs. A.M.M.C. Habets, biophysicist
Drs. M. Mirmiran, physician (IBRO fellowship)
Dr. H.J. Romijn, biologist (head section of electron microscopy)
M.T. Mud
A.Ph.J. Richter
P. Wolters

III. Interaction of the nervous system and hormones during maturation and adaptation

Prof. D.F. Swaab, physician (leader)
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Dr. G.J. Boer, biochemist
Dr. K. Boer, physician (until 1-10-79)
Drs. R.M. Buijs, biologist (prom.)
Dr. J. Dogterom, biochemist (until 1-9-79)
Drs. F.W. van Leeuwen, biologist
Dr. P. Pevet, biologist (university of Amsterdam)
Drs. P.J. van der Sluis, biochemist (from 1-6-79)
Drs. C.W. Pool, biologist (from 1-7-79)
Drs. M.G.B. Madlener, biologist (from 1-9-79)
B. Fisser
C. de Raay (FUNGO)

P. de Groot (FUNGO, until 1-4-79)
C.M.F. van Rheenen-Verberg
J.J. van Heerikhuizen (from 17-9-79)

IV. Development and correctibility of behaviour

Dr. N.E. van de Poll, psychologist (leader)
Dr. J.P.C. de Bruin, biologist
Drs. J.G. van Oyen, psychologist (prom.)
V.D.J. Nolten
S.M. van der Zwan
S.M. de Jong-van Zanten (from 13-12-79)

V. Neurophysiological aspects of maturation and adaptation of the nervous system (including computer department)

Dr. H.L. Walg, bioinformaticist (acting leader, head computer dept.)
Drs. C.V. de Blécourt, physician (military service)
Dr. J. van Pelt, physicist
H. Pronker

Monoaminergic mechanisms in rat and mouse

Drs. M. van Wijk (biochemist)

Secretaries

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W. Chen-Pelt (temp.)
E.M. Verbraak (temp.)

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F. Harkema (head)
R. Hofer
J.N. Roosien (temp.)

Library

Drs. C. Winkler, physician

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Mechanical workshop

A.W. Kamstra (head)
E.W. Moes
M. Westdorp (from 22-11-79)

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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 be placed on an international footing. This resulted in the formation in 1904 of the International Academics 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 civilised 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 Academy of Arts and Sciences (K.N.A.W.) applied to the government for permission to found an institute for brain research, and on June 8th 1909, the Netherlands 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. Ariëns Kappers, who gained international fame with his work on 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 kept 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. Ariëns 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 analysis of the brain, especially the cerebral cortex, was appointed director. Upon his retirement in 1962, he was succeeded by Prof. Dr. J. Ariëns Kappers, whose special fields of study were the circumventricular organs and, more particularly, the pineal gland. Under his direction, research into the structure and function of the pineal gland became an important part of the Institute's work.

Recent History

When, in August 1975, the government unexpectedly decided to close the Institute, the staff set up a committee which set out to do everything possible to have the decision reversed. The Institute received a large number of letters of support from universities, scientific institutions and individual researchers from all over the world. After much effort, unanimous parliamentary support for the Institute was obtained and, after an internal reorganization, investigations continued along one central research theme entitled 'Maturation and Adaptation of the Nervous System'.

On the 1st of October 1978, Dr. D.F. Swaab, acting-director since November 1975, was appointed director, and in December 1979 he was appointed 'extraordinary professor' of neurobiology at the University of Amsterdam.

Construction is now in progress of the Medical Centre of the University of Amsterdam, a vast complex within which the Institute will be definitively housed.

The organization of research

As of the 1st of January 1977, the central research theme 'Maturation and Adaptation of the Nervous System' is being investigated by five multi-disciplinary research groups as follows:

I. Adaptability of the nervous system of adult organisms

During periods of rapid brain development, hormones, nutrition and environment are important influential factors. Research is directed towards the capability of brains to recover from retardation as a result of hormonal, nutritional or environmental stunting factors acting during development. Normal development, retarded development and recovery potentialities are being investigated in several components of the central nervous system, such as dendrites and synapses. Light- and electron-microscopic qualitative and quantitative techniques are in use, while in collaboration with the other groups, further procedures (such as biochemical) are being utilized. In addition, histological services are made available to the other groups.

II. Interaction of the nervous system and behaviour in development

During maturation, the nervous system shows complex rhythmical patterns of activity at a time when specific synaptic connections are being established. The question is whether this spontaneous bio-electrical activity plays a role in normal development and in the formation of neural networks. The work on mechanisms of synaptogenesis was first performed on the frog, using behavioural responses of early operated animals, but attention is currently focussed on tissue cultures using electron microscopic and electrophysiological techniques. Sleep deprivation experiments on infant rats have been started in order to study this problem in the intact organism. As regards the physiology of early motor rhythms, the emphasis has shifted from the chick embryo to the study of postnatal development in the rat, using electrophysiological and anatomical techniques along with behavioural parameters. The electron microscopy section of this group has, in addition, a service task for the entire Institute.

III. Interaction between the nervous system and hormones during maturation and adaptation

An increasing number of neurons is found to produce hormones, which act not only on the pituitary and other peripheral target organs, but also on the brain

itself. Neuroendocrine mechanisms are thought to be involved in diuresis, reproduction, brain development, behaviour and other central processes as well as in diseases such as diabetes insipidus, disturbances of pregnancies, lactation and parturition and in certain mental disorders. Emphasis has been placed on: 1) the study of fundamental aspects of neurosecretion (e.g. the sites and mechanisms of hormone production, transport and release); 2) the function of neuroendocrine systems in brain maturation, and the role of foetal neuroendocrine systems in parturition; and 3) the involvement of neuroendocrine systems in reproduction, learning and behaviour. The main disciplines include immunological techniques, biochemistry, electrophysiology, light- and electron microscopy and clinical observations.

IV. Development and correctibility of behaviour

During development, specific motivational and emotional aspects of behavioural functions are organized in the brain. In this project, behavioural and morphological consequences of gonadal hormones and social factors acting during early postnatal development are being studied. Emphasis is placed upon sex differences in social categories of behaviour (aggression and sexual behaviour) and in emotions, activity and learning. In addition, behavioural experiments and observations for the other groups are performed by this department.

V. Neurophysiological aspects of maturation and adaptation of the nervous system

Incorporated in this group is the computer department which provides essential experimental and mathematical support to all five research groups. The scientific direction of group V will be determined in 1980.

An increasing amount of collaborative research is taking place among the five research-groups. An example is the project on the possible involvement of the hormone vasopressin in brain development. Rats, both homozygous and heterozygous for vasopressin deficiency, are currently being investigated during development by the various research groups using quantitative anatomical procedures for measurements on brain structures such as dendrites (group I), and electrophysiological techniques for the determination of the sleep-wake cycle (group II). Hormone assays and biochemical determinations of brain size, cell size and cell number are being performed by group III. As cerebellar development was found to be seriously stunted, an exploratory study on the motor-

coordination of these animals is being performed by group IV. The computer department of group V is assisting the other groups in data acquisition and computation. Another example of a project being jointly conducted by several groups is the study of the significance of REM-sleep for brain maturation and behavioural development.

Apart from the research data obtained by the research groups, servicing departments, assistants, guest workers, students and apprentices, several other activities are mentioned in this report, such as the

papers, seminars and lectures that were presented, and conferences that were attended.

The Institute's supervisory committee consisting of Prof. Dr. J. Joosse (chairman), Prof. Dr. H.B.G. Casimir, Prof. Dr. A.H.M. Lohman, Prof. Dr. R.A. Crone, Dr. F.H. Lopes da Silva and Dr. J.L. Slangen convened frequently in 1979 together with Dr. D.F. Swaab, K.E. de Roos and Dr. D. van der Mei (K.N.A.W.), while Prof. R. Balász (MRC Unit on developmental Neurobiology, London, England) was advising the committee in biochemical matters.

I. Adaptability of the nervous system of adult organisms

In this project, the plasticity and recovery of the 'mature' central nervous system after retardation due to environmental perturbations during development are studied. The adverse conditions involved in our studies are *undernutrition* and *neuropeptide* deficiency. Emphasis is placed on the study of (a) normal development, (b) impairment in this development and (c) the recovery potential. Neurons, dendrites and synapses are studied, mainly quantitatively, using light and electronmicroscopic techniques. This project is partly represented in the FUNGO workgroup Development and Aging of the Brain and Behaviour.

THEME 1. EFFECTS OF NUTRITIONAL REHABILITATION AND ENVIRONMENTAL ENRICHMENT ON CEREBRAL AND CEREBELLAR CORTEX OF PREVIOUSLY UNDERNOURISHED RATS

An initial experiment is underway to determine the effects of severe undernutrition, acting at the time of the brain growth, spurt, upon neuronal growth in the cerebral cortex, and to determine whether any alterations can be reversed by subsequent nutritional rehabilitation. Rat pups were undernourished from birth until 30 days by restricting their access to the lactating dam. Thereafter, the pups were separated from the dam and given *ad libitum* food supplies until the end of the experiment at 150 days. At 10, 15, 20, 30 and 150 days post partum (dpp), ten such experimental animals and ten control animals (fed *ad libitum* throughout) were killed and Golgi Cox or cresyl violet stained, frontal sections of their forebrains were prepared. The method of undernutrition produced a marked reduction in body weight, which was statistically highly significant from 10 days. By 30 days there was a deficit of 75.8%, and body weight remained reduced despite the subsequent refeeding (i.e., at 150 days the body weight of the refeed animals was 29.6% lower than that of the controls). Whole brain weight and forebrain weight were also significantly depressed due to undernutrition and the deficits again

persisted in the refeed animals (experimental animals showed whole brain and forebrain weight deficits of 27.1% and 25.5% respectively at 30 dpp, and 13.7% and 10.7% at 150 dpp. Measurements of neocortical area and thickness, carried out on cresyl violet sections taken at various standardised points along the length of the corpus callosum, indicated that these parameters, too, were reduced in 30 days undernourished animals. The effect appeared to be a differential one, with the more posterior sections (approximating to Krieg's areas 17 + 18) showing the greatest deficits. The degree to which these abnormalities can be reversed by refeeding to 150 days is currently under investigation. A study of the effects of this experimental treatment on pyramidal and non-pyramidal cell dendritic fields, particularly in the more vulnerable posterior region of the forebrain, is also in progress.

An electron microscopical study has been started to analyse the changes in the density of synaptic contacts in the cerebellar molecular layer of the rat after undernourishment and subsequent refeeding. The ultrastructural morphology of undernourished rats at 30 dpp has been compared qualitatively with that of the control animals. However, apart from some very striking differences, such as thickness of molecular layer and dendritic size, no obvious ultrastructural changes could be detected. A quantitative study is therefore necessary, and the first stage in preparation for doing this - i.e., the mastering of the criteria for distinction of different neuronal processes prior to the determination of synaptic densities - is already underway. The determination of the number of synapses on the basis of their profiles in tissue sections requires a simple model for the shape of the synapses in order to become amenable to statistical analysis. Thus, the synapse may be regarded either as a flat disc or as the cap of a sphere. For the first case several methods have been published for assessing the number of synapses per unit tissue volume. None of these methods is completely satisfactory, however, since they either assume special distributions of synapse size (e.g., a normal distribution) or are very time-consuming and yet be relatively inaccurate. Review of the literature has

not uncovered an accurate method that could be applied to our kind of data. For the second case no methods are available, as far as we know. In consequence, we are developing a Kolmogorov-Smirnov type of analysis to estimate the number of synapses per tissue volume in our material.

In addition, a study has been begun of the efficacy of environmental enrichment in the rehabilitation of undernourished rats. After day 30, rats are placed in groups of 9 'age-mates' in large cages 'enriched' with different toys. These cages are on loan from the Dept. of Comparative and Physiological Psychology of the Catholic University of Nijmegen (W. Raaymakers).

THEME 2. THE INFLUENCE OF HORMONES ON THE DEVELOPMENT OF THE CORTEX

Influence of vasopressin deficiency on the rat brain

In collaboration with group III, a start has been made with the follow-up of the pilot study reported last year. In the more recent study, maternal influences on the postnatal development of Brattleboro rats have been kept constant by using only HOM-DI mothers to rear both HET-DI and HOM-DI pups. A more detailed analysis is in progress by dissecting the brain in 8 different parts in a developmental series of animals (aged 0, 8, 12, 16, 20, 24, 30 and 180 dpp).

Cerebellum, medulla oblongata plus pons, bulbus olfactorius, colliculi, hypothalamus, hippocampus, cortex cerebri and the rest of the brain (mainly formed by the basal ganglia and the thalamus) were dissected and prepared for biochemical analysis. A similar analysis has been performed in adult Brattleboros which received intraventricular and subcutaneous vasopressin suppletion for a period of one month (150 - 180 days). No changes were measured in the brains of these adult HOM-DI rats on the basis of weight and DNA and protein estimations. Thus, if vasopressin does have a direct influence on Brattleboro brain size, its presence appears to be necessary in early development, rather than in later life. A preliminary study of the cerebellum reveals that, in both HOM-DI and HET-DI rats, the external granular layer has dispersed by 21 dpp, suggesting that the indication from the pilot study of a recovery in brain cell number in HOM-DI rats after 24 days is probably due to a glial response. A quantitative study of Purkinje cell dendritic trees in 180 days HOM-DI and HET-DI rats is in progress, along with preparation and area measurement of midsagittal Nissl-sections of cerebellum of 180 days Brattleboro rats.

THEME 3. THE STRUCTURAL DEVELOPMENT OF THE CORTEX

The general growth characteristics of the non-

pyramidal neurons in layer IV of the visual cortex of female albino rats were reported in last year's progress report. The study was carried out in collaboration with Dr. J.G. Parnavelas (University of Texas) and with group V. Three subgroups of non-pyramidal cells can be distinguished on the basis of the position of dendritic origin mainly: multipolar, bitufted and bipolar cells. The frequency with which each of these morphological celltypes is represented in our samples is 72%, 24% and 4%, respectively. The number of bipolar neurons measured at each developmental stage was too small to describe their growth-rate with accuracy. From the analysis of multipolar and bitufted cells it appears that the shape of the 'growth curves' is similar for all of the measured parameters. The projected cell body surface of bitufted cells is generally smaller than that of the multipolar neurons. The total number of dendritic segments per neuron is approximately similar for bitufted and multipolar cells. After day 18, however, total dendritic length per neuron of bitufted cells was larger than that of the multipolar ones, caused mainly by the longer intermediate segments of the bitufted cells; the terminal segments of the two cell types do not differ significantly. The lengths of the proximal (first order) intermediate segments in both multipolar and bitufted cells show strikingly smaller values than those of the higher orders. Increase in length of terminal segments after day 24 has been found only for the multipolar neurons. The radial distances of terminal tips of bitufted neurons are generally larger than those of multipolar cells from day 18 onwards.

In conclusion, these data indicate that in any future morphometrical studies of non-pyramidal neurons in layer IV a distinction must be made between bitufted and multipolar cells.

Collection of histological slides and preparations of Prof. C.U. Ariëns Kappers

The C.U. Ariëns Kappers collection of microscopic and macroscopic brain preparations of numerous vertebrates (fish, amphibians, reptiles, birds and mammals) has been cleaned and catalogued. Obsolete taxonomic names have been replaced by current nomenclature. The documentation was completed this year by J. Blaauw. An alphabetic register in Dutch, German, English and French of common names, a card system with the 'scientific' (i.e., latin) names, and a phylogenetic register — in which all of the species found in the collection are arranged taxonomically (i.e., in order of class, order, family, genus) — have been prepared. The staining methods used and the plane of cutting are specified in the card system. In addition, a Dutch and English user's manual has been written.

Golgi staining

The effects of diluted versus 'normal' Golgi-Cox staining of rat cerebral cortex have been analyzed. This study arose from the observation that, unlike normal Golgi-Cox fluid, the diluted fluid stained myelinated axons in the optic tectum of the goldfish. An experiment was therefore undertaken to determine whether a similar phenomenon occurred in normal cerebral cortex. In the $\frac{1}{4}$ diluted Golgi-Cox preparations of rat cerebral cortex, however, no staining of myelinated axons was observed, although the mode of dendritic/somatic impregnation appeared to differ considerably from the normal picture. Whereas with normal Golgi-Cox fluid the impregnation of the neuron starts in the cell body and proceeds progressively to the peripheral dendritic segments, with the diluted Golgi-Cox the impregnation started simultaneously in the neuron soma and a large part of the primary dendrites, and within several hours nearly the whole cell was impregnated. The time required for optimal impregnation of neurons with the diluted Golgi-Cox

solution was only 7 days, as opposed to 21-28 days with the normal Golgi-Cox solution. After this optimal time, however, the quality of the staining in the diluted Golgi-Cox deteriorates within a few days, while with the normal Golgi-Cox there was a margin of several weeks. In addition, it appeared that the diluted Golgi-Cox did not consistently result in the complete impregnation of neurons. We concluded therefore, that the diluted Golgi-Cox staining could be useful in obtaining a first, rapid impression of the different neurons present, but that it is not a reliable method for quantitative analysis of rat cortex.

For use of formalin-fixed human brain tissue, different silver-Golgi and Golgi-Cox methods have been explored in an attempt to identify a reliable stain. It appeared that Valverde's silver-Golgi method was the best one, while the diluted Golgi-Cox method appeared to be a good one for qualitative inspection. We were not particularly successful with the other methods. For human tissue of 20 weeks of gestation the normal Golgi-Cox was found to be too capricious.

II. Interaction of the nervous system and behavior during maturation

The group's research project has continued along the general lines of the past few years. There are three main themes, which can be characterized as follows: 1. neuronal basis of spontaneous behavior rhythms in early development; 2. influence of neuronal and behavioral rhythms on the development of the nervous system; and 3. mechanisms underlying the formation of selective nerve connections. Under theme-1, the organizational principles are being studied which account for the development of integrated functioning in the central nervous system. It is subdivided into (A) a portion dealing with the regulation of sleep/wake rhythms in infant rats, and (B) a portion exploring the culture of neural tissues *in vitro* as a 'model' system for studying physiological mechanisms at the cellular level. Theme-2 represents the reciprocal question, viz. what are the effects of endogenous activity rhythms upon developing organization in neuronal networks. It too is subdivided into (A) a portion dealing with the possible role played by sleep rhythms in brain maturation, and (B) an *in vitro* model system for studying the effects upon synaptogenesis of manipulating functional activity levels during early development. The third theme is a continuation of a long-standing project in our group, in which an experimental embryological approach is applied for the purpose of investigating the origin of sensory reflexes in frogs. This project is represented as a whole in the FUNGO workgroup 'Development and aging of brain and behavior'.

THEME 1A. CHARACTERIZATION OF BRAINSTEM 'SLEEP-GENERATOR' SYSTEMS IN DEVELOPING RATS

Baseline studies of sleep motility

Spontaneous movements during active sleep (AS) decline strikingly in vigor during the third postnatal week. The two ages, 13-14 days and 21 days, were therefore chosen for making a quantitative analysis. Amplitude and frequency measures appeared to vary continuously at both ages, without much indication for distinct categories of movements. Clustering of 'motility units' was observed, which allowed us to define motor 'bouts' (i.e., a sequence of motility-units separated by intervals not longer than 0.1 sec). Between two and three weeks of age a decline occurred in the frequency of bouts and in the amplitude and duration of motility units. The duration of individual bouts and the density of motility units within each bout remained the same. The overall amount of AS declined during this period from ca. 40% to 25% total-sleep-time, but the mean duration per AS epoch increased from ca. 65 sec to 145 sec.

Activity of reticular neurons

Attempts were abandoned to record multi-unit firing in restrained rat pups during the sleep/wake cycle. Not only was the microelectrode tip continually displaced by vigorous movements, but it also proved impossible to obtain normal sleep patterning. Considerably improved recordings could be obtained by using fine microwires (50 μ m in diameter), which were implanted in bundles into the n. reticularis gigantocellularis under

stereotaxic control and then cemented to the skull. This technique opened the possibility of recording successfully from neurons in free-moving rats from as early as 10 days of age. The results so far are in agreement with literature reports on adult rats: relatively high firing rates during active waking and active sleep, with much lower levels occurring during quiet wakefulness and quiet sleep. Selective firing only during AS was never observed in any unit. Quantification of state differences in reticular neuronal activity as a function of age is now underway.

Activity of midbrain raphe neurons

Because of their possible importance in control of the sleep cycle, our interest has also become focussed upon the activity of serotonergic neurons in the midbrain. Stereotaxic implantations into the nucleus raphé dorsalis in adult rats under anaesthesia revealed the presence of the slow regular firing which is typical for the anaesthetized state. Microwire recordings from this region in free-moving animals showed more complicated firing patterns, the general picture being similar to that described above for the, more laterally situated, reticular giant cells. In addition, however, spontaneous activity was at a minimum during the transitional phase from deep quiet sleep to AS (i.e. 'pre-REM' phase) and during the initial portion of AS.

THEME 1B. STRUCTURAL CORRELATES OF SPONTANEOUS BIOELECTRIC ACTIVITY IN NEURONAL NETWORKS CULTURED IN VITRO

Firing patterns of spinal cord explants

Out of 43 cultures tested, 14 were found to possess spontaneous neuronal activity, as measured by extracellular micropipettes. Active cultures ranged in age from 10-70 days in vitro, and their activity was more prominent in serum-free medium than in growth medium containing fetal calf serum. Horse serum was therefore tried, and has been found to have little or no suppressant action. The discharges ranged from infrequent action potentials to almost continuous firing, often over periods of several hours. Evoked bioelectric potentials were observed in only 6 cultures, even in serum-free medium. Further studies will be made this year in order to satisfactorily establish the baseline characteristics for neuronal discharges in this type of neural tissue.

Firing patterns of cerebral cortex neurons

Spontaneous bioelectric potentials were recorded in 20 out of 31 cultures of dissociated cortex tissue taken from fetal rats. The ages ranged from 9 to 38 days in vitro, and the recordings were made in growth medium

containing 20% horse serum. An outline of the normal developmental sequence in culture has begun to emerge, and is to be established definitively this year. The first spontaneous action potentials were seen in the second week, consisting of isolated spikes at about 1 min intervals on the average. Brief bursts of spikes then appeared which, by the end of the second week, were occurring every 5-10 seconds. Interspike intervals during these bursts measured about 10 msec at this time. During the third week, the interspike intervals decreased even further, and the bursts were generally much more frequent (every 1-2 sec). During this period of development, fluctuations in overall firing level appeared which followed an irregular time-course at several minutes per cycle.

Structural parameters of cortex development in vitro

It proved possible this year to standardize the culture conditions for dissociated cerebral neurons such that the quantitative development of six classes of synapses could be studied electron microscopically. A sigmoid curve of the numerical density of synapses emerged over the first 3 weeks of culture, and approximated the known time-course of cerebral development in vivo. Thereafter a decline in numbers set in, which is to be the subject of further investigation this year. The initial part of the growth curve also showed a good parallel to the development of bioelectric activity reported above; this correlation will be examined more precisely as more cultures are studied quantitatively. In addition, some interesting early growth phenomena were found, viz.: (1) preferential clockwise outgrowth of neurites (upon certain substrates), and (2) death of those nerve cells which, during the first few days in culture, fail to make extensive contact with glia cells or with other neurons. In addition, a 16 mm time-lapse film was made in which the dynamic character of outgrowing neurons and glial cells was vividly demonstrated.

THEME 2A. SIGNIFICANCE OF REM ('ACTIVE') SLEEP FOR BRAIN AND BEHAVIOUR MATURATION IN RATS

Effects upon adult sleep patterns

Polygraphic studies were made in 1 year old rats which had been treated with chlorimipramine (a blocker of monoamine re-uptake at synapses) for two weeks during early infancy. During treatment, the only apparent effect was a major loss of active sleep (AS) (these were the same animals in which a variety of behavioral abnormalities in adulthood had been reported in last year's Progress Report). Although circadian rhythmicity and the proportions of sleep and waking were the same in experimental and control groups, the former showed a significant increase in the amount of

AS per total sleep time: 11-16% vs. 5-10%. This last phenomenon was due primarily to a higher incidence of AS epochs (3.6 vs. 1.9 per hour recording time) but also to a longer mean duration of the epochs (68 vs. 46 sec) measured over an 8 hr period (in the light). These findings were confirmed in a second series of experiments, in which it turned out to be true only for the *male* of the species. Furthermore, many of the AS epochs in the drug-treated male animals were abnormal, with phasic movements being much more frequent and stronger than in controls. This particular sleep disturbance was also seen in several of the experimental female rats but only very sporadically. The hippocampal EEG during AS was quantified in the males, and the amplitude of theta wave activity was found to be almost twice as high in the experimental as in the control group. This finding too is suggestive of hyperactive AS mechanisms.

Effects upon adult brain morphology

Regional brain weights were measured in collaboration with H.B.M. Uylings (group I) and G.J. Boer (group III) in a series of male and female rats which had been treated chronically with chlorimipramine during early infancy (vide supra). In comparison with control males, the male experimental animals had a significantly enlarged hypothalamus. The cerebral cortex, on the other hand, showed a considerably lower total cell count (ca. -20%), based upon DNA measurements. Weight and total protein were normal, however, suggesting that some cells had undergone a compensatory hypertrophy. Female animals, in contrast, showed enlargement of both the cerebral cortex and the hippocampus, along with a concomitant increase in total DNA and protein, in comparison with control females. The results suggest a possible interaction early in development between monoamine systems or AS mechanisms on the one hand, and sexually dimorphic hormone levels on the other hand.

Neuronal activity in developing cerebral cortex

An improved method of chronic registration of spontaneous multiunit firing, using microwire bundles, has enabled us to make stable recordings up to two days post-op. Action potentials were observed only sporadically during all states of sleep and waking prior to 10 days of age. By 12 days after birth a state-related pattern of firing had become established, with an average firing rate which was 2-3 times higher during AS than during QS (quiet sleep). During sleep after chlorimipramine treatment, neurons fired continuously at the QS level, and also the quantified EEG activity showed a continuous QS character.

THEME 2B. SIGNIFICANCE OF BIOELECTRIC ACTIVITY FOR THE DEVELOPMENT OF NEURAL TISSUES IN CULTURE

Synaptogenesis in dissociated cerebral cortex neurons

In order to study the possible importance of spontaneous neuronal activity in the formation of different classes of synapses, the local anaesthetic xylocaine was added chronically to the culture medium. Absence of bioelectric discharges was verified by electrophysiological recordings. The first results of these experiments have shown that synapse formation is delayed in treated cultures (which may be due to a direct effect of xylocaine upon the outgrowth of neurites) but can reach control values despite the total absence of bioelectric activity. Furthermore, synapses in the treated cultures were perfectly normal in appearance, while complex functional interactions among the neurons appeared as soon as the anaesthetic was washed out of the tissue. Further details of the xylocaine effects are currently being worked out. It is noteworthy that the capacity of the cultured neurons to generate increasingly complex patterns of activity was delayed in xylocaine treated cultures, which is in close agreement with the morphological delay.

Selective innervation patterns by sensory ganglion cells in vitro

Improvements have been made in the culture techniques, so as to allow routine culturing of dorsal root ganglion (DRG) cells for at least 3 months, together with organotypic explants of spinal cord or medulla. Difficulties are still being encountered in staining the sensory fibers to enable their being traced into the 'target' neural tissues: neither degeneration histology, immunocytology (Van Leeuwen, group III) nor horseradish peroxidase (HRP) application have proven completely satisfactory. More experiments with HRP are planned, using modified techniques, and evoked potential methods are also being explored in order to trace the functional projections of the DRG neurons.

THEME 3. DEVELOPMENTAL MECHANISMS IN THE FORMATION OF CUTANEOUS AFFERENT CONNECTIONS IN FROGS

Topography of sensory ganglion cells

Cobalt labelling of neurons projecting to the skin on the back was carried out in skin-rotated and in normal frogs (in collaboration with the Anatomy Dept. of Debrecen Medical University, Hungary). No difference from normal distribution patterns was noted in the experimental frogs, which nevertheless all displayed well-developed misdirected wiping reflexes. In both groups of animals most of the back-skin neurons were located in the dorsal portion of the

ganglion, but the experimental group showed a significantly different cell size distribution. These experiments support our earlier electrophysiological mapping of the ganglia, and thus argue against selective peripheral outgrowth as the mechanism for correct skin innervation patterns. In addition, they suggest that competition among redundant nerve cells during ontogeny is probably the way in which sensory reflex connections are normally established.

Functional innervation patterns of the skin

Cutaneous receptive fields for ventral and lateral nerve trunks were mapped behaviorally (using nerve crush experiments) in skin-rotated and in normal frogs. Misdirected reflexes in the former group were completely abolished in most cases by crushing the dorsal nerve connections to the skin, proving that the

predominant innervation of belly skin on the back goes by that route. The appearance of misdirected wiping responses in the experimental animals therefore cannot be explained by a displacement of ventral receptive fields, i.e. by selective regrowth of those nerve fibers which had originally innervated the belly skin.

SERVICE FOR WORKGROUP III

A developmental series of cortical and hippocampal EEG recordings was made in a group of rats suffering from hereditary diabetes insipidus, along with their heterozygous littermates as controls. The aim was to detect possible differences in EEG parameters during the sleep-cycle which might shed light on the role of vasopressin in brain development. The data have been collected but not yet analyzed.

III. Interaction between the nervous system and hormones during maturation and adaptation

In this project the production and secretion of hormones by nerve cells (neurosecretion) and their action on various target organs, including the brain are studied. Neuroendocrine mechanisms are thought to be involved, among other things, in diuresis, reproduction, brain development, memory, behaviour and also in the central regulation of temperature, blood pressure, and osmolality. Furthermore they may play a role in diseases such as diabetes insipidus, disturbances of pregnancy, lactation, parturition and certain mental disorders. Emphasis is laid upon (1) fundamental aspects of neurosecretion in adults, i.e. on the sites of hormone production, transport and release in rat and man, in relation to learning, behaviour and other central processes, and upon neuroendocrine involvement in (2) maturation including that of the brain itself; and (3) reproduction. Furthermore (4) new methods are being developed to enable the study of specificity in immunocytochemistry, and (5) special attention is paid to the pineal gland by Dr. P. Pévet. The main disciplines include immunological techniques, electrophysiology, light- and electronmicroscopy, biochemistry and clinical observations, the last being performed in collaboration with various university clinics in the Netherlands and the United Kingdom. (6) In collaboration with the Free University (Amsterdam) studies were initiated on the immunocytochemical localization of (a) peptides in the nervous system of *Lymnaea stagnalis* and of (b) astrocytes in the human brain and in brain tumors. Parts of this project are represented in the FUNGO projects nrs. 13-35-07 (with financial support) and 13-35-20.

THEME 1A. THE SITES OF PRODUCTION, TRANSPORT AND RELEASE OF VASOPRESSIN AND OXYTOCIN

Various hypothalamic peptides, such as vasopressin and oxytocin, influence central processes. Transport of these hormones from the hypothalamic cell bodies to their sites of action in the brain is currently thought to occur mainly by direct transport via exohypothalamic peptidergic fibers. To study this route, vasopressin and oxytocin pathways have been specifically revealed by immunohistochemical techniques using the unlabeled antibody enzyme method and purification of the first antiserum. Vasopressin (AVP) and oxytocin (OXT) are synthesized in the paraventricular (PVN) and supraoptic nucleus (SON), while AVP-producing neurons are also found in the suprachiasmatic nucleus (SCN). From these sites of synthesis, extensive projections have been found to the limbic system and to other parts of the central nervous system.

Three types of peptidergic fibre terminations are suggested by light microscopical observations: 1. the 'classical' neurosecretory endings in the neurohaemal regions, i.e. in the neurohypophysis (semithin serial sections stained for AVP and OXT revealed, that AVP-containing fibres enter the neurohypophysis centrally in a bilateral way, surrounded by oxytocin fibres), the median eminence and organum vasculosum laminae terminalis; 2. boutons, ending on dendrites in the lateral septum, lateral habenula and amygdala, and on the dendritic tree of granule and pyramidal cells in the ventral hippocampus and 3. punctate perineuronal structures in e.g. the lateral septum, lateral habenula and amygdala. However, due to the limitations of the light microscopical technique it was impossible to demonstrate conclusively, that these

fibres did indeed terminate on neuronal structures in the CNS. Immunocytochemistry on the electron-microscopic level was employed, using the preembedding staining technique, to establish the shape and mode of termination of these AVP or OXT containing fibers within the limbic system. Thus, synaptic structures, positively stained for vasopressin, with a widened synaptic cleft, were frequently observed in the lateral septum, lateral habenula and nuclei of the amygdala. In addition, an oxytocin containing synapse could sometimes be demonstrated in the nuclei of the amygdala. Although some of the endings were demonstrated on cell bodies, most synapses were seen on dendrites (sometimes terminating 'en passage'). The morphological appearance of these peptide synapses does not seem to differ in any respect from that of the 'classical' transmitter containing synapses. Immunoelectronmicroscopy revealed a fourth type of termination of vasopressin containing fibers. In the medial nucleus of the amygdala such fibers were found to terminate sympathetically on oligodendrocytes suggesting that the neurosecretory fibers operate interdependently with glial cells that may be involved in, e.g. the release or removal of peptidergic neurotransmitters or the uptake of ions.

In order to see whether changes in peptide content could be measured during a behavioral paradigm in which neurohypophyseal hormones are involved, AVP and OXT were measured in 11 punched-out regional brain samples from rats which had been submitted to training for passive avoidance behavior and were sacrificed 5 min. after the application of the footshock. The previous immunocytochemical findings showed that AVP concentrations are generally higher than OXT concentrations in brain tissue, while in the medulla oblongata the reverse is true. However, in no case were the AVP and OXT levels significantly different from those found in the corresponding brain regions of control animals. The time between the learning trial and the moment of sacrifice is presumably a crucial factor in this paradigm.

An alternative approach, i.e. perfusion of specific brain areas by means of a push-pull procedure encountered serious technical problems. We are therefore currently investigating whether or not central release of neurohypophysial hormones can be demonstrated, using biochemical techniques in collaboration with the MRC Unit on Developmental Neurobiology in London.

THEME 1B. THE PRESENCE OF OTHER PEPTIDE HORMONES IN THE NEUROHYPOPHYSIS AND INTERMEDIATE LOBE OF THE PITUITARY

Castel claimed the presence of AVP on receptors in pars intermedia cells on the basis of the positive stain-

ing of these cells with antibodies raised against vasopressin. In the Brattleboro rat (homozygous for diabetes insipidus) we found, however, a similar staining using anti-AVP serum applied to semithin sections of the pituitary. In serial sections the same cells appeared to stain with anti- α -MSH. Since the staining with anti-AVP could be prevented by pre-absorption with α -MSH, the staining reported by Castel seems rather to be based on a cross-reaction to α -MSH than the presence of AVP on receptors.

Since hypothalamic hormones, that were thought to be released in the median eminence, like somatostatin, LHRH and TRH have been reported to be present in the neurohypophysis, in addition to AVP and oxytocin, their respective distributions have been studied using serial semithin sections.

- Somatostatin (SOM) fibers appeared to be present peripherally in the proximal neurohypophysial stalk, and mainly lateral in the more distal parts of the stalk. Although SOM fibers could be observed in the most distal parts of the neural lobe, they seem to terminate rapidly upon entrance. Similar to the AVP fibers SOM fibers were found to enter the neural lobe bilaterally. The staining of anti-SOM in the SON and PVN, as reported in the literature, could be explained by cross-reaction with neurophysins.

- LHRH fibers appeared not to enter into the neural lobe but to terminate mostly on the tubero infundibular sulcus.

- TRH fibers were observed in the external zone of the median eminence and the neurohypophysial stalk. However, in the most distal part of the stalk also fibers containing neurohypophysial hormones were stained with anti-TRH. On the basis of model experiments on agarose beads this most probably non-specific staining could be attributed to binding of anti-TRH to AVP, OXT and bovine neurophysin I and II. However, solid phase absorption of anti-TRH to these compounds did not result in any diminution of this reaction, so that additional factors are probably involved.

THEME 2A. DEVELOPMENT OF NEUROENDOCRINE SYSTEMS

Neurohypophysial hormones might also be involved in brain development (see below). The developmental aspects of the hypothalamo-neurohypophysial system were therefore followed both quantitatively by means of radioimmunoassay, and qualitatively by means of immunocytochemistry. The radioimmunoassayable content of AVP in the fetal rat brain showed a steady rise from day 16 of pregnancy onwards, whereas the OXT content followed a U-shaped curve with high — even adult — concentrations already at day 16, followed by decreasing levels up to day 18 and a gradual rise

later in gestation. The pituitary AVP content increased suddenly from fetal 19, while the OXT content showed its first rise between days 16 and 17.

Immunocytochemistry revealed AVP containing cells in the rat hypothalamus from day 16 onwards in the region of the SON, and from day 17 in the region of the PVN. Exohypothalamic fibers were also visible from day 17 onwards. The AVP producing cells of the SCN, on the other hand, become stainable only shortly after birth. The first AVP containing SCN cells were demonstrated 2 days postnatally. The SCN becomes clearly recognizable from the 7th day, with positively staining neurons and fibers of fine caliber, and by the 12th postnatal day has an essentially adult appearance. In the second postnatal week extrahypothalamic fibers were found to grow from the SCN into the limbic system. It lasts up to three weeks postnatally, however, until its extensive projections into the limbic system are fully mature.

A start was made with the study of the development of the vasopressinergic and oxytocinergic system in the human brain using conventionally formalin fixed samples, which come mainly from the Free University (Prof. F.C. Stam, Dept. of Neuropathology). In the brains of 24 and 35 weeks old fetuses, the various parts of the supraoptic and paraventricular nucleus could be well distinguished. Extrahypothalamic fibers containing AVP and/or OXT were found in the fetal spinal cord from 17 weeks of pregnancy onwards. In a 34 weeks old fetus neurohypophyseal hormones containing fibers were observed in the septal area. In the brain of an 80 years old man exohypothalamic oxytocinergic and/or vasopressinergic fibers were found in the amygdala, hippocampus (in which many branching fibers were visible) and in the septal area, in which many perineural structures were observed.

α -MSH is thought to stimulate intrauterine body- and brain development. Radioimmunoassayable α -MSH was found both in the foetal brain and the pituitary from day 16 of pregnancy. A rapid rise in pituitary α -MSH was found around fetal day 19, which is the day of the intrauterine growth spurt. The α -MSH content and concentration revealed in the fetal rat brain a U-shaped curve with high, essentially adult concentrations on day 16 of pregnancy, and a minimal concentration on day 18 after which the α -MSH content of the fetal brain begins to rise again.

THEME 2B. NEUROPEPTIDES AND BRAIN DEVELOPMENT

Earlier findings on Brattleboro diabetes insipidus (DI) rats have revealed a pre- and postnatal retarded brain development in this mutant, which persists into adulthood. The biochemical data of this study (brain DNA, protein and lipid) could not be related to any other known circumstance of disturbed brain develop-

ment, so that the vasopressin deficit in these animals is thought to be the causal factor of this deficit.

Because in this first study not enough attention was paid to possible differences in the pre- and postnatal environment of the mutants and controls, we have set up a systematical breeding by mating homozygous (HOM)-DI females with HOM-DI and HOM-normal (N) males. HOM- and heterozygous (HET)-DI (control) litters were subsequently raised and nursed under complete identical conditions i.e. with a HOM-DI mother. Postnatal body and brain growth appeared again to be retarded in HOM-DI rats, although the differences between HOM- and HET-DI animals were less striking than in the first study. A specific factor of the HOM-DI mother as cause of the growth retardation in the newborn could moreover be excluded since HOM- and HET-DI pups delivered by HOM-DI mothers and nursed by HOM-N mothers show the same differences.

A series of brain areas have been sampled in collaboration with group I of both HOM-DI and HET-DI litters between postnatal days 1 and 30 (cerebral cortex, cerebellum, bulbus olfactorius, hippocampus, hypothalamus, colliculum, medulla and residue). Weighing and assaying of these areas for DNA and protein is in progress in order to determine which sites, when and how are influenced most strikingly. The most affected zones will be chosen for further investigation on the replenishment of vasopressin in HOM-DI pups.

If vasopressin has a direct effect on brain development, it has to be executed early postnatally, since daily supplementation of vasopressin for one month in 150 days adult male and female HOM-DI Brattleboros either peripherally (subcutaneous injections of Pitressin tannate in a dose normalizing the diuresis), intracerebroventricularly (50 ng/2 μ l via permanent canule in lateral ventricle) or a combination of both treatments, appeared not to restore the deficits in brain weight and cell content of cerebral cortex and cerebellum.

In an approach to elucidate biochemically the mechanism of retarded brain growth of HOM-DI Brattleboro, a first [3 H]-thymidine incorporation study has been set up in collaboration with Dr. A. Patel and Dr. R. Balász (MRC, Developmental Neurobiological Unit, London). Preliminary data point to decreased rates of cell proliferation in cerebral cortex, cerebellum as well as in the bulbus olfactorius at postnatal days 5, 10, and 20.

THEME 3. NEUROHYPOPHYSEAL HORMONES AND REPRODUCTION

The spontaneous electrical activity of neurosecretory cells, which is thought to be essential for hormone release, is usually very low (1-2 action potentials/sec).

Even during activated circumstances, i.e. late gestation and around parturition, paraventricular nucleus cells were found to show an overall mean firing rate of only 3-4 per sec. In order to see if such low frequencies might be of physiological importance, the effect of 6 per sec electrical stimulation of the pituitary stalk *in vivo* upon plasma oxytocin concentration, uterine contractions and intramammary pressure was studied. Stimulation for 30 min at this rate increased the plasma oxytocin concentration. The same type of stimulation elicited uterine contractions in early *post partum* rats, and milk ejection in the lactating rat, but the latter phenomenon did not faithfully mimic physiological events (in contrast with the former). It was concluded that, in contrast to previous literature data, low frequency stimulation of the pituitary stalk *in vivo* is also effective in producing hormone release and, furthermore, that the low mean firing rates of neurosecretory cells may be of physiological importance.

Neurohypophyseal hormones might play a role in fertility, although little experimental data is available on this subject. The Brattleboro rat, in its homozygous recessive form, suffers from hypothalamic diabetes insipidus, caused by the absence of vasopressin. In contrast, the oxytocin release is abnormally high. This mutant therefore seems to be a suitable model for studying this aspect, especially since it appears to show subnormal fertility. From a study of the reproductive capabilities of Brattleboros, it appeared that this subfertility is at least partly of male origin, although the characteristically small litter size in this strain seems to be due to maternal factors (and not to decreased viability of vasopressin deficient fetuses). Moreover, an advancement of the onset, and an acceleration of the course of labour could be demonstrated, which data can be explained in terms of vasopressin deficiency and oxytocin surplus.

Fetal neurohypophyseal hormones were thought to be important for a speedy time-course of labour, as suggested by the protracted course of labour found in human anencephalics and in brain-aspirated rat fetuses. Fetal and new-born pituitary content of oxytocin, vasopressin and α -MSH were measured, in order to assess possible changes in their release during the process of labour. In the 24 hour period during which delivery is likely to occur in the Wistar rat, the fetal pituitary oxytocin and vasopressin, content increased both, whereas the α -MSH content remained the same. However, during and/or just prior to labour, the oxytocin content was found to decrease by 30%, indicating an enhanced fetal release of the hormone at this stage. The actual expulsion of each fetus was concluded not to provide an important extra stimulus for oxytocin release.

In addition, intrauterine residence of the fetus

following decapitation of the mother, was found to elicit a further 30% decrease in fetal pituitary oxytocin content. Neither vasopressin nor α -MSH content were demonstrated to be altered by the process of labour or intrauterine residence following decapitation. This does, however, not exclude the possibility of some release of these hormones, given the fact that their pituitary content is twenty times higher than the oxytocin content in the fetus and newborn.

Neurohypophyseal hormones thus seem to be involved not only in lactation and parturition as far as the maternal neurohypophysis is concerned, but also the fetal posterior lobe plays a role in the latter process, while the neurohypophysis appears, in addition, to be involved in fertilization.

Because of the disturbances found in growth, development and labour, which were found in the Brattleboro rat, a retrospective study was performed concerning these aspects in an Amsterdam family with a hereditary form of hypothalamic diabetes insipidus. Parturition appeared to have taken place at term, the course of labour was not significantly accelerated; neither birthweight, length of the children, head circumference nor postnatal growth were different from the controls. These observations raise the question of whether the small amount of vasopressin circulating in these patients is sufficient to prevent disturbances similar to those described in the homozygous Brattleboro rat, which is not producing any AVP at all.

THEME 4. METHODOLOGICAL DEVELOPMENTS CONCERNING SPECIFICITY IN IMMUNOCYTOCHEMISTRY

The definition of specificity in immunocytochemical localization is that one known compound is stained in the tissue section by the immunocytochemical technique and all the other factors are negative. One aspect of specificity is the specificity of the antiserum for the antigen. To determine impurities, unwanted immunoreactivity and cross reacting antibodies, the antiserum has to be tested in the immunocytochemical localization procedure on the spectrum of compounds derived from the tissue of investigation. Moreover, the immunoreactivity of the antiserum for a known compound has to be determined quantitatively.

Isoelectric focusing on polyacrylamide gels has been chosen as the most appropriate method for making such a spectrum of tissue peptides. We have been successful in extending the pH-gradient up to pH = 11, necessary to separate all possible peptides. After separation the compounds have to be immobilized for the immunocytochemical procedure. We previously described to be able to fix oxytocin in horizontal sections of gels by means of 80°C paraformaldehyde vapour. The concomitant reduction in immunoreactivity ($\leq 5\%$ on the basis of radioimmunoassay)

however, makes this fixation procedure unfavourable. In a further attempt peptides were coupled by carbodiimide prior to isoelectrofocusing hydrolyzed polyacrylamide gels. This resulted in a 60% binding for oxytocin, but deformation of the gel and a shift of the pH-range towards the acid region during focusing makes this approach useless. Since binding to polyacrylamide gels was repeatedly unsuccessful, and also coupling to polystyrene tubes failed, binding of the peptides extracted from the gel onto CNBr-activated Sepharose beads will be tried. It has, however, the disadvantage of giving a discontinuous instead of a continuous spectrum of the tissue compounds.

A quantification of the immunocytochemical reaction both in tissue sections and in a model system, using isolated antigens, is another aspect on the way to 'monospecificity' in immunocytochemistry. In order to reduce the influence of distributional errors on the outcome of the quantification of the coloured end product after an immunoperoxidase (PAP) reaction in an tissue section, the use of a computer assisted microscope cytospectrophotometer with scanning object stage is inevitable. For this reason the FLUOST-microscope spectrophotometer measuring software, as originally written in FOCAL-12, was converted into FORTRAN-IV to enable in the near future their processing on the institute computer. In the meantime, the measurements will be done using the cytospectrophotometer of the Zoological laboratory (University of Amsterdam).

A matrix of neuropeptide-coupled beads is used as a quantitative model system for the immunocytochemical PAP reaction. Instead of the (precipitating) diaminobenzidine as used on the tissue sections, in this model system soluble o-phenyldiamine is used as electron donor. This enables spectrophotometric quantification on the supernatant, which is much simpler in practice than the cytospectrophotometer measurement on DAB-stained beads. The moment the relation between the reaction in the model system and in tissue sections has been settled, purification of the antisera up to more specific coupling agents, can be started.

THEME 5. PINEAL HORMONES

Mammals have evolved neuroendocrine mechanisms which ensure that, under natural conditions, their young are born only during a restricted period of the year, thus permitting the survival of the species. The pineal acts as intermediary between the environment and such reproductive processes, which makes it a good model for studying this particular adaptive process of the brain to the environment.

In this framework, especially the peptidergic hormones that are synthesized by the pineal gland, are be-

ing studied. The results obtained by means of immunocytochemistry and radioimmunoassay (RIA) permit us to assert that arginine vasotocin (AVT) which was regarded by various authors as *the* antigonadotropic hormone, is in fact not present in the mammalian pineal. Moreover, using a new bioassay for vasotocin activity (in collaboration with Dr. J.M. Holder and Dr. B. Vivien-Roels, France), a vasotocin-like activity has been observed in bovine and ovine pineal (3-5 pg/pineal). A fraction called E5, isolated from bovine pineal (Dr. N. Neașu, Romania), and free of vasotocin as determined by RIA, presents a biological activity similar to that of AVT, at least in the test which we used. It appears (collaboration with Dr. A. Reinharz, Switzerland), that a compound of this fraction has a carboxyterminus, similar to AVT and AVP (Pro-Arg-Gly (NH₂)). This tripeptide (graciously synthesized for us by Organon, Oss, The Netherlands) proved to be devoid of biological AVT-activity.

The identification of AVP and OXT containing fibers in the SCO, the pineal stalk and the anterior part of the pineal demonstrates clearly that AVP and OXT measured by means of RIA in the pineal gland and SCO of mammals can be explained by the presence of extrahypothalamic AVP or OXT containing fibers.

AVP, OXT, neurophysin I and II (collaboration with Dr. A. Reinharz, Switzerland) have been measured in the bovine pineal gland. The ratio of OXT and AVP appears to be similar to that of neurophysin I to neurophysin II. This similarity suggests that neurophysins present in the pineal are the carrier proteins of the neurohormones AVP and OXT, rather than of AVT.

Using immunocytochemistry and antibodies against AVT, α -MSH, LHRH and somatostatin, immunoreactive cells were found in the rat pineal gland. All of these antibodies reacted with the same cells, which could also be stained by means of an antibody raised against the UMO5R sheep pineal fraction (a fraction that presents antigonadotropic properties *in vivo*, which was prepared by Dr. I. Ebels, Utrecht). Relatively more immunoreactive cells were found in the pineals of young rats than in the pineal of adult animals. Comparison of the results obtained with different potent antibodies against each of the peptides, and a study of the staining properties of the antibodies in the pineal after solid phase adsorption to different peptides or to different sheep pineal fractions led to the suggestion that the immunoreactivity found in the rat pineal is not due to the presence of genuine AVT, α -MSH, LHRH or somatostatin, but rather to an unidentified compound reacting with each of these antibodies. This factor is synthesized in the pineal gland, as was demonstrated using cultured pineals. The

UMOSR fraction and the progonadotropic Prot. 4 fraction of the sheep pineal seem to be chemically related to this unknown compound. That this unknown compound is a biologically active principle is at present only suggested by its more pronounced presence in the young rat. From our immunocytochemical study we suggest that the different biological activities of the mammalian pineal gland could depend on a yet unknown compound, a large common precursor that is cleaved to give rise to a number of small biologically active compounds. The different secretory processes described for the mole fetus possibly represents the morphological basis of such a phenomenon. Considering their synthetic/secretory activity, the pinealocytes of the mole fetus are characterized by the presence of accumulations of proteinaceous material (APM) in the cisternae of the granular endoplasmic reticulum (GER). Two types of APM were found. One type shows a paracrystalline organization as has also been described in the adult mole. In the fetus, however, the paracrystalline-organized APM occur only infrequently. A progressive transformation, via an increase in size or by fusion, of vacuoles containing material originating from the cisternae of the GER in APM has been observed. Moreover, granular vesicles, (GV) originating from the Golgi saccules were sometimes found, together with APM, in the same cell. GV and APM are the characteristic final morphological products of two secretory processes involved in the synthesis and release of proteic compounds. The fact that both of them can occur simultaneously in a given cell leads to the conclusion that the two above mentioned processes are not necessarily independent, at least in the mole, but are different morphological aspects for elaboration of a protein complex.

THEME 6A. PEPTIDERGIC NEURONS IN THE POND SNAIL, *LYMNAEA STAGNALIS*

In collaboration with members of the Biological Laboratory of the Free University of Amsterdam (L.P.C. Schot, H.H. Boer, E.W. Roubos, A. ter Maat, J.C. Lodder and D. Reichelt) a study was initiated on the presence and possible relevance of peptides in the central nervous system (CNS) of the pond snail, *Lymnaea stagnalis*. CNS were fixed for 24 hours in a mixture of glutaraldehyde, picric acid and acetic acid or in Bouin Hollande and sections (6-8 μm) were stained with the unlabelled antibody enzyme technique using antisera to various vertebrate peptides.

The CNS of *Lymnaea stagnalis* consists of paired buccal, cerebral, pedal, pleural and parietal ganglia and of a single visceral ganglion. With anti-AVP two groups of about 20 cells were stained in each cerebral ganglion. These cells have small projections to the

neuropile of the cerebral ganglion. In the pedal ganglia two groups of about 15 cells were stained. The cells of one group have fibres running to the cells of the other group, while there are also fibres running via the pedal commissure to the contralateral pedal ganglion via the pedo-cerebral connective to the cerebral ganglion and via the pedopleural connective to the pleural ganglion. In the cerebral ganglion many thin fibres are present. Some fibres to terminate while others are running to the contralateral cerebral ganglion. From the pleural ganglion some fibres run via the parietal ganglion to the visceral ganglion. In the buccal ganglia two positive cells and many thin fibres were observed. Some fibres seems to terminate while others are running to the contralateral cerebral ganglion. From the pleural ganglion some fibres run via the parietal ganglion to the visceral ganglion. In the buccal ganglia two

In addition to the cells that also stained with anti-AVP, a few other cells were found to stain with anti-AVT in the pedal ganglion. In the buccal ganglia 4 neurons were stained, in the cerebral ganglia about 6 and in the visceral ganglion about 30 with anti-AVT. Positive fibres were found in all ganglia. Some fibres seem to end on other neurons. Also outside the CNS positive fibres were observed e.g. between muscle fibres of the dorsal aorta.

With anti-oxytocin about 10 neurons were stained in the visceral ganglia and with anti- α -MSH about 15 cells in each pedal ganglion. With anti-gastrin about 20 cells were identified in each cerebral ganglion. These cells have fibres running through the cerebral commissure to the contralateral cerebral ganglion and through the cerebro-pleural connective to the pleural ganglion. In the pedal ganglion tree groups of about 30 cells were identified.

Radioimmunoassay revealed the presence of small amounts of the peptides. Per CNS, 50 pg vasopressin, 10 pg oxytocin, 59 pg α -MSH, and 20 pg ACTH were found. Isoelectric focusing separation followed by oxytocin radioimmunoassay (H. van Pelt-Heerschap) suggested, however, that the snail nervous system does not contain genuine oxytocin. The immunocytochemical study suggests that many peptides might be present in the snail nervous system that are related to those known to be produced by the mammalian brain. In addition, it provides a useful technique for distinguishing types of nerve cells in the snail nervous system, and for tracing their neurites throughout the nervous system. It is hoped in this way to find a preparation for a neuron that is innervated by peptidergic synaps which can then be studied in a more fundamental way, e.g. by means of electrophysiological techniques, than would be possible in the mammalian brain.

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 W. Kamphorst (Dept. of Neuropathology, Free University, Amsterdam), a study was therefore initiated to apply antibodies raised to glial fibrillary protein acid (GFA), a marker for astrocytes, using the unlabelled antibody enzyme technique. Brains from three aged men were systematically stained by this procedure and the distribution of the 5 types of positively staining glial cells (sclerotic-, large stellate-, smaller gracile-, elongated-, and Bergmann cells) are described. However, also positively staining neurons were observed in the visual cortex and in many axons in the white matter, so that the antibodies used can not be con-

sidered as absolutely specific for astrocytes. The staining appeared also to be positive in a number of brain tumors, and was found to be more sensitive in staining fibers than the conventional techniques.

MISCELLANEOUS

As in previous years the radioimmunoassays for vasopressin, vasotocin, oxytocin and α -MSH have been applied to a great number of studies of members of the group itself as well as to collaborative studies with investigators in other laboratories. The projects with Dr. D.J. Drewett on human lactation were continued, as was the project with Prof. B.T. Pickering and Dr. R.W. Goodfellow on human labour. A project was started with Drs. Ruis and Corbey of the 'Groot Gasthuis' at 's Hertogenbosch on the effect of oxytocin administration per spray upon lactation in women.

IV. Development and correctability of behaviour

Research in this project is concerned with the study of social behaviour (aggression and sexual behaviour), emotional and learning behaviour. These modes of behaviour, which generally exhibit clearcut differences between the sexes, are being studied in relation to the consequences of gonadal hormones and environmental factors acting during pre- and early postnatal development, and with the neural substrate involved in their regulation.

During 1979 the work continued along 3 lines: (1) analysis of motivational and emotional aspects of sex-differences in behaviour, (2) sex-differences in aggression and learning and the effects of gonadal hormones, and (3) 'behavioural functions' of the prefrontal cortex. Moreover, an increasing interest on the part of other groups at the Institute in the study of behaviour, stimulated experiments of behavioural effects of REM sleep deprivation and studies on motor skills and learning in Brattleboro rats.

Parts of this project are represented in FUNGO (Development of Brain and Behavior as well as Behavioral Mechanisms), BION (Ethology), and PSYCHONOMY (Comparative and Physiological psychology).

THEME I. DEVELOPMENTAL ASPECTS OF LEARNING AND OF SEXUAL AND AGGRESSIVE MOTIVATION

Experiments were started this year, aimed at an analysis of sexual motivation in the rat. As a first approach, receptivity, attractivity and proceptivity behaviour (indicating the urge to engage in sexual activity) was studied in females treated with estrogen (E),

estrogen + progesterone (EP), testosterone (TP), or oil as a control (O). Animals were tested both in a Y-maze, with sexual interaction as reward, and in a semi-open field where males could give an indication for the attractivity of these females. In addition, a sexual interaction of these females with a male of proven sexual vigor was analysed in detail using video and the 'White system' analysis method. The results indicate that these techniques can be reliably used to study sexual motivation in female rats. Estrogen + progesterone, but also testosterone increased attractivity and sexual motivation as well as receptivity in this experiment. The work will be extended to the male aspects of sexual as well as to aggressive motivation.

The analysis of mutual influences of specific components of aggressive and sexual behaviour was further studied in confrontations of male or female WEZOB and S3 rats. In pairs of animals, in which either one or both had been treated with testosterone, sexual and aggressive behaviour was registered in order to investigate a possible interaction, or interdependence, of these two systems. The now restructured and extended program of the 'White system' computer analysis of behaviour, worked out in the computer department (see group V) enabled a detailed analysis in this respect. No clearcut relationship between sexual and aggressive behaviour could be determined, either within a given individual or in the interaction between the two males or females, although some significant correlations and sequences were found. This work is now being further studied in pairs of animals treated with estrogen or testosterone, in which higher levels both of aggressive behaviour and of masculine and feminine sexual responses were induced.

The work on the ontogeny of sex-differences in passive avoidance behaviour was completed this year by the inclusion of groups of respectively 30 days old males and females trained with a high (1.6 mA) shock intensity. The results confirmed earlier observations that, in prepuberty, female rats surpass males in passive avoidance learning. When retested again in adulthood, however, males but not females showed better performance in passive avoidance learning. This finding confirmed the reversal with maturity of the sex difference in passive avoidance which had earlier been observed using lower shock intensities.

THEME 2. SEX-DIFFERENCES AND HORMONAL FACTORS IN THE DEVELOPMENT OF AGGRESSION, SEXUAL BEHAVIOR AND LEARNING

As in most mammalian species, male mice and rats are generally assumed to show more intraspecific fighting than females. This phenomenon is usually associated with 'organizing' and 'activating' effects of male gonadal hormones. This hypothesis was tested in male and female rats of two strains: the WEZOB and the S3 rat. Experimental procedures consisted of gonadectomizing the animals and subsequently treating them with either testosterone (TP) or oil (O). The hormonal status (i.e. oil or TP treatment) of the animals tested against each other was varied systematically, thus resulting in four groups for both males and females of the two strains: oil vs oil, oil vs TP, TP vs oil and TP vs TP. Behaviour was recorded on video tape, and for each animal 9 aggressive and other social parameters of behaviour (including mounting) were scored. Aggressive behaviour occurred in all four types of encounters between males and females of both strains. In females (WEZOB and S3) and in S3 males, TP treatment resulted in an increase in aggression as compared with oil-treated groups. In WEZOB males the relatively high level of aggression in the oil-treated group was not further increased by TP treatment. A further analysis of differences in aggressive behaviour (using discriminant analysis) pointed to sex-differences in aggression in oil-treated animals of the WEZOB strain only. These results thus cast serious doubt on the hypothesis of organizing effects of testosterone during development as regards intraspecific aggression in the rat.

The results of these first series of experiments without doubt indicate that females as well as males readily show aggression when treated with testosterone in adulthood. Recent experiments on sexual behaviour reported in the literature emphasize the relevance of aromatization processes in specific regions of the brain and give evidence that the observed behavioural activation was in fact due to estrogen produced by the aromatization of testosterone. Earlier experiments on

sexual behaviour at our lab indicated that activating effects of estrogen and testosterone could be dependent upon the presence or absence of testosterone during early postnatal development (i.e. on an 'organizing' action of testosterone). Based on these results activating effects of estrogen and testosterone in intraspecific aggression are now being investigated in male and female rats. These experiments were started in collaboration with Dr. N. Bowden and Dr. P. Brain (Swansea, U.K.). To enable a separation of peripheral and central effects, whereas effects of these hormones on pheromonal glands will also be assessed.

Females confronted with other females often show masculine sexual responses. This phenomenon is of special interest since these animals cannot possibly have been affected by endogenous testosterone during development. It is hypothesized, that this behaviour, which occurs in some but not all females, could be the reflection of a slight degree of masculinization of the nervous system, resulting from testosterone stemming from male fetuses in utero. This hypothesis was investigated using female rats from 'all-female' litters versus females from litters predominantly consisting of males. These litters were kindly selected by the Central Institute for the Breeding of Laboratory Animals - TNO Zeist. The groups were treated in adulthood with minimal dosages of estrogen + progesterone or with testosterone.

The results in no way support the hypothesis about prenatal masculinization as a result of hormonal stimulation stemming from male littermates: masculine responses occurred frequently in both groups, and equal levels of lordosis were established during estrogen as well as testosterone stimulation in these groups.

This year, several parameters of behaviour were analysed in an attempt to shed light on the sex-differences in passive avoidance learning. Results of previous experiments were reanalysed with the aid of non-parametric point bi-serial correlation coefficients, in order to find out if sex-differences in response inhibition on the retention trial (i.e. successful learning) could be related to individual differences in response latencies during the pre-shock trials. The low correlations obtained suggest that sex-differences in pre-shock trial latencies make at best only a minor contribution to the sex-differences on retention trials.

In further experiments, male and female Wistar rats were tested during five daily sessions in an open field and in a special testcage for measuring dark preferences. These animals were subsequently run in a passive avoidance learning experiment. In the open field, females showed much higher levels of ambulatory activity and rearing, and less defecation than did males. No sex-differences in dark preference were

observed: equal amounts of time were spent in the dark compartment by male and female rats. Females visited this compartment more frequently, however, in the passive avoidance test, the percentage of male rats showing response inhibition was much higher than in the females. Correlational analysis is currently being performed in order to find out whether any parameters can be found in the open field and the dark preference tests that are strongly related to the sex-differences in avoidance learning (and thus might account for the sex-differences in this learning task). In addition, possible relationship between the stage of estrous cycle and the behavioural results in the female are under study. A preliminary experiment showed that the amount of preshock experience (trials in the passive avoidance test without shocks) is an important, positively correlating determinant of response inhibition on the retention trial in the males. It is still unclear if it is also important for the sex-differences in passive avoidance as such.

The results on passive avoidance – conceived as an approach/avoidance conflict – indicate that avoidance tendencies dominate in males in this test whereas in females approach tendencies are dominant. Memory deficits per se, do not account for the differences in learning, since our earlier experiments clearly showed that the majority of females do not inhibit the response even when tested immediately after receiving the shock. On the basis of these findings, experiments were started to test the hypothesis that males possibly attend more to the spatial aspects of the test, whereas females generalize fear to the test situation as a whole. For this purpose, a test in the Skinner box has been created in which an approach/avoidance conflict can be studied in which spatial aspects are irrelevant for learning.

Male and female rats were tested in the Skinner box under a learning schedule in which food as a reinforcer could be obtained by continuously depressing a lever during 10 secs. After sixteen daily sessions, the program was changed in such a way that the animal received a mild shock also when the criterion for food reinforcement was met. This stage of experimentation lasted for two daily sessions. The original program was resumed thereupon, for 24 days. At the end of the preshock stage, about seventy percent of the responses met the criterion in both males and females. In this way about 80 reinforcers per session were earned. Shock treatment (although much lower than normally used in passive avoidance learning) proved to be a very effective response suppressor. Responding resumed only gradually, and many animals still had not reached pre-shock levels at the end of experimentation. Females, however, resumed responding significantly sooner than did males. Further analysis of these data with the aid of the computer will be started as soon as

the necessary programs are available. Attention will then be paid to the so called abortive responding (i.e. termination of the response before reinforcement criteria are met), which is especially present in the post-shock sessions.

THEME 3. THE NEURAL SUBSTRATE UNDERLYING AGGRESSIVE AND SEXUAL BEHAVIOURS

The prefrontal cortex (pfc), defined as the projection area of the nucleus dorsomedialis thalami, is involved in a variety of behavioural functions. Of special interest for our research are its possible functions with regard to social interactions (aggressive and sexual) and sexually dimorphic learning tasks. Possible structural differences between the sexes during development are also being studied.

In male rats of the WEZOB strain thermal RF lesions were made in either the orbito-frontal or the (dorso-)medial part of the pfc. Following a post-operative recovery period, aggressive behaviour was studied in encounters, both with male Wistar rats and with sham-operated WEZOB rats. Sexual behaviour was studied in encounters with female Wistar rats, hormonally induced into oestrus. Analysis of both experiments revealed that sexual behaviour is affected neither by orbito-frontal nor by (dorso-)medial pfc lesions. In contrast, in our testing situation of male aggressiveness, rats which had received orbito-frontal pfc lesions, either at a prepubertal or postpubertal age, showed an increased aggression level resulting in a dominant position over their sham-operated controls. In encounters between WEZOB and Wistar males, both lesioned and sham-operated WEZOBs usually became dominant over their Wistar opponents, while no differences in levels of aggression between lesioned and sham-operated WEZOBs were observed in these tests. Lesions of the (dorso-)medial pfc did not result in noticeable differences in aggression from the control (WEZOB) rats. Histological analysis of orbito-frontal and (dorso-)medial pfc lesions is in progress, and involves a precise determination of lesion site and size, using surface measurements from 100 μ Nissl-stained sections.

In a subsequent experiment, started at the end of 1979, both male and female rats of WEZOB strain are being used, which were lesioned in the orbito-frontal pfc at a postpubertal age. Aggressive behaviour will be studied as in the previous experiments, while additional behavioural tests will involve observations of inter-male social interactions following a severe aggressive experience, i.e. defeat by a more aggressive rat.

As an alternative to thermal RF lesions, a preliminary experiment has been carried out (with the help of H. Pronker) to study the effect of cortical administration of kainic acid. This neurotoxin is

reported to destroy cell bodies, leaving passive nerve fibers undamaged. The first results look promising and justify additional experiments.

The analysis of the histological material prepared to study the structural development of the pfc in male and female rats (cf. the 1978 progress report) is in progress. The structural differences in the size of the medial preoptic nucleus between male and female rats, recently reported in the literature, could be ascertained in this material.

BEHAVIOURAL EXPERIMENTS IN COLLABORATION WITH OTHER WORKGROUPS

Together with group II, the behavioural consequences of chronic deprivation of active sleep (AS) by means of chlorimipramine, during postnatal development were investigated. In an earlier experiment, AS deprived animals and controls were tested in a series of behavioural tests: open field, sexual behaviour, mouse-killing, and aversively and appetitively motivated learning. A significant difference between drug injected and saline control groups was observed both for ambulation in the inner part of the field and for rearing, suggesting that drug treated animals show less exploratory behaviour in the open field test. In addition, the low levels of intromissions and, even more so, of ejaculations in the experimental group suggested that consummatory (as opposed to motivational)

aspects of sexual behaviour were affected. Temporal and spatial discrimination was tested in a Skinner box. No differences were found between the groups, either on differential reinforcement of low rate, or on spatial alternation learning. However, higher response rates were observed in the experimental group, with an accuracy equal to that of the controls. This effect was increased further when training was resumed two months later. In a second experiment, the results on open field and sexual behaviour were replicated with groups of males treated with lower dosages of the drug. Further experiments on male and female rats are planned for 1980.

In order to assess behavioural consequences of the congenital absence of vasopressin during development in 'Brattleboro' rats, exploratory investigations were performed on motor coordination. Tests for motor behaviour were developed, since the cerebellum of homozygous Brattleboro rats lags far behind that of the heterozygous conspecifics in development. Slight effects could be established with some of the tests used but further research will be necessary, as weight differences are possibly involved, and undernutrition in diabetes insipidus rats could thus be a confounding factor. These tests will be further developed for use in experiments concerning the rehabilitation of effects of undernourishment by enriching the environment during early life (group I).

V. Neurophysiological aspects of maturation and adaptation of the nervous system

Group V is currently in a transitional phase, in which (1) the cerebral bloodflow project, which is rounded off, has not proceeded because De Blécourt had to fulfill his military service and (2) the several vacancies of this group are temporarily used in the other groups. Therefore account will only be given concerning the computer department.

COMPUTER DEPARTMENT

INSTRUMENTATION

The department uses two configurations, the first of which is an IBM 1130 with 16K words core memory, card reader/punch and papertape reader/punch combinations, interfacing for A/D and D/A conversions, printer, graphic display, plotter, console and 2 disk-drives. The system is running under D.M-2 mod. 12. The WDV-Ampex magnetic tape subsystem was taken out of operation this year.

The second installation is an Interdata model 70 with 64K bytes core memory, printer, card-reader, papertape reader and punch, console display and 2

local terminals, dual disk-drive and magnetic tape subsystem. The plotter and the graphic display can be shared with the IBM 1130. The operating system used is OS16/MT2. The WDV A/D and D/A interfacing, together with the SAC-channel splitter/multiplexer to the Ampex tapeunit has been taken out of operation because of poor quality and will be replaced in future. Several real-time on-line experiments are connected to the Interdata.

As the current needs for automation of experiments and computational power greatly exceed the capacities of the present systems, and since the IBM 1130 will soon be taken out of operation because the costs of maintenance and technically unwarranted exploitation, a market survey was started in order to select an appropriate computer configuration for the next decade. Hence, a report was composed for submission to the relevant committees.

CONVERSION FROM IBM TO INTERDATA

White system

The conversion of the White system from IBM to Interdata has reached its final stage. The program has thoroughly been modified to meet the current used re-

quirements and to utilize all facilities as offered by the Interdata computer. Thus, the possibility was created to do a simultaneous recording from all 7 channels from the Analog/7 instrumentation recorder. The White system software includes the following features: 1. time-optimal parallel decoding of up to 7 channels of instrumentation tape, 2. selection of groups according to given criteria, 3. manipulations with key-settings and corresponding intervals before advanced analysis, 4. file management capacities, 5. frequency, latency and duration values can be used as input for a. factor analysis, b. discriminant analysis and c. parametric and non-parametric tests, and 6. analysis and display of time patterns of behavioral items such as transitions and coincidences.

The interface, as developed by the electronics department, has been connected to the institute's digital multiplexer. The acquisition software uses a special interrupt handler, ignoring the operating system in this respect, in order to reduce the interrupt-response time 15-fold.

Statistics

Several stand-alone hypothesis testing programs for t-tests, Kruskal-Wallis and Mann-Whitney tests have been converted from the IBM to the Interdata, using a new free-format reading procedure that allows disk-files to be used as input.

COMPUTER-AIDED SCANNING OF DENDRITES

Acquisition

After the extension of the number of labels to 16 over the year 1978, the last remaining bugs with respect to the correction procedure were solved. Moreover, the number of error messages has been increased in order to obtain a fail-safe performance.

Analysis

The data processing possibilities have largely been increased to include a number of features offered by the original IBM Rochester-data program. At this stage, 1 to 5 groups (selected according to various criteria) can be formed, which are to be subjected to statistical post-processing. The following items can now be selected for statistical post-processing: cell surface, radial distance of any labelled point, segment lengths and summed segment lengths. In the case of segment length and radial distance, a choice can be made from intermediate segments, end segments, and cut segments or all together. The segments in turns can be chosen either from basal or radial dendrites, but this selection can be skipped on request. Work on this subject is still in progress. The statistical post-processing used on the selected data is performed by means of the

hypothesis testing procedure as mentioned under the chapter CONVERSION.

DIGITIZER TABLEAU

After the coupling between the digitizer system Kontron MOP AM/02 and the Interdata computer was established, an elementary data-acquisition program was developed in order to get automatic data storage on mass memory. Data are separately recorded according to their functional significance. A file-management section will soon be achieved; work with respect to the processing of these data is in progress.

RADIO-IMMUNOASSAY

The RIA-program has been modified with respect to the data-reading section. Reader-device status-sensing has been used to circumvent the procedure of the feeding of 'zero-tapes'. This in order to minimize awkward operator intervention as usual before.

GRASON-STADLER SYSTEM

Acquisition

After numerous problems showing up with the data acquisition, the principles of operation were modified and the program code was thoroughly revised. The modifications implied a minimization of the number of disk-actions and seek time, further a changed I/O-wait procedure to keep the program susceptible for external interrupts, and finally a redefinition of the duration of an animal trial.

Operator intervention was decreased in this way. Moreover, the program had to be corrected for situations with more than 42 animals to be tested during one session.

Analysis

The analysis program has been largely extended to include the possibility of statistical analysis of multiple events. These events can be defined as a flexibly browsed complex combination of cage parameters. Statistics can be applied to data per animal as well as to groups. At this stage, data are reduced to histograms in an interactive manner. Processing has been based upon a great variety of selection possibilities. The features of this system will gradually be increased in future.

STATISTICS

The integration of the statistical subroutine library into a consistent package, as started in 1978, has been considerably increased. The system has been designed for interactive use with free-format input. As the package is still running on the IBM, the use of punch-

ed cards as an input medium is mandatory; however, on the future computer system, any input device will be usable, inclusive disk-files, created as output by other (acquisition) programs.

The next chapters have been added to the already-existing variance analysis section: 1. polynomial regression, 2. multiple linear regression, 3. a data-screening package, containing a characterization of distributions, one- and two-way tabulation and a histographic representation, 4. a hypothesis testing package for ordinally scored data, containing a. one sample Kolmogoroff-Smirnoff analysis, b. the following unrelated sample tests: Kruskal-Wallis, Mann-Whitney and Kolmogoroff-Smirnoff (more samples), c. the following related sample tests: Friedman, Wilcoxon, and L-test, d. the following correlation analysis features: Spearman rank-order correlation, Kendall rank-correlation and partial rank-correlation, and Kendall coefficient of concordance, 5. factor analysis (max 19 variables), and 6. discriminant

analysis (max 16 groups, max 19 variables). The last two programs contain greatly improved mathematics as compared with the IBM SSP program library.

HARDWARE DEVELOPMENTS

See progress report of the electronics department with respect to the White-system interfacing and the coupling of the Kontron MOP AM/02 digitizer.

PRODUCTION SERVICE

Acquisition and analysis programs for observations using the White system and Grason-Stadler system have almost daily been used. Also radio-immunoassays and dendrite data were frequently processed. The IBM statistical package has been of extreme value for the interpretation of (especially behavioral) data. The semi-automatic dendrite tracking system could not be used because of mechanical trouble in the control of the Zeiss-table.

Monoaminergic mechanisms in the brain; action of tricyclic antidepressant drugs

The main line of investigation that has been performed at the Department of Biological Psychiatry of the State University of Groningen, in collaboration with Dr. J. Korf, has been the influence of chronic administration of tricyclic antidepressant drugs on serotonergic mechanisms in rodent brains.

A. Rapid postmortem changes in mouse cerebral 5-HT and 5-HIAA levels

As was reported in the 1978 Progress Report, postmortem manipulations of brain tissue of decapitated mice can produce a decline in the levels of 5-HT and a rise in the levels of 5-HIAA. Both enzymes for the synthesis and the degradation of 5-HT might play a role in this phenomenon. The administration of the MAO inhibitor Pargyline appeared to prevent postmortem changes. This effect might be of importance for turnover measurements of 5-HT, especially if MAO inhibitors are used on a short-term basis of these drugs, since suppression of postmortem artefacts may not be constant over time.

We have now reestablished 5-HT and 5-HIAA time courses after Pargyline administration (75 mg/kg, i.v.) in mice under various conditions, using time intervals of 15 min. The animals were killed either by decapitation or by microwave irradiation, and one brain half was given a treatment that facilitated postmortem changes (*i.e.* a combination of freezing and thawing, mincing, and exposure to air at room temperature). The results show that involvement of postmortem changes leads to high turnover values for 5-HT in

decapitated animals. This could at least partly explain the divergent values reported in the literature for such measurements. Our observations stress the importance of rapid postmortem handling of animals and brain tissue and the use of microwave irradiation in such investigations.

B. Distribution of imipramine in the brain

Another aspect of our investigations is the cerebral distribution of tricyclic antidepressant drugs and notably its relation to the various actions of these drugs in the brain (e.g. the classical effects on uptake in nerve endings and turnover of NA and 5-HT, and also recent findings such as in vitro specific high-affinity labelling to brain membranes and the preferential localization in synaptic areas).

We injected (*i.v.*) small amounts of ³H-imipramine of high specific activity. Several brain parts and whole blood were counted. An uneven localization of the radioactivity was demonstrated. The activity was relatively high in the frontal and occipital cortex, intermediate in the striatum, and low in the hypothalamus, brain stem and cerebellum, with a ratio of the highest to the lowest activity of about 1.7. This distribution was apparent within two minutes, and persisted up to at least four hours after injection, although the ratio of activities tended to decrease with time. The distribution of radioactive imipramine was different from that reported for several neurotransmitters including NA, DA, 5-HT and GABA. Blood levels were roughly one tenth of those in the brain. In order to see if receptor binding was involved, it was tested whether the uptake of radioactive imipramine

was saturable. A dose-response experiment showed that the uptake and distribution of radioactivity were unchanged over a range from 0.5 and 5000 micrograms/kg. Apparently the distribution did not depend on receptor binding.

Electronics workshop

A description of some of the larger projects developed in this department is given below. The mechanical parts of the apparatus were built in the mechanical workshop.

1. INTERFACE

The construction of an interface which started in 1978 and was designed for rapid feeding of data from magnetic tape into the computer system, has been completed and tested in the first half of 1979. By means of this interface we are now in a position to simultaneously feed 7 channels of data into the computer, thus reducing the time required for such an operation by 150 minutes. The existing programs have been ably converted by J. van Pelt to conform to this new interface.

2. MICRO-PIPETTE DEFLECTOR

By means of a slender spiral heating element, the glass micro-pipette is heated to pliability, after which graduated weights press the malleable tube into a predetermined gradient. The mechanical workshop designed and constructed the deflector, while the electronics workshop realised the electronic steering device involved.

3. LEVEL-DETECTOR

For a selective passage of EEG-signals containing action-potentials of various amplitudes, two level-detectors were constructed designed to allow the pass-

Presently, we are examining which other factors might control this distribution. Since the distribution of imipramine is similar to the regional pattern of cerebral blood flow, attention is being focussed on this possibility.

ing of these signals only in case their potential-differences exceed pre-determined and adjustable levels. At the output-phase of the detector, the following signals are available to the operator:

- a. the input signal as such.
- b. the above-level input signal.
- c. the right-angular p.d. of adjustable amplitude.
- d. the various signals can be presented audibly by means of amplifier and loudspeaker.

4. SEMI-AUTOMATIC MEASURING SYSTEM FOR MICROSCOPES

In co-operation with the mechanical workshop, an extensive investigation as to the cause of the deviations in Zeiss semi-automatic measuring-systems was conducted. It transpired that Zeiss construction works at Göttingen were incapable of reproducing backlash-free (with a tolerance of 1 micron) scanning-tables from a master-model designed by Zeiss engineers at their research laboratory at Oberkochen. Having succeeded in convincing Zeiss research department of the discrepancies in the measuring methods in regard to checking the accuracy of the scanning-tables manufactured by their construction works, Zeiss engineers appeared incapable of offering a solution. Within the NIH technical services, a start has now been made to design a prototype scanning-table with an accuracy of displacement in the x, y and z-axis of .5 micron, whereby among other refinements, the 24 volt stepping electro-motor will be replaced by a low voltage DC-motor, and incorporating a feed-back loop for continuous monitoring of the actual displacement of the axes.

Mechanical workshop

An enumeration of some of the major projects developed in this department is given below. Electronic equipment for these projects was developed in the Electronics Workshop.

WORKGROUP I

1. *Semi-automatic dendrite measuring system.* After investigating the cause of mechanical errors in the 0.5 μm scanning-stage of the Zeiss semi-automatic den-

drite measuring system, the Zeiss production works in Göttingen were visited and a solution was found. Movement in the Z-axis direction of the measuring microscope has been improved by tempering the ball-bearings.

To prevent future problems with the measuring system as a result of wear and tear of components, a prototype for an improved system was made in collaboration with J. Overdijk of the Electronics Department.

2. Several gadgets were made for the 'enriched environment cages'.

3. About 2000 aluminium slide holders were made to store the histological slides of the collection of Prof. C.U. Ariens Kappers.

WORKGROUP II

1. Construction of four 6-channel mercury turning-contacts, to be used in making recordings from freely-moving rats.
2. Construction of several holders.
3. Construction of a rat-cage for use during recording experiments.
4. Modification of four culture-ovens to make them suitable for CO₂ gassing.

WORKGROUP III

1. Construction of two plastic incubation boxes.
2. Construction of a cooling-spiral in the vibratome.
3. Construction of three incubation-plates suitable for use with small volumes of fluid.
4. Construction of a device enabling small quantities of microporous plastic powder with peptide solutions under vacuum to be loaded discretely.
5. Modification of two circulation-pumps with delay for microscopic work.

WORKGROUP IV

1. Four test cages were built for measuring sexual motivation in rats:
 - a. two 'semi open field' cages to measure sexual preference or attractivity; on all four sides of these small metal cages, a partition grid can be placed to

divide the field. Walking patterns of the experimental animal in the open field are scored by dividing the field into 9 equal parts.

- b. two Y-mazes with a Y-formed runway leading to two goal boxes in which sexual stimulation can be given by a second animal as a reward.
2. Two test cages were built to determine the behaviour of rats under varying conditions of ambient light: two square compartments, connected by an obtuse-angled runway, can be lighted in such a way as to produce a dark-light gradient in the alley.
 3. Two cages were built to measure passive avoidance learning in rats. These cages are formed by a rectangular goalbox with a grid floor for delivering electric shocks. One side of the box is made of plexiglass to enable behaviour observation, while the other sides are made of PVC.

MISCELLANEOUS

In addition to the routine repair- and maintenance-work and the construction of accessories such as:

- chassis and front panels for apparatus made by the electronics workshop,
- storage-trays (synthetic fabric) for storing small tubes,

the mechanical workshop carried out modifications on a variety of equipment including:

microscopes, centrifuges, shakers, Y-counters, micro-manipulators, micro-scissors, micro-pipettes and photographic equipment.

General technical service

This Department is also concerned with the repair and maintenance of:

1. the buildings;
2. the electricity-, heating-, gas- and watersupplies, including drainage and sewage;
3. the fixed installations such as the
 - a. air-conditioning units,
 - b. refrigeration- and cooling-units,
 - c. various compressors,
 - d. toxic fumes disposal cupboards,
 - e. isotope laboratory,
 - f. radio-active material storage cupboard,
 - g. auxiliary generator,
 - h. offset press.

In addition to the routine repair- and maintenance-work, the Department carried out several major projects, among others:

1. the complete renovation of the wooden building in which the research animals are kept to form eight stables, whereby the wooden external as well as inter-

nal walls have been replaced by masonry. Each stable in turn has been refurbished with a granite floor, tiles against the walls, aluminium doors, air-conditioning, water-supply and -drainage and adjustable lighting.

2. the construction of several teaching and exhibition aids used during the Summerschool held on the premises of the Royal Academy of Arts and Sciences in Amsterdam.

3. the Institute's relocation project, which aims at reorganizing the internal lay-out in such a manner as to centralize the area allocated to each individual department as much as possible, came nearer to its realization with the relocation of:

- a. the library, involving 200 linear metres of bookshelves,
- b. the director's office, now located in the centre of the building,
- c. the administration office next to the reception,
- d. the secretary's office, now adjoining that of the manager,
- e. the kitchen next to the canteen, the two being connected by a serving hatch.

Guestworkers and working visits abroad

Baker, R.E. at Jenkins Dept. of Biophysics, Johns Hopkins University Baltimore, U.S.A., June 1-23 and at Dept. of Anatomy, Medical University, Debrecen, Hungary, October 1-14 (FUNGO Travelling Fellowship).

Boer, G.J. at Medical Research Council Laboratories, Developmental Neurobiological Unit, R. Balász and A. Patel, London, England: Cell acquisition rate in developing brain of Brattleboro rats, October 6, subsidized by the Wellcome Trust, England.

Friedli, M. (guestworker from the Laboratoire de Physiologie Comparée, Genève, Suisse) was offered the opportunity to learn the oxytocinradioimmunoassay (group III).

De Jonge, F.H. (guestworker): Effects of victory and defeat on subaggressive behavior in WEZOB rats (group IV).

Martin, J.T. (guestworker from Stockton State College, Pomona, New Jersey): Vasotocin and α -MSH in the development of imprinting in ducklings (group III).

Matesz, K. (guestworker, supported by FUNGO, from the Dept. of Anatomy, Medical University, Debrecen, Hungary): To work on cobalt labelling of cutaneous sensory neurons in dorsal root ganglia of skin-rotated frogs, May 1-28 (group II).

McConnell, P. at Dept. of Anatomy, University of Birmingham, England, Dr. M. Berry, January and July.

Parnavelas, J.G. (guestworker from University of Texas, Dallas, USA): Development of non-pyramidal neurons in visual cortex of the rat, August 24-27 (group I).

Patel, A.J. (guestworker from MRC Developmental Neurobiological Unit, London, England): Brain

development in the Brattleboro rat.

Pévet, P. (from the University of Amsterdam, who is permanently placed in our institute, see group III) at Laboratoire d'Embryologie et Zoologie Expérimentale, Dr. Vivien-Roels, and at Laboratoire de Physiologie Comparée, Dr. F.C. Molder and Dr. J.M. Guerné, Strasbourg France, November 15-17.

Schober, W. (guestworker from the Karl Marx Universität, Paul Flechsig Inst. für Hirnforschung, Dept. of Anatomy, Leipzig, DDR): Determination of size of different brain structures of several macroscopical specimens from the C.U. Ariëns Kappers-collection, April-May (group I).

Schot, L.P.C. (guestworker from the Free University, Amsterdam): Immunocytochemical localization of neuropeptides in the central nervous system (*Lynmaea stagnalis*) (group III).

Swaab, D.F. at MRC Developmental Neurobiological Unit, London, England, R. Balász and A. Patel, October 31-November 2, and at Dept. of Pediatrics and Neonatal Medicine, Hammersmith Hospital, Prof. Dubowitz, London, England, October 31-November 2.

Uylings, H.B.M. at MRC Developmental Neurobiological Unit, Dr. A. Patel, London, England, January 16, at Dept. of Cell Biology, University of Texas, Dr. J.G. Parnavelas, Dallas, USA, April 26-May 2, and at Dept. of Anatomy, Boston University, Dr. E.L. White, Boston, USA, May 7.

Visser, M. (guestworker from St. Elisabeth Gasthuis, Haarlem): α -MSH and development in man.

Yadav, M. (guestworker from University of Kuala Lumpur, Malaysia): Preparation of a research program on the pineal in equatorial countries, December 14 (group III).

Publications (1979)

Baker, R.E. and M.A. Corner - Possible mechanisms underlying the development of cutaneous connections in the frog. In: Chemical Specificity of Neurons, M. Cuénod, G. Kreuzberg and F. Bloom (Eds.), Progress in Brain Research Vol. 51, pp. 117-121. Elsevier, Amsterdam.

Bakhuis, W.L. and J. van de Nes - The causal organization of climax behaviour in the domestic fowl (*Gallus domesticus*). Behav. 70, 185-230.

Berry, M., P. McConnell and J. Sievers - Dendritic growth and control of neuronal form. In: Current

Topics in Developmental Biology, R.K. Hunt (Ed.), Developmental Neurobiology, Vol. 15-16. Academic Press, New York, in press.

Boer, G.J., D.F. Swaab, H.B.M. Uylings, K. Boer, R.M. Buijs and D.N. Velis - Neuropeptides in rat brain development. In: Adaptive Capabilities of the Nervous System, Progress in Brain Research Vol. 53, P. McConnell et al. (Eds.), Elsevier, Amsterdam, in press.

Boer, H.H., L.P.C. Schot, E.W. Roubos, A. ter Maat, J.C. Lodder, D. Reichelt and D.F. Swaab - ACTH-like immunoreactivity in two electrotonically

coupled giant neurons in the pond snail (*Lymnaea stagnalis*). *Cell Tiss. Res.* 202, 231-240.

Boer, K., K. Cransberg and J. Dogterom - The effect of low-frequency stimulation of the pituitary stalk on neurohypophysial hormone release in vivo. *Neuroendocrinology*, in press.

Boer, K., J. Dogterom and H.F. Pronker - The rat fetal pituitary content of oxytocin, vasopressin and α -MSH during labour. *J. Endocrinol.*, in press.

Boer, K., D.F. Swaab and M. Visser - The fetal brain and parturition. *Animal Reprod. Sci.* 2, 63-75.

Bour, H.L.M. and M.A. Corner - Neural, and motor rhythms during development in the domestic chicken. In: *Neural and Endocrine Aspects of Biological Rhythms in Birds*, J. Tanabe, K. Tanaka and T. Ookawa (Eds.), Springer, Berlin, in press.

Buijs, R.M. - Immunocytochemical demonstration of vasopressin and oxytocin in the rat brain by light- and electronmicroscopy. *J. Histochem. Cytochem.*, in press.

Buijs, R.M. and P. Pévet - Vasopressin- and oxytocin-containing fibres in the pineal gland and the subcommissural organ of the rat. *Cell Tiss. Res.*, 205, 11-17 (1980).

Buijs, R.M. and D.F. Swaab - Immunoelectron-microscopical demonstration of vasopressin and oxytocin containing synapses in the limbic system of the rat. *Cell Tiss. Res.*, 204, 355-365.

Buijs, R.M., D.N. Velis and D.F. Swaab - Extra-hypothalamic vasopressin and oxytocin innervation of fetal and adult rat brain. In: *Adaptive Capabilities of the Nervous System*, Progress in Brain Research, Vol. 53, P. McConnell et al. (Eds.), Elsevier, Amsterdam, in press.

Corner, M.A., R.E. Baker and W.A.M. Veltman - Experimental studies on cutaneous afferent connections in frogs. In: *Biological Aspects of Learning, Memory Formation and Ontogeny of the CNS*, H. Matthies, M. Krub and H. Popov (Eds.), Abh. Akad. Wiss. DDR, Akademie-Verlag, Berlin, in press.

Corner, M.A., H.L.M. Bour and M. Mirmiran - Development of spontaneous motility, and the question of its physiological interpretation in rat, chick and frog. In: *Neural Growth and Differentiation*, E. Meisami and M. Brazier (Eds.), IBRO Monogr. Ser. Vol. 5, Raven Press, New York, pp. 253-267.

Corner, M.A., M. Mirmiran, H.L.M. Bour, N.E. van de Poll, H.G. van Oyen, G.J. Boer and H.B.M. Uylings - Does rapid-eye-movement sleep play a role in nervous development? In: *Adaptive Capacities of the*

Nervous System, Progress in Brain Research Vol. 53, P. McConnell et al. (Eds.), Elsevier, Amsterdam, in press.

De Bruin, J.P.C. - Telencephalon and behavior in teleost fish: a neuro-ethological approach. In: *Comparative Neurology of the Telencephalon*, D.O.E. Ebesson (Ed.), pp. 175-201. Plenum Publishing Corp., New York.

Dogterom, J. and R.M. Buijs - Vasopressin and oxytocin in distinct rat brain regions: basal levels and levels after training for passive avoidance behaviour. In: *Proc. of the IBRO-UNESCO Conf. on Neuropeptides and Neural Transmission*, Jablonna, Poland, 1979. IBRO monogr. series, Raven Press, New York, in press.

Dogterom, J., F.G.M. Snijdwint, P. Pévet and D.F. Swaab - Studies on the presence of vasopressin, oxytocin and vasotocin in the pineal gland, subcommissural organ and foetal pituitary gland: failure to demonstrate vasotocin in mammals. *J. Endocrinol.*, 84, 115-123 (1980).

Dogterom, J., F.G.M. Snijdwint, P. Pévet and R.M. Buijs - On the presence of neuropeptides in the mammalian pineal gland and subcommissural organ. In: *The Pineal Gland of Vertebrates Including Man*, J. Ariëns Kappers and P. Pévet (Eds.), Progress in Brain Research Vol. 52, pp. 465-470, Elsevier, Amsterdam.

Hodde, K.C. - The vascularization of the rat pineal organ. In: *The Pineal Gland of Vertebrates Including Man*. Progress in Brain Research Vol. 52, pp. 39-44, J. Ariëns Kappers and P. Pévet (Eds.), Elsevier, Amsterdam.

Hodde, K.C. and W.A.M. Veltman - The vascularization of the pineal gland (epiphysis cerebri). In: *SEM inc. Proceedings 1979*. O. Johari (Ed.).

McConnell, P.S. - Nutritional effects on non-mitotic aspects of central nervous system development. In: *Adaptive Capabilities of the Nervous System*. Progress in Brain Research Vol. 53, P.S. McConnell, G.J. Boer, N.E. van de Poll, H.J. Romijn and M.A. Corner (Eds.), Elsevier, Amsterdam, in press.

McConnell, P. and M. Berry - The effects of postnatal lead exposure on Purkinje cell development in the rat. *Neuropathol. Appl. Neurobiol.* 5, 115-132.

McConnell, P., G.J. Boer, N.E. van de Poll, H.J. Romijn and M.A. Corner (Eds.) - *Adaptive capabilities of the Nervous System*, Progress in Brain Research Vol. 53, Elsevier, Amsterdam, in press.

Mirmiran, M., H.L.M. Bour and M.A. Corner - Pharmacological suppression of paradoxical sleep during postnatal development in the rat. In: *Sleep 1978*, L.

- Popoviciu, B. Asgiu and G. Badin (Eds.), Editura Medica, Bucharest, in press.
- Mirmiran, M., M.A. Corner and H.L.M. Bour, -Pharmacological suppression of active (REM) sleep in infant rats: effect on adult sleep patterns. In: Ontogenesis of the Brain Vol. III, S. Trojan (Ed.), Charles University Press, Prague, in press.
- Mirmiran, M., N. van de Poll, H. van Oyen, G. Boer and M.A. Corner - Lasting sequelae of chronic treatment with chlorimipramine during early postnatal development in the rat. I.R.C.S. J. Med. Sci., in press.
- Parnavelas, J.G. and H.B.M. Uylings - The growth of non-pyramidal neurons in the visual cortex of the rat: a morphometric study. Brain Res., in press.
- Pévet, P. - Secretory processes in the mammalian pinealocyte under natural and experimental conditions. Progress in Brain Research Vol. 52, pp. 149-194. Elsevier/North-Holland Biomedical Press, Amsterdam.
- Pévet, P. - The pineal gland. An analysis of the number of papers published during the past years. EPSG-Newsletter 2, 11-16.
- Pévet, P. - The pineal gland of the mole (*Talpa europaea* L.). VI. The fine structure of fetal pinealocytes. Cell. Tiss. Res. 206, 417-430 (1980).
- Pévet, P. - Ultrastructure of the mammalian pinealocytes. In: The Pineal Gland: its Anatomy and Biochemistry, R.J. Reiter (Ed.), CRC Press, Palm Beach (Fla.) U.S.A., in press.
- Pévet, P., I. Ebels, D.F. Swaab, M.T. Mud and A. Arimura - Presence of AVT-, α -MSH-, LHRH- and somatostatin-like compounds in the rat pineal gland, and their relationship with the UMO5R pineal fraction; an immunocytochemical study. Cell. Tiss. Res., 206, 341-353 (1980).
- Pévet, P. and D.F. Swaab - Immunocytochemical evidence for the presence of an α -MSH-like compound in the rat pineal gland. J. de Physiol. (Paris) 75, 101-103.
- Romijn, H.J. - Het Slapend en Dromend Bewustzijn. AMBO, Baarn pp. 103.
- Romijn, H.J., M. Mud, P. Wolters and M. Corner - Neurite formation in dissociated cerebral cortex in vitro: evidence for clockwise outgrowth and autotopic contacts. Brain Research, in press.
- Swaab, D.F. - Neurohypophysial hormones and their distribution in the brain. Internat. Health Foundation Congress, Bordeaux, France '79, in press.
- Swaab, D.F. - Neuropeptides and brain development: A working hypothesis. In: A Multidisciplinary Approach to Brain Development, C. Di Benedetta (Ed.), Internat. Meeting, Bari, Italy, April 18-20, 1979, Elsevier, Amsterdam, in press.
- Swaab, D.F. and K. Boer - Function of pituitary hormones in human parturition: a comparison with data in the rat. In: Human Parturition: New Concepts and Developments, M.J.N.C. Keirse, A.B.M. Anderson and J. Bennebroek Gravenhorst (Eds.), pp. 49-71. Kluwer Acad. Publishers Group, Dordrecht, The Netherlands.
- Swaab, D.F. and K. Boer - Technical developments in the study of neuroendocrine mechanisms in the rat pregnancy and parturition. In: Animal Models in Fetal Medicine, P.W. Nathanielsz (Ed.), Elsevier, Amsterdam, in press.
- Swaab, D.F., M. Visser and J. Dogterom - The maturity of the foetal brain and its development in labour. In: Psychoneuroendocrinology in Reproduction, L. Zichella and P. Pancheri (Eds.), pp. 483-495. Elsevier, Amsterdam.
- Tilders, F.J.H., H.A. van der Woude, D.F. Swaab and A.H. Mulder - Identification of MSH-release inhibiting elements in the neuro-intermediate lobe of the rat. Brain Res. 171, 425-435.
- Uylings, H.B.M., J.G. Parnavelas, H.L. Walg and W.A.M. Veltman - The morphometry of the branching pattern of developing non-pyramidal neurons in the visual cortex of rats. In: Proc. Vth. Int. Congr. Stereol. Soc., Suppl. to Mikroskopie, G. Bernroider and H. Adams (Eds.), in press.
- Van de Poll, N.E. and H. van Dis - The effect of medial preoptic-anterior hypothalamic lesions on bisexual behavior of the male rat. Brain Res. Bull. Vol. 4, 505-511.
- Van Dis, H., M.A. Corner, R. Dapper, G. Hanewald and H. Kok - Intra-individual consistency and inter-individual differences in the EEG during quiet wakefulness in humans. Electroenceph. Clin. Neurophysiol. 47, 87-94.
- Van Leeuwen, F.W. - An introduction to the immunocytochemical localization of neuropeptides and neurotransmitters. Acta Histochemica, in press.
- Van Leeuwen, F.W. - Extrahypothalamic neuropeptide sites and pathways, J. Histochem. Cytochem., in press.
- Van Leeuwen, F.W. - Immunocytochemical specificity for peptides with special reference to oxytocin and arginine-vasopressin. J. Histochem. Cytochem., in press.

Van Leeuwen, F.W., C. de Raay, D.F. Swaab and B. Fisser - The localization of oxytocin, vasopressin, somatostatin and luteinizing hormone releasing hormone in the rat neurohypophysis. *Cell Tiss. Res.* 202, 189-202.

Van Oyen, H.G., N.E. van de Poll and J.P.C. de Bruin - Sex, age and shock intensity as factors in passive avoidance. *Physiol. Behav.* 23, 915-918.

Van Wijk, M., J.B. Sebens and J. Korf - Probenecid-induced increase of 5-hydroxytryptamine synthesis in rat brain, as measured by formation of

5-hydroxytryptophan. *Psychopharmacol.* 60, 229-235.

Van Wimersma Greidanus, Tj.B., G. Croiset, H. Goedemans and J. Dogterom - Vasopressin levels in peripheral blood and in cerebrospinal fluid during passive and active avoidance behavior in rats. *Hormones and Behavior* 12, 103-111.

Visser, M. and D.F. Swaab - Life span changes in the presence of melanocyte stimulating hormone containing cells in the human pituitary. *J. Develop. Physiol.* 1, 161-178.

Abstracts, etc. (1979)

Boer, G.J. - Development of a specificity test for immunocytochemistry of neuropeptides using high-voltage electrofusing in polyacrylamide micro slabs. *J. Endocrinol.* 80, 54P-55P.

Boer, G.J., D.F. Swaab, R.M. Buijs and H.B.M. Uylings - Neuropeptiden en hersenontwikkeling. *Ned. T. v. Geneesk.*, 124, 250 (1980).

Boer, G.J., D.F. Swaab, R.M. Buijs en H.B.M. Uylings - Neuropeptiden en hersenontwikkeling. *Proc. Vergadering Ned. Ver. voor Endocrinol., Acad. Ziekenhuis Utrecht*, March 17, 1979.

Boer, G.J., D.F. Swaab, R.M. Buijs, H.B.M. Uylings, D. Velis and K. Boer - Neuropeptides in rat brain development. 11th Int. Summer School of Brain Research, 'Adaptive Capabilities of the Nervous System', Amsterdam, p. 42.

Boer, G.J., D.F. Swaab and H.B.M. Uylings - Neuropeptides and brain development. *Proc. 7th Meeting of the Intern. Soc. for Neurochem.*, Jerusalem, p. 244.

Boer, G.J., C. van Rheenen-Verberg, H.B.M. Uylings and B. Fisser - Postnatal brain development in the absence of vasopressin. *J. Endocrinol.* 80, 64P-65P.

Boer, K., J. Dogterom, and H.F. Pronker - Foetal pituitary oxytocin in α -MSH content during labour in the rat. *Proc. Intern. Symp. on Fetal Medicine*, Venice, Italy.

Boer, K., J. Dogterom and F.G.M. Snijdwint - Neurohypophysial hormone release during pregnancy and parturition. *J. Endocrinol.* 80, 41P-42P.

Bour, H.L.M., W.L. Bakhuis and M.A. Corner - Cyclic motility patterns in relation to EEG fluctuations in hatching chicks. *Waking and Sleeping* 3, p. 72.

Bour, H.L.M., M. Mirmiran, M.A. Corner and S. de Boer - Spontaneous motor activity during sleep in the

rat at early stages of postnatal development. *Waking and Sleeping* 3, p. 72.

Buijs, R.M. and D.F. Swaab - Immunoelectron-microscopical demonstration of vasopressin and oxytocin synapses in the rat limbic system. *Proc. 11th Int. Summer School of Brain Research 'Adaptive Capabilities of the Nervous System'*, Amsterdam, August 13-17, Amsterdam.

Corner, M.A. - Book review of 'Cerebral Correlates of Conscious Experience', P. Buser and A. Rougeul-Buser (Eds.), *Trends in Neuroscience*, February issue, pp. X-XI.

Corner, M.A. and K. Boer - The Netherlands Institute for Brain Research, *Trends in Neuroscience*, July issue, pp. VIII-IX.

De Blécourt, C.V. and J. Smith - Correlations between EEG and cerebral blood flow in the rabbit, combining parameters of different EEG analysis methods. *Proceedings of the 20th Dutch Federation Meeting*, Groningen, April 18-20.

De Blécourt, C.V. and J. Smith - Correlations between EEG and cerebral blood flow in the rabbit. In: *Cerebral Blood Flow and Metabolism*, *Acta Neurol. Scandinavica*, Vol. 60, Suppl. 72, pp. 32-33.

De Bruin, J.P.C., N.E. van de Poll, S. van der Zwan and H.G. van Oyen - Ontogenetic aspects of sex differences in passive avoidance behavior. *Proc. 20e Federatieve Vergadering van Medisch Wetenschappelijke Ver.*, Groningen.

De Bruin, J.P.C., N.E. van de Poll, H.G. van Oyen, V.D.J. Nolten and S.M. van der Zwan - Behavioral functions of the prefrontal cortex in the rat. *Proc. 11th Int. Summer School of Brain Research*, Amsterdam, abstr. p. 77.

Dogterom, J. and R.M. Buijs - Vasopressin and oxytocin in the rat brain during passive avoidance behaviour. *IBRO-UNESCO Congress on Brain Peptides*, Jablonna, Poland.

- Dogterom, J., P. Pévet, R.M. Buijs, F.G.M. Snijdewint and D.F. Swaab - Vasopressin, oxytocin and vasotocin in pineal gland, subcommissural organ and foetal pituitary: failure to demonstrate vasotocin in mammals, *Acta Endocrinol., Kbh.* Vol. 91, Suppl. 225, p. 413.
- Hodde, K.C., and C.V. de Blécourt - Extracerebral supply by the internal carotid artery in the rabbit and the rat. In: *Cerebral Blood Flow and Metabolism. Acta Neurol. Scandinavica* Vol. 60, Suppl. 72, p. 636-637.
- Hodde, K.C. and C.V. de Blécourt - Extracerebral supply by the a. carotis interna in rabbit and rat. Proc. of the 20th Dutch Federation Meeting, Groningen, 18-19-20 April.
- Hodde, K.C. and C.V. de Blécourt - The rat nasal blood supply and arteriovenous anastomoses. Proceedings of the Anatomical Soc. of Great Britain and Ireland, *J. Anat.* 129, 1, pp. 206-207.
- Hodde, K.C., C.V. de Blécourt and W.A.M. Veltman - Extracerebrale bloedvoorziening via de a. carotis interna in het konijn. *Ned. T. Geneesk.* 123, nr. 33, p. 1451.
- Martin, J.T., J. Dogterom and D.F. Swaab - Vasotocin and α -MSH in the pituitary and hypothalamus of the duck in relation to the imprinting sensitive period. Proc. Annual Meeting of the American Soc. of Zoologists, Tampa, Florida, U.S.A., December 27-30.
- McConnell, P. and M. Berry - The effect of refeeding after various periods of undernutrition on the morphology of Purkinje cells in the cerebellum of the rat. *J. Anat.* (London) 129, p. 202.
- McConnell, P. and M. Berry - The effects of undernutrition and refeeding on the morphology of Purkinje cells in the rat cerebellum. Proc. 11th Int. Summer School of Brain Research 'Adaptive Capabilities of the Nervous System', Amsterdam, August 13-17.
- McConnell, P. and M. Berry - The effects of undernutrition and refeeding on the morphology of Purkinje cells in the rat cerebellum. *Neurosci. Lett. Suppl.* 3, S25.
- Mirmiran, M., H.L. Bour and M.A. Corner - Pharmacological suppression of paradoxical sleep during postnatal development in the rat. In: *Waking and Sleeping* Vol. 3, p. 205.
- Mirmiran, M. and M.A. Corner - Chronic pharmacological suppression of active sleep in infant rats: effects on adult sleep patterning. *Sleep Research*, in press.
- Parnavelas, J.G. and H.B.M. Uylings - Morphometrical analysis of developing non-pyramidal neurons in the visual cortex of the rat. Abstr. Soc. Neurosci., 9th annual meeting, Georgia, Nov. 2-6, p. 172.
- Pévet, P., J. Dogterom, R.M. Buijs, I. Ebels, D.F. Swaab and A. Arimura - Presence of α -MSH-, AVT- and LHRH-like compounds in the mammalian pineal and subcommissural organ and their relationship with the UMOSR pineal fraction. 10th Conference of European Comparative Endocrinologists, Sorrento, Italy, May 21-25, 1979. *General and Comp. Endocrinol.* (1980), in press.
- Pévet, P., J. Dogterom, R.M. Buijs and A. Reinharz - Is it the vasotocin or a vasotocin-like peptide which is present in the mammalian pineal and subcommissural organ? *J. Endocr.* 80, p. 49.
- Pévet, P., J. Dogterom, R.M. Buijs, B. Vivien-Roels, F.C. Holder and J.M. Guerné - l'arginine vasotocine, est-elle présente dans la glande pinéale et dans l'organe sous-commissural des mammifères? *J. de Physiologie* (Paris), in press.
- Reinharz, A., P. Pévet and M. Valloton - Presence of two neurophysins and two neurohormones in bovine pineal gland. *Experientia*, in press.
- Romijn, H.J. - Boekbespreking - A.M.L. Coenen: 'De Slaap. Een psychobiologische inleiding.' (Van Gorkum, Assen 1979) In: *Intermediair and Dagblad Trouw*.
- Romijn, H.J. - Boekbespreking - Dr. M. Gyselen, Dr. P. Mommaers and J.J.C. Marlet: 'Hoe menselijk is mystiek.' (AMBO, Baarn 1979) In: *Dagblad Trouw*.
- Romijn, H.J. - Boekbespreking - D. Schlüter and R. Jobse (red.): 'Frederik van Eden. Dromenboek' (Bert Bakker, Amsterdam 1979) In: *Intermediair and Dagblad Trouw*.
- Romijn, H.J. - Boekbespreking - T. Buzan and T. Dixon: Hersenen. (Zuidgroep B.V. Uitgevers, Den Haag 1979) In: *Dagblad Trouw*.
- Romijn, H.J., M.T. Mud, P.S. Wolters and A.M.M.C. Habets - 'Synapse formation in dissociated embryonal rat cerebral cortex in vitro.' Proc. 11th Int. Summer School of Brain Research, Amsterdam, August 13-17.
- Schot, L.P.C., H.H. de Boer and D.F. Swaab - Peptidergic neurons in the pond snail (*lymnaea stagnalis*). Proc. 11th Int. Summer School of Brain Research, Amsterdam, August 13-17.
- Swaab, D.F. - Immuno-neurocytochemie. Abstr. in klapper t.b.v. cursus 'Neurochemie', Universiteit van Utrecht, June 6-12.

Swaab, D.F. - The maturity of the foetal brain and its involvement in labour. Proc. 2nd Int. Symp. on Clinical Psycho-Neuro-Endocrinology in Reproduction 'Ovulation and Labour', Venice, Italy, June 3-5.

Swaab, D.F. Book review of Programs of the brain, J.Z. Young, Oxford University Press, Oxford, 1978. In: Theor. Appl. Gen.

Swaab, D.F. - Book review of 'The hypothalamus'. Research Publications: Association for Research in Nervous and Mental Disease Vol. 56, S. Reichlin, R.J. Baldessarini and J.B. Martin (Eds.), Raven Press, New York, 1978. In: Quart. Rev. Biol. 54, 119.

Swaab, D.F. and R.M. Buijs - De peptide synaps. 'Fyfar' symposium 'Neuropeptiden en gedrag', Winschoten, Zeist. Ned. T. Geneesk. 123, 1590.

Swaab, D.F., G.J. Boer, R.M. Buijs, H.B.M. Uylings and F.W. van Leeuwen - Neuropeptides and brain development - a working hypothesis. Proc. Int. Meeting 'A multidisciplinary approach to brain development', Brindisi, p. 50.

Uylings, H.B.M. - Morphometry of 3-dimensional neuronal branching structures. Conference on 'Mathematical trees in principle and practice'. Harvard University, Harvard Forest, Petersham, May 4-6.

Uylings, H.B.M., J. Overdijk, K. Kuypers and A.W. Kamstra - A semi-automatic system for measuring cellular tree structures in 3 dimensions. Acta Morphol. Neerl.-Scand. 17, p. 257.

Uylings, H.B.M., J.G. Parnavelas, W.A.M. Veltman and H. Walg - The morphometry of the branching pattern in developing non-pyramidal neurons in the visual cortex of rats. Proc. 5th Int. Congress Soc. Stereology, Salzburg, September 3-7, p. 46.

Van de Poll, N.E., M. Mirmiran, H.G. van Oyen, H.G. Bour and M.A. Corner - Behavioral consequences of chronic deprivation of active (REM) sleep during postnatal development in the rat. Proc. 20e Federatieve Vergadering Med. Wetensch. Verenig., Groningen, abstr. nr. 326.

Van de Poll, N.E., F. de Jonge, J. van Pelt, H.G. van Oyen, S.M. van der Zwan and J.P.C. de Bruin - Sex differences in aggression in rats. Proc. 11th Int. Summer School of Brain Research, Amsterdam, August 13-17.

Van Leeuwen, F.W., D.F. Swaab, C. de Raay and B. Fisser - Immunoelectron microscopical demonstration of α -MSH-like compounds in the rat brain. J. Endocrin. 80, 59P-60P; and Ultramicroscopy 4, 125.

Van Oyen, H.G., N.E. van de Poll, M. Mirmiran, H.G. Bour and M.A. Corner - Effects of chronic deprivation of active (REM) sleep during postnatal development in the rat. Neurosci. Lett., Suppl. 3, S269.

Van Wijk, M. and J. Korf - Rapid determinations of 5HT and 5HIAA; use in studies of postmortem effects in brain tissue preparations. Abstr. nr. 481, Proc. 20e Feder. Verg. van Med. Wetensch. Verenig., Groningen, abstr. nr. 481.

Velis, D.N., R.M. Buijs and D.F. Swaab - Neurosecretory cells and their exohypothalamic fibres in rat brain development. Proc. 11th Int. Summer School of Brain Research, Amsterdam, August 13-17.

Velis, D.N., R.M. Buijs and D.F. Swaab - Neurosecretore cellen en hun exohypothalame vezels tijdens de hersenontwikkeling bij de rat. Ned. T. Geneesk., in press.

Papers read

See also 'abstract' and 'teaching' sections.

Baker, R.E. - Development of skin flicks in frogs: mechanisms, underlying formation of somatosensory connections mediating wiping reflexes. Seminar at Jenkins Biophysics Dept., Johns Hopkins University, Baltimore, U.S.A., June.

Boer, G.J. - Brain development in the absence of vasopressin. Medical Research Council, Developmental Neurobiology Unit (Prof. Dr. R. Balász), London, October 2.

Buijs, R.M. - De lokalisatie van vasopressine en oxytocine in het centraal zenuwstelsel van de rat. Laboratorium voor Fysiologie, Leiden, March 29.

Buijs, R.M. - Licht- en electronenmicroscopische lokalisatie van vasopressine en oxytocine in het centraal zenuwstelsel van de rat. Medisch-Fysisch Instituut TNO, Utrecht, October 23.

Buijs, R.M. - The specific localization of vasopressin and oxytocin pathways in the rat central nervous system. 30th Annual Meeting Histochemical Society, Keystone, Colorado, U.S.A., April.

Corner, M.A. - Experimental analysis of the formation of cutaneous reflex connections in the frog. Seminar at Dept. of Physiology, School of Medicine, Nagoya University, Japan, July.

Corner, M.A. - Suppression of active sleep in rats by means of chronic chlorimipramine treatment in early development. Round table presentation at Segawa

- Neurological Clinic for Children, Tokyo, Japan, July.
- Corner, M.A. - Development of active sleep in the rat, and the possible effects of its suppression during early infancy. Seminar at Institute for Medical Physics TNO, Utrecht, November.
- Corner, M.A. - Nerve tissue culture as a model system for studying synaptogenesis in the rat cerebral cortex. Lecture at FUNGO Meeting on 'Development of Brain and Behavior', Groningen, December.
- De Bruin, J.P.C. - Sulcal prefrontale cortex lesions, agressief en sexueel gedrag bij de Wezob-rat. FUNGO-bijeenkomst Werkgemeenschap 'Ontwikkeling Zenuwstelsel en Gedrag', Groningen, October.
- De Bruin, J.P.C. - De prefrontale cortex en gedrag bij de rat. BION-bijeenkomst Werkgemeenschap 'Ethologie', Woudschoten, December.
- McConnell, P. - The effects of starvation and refeeding on dendritic morphology of cerebellar Purkinje cells. Verhaart Meeting of Dutch neuroanatomists, Amsterdam, May 15.
- Uylings, H.B.M. - Morphometry of visual cortex dendrites. Boston University, Dept. Anatomy, Boston, May 7.
- Uylings, H.B.M. - De invloed van vasopressine op de ontwikkeling van de cortex van de rat. FUNGO-bijeenkomst Werkgemeenschap 'Ontwikkeling Zenuwstelsel en Gedrag', Groningen, October 5.
- Uylings, H.B.M. - Analyses van dendrieten in ratten in verschillende condities en tijdens hun ontwikkeling. Medisch-Fysisch Instituut TNO, Utrecht, October 23.
- Uylings, H.B.M. - Semi-automatisch systeem voor het meten van vertakkingsstructuren in lichtmicroscopische preparaten. Werkgroep 'Cytometrie en cytometrie', Ned. Ver. voor Celbiologie, Leiden, December 14.
- Van de Poll, N.E. - Sexual behavior in female rats born in mixed or 'all female' litters. FUNGO-bijeenkomst Werkgemeenschap 'Hormonen en Hersenfuncties', Rotterdam, February.
- Van de Poll, N.E. - Aggression in males and females of the Wezob and S3 strain. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Van de Poll, N.E. - Sexual behavior in female rats born in mixed or 'all female' litters. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Van Leeuwen, F.W. - Immunocytochemical localization of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system, 30th Annual Meeting of the Histochemical Society, Keystone, Colorado, U.S.A., April 11.
- Van Leeuwen, F.W. - Immunocytochemistry of neuropeptides and neurotransmitters (including recent technical developments), XXIst Symposium of the 'Gesellschaft für Histochemie', Gargellen, Austria, September 20.
- Van Leeuwen, F.W. - Immunocytochemische lokalisatie van neuropeptiden op licht- en electronen-microscopisch niveau, EM Werkgroep Zenuwstelsel en de Verhaartgroep, Amsterdam, IOI, December 11.
- Van Oyen, H.G. - Behavioral analysis of sex-differences in aversively motivated learning in rats. BION-bijeenkomst Werkgemeenschap 'Ethologie', Leiden, June.
- Van Oyen, H.G. - Sex-differences in learning of rats. FUNGO-bijeenkomst Werkgemeenschap 'Hersenen en Gedrag', Utrecht, November.
- Van Oyen, H.G. - Possible mechanisms of hormonal determinants of behavior. Vakgroep Psychofysiologie, Utrecht, November.
- Van Oyen, H.G. - Gonadal hormones development and behavior. MFI, November.
- Van Oyen, H.G. - Acquisition and longterm retention of spatial discrimination in the male and female rat. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Van Oyen, H.G. - Sex-differences in passive avoidance learning. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Van Oyen, H.G. - Passive avoidance in rats with hereditary hypothalamic diabetes insipidus. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Van Oyen, H.G. - Effects of chronic deprivation of active sleep during postnatal development. Joint Dutch-Belgian Meeting of the Psychonomic Society, Tilburg, November.
- Pévet, P. - Pineal peptides. FUNGO-Workshop, Utrecht, October.
- Swaab, D.F. - Hormonen, transmitters en missers (Didactische themadag 'Peptiderge cellen, immunocytochemie en electrofysiologie'), Ned. Ver. voor Endocrinologie i.s.m. BION-Workshop voor Vergelijkende Endocrinologie, Vrije Universiteit, Amsterdam, May 11.
- Swaab, D.F. - Neurohypophysial hormones and their distribution in the brain. International Health Foun-

Teaching

a. Students

E.L. Baruch-Virransalo (biochemist, Apprentice): Neuropeptides in early fetal development (Group III)

S. de Boer (biology student, Agriculture College, Wageningen): Completion of her subsidiary subject on 'Phasic motility during paradoxical sleep in rats at two and three weeks of postnatal age' (Group II)

H.K. Boekema (H.T.S.-volunteer): Modification of the data acquisition programmes for the semi-automatic dendrite measurement system (Group V)

J.P. ter Borg (biology student, Free University, Amsterdam): Exohypothalamic neurosecretory fibres in the human brain (Group III)

F.H. de Jonge (biology student, University of Amsterdam): Aggressive behavior in male and female WEZOB and S3 rats (Group IV)

Oosting, P.R. (medical student, University of Amsterdam): Hereditary hypothalamic diabetes insipidus in man (Group III)

H.M.L. van Pelt-Heerschap (biology student, Free University, Amsterdam): Identification of oxytocin immunoreactivity in *Lymnea stagnalis* using isoelectric focussing (Group III)

G.J. de Vries (biology student, Free University, Amsterdam): Maturation of the suprachiasmatic nucleus and its exohypothalamic pathways (Group III)

J. Scholtens (biology student, University of Amsterdam): Sexual motivation in the female rat (Group IV)

L.P.C. Schot (biology student, Free University, Amsterdam): Immunocytochemical localization of neuropeptides in the central nervous system (*Lymnaea stagnalis*) (Group III)

D.N. Velis (medical student, Northwestern University, Chicago, U.S.A.): Maturation of the supraoptic- and paraventricular nucleus and their exohypothalamic pathways (Group III)

E. Welker (medical student, University of Amsterdam): Properties of diluted Golgi-Cox and normal Golgi-Cox methods for staining of neurons (Group I)

b. The 11th International Summer School of Brain Research

This Summer School was held in the 'Trippenhuis' of the Royal Netherlands Academy of Sciences, Amsterdam, August 13-17. Since the research at the Institute is organized around the central theme 'Maturation and Adaptation of the Nervous System', and the maturation facet was the theme of our previous Summer School, 'Adaptive Capabilities of the Nervous System' was chosen as the subject for this year's Summer School. The organizing committee (G.J. Boer, K. Boer, J. Dogterom, P.S. McConnell, J. van Pelt, H.J. Romijn, J. Sels, D.F. Swaab, H.B.M. Uylings, N.E. van de Poll and M.A. Corner) compiled a programme to which 26 scientists have agreed to contribute. Since this year's Summer School coincided with the 70th anniversary of the founding of our Institute some special events were included in the social programme, e.g. a laboratory visit to the Netherlands Institute for Brain Research by boat and two public lectures which were presented in the 'Koningszaal' of Artis (the Zoological Garden of Amsterdam). Dr. M.G. ter Haar (Cambridge) staff editor of 'Trends in Neurosciences' gave a lecture on 'Mind, the future', while Prof. H. van der Loos (Lausanne) spoke on 'The brain adapts to what, and how?' The Summer School lectures will be published in the series 'Progress in Brain Research'.

c. Lectures and thesis

De Bruin, J.P.C. - Integrated lecture 'Ethology and Neurobiology, University of Amsterdam, Biology, January 19

De Bruin, J.P.C. - member committee for PhD thesis of A. Nijssen, University of Amsterdam, Social Sciences, 'Relaties tussen gedragsystemen', Amsterdam, June 15.

De Bruin, J.P.C. - member committee for PhD thesis B.A. Heuts, University of Amsterdam, Social Sciences, 'De beïnvloeding van het agonistische gedrag van enkele zoetwatervissen voor soortgenoten en door de bekendheid met de omgeving', Amsterdam, December 21.

Swaab, D.F. - Integrated lecture 'Growth and development of the brain', Medical School, University of Amsterdam, January 25.

Swaab, D.F. - Integrated lecture 'Neuroendocrine

aspects of the menstrual cycle', Medical School, University of Amsterdam, February 5.

Swaab, D.F. - lecture 'Immunoneurocytochemistry', June 11, during the course on 'Neurochemistry', W.H. Gispen, Univ. of Utrecht. Demonstrations were given by G.J. Boer, J. Dogterom and F.W. van Leeuwen.

Swaab, D.F. - lecture 'Morphological and biochemical changes in the brain in aging and dementia', Boerhaave Committee for postgraduate medical teaching, course on Dementia, general medical and neurological aspects, Leiden, November 15-16.

Miscellaneous

Boer, G.J. - Supervisor of the radio-isotope laboratory of the Netherlands Institute for Brain Research.

Corner, M.A. - Program committee 1st Meeting of the International Society for Developmental Neuroscience, Strasbourg, July 1980; regional secretary for the Netherlands and Denmark International Society for Developmental Neuroscience; advisory boards of Developmental Psychobiology, Developmental Neuroscience, Neuroscience and Biobehavioral Reviews, The Behavior and Brain Sciences and IRCS Medical Sciences; project leader in the FUNGO Workgroup 'Development and Aging of the Brain and Behavior'; research advisor at Dept. of Clinical Psychology, University of Amsterdam.

De Bruin, J.P.C. - Chairman of the session on 'Aggression', Conference of the International Association of Fish Ethologists; secretary of the Institute's Council; leader of BION project (BION Workgroup 'Ethology', projectgroup 'Gedragmechanismen').

McConnell, P. - Referee for *J. Develop. Physiol.* (1 ×) and *J. Neurosci. Methods* (1 ×).

Pévet, P. - Referee for *Acta Endocrinologica* (1 ×); co-editor of the European Pineal Study Group Newsletter; secretary-treasurer of the European Pineal Study Group; leader of FUNGO-project 13-53-33 in Workgroup 'Regulation of Pituitary Functions'.

Swaab, D.F. - Member of the advisory boards of *Acta Endocrinologica* and *J. Neural Transmission*; member of the editorial boards of *J. Developmental Physiology* and *J. Neuroscience Methods*; member of FUNGO evaluating committee for 'Endocrinology'; leader of FUNGO-project 13-35-07; vice-president of the Dutch Society for Endocrinology; secretary of the Dutch Committee of the International Brain Research Organization; referee for *J. Endocrinology* (2 ×), *J.*

Swaab, D.F. - co-referent for PhD thesis J. Assies, University of Amsterdam, 'Neuroendocrine aspects of prolactinomas. A clinical study', October 25.

Swaab, D.F. - member committee for thesis O.P. Bleker, University of Amsterdam, 'On human intrauterine growth', November 22.

Swaab, D.F. - member committee for thesis A. ter Maat, Free University, Amsterdam, 'Integrative properties of a group of neuroendocrine cells in the Pond snail', December 6.

Developmental Physiology (1 ×), *Europ. J. Pharmacology* (1 ×); member of the International Scientific Committee of the European Science Foundation (European Training Programme in Brain and Behavior Research).

Uylings, H.B.M. - Co-organizer of the program-committee for the meetings of Dutch neuromorphologists; workgroup leader, FUNGO Workgroup 'Development and Aging of Brain and Behaviour'; referee for *Bull. Math. Biology* (2 ×), *J. Develop. Physiology* (1 ×), and *J. Neurosci. Methods* (1 ×).

Van de Poll, N.E. - Member of the Dobberke Foundation for Comparative Psychology; member of the Advisory Committee for the project 'Neural mechanisms of appetitive and aversive behavior in rats' of the Workgroup 'Comparative and Physiological Psychology, Psychonomy'; advisor of the project 'Hormonal regulation of sexual behavior in the male stump-tail macaca'; secretary of the Workgroup 'Hormones and Brain Function', aspirant Workgroup to FUNGO; leader of projects in BION, FUNGO and Psychonomy.

Van Leeuwen, F.W. - Organizer of Workshop on Extrahypothalamic and Neuropeptide Sites and Pathways, 30th Annual Meeting of the Histochemical Society, Keystone, Colorado, USA, April 7-13, organizer of the Workshop on Immunocytochemistry of Neuropeptides and Neurotransmitters (including recent technical developments), 21st Symposium of the 'Gesellschaft für Histochemie', Gargellen, Austria, September 19-22; chairman of the Workgroup 'Immunoelectron microscopy' of the Dutch Society for Cell Biology, in collaboration with the Dutch Society for Electronmicroscopy, University of Utrecht, November 9.

Van Oyen, H.G. - Member of the Advisory board of the project 'Odor intensity discrimination in the rat' of the Workgroup 'Comparative and Physiological Psychology, Psychonomy'.

Seminars given at the Institute in 1979

(organization Dr. G.J. Boer)

January 17 - Dr. G.A. Groos (Laboratorium voor Fysiologie, Rijksuniversiteit Leiden): Electrofysiologisch onderzoek aan de Nucleus Suprachiasmaticus.

January 26 - Dr. H. Distel (Institut für Medizinische Psychologie, Universität München): Autoradiographic tracing of developing subcortical projections in rabbit embryos.

February 7 - Dr. D. Mellon Jr. (Dept. of Zoology, University of Cambridge, Cambridge, England): The control of muscle transformation in Crustaceans.

February 14 - Dr. M.E.A. Reith (Institute for Neurochemistry, Ward's Island, New York, U.S.A.): Afbraak van ACTH₁₋₂₄ door cytosol van muizehersenen.

February 28 - Prof. Dr. J.R.G. Challis (Dept. of Obstetrics and Gynaecology, University of Western Ontario, London, Canada): Development of fetal adrenal function in relation to the role of the fetal brain in labour.

March 14 - Prof. Dr. M.D. van Hof (Laboratorium voor Fysiologie, Erasmus Universiteit, Rotterdam): Speelt het milieu exterieur een rol in de postnatale ontwikkeling van het visuele systeem bij het konijn?

March 28 - Dr. I. Divac (Instituut voor Neurofysiologie, Universiteit van Kopenhagen, Denemarken): Studies on anatomy and function of the prefrontal cortex.

March 29 - Dr. K.S. Bedi (Dept. of Children Health, University of Manchester, Manchester, England): Stereological analysis of the neuronal and synaptic content of the frontal and cerebellar cortex of rats undernourished during early life.

May 16 - Dr. W.K. Paull (Dept. of Anatomy, Medical

College of Georgia, Augusta, Georgia, U.S.A.): Perinatal neuroendocrine morphology. Transmission and scanning electron microscopy, with light microscopic immunocytochemistry of the LHRH pathway in intact, ovariectomized, estrogen-treated prepubertal female rats.

May 18 - Dr. H.W.M. Steinbush (Laboratorium voor Anatomie en Embryologie, Katholieke Universiteit, Nijmegen): Immunohistochemische lokalisatie van serotonine en noradrenaline in het centraal zenuwstelsel van ratten.

May 23 - Dr. K. Matesz (Dept. of Anatomy, Medical University, Debrecen, Hungary): Central projection of the cranial nerves in the frog.

June 6 - Dr. W. Schober (Paul-Flechsig-Institut für Hirnforschung, Karl Marx Universität, Leipzig, DDR): The primary optic projections in the albino rat.

June 8 - Dr. D. Goldfoot (Wisconsin Primate Center, Wisconsin, USA, visiting scientist Erasmus Universiteit, Rotterdam): Development of sex differences in infant Rhesus monkeys: hormonal and social interactions.

September 26 - Dr. V.M. Pickel (Dept. of Neurology, Cornell University Medical College, New York, U.S.A.): Immunocytochemical localization of catecholamines and neuropeptides.

November 21 - Prof. Dr. G.P. Baerends (Zoologisch Laboratorium, Rijksuniversiteit Groningen): De invloed van de activatietoestand van gedragssystemen op de verwerking van informatie over de buitenwereld.

December 4 - Dr. B.S. Hetzel (CSIRO Division of Human Nutrition, Adelaide, South Australia): Iodine deficiency in sheep.

December 19 - Dr. S. Leung (Instituut voor Medische Fysica TNO, Utrecht): The hippocampal CA 1 region in the rat: a study of neurophysiology and behavior.

Hubrecht Laboratory International Embryological Institute

Progress Report 1979

Edited by J. Faber

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

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Management and staff

Director

Vacancy as from November 19th

Acting Director

D. van der Mei, Ph.D.
(as from August 15th)

Deputy Director

J. Faber, Ph.D.

Laboratory Manager

Elizabeth A. Berends

Chief Librarian

B.Z. Salomé, M.Sc.

Supervisor of Animal Care

Romee Verhoeff-de Fremery,
M.Sc.

Prof. of Exper. Embryol., University of Utrecht

P.D. Nieuwkoop, Ph.D.

Experimental Morphology; Curator of the Central Embryological Collection

Elze C. Boterenbrood, Ph.D.

Experimental Morphology; Cinematography

K. Hara, Ph.D.

Histo- and Cytochemistry

Geertje A. Ubbels, Ph.D.

Ultrastructural Research

J.G. Bluemink, Ph.D.

Tissue and Organ Culture

Kirstie A. Lawson, Ph.D.

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

Honorary research associate

W.P. Luckett, Ph.D. (Omaha,
Nebr., U.S.A.)

Temporary research associates

J. Boonstra, Ph.D.
S.A. Nelemans, M.Sc.
C.J. Weijer, M.Sc.
E.J.J. van Zoelen, Ph.D.

Guest worker (Univ. of Leiden)

W.H. Moolenaar, Ph.D.

Visiting scientists

Rosine Chandebais, Ph.D., Prof.
(Marseille, France)
J. Klag, Ph.D. (Kraków, Poland)
Prema Michael, M.Sc. (Shillong,
India)
Christine L. Mummery, Ph.D.
(London, England)
K. Rzehak, Ph.D. (Kraków,
Poland)
P. Sura, M.Sc. (Kraków, Poland)

Graduate Students (Univ. of Utrecht)

A. Bierman
H. Spanjer

OTHER STAFF (partial)

Technicians (semi-scientific staff)

A.R.J. Bleumink
Alie Feijen
W.J. Hage
Frida C. Haring-Vork
C.H. Koster
W.A.M. van Maurik
P. Meyer
Jenny Narraway
P. Poot (temporary)
L.G.J. Tertoolen
P. Tydeman

F.J.M. Vervoordeldonk
Willeke M. Miltenburg-Vonk
R. Willemsen

Secretariat

Sylvia J. de Vos (on leave)
Dorothy J.S. Parsons

Administration

A. van den Breul
B.H.H. de Deugd

Library

Oeke E.H. Kruythof
Nora Pulle-Starke
Anne-Miek Vernooij-Wiss
Engelina C. Ekelaar (GEIS)

Photography and Art

L. Boom
Carmen Kroon-Lobo

Head of Domestic Service

G. van Garderen

Technical and Electronics Workshop

W. Leurink
J.H. Beeker
L.J. de Kam
H.L. Krielen
J.L. van Lun
C. Mook
K.R. Reitsma

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 multidisciplinary approach to the many problems of development seven disciplines are being practised, each applying a variety of experimental procedures (see p. 3).

The laboratory aims at stimulating international co-operation and understanding by, among other things, organising International Research Groups in Developmental Biology at more or less regular intervals, and by the biennial publication of an international directory of investigators active in developmental biology (General Embryological Information Service).

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 central theme of the research carried out at the Laboratory is the *origin of patterns of cellular differentiation*. This theme is being approached in four *research groups*. Their current work is described in sections I-IV, which together represent the main research programme of the Laboratory. Projects car-

ried out outside the research groups proper (though often having a distinct relation to the work in the research groups) are described in sections V and VI.

The main research programme encompasses three main levels of biological organisation, the organismal, the cellular and the molecular level. Wherever possible the material in this Report is arranged roughly according to levels of organisation in the order named.

I. Research Group: Embryogenesis in Amphibians

Members: J.G. Bluemink (leader), E.C. Boter-enbrood, K. Hara, P.D. Nieuwkoop, G.A. Ubbels, R. Verhoeff-de Fremery; W.J. Hage, C.H. Koster, W.A.M. van Maurik, J.M. Narraway, P. Tydeman, F.J.M. Vervoordeldonk.

Guest workers: J. Klag, K. Rzehak.

Long-term collaboration with: 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).

The major focus of interest of this research group is the analysis of *early cell diversification* during cleavage and in the blastula and gastrula stages. Features of,

and events in the uncleaved egg form an integral part of this analysis. Apart from the work described under A-C below, nucleo-cytoplasmic interactions will also be studied in the future.

A. MORPHOGENETIC MOVEMENTS AND CELL INTERACTIONS

*1. Pre-gastrular surface movements in the morula and blastula (*Xenopus laevis*, *Bombina orientalis*, *Discoglossus pictus*)*

During gastrulation a drastic topographical rearrangement of cell layers takes place, opening the way for new cellular interactions essential to the formation of a complete, organised embryo. The immediate cause of the initiation of gastrulation is still unknown. On the one hand, a relation has been found between the time of inception of invagination and the duration of the cell cycle in the preceding synchronous cleavages¹;

on the other hand, pregastrular movements have been observed on the surface of the advanced blastula: epiboly in the animal hemisphere, sometimes coupled with ingression in the vegetative hemisphere². Since these movements can be regarded as a preliminary to gastrulation, a precise characterisation of their temporal and spatial aspects and their relation to the cell cycle will contribute to the elucidation of the initiation of gastrulation.

We have started a time-lapse cinematographic study of the surface area taken up by individual blastomeres and their descendants after successive synchronous cleavages. Preliminary observations revealed enlargement of the total external surface area of the animal blastomeres and reduction of that of the vegetative blastomeres in successive stages. There was no egression of internal cells to the external cell layer, nor ingression of external cells.

¹ Signoret, J. and Lefresne, J. – 1973, *Ann. Embryol. Morphogen.* 6, 299-307

² Keller, R.E. – 1978, *J. Morphol.* 157, 223-247

2. Mechanisms of mesoderm induction (*Ambystoma mexicanum*)

In amphibians the mesoderm arises as a result of an inductive action emanating from the vegetative yolk mass (see previous report, introduction to sect. II). There are many indications that the variety of mesodermal structures formed may be related to a regional variation in the intensity of the inductive action. Morphological data are consistent with the possibility that a diffusion mechanism is responsible for such regional variation¹. However, little is known of the cellular and molecular basis of mesoderm induction. What is known comes from experiments where heterospecific denatured inducers were used, which makes their significance for the normal mesoderm induction process doubtful.

Pilot experiments to test the inductive capacity of the vegetative yolk mass (the natural inducer) have been performed using disaggregated cells. The histological analysis of the material obtained is in progress.

¹ Weijer, C.J., Nieuwkoop, P.D. and Lindenmayer, A. – 1977, *Acta Biotheor.* 26, 164-180

B. THE ROLE OF HETEROGENEOUS CYTOPLASMIC DISTRIBUTION

1. Egg rotation experiments and the establishment of dorso-ventral polarity (*Xenopus laevis*)

In normal development the establishment of dorso-ventral polarity in the uncleaved anuran egg is the result of sperm penetration. By marking the point

180° opposite the sperm entrance point (SEP) with a small crystal of Nile Red (see previous report, sect. I.A.I.1 and this report, sect. VIII, publ. 19) we have now shown that the centre of the grey crescent (the site where the dorsal blastoporal lip will appear later) appears opposite the SEP (in most cases at 180°, in almost all cases between 140° and 220°).

However, under experimental conditions gravity may offset the orienting effect of the sperm (see previous report, sect. I.A.I.5 and this report, publ. 18). If eggs having their SEP in the equatorial region are kept rotated 90° for some time in a 5% solution of Ficoll-400 (which prevents back-rotation as a result of shrinkage of the perivitelline space), the eggs having their (vitaly stained) SEP up will have their dorso-ventral polarity reversed by 180°, whereas the eggs with the SEP down will show no polarity reversal. Cytological analysis of 22 such eggs has now shown that in both classes of eggs the position of the 'dorsal cytoplasm' (see previous reports) and of the pronuclei remains unchanged. However, both in 'polarity-reversed' and in 'non-reversed' eggs a distinct yolk ridge ('mur vitellin') is found under the cortex on the future dorsal side (being the original ventral side in 'reversed' eggs). Our interpretation is that the overall distribution of the internal cytoplasm is maintained by a web of microtubules (of which the sperm aster and the female pronuclear monaster form part), whereas in the periphery of the egg the yolk distribution is altered under the action of gravity. These results show that neither the position of the grey crescent nor that of the 'dorsal cytoplasm' is the ultimate determinant of the future dorsal side, but that it is determined by the sub-cortical arrangement of the vegetal yolk (or of other cytoplasmic constituents shifting together with the yolk)¹. This supports our suggestion made elsewhere (see publ. 18) that the dorso-ventral reversals obtained by Curtis² were due to general operational conditions rather than to the transplantation of grey crescent cortex as such. Our conclusions are supported by time-lapse films made of eggs developing after rotation with the SEP up or down.

¹ Gerhart, J.C. – Chapter in *Biological Regulation and Development*; Ed. R.F. Goldberger, New York, Plenum (in press)

² Curtis, A.S.G. – 1960, *J.Embryol. exper. Morphol.* 8, 163-173

2. Surface activity of the egg as related to internal cytoplasmic displacements and the establishment of dorso-ventral polarity (*Xenopus laevis*)

The work started last year (see previous report, sect. I.A.I.1) was continued. The asymmetrical post-fertilisation waves (PFWs) which occur on the surface

of the egg¹, were analysed in relation to the coincident internal cytoplasmic displacements, using time-lapse cinematography and cytological techniques. The basic assumption was that microtubules are instrumental in both phenomena and that cytoplasmic displacements are initiated by the sperm centriole. Vinblastine and colchicine were used as inhibitors of microtubular activity in fertilised eggs, and unfertilised eggs were activated by pricking. After both kinds of treatment the contents of the animal half of the egg, including the so-called 'clear cytoplasm' (the later 'dorsal cytoplasm', see previous reports), are rearranged under the influence of gravity only. The normally observed asymmetrical cytoplasmic displacements (see previous reports) fail to occur; moreover, the PFWs are absent, as shown by time-lapse cinematography. Vinblastine also interferes with the cortical pigment movements normally leading to grey crescent formation.

This evidence supports the assumption that the asymmetrical PFWs and the coincident internal cytoplasmic displacements have a common basis in microtubular activity associated with the presence of the sperm. We have suggested elsewhere (see publ. 18) that the sperm, by way of its centriole, acts on some target in the egg, which in its turn directly determines an asymmetrical contraction of the cortex; this would then lead to the asymmetrical arrangement of the vegetal yolk which ultimately determines the dorso-ventrality of the egg. It now seems likely that the target in question is the cytoskeleton of the egg. This would also explain why neither the grey crescent nor the 'dorsal cytoplasm' are determinants of the dorsal side (see 1 above). Grey crescent formation may reflect the movement of pigment granules trapped in a contractile matrix of filamentous proteins, carrying with it associated materials of the deeper cytoplasm². In conjunction with this study a 'normal table' of the external and internal development of the egg from fertilisation till first cleavage has been established (see publ. 18). This is being refined using a combination of video-time-lapse recording and cytology.

¹ Ubbels, G.A. – 1977, *Mém. Soc. Zool. France* 41, 103-115

² Gerhart, J.C. – Chapter in *Biological Regulation and Development*; Ed. R.F. Goldberger, New York, Plenum (in press)

3. Development of a method for artificial fertilisation with a high yield of bi- and trispermic eggs (*Xenopus laevis*)

Bi- and trispermic eggs can be used to study the role of the sperm in symmetrisation of the anuran egg¹ (see also report for 1977, sect. I.A.i.3 and 4). Therefore, a

laboratory method for artificial fertilisation was elaborated that yields a relatively high number of bi- and trispermic eggs. As in some other animal groups, in anurans the first block to polyspermy results from depolarisation of the egg plasma membrane immediately upon sperm entry of artificial activation²; it is due to an efflux of Cl⁻ ions. Therefore, next to the application of high sperm concentrations and prolongation of the insemination period, elevation of the Cl⁻ concentration in the external medium was used. The highest percentage of bi- and trispermic eggs was obtained using 60-70% MMR as the external medium (see publ. 18) and restricting the insemination period to 5 min.

¹ Ubbels, G.A. – 1977, *Mém. Soc. Zool. France* 41, 103-115

² Cross, N. and Elinson, R. – 1978, *Amer. Zool.* 18, 642

4. Ooplasmic segregation during egg maturation (*Xenopus laevis*)

Oocytes artificially brought to maturation *in vitro* were analysed cytologically. It was found that when the germinal vesicle breaks down at least part of its content forms the so-called 'yolk-free' or 'clear' cytoplasm (the later 'dorsal cytoplasm'; see previous reports).

C. STRUCTURAL HETEROGENEITY IN THE CELL MEMBRANE

1. Freeze-fracture electron microscopy of the egg plasma membrane in relation to the establishment of dorso-ventral polarity (*Xenopus laevis*)

In *Fucus*¹ and in *Xenopus*² egg polarity can be correlated with topographical differences in the plasma membrane, which may represent differences in functional membrane properties. Cell partitioning will equip the daughter cells with unequal sets of functional membrane units. If it is assumed that such units act as circuits for plasma membrane/cytoplasmic interaction, membrane partitioning as such may bear on the mechanism that elicits the spectrum of gene activities responsible for early cell determination. In the amphibian zygote a dominant morphogenetic role has been ascribed to the cortex of the future dorsal side. In this context it is our aim to compare the dorsal with the ventral egg surface membrane, using freeze-fracture electron microscopy. A new method has been elaborated to isolate ca. 0.5 × 0.5 mm sheets of plasma membrane from selected areas, to fix them to a polylysine-coated specimen holder, and to quickly freeze them in a 'sandwiched' configuration. In freeze-

etch replicas dorsal and ventral parts of the plasma membrane will be analysed for regional differences in the distribution of intramembranous particles.

¹ Peng, H.B. — 1976, Ph.D.thesis. Purdue Univ., West Lafayette

² Bluemink, J.G. and Tertoolen, L.G.J. — 1978, *Cytobiologie* 16, 358-366

2. Lectin binding properties of the egg plasma membrane and of newly formed (cleavage) membrane (*Xenopus laevis*)

In the dividing egg new membrane is formed along the cleavage furrow¹. The structural and functional properties of the new membrane are different from those of the pre-existing membrane². Freeze-fracture electron microscopy has shown that the old membrane is densely populated with intramembranous particles (IMPs), whereas there are few IMPs in the new membrane³. The surface properties of both membrane regions were analysed by means of various ferritin-coupled lectins. Concanavalin A binds moderately,

Wheat Germ Agglutinin more strongly to both old and new membrane. Soy Bean Agglutinin does not bind to old membrane but binds positively to new membrane. There was no aspecific binding in the controls. The ferritin density along smooth and rough-surfaced areas as seen in ultrathin sections did not differ noticeably. It was concluded that there is no correlation between lectin binding capacity and IMP frequency. The regional differences in the intensity of surface-bound fluorescence reported by others⁴ in light microscope observations are presumably due to variations in surface roughness.

¹ Bluemink, J.G. and Laat, S.W. de — 1973, *J. Cell Biol.* 59, 89-108

² Laat, S.W. de and Bluemink, J.G. — 1974, *J. Cell Biol.* 60, 529-540

³ Bluemink, J.G., Tertoolen, L.G.J., Ververgaert, P.H.J.Th. and Verkleij, A.J. — 1976, *Biochem. Biophys. Acta* 443, 143-155

⁴ Tencer, R. — 1978, *Exp. Cell Res.* 116, 253-260

II. Research Group: 'Organogenesis in Mammals'

Members: K.A. Lawson (leader); R. Willemsen.
Long-term collaboration with: A.A.W. ten Have-Opbroek (Leiden, Netherlands).

D. EPITHELIAL-MESENCHYMAL INTERACTIONS IN MAMMALIAN ORGANOGENESIS

Organs such as the lung and salivary gland, in which the mesenchyme is essential for the growth and development of the epithelium, are being used to study

1. mesenchymal control of epithelial morphogenesis,
2. the extent to which cell proliferation and/or morphogenesis are necessary for the expression of overt cell differentiation in the epithelium, and
3. the question whether there are instructive interactions during very early stages of organogenesis.

1. Specificity in mesenchyme requirement during early lung morphogenesis (*Rattus norvegicus*)

Whereas 14-day fetal lung epithelium, of which the primary bronchus has begun to form secondary buds, will continue to show branching morphogenesis *in vitro* when recombined with salivary mesenchyme,

primary bronchi from 13-day fetuses fail to develop in salivary mesenchyme, although they develop normally in their own mesenchyme (see previous report, sect. II.B.2). This failure is not due solely to the small initial size of the bronchus, since distal epithelial tips of comparable size from 14-day lung rudiments will continue morphogenesis in salivary mesenchyme. The failure can be overcome by removing the salivary mesenchyme after 3 days' culture and recombining the lung epithelium with salivary mesenchyme freshly obtained *ex vivo*. It has now been found that mechanical removal and replacement at 3 days of the same piece of salivary mesenchyme with which the lung epithelium was originally combined, is sufficient to initiate branching morphogenesis in the epithelium within 20 h. The effect is therefore not due to ageing of the salivary mesenchyme. The effect of altered spatial relationships of epithelium and mesenchyme at 3 days on the rate of cell division in the epithelium is being investigated.

2. Possible role of mesenchyme in determining cell differentiation in lung epithelium (*Mus musculus*, *Rattus norvegicus*)

This work is the continuation of that started last year (see previous report, sect. II.B.3). The influence of the mesenchyme and the role of epithelial morphogenesis in lung epithelial differentiation is being investigated

using a lung-specific antiserum which reacts with the cuboidal epithelium of the future respiratory portion of the lung of 15-day and older mouse fetuses¹.

i. Optimal conditions for the development *in vitro* of the respiratory portion of the lung from early lung rudiments have been established, using the mouse left lung as standard. The unbranched left primary bronchus from the 11-day fetus develops better in association with the right lung rudiment than alone, and slightly better on an agar substrate than direct on the surface of a plasma clot. Twelve-day lungs grow and branch faster directly on the surface of the clot. The addition of extra bronchial mesenchyme was already known to stimulate lung epithelial growth²; it has now been shown that such stimulation is effective across a Nuclepore filter with pores of 0.05 μm diameter, into which cytoplasmic processes are unable to penetrate. The effect of additional lung mesenchyme is therefore due to diffusible material, and is not dependent on cell contact. Cuboidal and flattened epithelia are formed in the distal portions of the rudiments within 7 and 10 days of culture, respectively. Preliminary results with rat material show that the cuboidal cells *in vitro* react

with the specific lung antiserum. Attempts to culture presumptive lung regions from early somite embryos are in progress.

ii. Supernumerary buds induced on the tracheal portion of the lung rudiment by bronchial mesenchyme³ continue to branch in culture and form distal cuboidal cells indistinguishable from those in the lung. The possible appearance of specific lung antigen (lung differentiation product) in these tracheal-derived cuboidal cells is being investigated.

iii. Rat lung epithelium branches in a salivary pattern in submandibular mesenchyme (see previous reports). After prolonged culture 'cuboidal' cells are found in the distal parts of the epithelium. These cells will also be examined for the appearance of specific lung differentiation product.

¹ Have-Opbroek, A.A.W. ten – 1979, *Devel. Biol.* 69, 408-423

² Alescio, T. and Colombo Piperno, E. – 1967, *J. Embryol. exper. Morphol.* 17, 213-227

³ Alescio, T. and Cassini, A. – 1962, *J. Exper. Zool.* 150, 83-94.

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

Members: A.J. Durston (leader), C.J. Weijer (res. assoc.); A.R.J. Bleumink, F. Haring-Vork, P. Poot. *Long-term collaboration with:* S.K. Brahma (Utrecht, Netherlands), D.O'Day (Toronto, Ont., Canada).

The aim of this research group is to seek general principles underlying morphogenesis and pattern formation. There is good evidence that such general principles exist, in the sense that different organs in particular embryos are mapped out via homologous mechanisms. It is also clear that morphogenesis and pattern formation share important common features in a variety of embryos, and a few explicit homologies have been demonstrated between equivalent morphogenetic events in quite distantly related organisms. To date there is virtually no evidence as to the nature of any general morphogenetic principles, but in multicellular embryos it is reasonable to think of universal cell interactions. Our approach to the problem is twofold: a. To identify cell interactions underlying morphogenesis and pattern formation in a very simple multicellular organism, *Dictyostelium discoideum* (Dd), we try to obtain a fairly complete

cell-level understanding of morphogenesis in this model system and to identify any generalities (i.e. multiple roles for single interactions) (see E below); b. Comparative studies to look for universal cell interactions; in particular, to determine whether cell interactions in vertebrate embryos and other developing systems are analogous to Dd cell interactions (see F below).

E. MORPHOGENESIS AND PATTERN FORMATION IN *Dictyostelium discoideum*

We are mainly concerned with later (post-aggregation) stages of Dd development, during which the multicellular 'slug' develops into a fruiting body containing a proportionate pattern of two cell types (stalk cells and spores).

1. Cell movement

As reported last year (see previous report, sect. II.C.1), later Dd cells make non-decremental waves of movement (period 2-10 min, velocity ca. 50 $\mu\text{m}/\text{min}$) resembling waves seen during Dd aggregation (see publ. 1). We also showed that one cell competence underlying aggregation waves (chemotaxis to 3', 5', cyclic AMP (c-AMP) persists in later Dd development and that prestalk and prespore cells in tissues differ in chemotactic behaviour. They sort out via chemotaxis

during normal development and in response to artificial c-AMP sources (see publ. 7). The following approaches were made to pursue these results.

1a. Relaying of c-AMP pulses

We are checking whether slug cells amplify a c-AMP pulse. In the first experiments (see previous report, sect. II.C.1a) c-AMP pulses (peak concentration 10^{-7} – 10^{-9} M) had been added to an aerated cell suspension and total c-AMP and c-GMP were measured as a function of time, using competitive binding assays. This method showed a small c-GMP increase but no c-AMP increase above background following the input pulse. (Aggregation-stage cells make sizeable c-AMP and c-GMP pulses following stimulation.) In subsequent experiments we have used a method which enables detection of a small response to a high-amplitude signal. Slug cells were pre-labelled with 32 P-phosphate or 3 H-adenine, stimulated with unlabelled c-AMP, and separated from secreted products by perfusion (in experiments using cells supported on a high-porosity membrane filter) or by centrifugation through mineral oil (in experiments using cell suspensions). Secreted c-AMP was then purified on neutral alumina and Dowex, and counted. Preliminary results indicate a small relaying response to peak concentrations of 10^{-6} M c-AMP.

1b. Cell sorting, chemotaxis and adhesion

Dissociated prestalk and prespore cells were examined for differences in their chemotactic response to c-AMP. So far no difference was found, suggesting that the cell sorting seen in tissues occurs via a cell density-dependent effect: e.g. via a difference in adhesiveness between prestalk and prespore cells. We are therefore testing reagents which block Dd cell contact, to see if these affect sorting. TRITC-labelled prestalk cells (see 3a below) and unlabelled prespore cells, from slugs, are dissociated and the cell suspension is allowed to form mixed aggregates by incubating for 30 min in the presence of a test substance. The suspension of mixed aggregates is then deposited on 10^{-4} M c-AMP agar and the time course of c-AMP-induced cell sorting is followed (see publ. 7). So far, we have tested Fab fragments (i.e., univalent antibodies) made against membrane preparations from Dd cells at 0 hr and 9 hr of development. These are reported to block adhesion via two early classes of contact site (B sites in the case of 0-hr Fab, and A and B sites in the case of 9-hr Fab: see 2b below). Neither Fab preparation had a dramatic effect on cell sorting. Anti-0-hr Fab at 8 mg protein/ml had no effect and the same concentration of anti-9-hr Fab caused a slight delay (< 30 min) in comparison with treatment by aspecific Fab. It thus seems unlikely that A or B sites have a role in cell sorting. This conclusion is

tentative because the effective concentration of Fab (i.e., that within the mixed aggregates) is unknown.

2. Cell contact

2a. Cell coupling

Previous electrophysiological measurements (see previous report, sect. II.C.2a) showed an absence of electrical coupling between cells in Dd aggregates and slugs. However, Dd cells are small (7 μ m diameter), leading one to suspect they may be damaged by penetration and consequently uncouple. The studies started last year to check the membrane potential (by a distribution method, using TPP⁺) and the K⁺ resistivity of the membrane (by 86 Rb⁺ and 42 K⁺ flux measurements) were continued. Fluorescein was also injected successfully into slug and early aggregation stage cells: these experiments showed no evidence of intercellular dye diffusion, even though the injected cells remained intact, motile and in contact with neighbours.

2b. Blocking of contact sites

There is a report in the literature¹ that univalent antibodies (Fab's) made against crude particulate fractions from vegetative Dd cells (i.e., 0 hr of development) and against aggregation-stage Dd cells (9 hr of development) effectively block cell adhesion via two classes of contact site. Anti-vegetative Fab's are reported to block contact via B sites, which mediate adhesion between vegetative cells and side-to-side adhesion between the polarised aggregation-stage cells. Anti-aggregative Fab's are reported to block adhesion via B sites and also via A sites, which mediate end-to-end adhesion between aggregation-stage cells. We made similar Fab preparations, to use as a first line of investigation to examine the effects of blocking A and B contact sites on cell sorting and differentiation (see 1b above and 3 below). Antisera were made against particulate fractions from 0 and 9-hr cells, similarly as described by Beug *et al.* IgG's were purified from these, using affinity chromatography to protein A.

The IgG's were split into Fab and Fc fragments, using sepharose-papain and Fab fragments were then separated from the Fc fragments on the protein A column. Molecular weights of the IgG's and Fab's were confirmed using SDS gel electrophoresis. Our antisera proved highly polyspecific and similar to each other. Immunoelectrophoresis against the homologous and heterologous antigens showed about 12 major precipitation lines, which were all common to both antisera. The Fab preparations show at least some of the biological effects previously reported and show differences from each other which are presumably due to minor components. Anti-aggregative Fab totally

blocked or delayed adhesion between aggregation-stage cells or cells of earlier stages at concentrations down to 0.7 mg protein/ml. Anti-vegetative Fab had a weak effect on adhesion of aggregation-stage cells. Neither preparation strongly inhibited chemotaxis and neither affected reaggregation of slug cells. Tests of effects on vegetative cell (B-site) adhesion (which is weak) require quantitative measurements and will be performed during 1980, once equipment for measuring cell adhesiveness becomes available.

¹ Beug, H., Katz, E.F. and Gerisch, G. — 1973, *J. Cell Biol.* 56, 647-658

3. Cell differentiation

During Dd development, one cell type (vegetative amoeba) differentiates to a proportionate pattern of two new cell types (stalk cells and spores). The final pattern is foreshadowed by a regulative 1-D axial pattern of 'embryonic' cell types (prestalk and prespore cells) in the slug. This organism thus poses, in a very simple way, the universal problem of how embryos make regulative patterns of differentiation. Our investigations fall under three headings.

3a. Testing models for pattern formation

Virtually all models for regulative pattern formation postulate a differentiation-controlling axial gradient (e.g. a concentration gradient for a morphogen), which controls proportionality via a threshold.

However, our earlier results (see previous reports) and those of others show that formation and regulation of the slug's pattern involves cell sorting between two cell populations. It is possible that position of cell types along the slug's axis is determined entirely by movement, while cell differentiation (and the ratio of cell types) is controlled by a non-positional homeostatic mechanism. We have begun testing various models (see publ. 15) as follows.

i. Positional models and particular classes of non-positional models make different predictions about differentiation and movement of marked cells of a particular type grafted into various positions in slugs and slug pieces. We have marked Dd cells by conjugating them to TRITC (tetra-methyl rhodamine iso-thio-cyanate). This dye binds to the cell surface, is then internalised and acts as an excellent non-diffusible, non-cell type-specific marker. We have used TRITC in a series of experiments to follow differentiation of marked cells grafted to various positions. The results favour a certain class of non-positional models.

ii. Particular models make specific predictions about the kinetics of cell type conversion during regulation

of prestalk and prespore regions from slugs.

Preliminary measurements have been made, using cell type-specific FITC-immunofluorescent staining (see also 3c below) to quantify the prespore cell fraction. (FITC = fluorescein iso-thio-cyanate).

iii. We have been able to separate pre-aggregation Dd cells into two cell types by Percoll density gradient centrifugation. These may be forerunners of the prestalk and prespore types: if so, this would be strong evidence for non-positional differentiation. We are investigating the properties of these early cell types.

3b. Cell markers

We have examined adsorbed antisera, lectins and other reagents to obtain new stage-specific and cell type-specific markers for Dd differentiation. Specific goals are: to obtain a good prestalk cell marker (for 3c below) and early differentiation markers (for 3a.iii above). So far, we have two new prestalk cell markers: cell surface staining with Wheat Germ Agglutinin, and with an antiserum made against aggregation-stage cells and adsorbed against vegetative cells (see publ. 15). Neither is good enough to use in quantitative studies of differentiation.

3c. Morphogens

We are seeking morphogens regulating the transitions amoeba $\begin{matrix} \swarrow \text{prestalk} \\ \searrow \text{prespore} \end{matrix}$ and prestalk = prespore. The test system used is differentiation of Dd cells in submerged culture, in microtest trays under pure oxygen.

Prespore cell differentiation is assayed by indirect FITC-immunofluorescent staining, using an antiserum made against homogenised Dd spores and adsorbed against a homogenate of Dd aggregation-stage cells, to remove aspecific binding. This is a specific stain for a prespore cell-specific organelle (the prespore vacuole), which contains precursors of the spore wall. Prestalk cell differentiation is assayed by measuring the prestalk enzyme alkaline phosphatase. The tests performed have been, and will be 1. adding agents early in development, and looking for shifts to prestalk or prespore cells, and 2. starting with pure populations of prespore or prestalk cells and looking for effects on regulation. The agents tested so far are known and suspected slime mould morphogens, such as NH_4^+ and c-AMP, and stage-specific anti-slime mould-cell surface Fab fragments.

F. MORPHOGENESIS IN OTHER SYSTEMS

1. Pilot studies on cell movement in vertebrates

A start was made with filming movements of marked cells in intact structures as well as during cell migration from explants, both in embryonic development of the

chick (with K.A. Lawson) and in *Ambystoma* limb regeneration (with J. Faber).

2. Films of culmination in *Polysphondylium pallidum*

Polysphondylium is a genus of the cellular slime moulds in which the fruiting bodies make metameric whorls of side branches (i.e., whorls of miniature fruiting bodies standing at right angles to the main stalk). This spatially periodic branching process may have features in common with periodic pattern formation in other organisms, and we have filmed it in *Polysphondylium pallidum* to know something of its basis.

The films and other experiments showed the following main points. Branching was initiated by the appearance of a ring of tips (organisers) near the rear end of the main sorogen. Each tip organised a territory, which split off from the main cell mass and its neighbours, and then developed into a small fruiting body. Since it was known for *Dictyostelium discoideum*¹ that new tip formation is inhibited by the

tip of a developing slug (or sorogen), we examined the effect of excising the tip of the main *Polysphondylium* sorogen on side branching. The result was immediate formation of multiple side branches from every part of the sorogen.

We tentatively infer that a *Polysphondylium* sorogen resembles a *Dictyostelium discoideum* slug in containing a posteriorly decreasing axial gradient in capacity to inhibit tip formation, and that this gradient fluctuates, so that inhibition sometimes becomes ineffective at the low point of the gradient (i.e., at the rear end of the sorogen). The fact that this fluctuation results in formation of whorls of new tips at regular intervals in space (and, as the films show, in time) suggests that the amplitude of the inhibitory gradient oscillates. The oscillation in branching is accompanied by a synchronous oscillation in rate of erection of the sorogen (T - 1 hr).

This work has been submitted for publication.

¹ Durston, A.J. - 1976, *Nature* 263 (5573), 126

IV. Research Group: 'Regulation of the Cell Cycle'

Members: S.W. de Laat (leader), P.T. van der Saag, J. Boonstra (res. assoc.), S.A. Nelemans (res. assoc.), E.J.J. van Zoelen (res. assoc.); A. Feijen, G. Hendriks, P. Meyer, W.M. Miltenburg-Vonk, L.G.J. Tertoolen.

Guest workers: W.H. Moolenaar, C.L. Mummery.
Long-term collaboration with: E.L. Elson (St. Louis, MO, U.S.A.), J. Schlessinger (Rehovot, Israel), M. Shinitzky (Rehovot, Israel).

G. REGULATION OF THE CELL CYCLE: ROLE IN DEVELOPMENT AND DIFFERENTIATION

In this research group attention is focussed on the molecular regulation of cell multiplication and cell differentiation. The development of a multicellular organism results from the temporally and spatially controlled multiplication and differentiation of its cells. Terminal differentiation, i.e. the developmental process by which cells attain their definitive form and function, is often characterised by growth arrest in a specific phase of the cell cycle. This indicates a link between the regulation of the cell cycle and the initiation of cell differentiation.

1. Role of the plasma membrane in the regulation of the cell cycle and cell cycle-dependent differentiation

This study focusses particularly on the plasma membrane, because of 1. the transducer role of the plasma membrane in interactions between the extracellular environment and the cell; 2. the communicative role of the plasma membrane in interactions between cells; and 3. the regulatory role of the plasma membrane with respect to cytoplasmic factors that are involved in gene regulation.

Murine neuroblastoma cells, which can be cultured *in vitro*, are being used as a model system for neuronal differentiation. Originally these cells were isolated from a mouse embryonic tumor derived from neural crest cells. Neuroblastoma cells therefore resemble neuroblasts, i.e. embryonic, undifferentiated but determined precursors of sympathetic nerve cells. *In vitro*, differentiation can be induced synchronously in almost unlimited numbers of cells; the cells are arrested in the G₁-phase of the cell cycle and develop the characteristic morphological, biochemical and electrical properties of a nerve cell. The availability of large synchronous populations of cells, as well as the presence of well-defined and well-detectable differentiation markers, make these cells suitable for studying the regulation of the cell cycle and cell-cycle-dependent differentiation.

(Note: project 1i below uses pheochromocytoma cells.)
At present our research is directed mainly at the

analysis of 1. the molecular mechanisms by which the plasma membrane controls cytoplasmic factors involved in growth control and gene regulation, and 2. the molecular mechanisms by which external factors mediated by the plasma membrane are involved in the control of cell proliferation and differentiation. To this end the causal relationships are being studied between the physico-chemical properties, the composition, and the ultrastructure of the plasma membrane on the one hand, and on the other the functioning of membrane-bound enzymes responsible for the production of molecules and the transport of ions and molecules involved in growth control and gene regulation. In addition, the mechanisms are being investigated by which external factors, through binding to plasma membrane receptor sites, can modulate these properties and processes. The following methods are being employed in this study: a. *biochemical methods* for the analysis of 1. the composition of the plasma membrane, 2. the functioning of membrane-bound enzymes involved in membrane ion transport (($\text{Na}^+ - \text{K}^+$)-ATPase) and cyclic nucleotide metabolism (adenylate cyclase), 3. membrane transport of ions and nutrients (tracer fluxes), 4. properties of specific membrane receptors. 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), 3. electrical membrane properties and ion transport (electrophysiology). c. *freeze-fracture electron microscopy* for the analysis of the ultrastructure of the plasma membrane, and scanning electron microscopy for the analysis of cell surface architecture. d. *time-lapse cinematography* for the analysis of cell cycle kinetics.

1a. Membrane ion transport and the cell cycle

The properties of the cation transport systems and the related membrane potentials have been shown to be involved in the regulation of cell proliferation^{1,2}. A particular role for the K^+ transport system in these processes has been suggested. The study of the relationships between these properties requires accurate methods of analysing flux kinetics. Therefore, K^+ transport was studied in exponentially growing neuroblastoma cells (clone Neuro-2A), using $^{42}\text{K}^+$ and $^{86}\text{Rb}^+$ as radioactive tracers. K^+ efflux studies in particular are very useful for studying both K^+ flux kinetics and the permeability properties of the plasma membrane. Two different methods were used to measure K^+ efflux; one, a sampling method, where samples of the external medium were taken at various times after removal of the initial labelling medium, and the other, a washing method, where all external medium was removed and replaced by fresh medium

at 6-sec intervals. Using either method it was found that K^+ efflux could not be described as from a one-compartment system under steady state conditions; however, the two compartments could only be completely resolved using the washing method.

The compartment from which K^+ efflux was most rapid (the 'fast' compartment) was found to be associated with the cell surface. The 'slow' compartment represented the intracellular K^+ . This compartment contained 151 ± 5 nmoles $\text{K}^+ / 10^6$ cells, the rate constant was $0.0164 \pm 0.0005 \text{ min}^{-1}$. From these parameters the unidirectional K^+ efflux was calculated to be 2.46 ± 0.14 nmoles $\text{K}^+ / \text{min} / 10^6$ cells. These conclusions were based mainly upon experiments where increasing the external K^+ concentration was seen to have no significant effect on the slow compartment, although the content of the fast compartment was increased. Addition of $1 \mu\text{M}$ valinomycin also had no effect on the slow compartment, but the fast compartment was rapidly released. The observations were confirmed by measurements of the intracellular K^+ activity by means of an ion-selective microelectrode.

Comparison of flux measurements made with the radioactive isotopes $^{86}\text{Rb}^+$ and $^{42}\text{K}^+$ as tracers for K^+ showed that Rb^+ was specifically bound on both the inside and the outside of the cells, and hence was not a suitable analogue for K^+ in studying K^+ flux kinetics in Neuro-2A cells. Measurements made with conventional and ion-selective microelectrodes showed that the membrane potential was -42.9 ± 1.3 mV and the intracellular K^+ activity was 108.1 ± 3.0 mM. It was also demonstrated that the membrane potential in these cells could be described by the constant field equation³, with Na^+ and K^+ as the permeable ions and Cl^- in thermodynamic equilibrium with the membrane potential. It was then possible to calculate the permeabilities of K^+ and Na^+ and the K^+ conductance from the flux and electrophysiological studies, using the equations from electrodiffusion theory³.

Similar studies were subsequently carried out in synchronised cells. It was found that the K^+ efflux rate was high in mitosis, decreased rapidly in the G_1 -phase, and rose at the onset of the S-phase. A small decrease was observed in the G_2 -phase. Similar modulations through the cell cycle were found for intracellular K^+ content, membrane potential and intracellular K^+ activity. Calculation of the K^+ and Na^+ permeabilities and the K^+ conductance revealed that these parameters were high in mitosis, decreased in G_1 , and increased at the onset of S. The calculations were confirmed by measurements of the specific conductance using conventional microelectrodes.

Using the cardiac glycoside ouabain as an inhibitor of ($\text{Na}^+ - \text{K}^+$)-ATPase, it was demonstrated that the

active K^+ influx was also high in mitosis, low in G_1 and high at the onset of S. From these results the question arises whether the modulation of active K^+ transport results from intracellular substrate concentration changes or from changes within the plasma membrane itself. Therefore, $(Na^+ - K^+)$ -ATPase activity was measured in cell homogenates through the cell cycle under optimal substrate conditions. The enzyme activity appeared to be modulated similarly to active K^+ transport *in situ*. It was concluded that through the cell cycle of Neuro-2A cells K^+ transport is regulated on at least two levels: 1. on the level of the passive permeability properties of the plasma membrane, due to intrinsic variations of the membrane during the cell cycle (as shown by the modulation of the K^+ and Na^+ permeabilities), and 2. on the level of the $(Na^+ - K^+)$ -ATPase. The mechanism of the latter regulation has not yet been established. However, since $(Na^+ - K^+)$ -ATPase activity measurements in cell homogenates under optimal conditions show a similar modulation through the cell cycle as found for K^+ influx, it is likely that the number of functional units of this enzyme and/or conformational changes of the enzyme are modulated through the cell cycle. Two papers on this research have been submitted for publication.

¹ Jung, C. and Rothstein, A. – 1967, J. Gen. Physiol. 50, 917-931

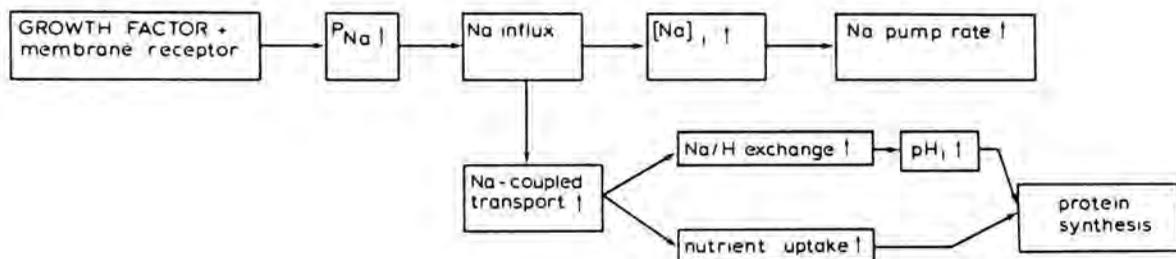
² Cone, C.D. – 1969, Trans. N.Y. Acad. Sci. Ser. 2: 31, 404-427

³ Hodgkin, A.L. and Katz, B. – 1949, J. Physiol. 108, 37-77

1b. Membrane ion transport and growth stimulation

Many mammalian cells stop growing and remain in a resting state when growth factors are removed from the culture medium. Addition of fresh serum or purified growth factors to such cells triggers a series of 'early' events which ultimately lead to the initiation of DNA synthesis and cell division. This project focusses on the dynamic membrane permeability changes following growth stimulation of serum-deprived neuroblastoma cells, using microelectrode and tracer

flux techniques. Within seconds of the addition of dialysed serum to neuroblastoma N1E-115 cells the membrane gradually depolarises to a maximum level reached after 40-70 sec. Next, the membrane potential slowly declines to a new steady level (after 5-10 min), which is depolarised with respect to the original resting potential. This depolarisation largely reflects an increase in Na^+ permeability, resulting in an enhanced Na^+ -influx (see publ. 8). A direct result of the serum-induced Na^+ -influx is a two-fold stimulation of the rate of the Na^+ , K^+ -pump within minutes of serum addition, as inferred from ouabain-inhibitable $^{86}Rb^+$ -uptake measurements. In the absence of serum the pump stimulation can be mimicked by increasing net Na^+ -influx with neurotoxins (veratridine, scorpion venom) or the Na^+ -ionophore monensin. DNA synthesis starts to increase at 10 hr after serum addition. Growth-depleted serum, incapable of stimulating DNA synthesis, fails to elicit any of the electrical and ionic events. Serum-induced Na^+ -influx, Na^+K^+ -pump stimulation and DNA synthesis are completely blocked by the diuretic amiloride (0.4 mM), but the membrane potential response is not significantly affected by the drug. This suggests that serum-stimulated Na^+ -influx occurs as part of an electroneutral exchange mechanism. The counter-transported ionic species is likely to be hydrogen: addition of NaCl (25 mM) to a stirred suspension of cells in Na^+ -free solution immediately enhances the rate of acidification of the medium, but *not* when amiloride (0.4 mM) is present. Na^+ / H^+ exchange has been shown to be amiloride-sensitive and to be involved in the regulation of intracellular pH in mouse skeletal muscle and in fertilised sea urchin eggs. A rise in intracellular pH appears to be a trigger for the onset of protein synthesis preceding DNA replication in the fertilised egg. Our results suggest a scheme for Na^+ -influx-dependent events triggered by serum growth factors as given below. Removal or depletion of serum from the medium initiates neuronal differentiation in N1E-115 cells. In view of the scheme such a differentiation program would then be induced by an accumulation of protons in the cell, i.e. by a decrease in the intra-



cellular pH. Interestingly, lowering the pH of the medium, which might result in a decrease of the intracellular pH as well, has been reported to induce morphological differentiation in a neuroblastoma cell line¹. The proposed role for intracellular pH as a 'second messenger' of growth factor-membrane interaction provides an attractive working hypothesis on which future experiments will be based.

¹ Bear, M.P. and Schneider, F.H. – 1977, *J. Cell. Physiol.* 91, 63-68

1c. Regulation of (Na⁺–K⁺)-ATPase activity at the molecular level

Investigations have been started in order to elucidate the regulation of (Na⁺–K⁺)-ATPase activity during the cell cycle of Neuro-2A cells at the molecular level. To this end the dependence of both the enzymatic and the pumping activity of the protein upon the concentrations of Na⁺, K⁺ and ATP were investigated, while the effects of these components on inhibition by ouabain were also studied. It has been shown that at physiological K⁺ concentrations (~4.5 mM) an external ATP concentration of 4 mM stimulates the pumping activity 1.7-fold (as measured by ⁸⁶Rb⁺ uptake). Under these conditions the pump itself shows half saturation with respect to external potassium at [K⁺] = 0.8 mM. The sensitivity of the pump to inhibition by ouabain is also strongly influenced by the presence of external ATP. In the presence of low potassium (0.4 mM) the addition of 4 mM ATP lowers the K_d for ouabain from 24 to 7 μM. Extrapolation to zero potassium yields a K_d of 2.6 μM for inhibition by ouabain in the presence of ATP, and a competition by K⁺ on this ouabain binding site with a K_i of 0.26 mM.

The high affinity of ouabain for (Na⁺–K⁺)-ATPase in the presence of ATP and in the absence of external K⁺ makes it possible to determine the number of active enzyme copies per cell by direct binding studies with ³H-ouabain. In all cases the difference in binding between zero K⁺ and high K⁺ conditions was measured. In this way it could be shown that Neuro-2A cells in an exponentially growing culture contain an average of 3.5 × 10⁵ active copies per cell, with a dissociation constant of 2.2 μM. Experiments are in progress to investigate to what extent the (Na⁺–K⁺)-ATPase is saturated with respect to internal Na⁺ under physiological conditions. From this a correlation can then be made between the pumping activity measured under physiological conditions and the rate of ATP hydrolysis measured under optimal conditions. In the near future the above experiments will be extended to cells in various phases of the cycle to determine variations in the number of active copies per

cell. We will also investigate possible variations in affinity towards K⁺, ouabain and ATP, which would indicate conformational changes in the enzyme during the cell cycle.

1d. Lateral mobility of membrane components

The assembly for measuring the lateral mobility of membrane components by the fluorescence photobleaching recovery (FPR) technique (see previous report, sect. III.D.1c) has recently been completed (see also section VI.2). We will start by studying the mobility characteristics of growth-stimulating peptide hormones and membrane-bound enzymes during the cell cycle of Neuro-2A cells. For the latter type of study specific fluorescently labelled antibodies are required. We will also investigate whether a relation exists between the activity of (Na⁺–K⁺)-ATPase and its mobility characteristics.

1e. Role of the lipid composition of the plasma membrane

i. Compositional changes during differentiation.

Earlier observations made it clear that during the cell cycle and differentiation of neuroblastoma cells considerable changes occur in the physico-chemical properties of the plasma membrane as determined by fluorescence polarisation (membrane fluidity)^{1,2} and photobleaching recovery (lateral mobility of lipid and protein membrane components) (see this report, publs. 6 and 20). To obtain a better understanding of the molecular nature of these changes the isolation and characterisation of the plasma membrane is a necessary step.

In the previous report (sect. III.D.1f) the isolation procedure and the lipid composition of plasma membranes of exponentially growing Neuro-2A cells were described. We can now report the purification and the lipid composition of plasma membranes of differentiated Neuro-2A cells. Differentiation was induced by lowering the serum concentration in the medium from 10% (v/v) to 0.5% (v/v), and by adding the phosphodiesterase inhibitor IMBX (0.2 mg/ml) and dibutyryl c-AMP (0.5 mM) for 72 hr. As compared with the plasma membrane isolation procedure used for exponentially growing cells some modifications were introduced. To preserve the plasma membrane marker enzyme (Na⁺–K⁺)-ATPase the addition of 2 mM dithiothreitol proved essential. Furthermore, a single-step sucrose gradient appeared to be sufficient to obtain maximal purification. In the purified plasma membrane fraction the marker enzyme was enriched nine-fold. As expected, the apparent membrane viscosity was maximal in this fraction. The phospholipid composition of plasma membranes of differentiated Neuro-2A cells was found to be: phosphatidylcholine, 49.6%, sphingomyelin, 14.7%;

phosphatidylserine + phosphatidylinositol, 10.3%; phosphatidylethanolamine, 20.7%; others, 3.6%.

A comparison with the composition of plasma membranes of exponentially growing cells shows a significant decrease in the negative phospholipids phosphatidylserine and phosphatidylinositol. Furthermore, the molar cholesterol/phospholipid ratio decreased drastically upon differentiation: from 1.02 to 0.69. This decrease in relative cholesterol content could well explain the previously observed decrease in the apparent membrane viscosity and the increase in the lateral mobility of plasma membrane lipids and proteins during differentiation (for references see above, earlier observations).

A start has been made with the analysis of plasmalogens and fatty acids in plasma membranes of both exponentially growing and differentiating cells. Experiments to analyse the significance of the observed changes in membrane lipid composition are in progress.

¹ Laats, S.W. de, Saag, P.T. van der and Shinitzky, M. – 1977, Proc. Natl. Acad. Sci. USA, 74, 4458-4461

² Laats, S.W. de, Saag, P.T. van der, Nelemans, S.A. and Shinitzky, M. – 1978, Biochim. Biophys. Acta 509, 188-193

ii. *Fatty acid supplementation.* In principle the lipid composition of the plasma membrane can be modified directly (see previous report, sect. III.D.1g) or indirectly by growing the cells in lipid-defined culture media. In order to obtain such media, serum was delipidated by butanol/ether extraction and Neuro-2A cells were grown and subcultured on media supplemented with this serum, to which different fatty acids were added. The generation time (Tg) of cells grown in delipidated serum was doubled (20 hr) compared with control cells. Addition of 10 µg/ml palmitic, stearic or oleic acid reduced Tg to 14 hr. Therefore, the growth kinetics of these cells can be markedly influenced by the presence of extracellular fatty acid supplies. This may be expected to be due to altered lipid-enzyme interactions within the membrane as a result of metabolic incorporation of the fatty acids.

We therefore analysed the kinetic behaviour of (Na⁺–K⁺)-ATPase and adenylate cyclase in crude membrane preparations under these circumstances. The specific activity of (Na⁺–K⁺)-ATPase in cells grown on delipidated serum without supplementation by fatty acids was 20% reduced compared with control cells, whereas stearic, palmitic and oleic acid supplementation resulted in no, 15% and 60% inhibition of enzyme activity, respectively. Enzyme-kinetic analysis of the basal activity of adenylate cyclase in stearic and oleic acid-supplemented cells showed that

the apparent affinity constant for ATP was unaltered compared with control cells (K_m = 0.18 mM).

However, the maximum velocity of the enzyme reaction in stearic and oleic acid-supplemented cells was 170% and 39% of the control value, respectively. These results demonstrate the importance of lipid-protein interactions in controlling the activity of membrane-bound enzymes. The implications for growth regulation are at present under investigation.

1f. Growth of neuroblastoma cells in defined media

Recently methods have become available for the cultivation of mammalian permanent cell lines and primary cultures under conditions in which no (bovine) serum is required for cellular growth¹. Replacement of the serum by a (small) number of defined components such as hormones, trace elements, proteins and other components can lead to sustained growth under serum-free conditions. As growth and differentiation are usually under the direct influence of the presence of serum, and this medium component is to a large extent undefined, it is of great importance for the analysis of the underlying regulatory mechanisms to grow the cells in a completely defined medium.

Experiments with Neuro-2A cells have enabled us to define a medium in which these cells grow at an equal or even faster rate than in media containing 10% fetal calf serum. A detailed analysis of the required minimum levels of some of the key components involved (insulin, progesterone, putrescine, selenite, transferrin) and of their role in controlling cell proliferation is in progress. It has also been possible to induce morphological differentiation of the cells in this medium (using c-AMP-elevating agents), and to supplement the medium with cholesterol or fatty acids to modify the plasma membrane lipid composition, a method used to study the role of membrane lipids in the regulation of growth and differentiation (see also 1e. ii above).

¹ Bottenstein, J.E. and Sato, G.H. – 1979, Proc. Natl. Acad. Sci. USA 76, 514-517

1g. Kinetics of the Neuro-2A cell cycle

A study was made of the cell-kinetic behaviour of Neuro-2A cells in sparse cultures (15-30,000 cells/cm²). By means of time-lapse cinematography the variation in intermitotic time between individual cells, and more specifically the differences in intermitotic time between sister cells, were measured and analysed in terms of the Smith and Martin model for the cell cycle¹. According to this model cells can stay in the G₁-phase of the cycle (A-state) for a variable period of time, and require a random trigger before they can proceed through the other phases of the cycle

(B-phase). Differences in intermitotic time between sister cells are ascribed entirely to differences in the time each of the cells stays in the A-state. It has been shown that the cell-kinetic behaviour of Neuro-2A cells can be fully described in terms of the above model, if it is assumed that variation in the length of the B-phase occurs between individual cells in the culture such that the mean variation in length of the B-phase increases with decreasing cell-cell relationship. A method was elaborated to quantify the amount of variation in each of the system parameters. As a result of mathematical description it could be shown that in an exponentially growing culture under optimal conditions (10% serum, 37°C., pH 7.6) approximately 10% of the cells are in the A-state. This analysis provides the possibility to investigate whether variation in external growth conditions results in a redistribution of cells over the various phases of the cycle.

This work has been submitted for publication.

¹ Smith, J.A. and Martin, L. – 1973, Proc. Natl. Acad. Sci. USA 70, 1263-1267

1h. Interaction of epidermal growth factor with neuroblastoma cells

A new project was started in collaboration with Dr. J. Schlessinger and Dr. Y. Shechter (Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel). Epidermal growth factor (EGF) is one of the biologically most potent peptide hormones and one of the best characterised as to physical, chemical and biological properties. It behaves as a potent mitogen for epithelial and epidermal cells and for many cells in culture. EGF binds specifically to homogeneously distributed receptors on the membrane of mouse 3T3 cells. The dispersed receptors are laterally mobile in the plane of the membrane and the hormone-receptor complexes can aggregate into visible, immobile clusters ('patches') which are collected over coated regions of the plasma membrane. Patch formation on the cell surface is inhibited by primary amines. The clusters become internalised as endocytic vesicles, leading to receptor loss, i.e. down-regulation of receptors. This process is dependent of EGF concentration, time and temperature^{1,2,3}. Our experiments with Neuro-2A cells have demonstrated the following: 1. EGF binds specifically to saturable sites on the cells; 2. EGF induces the loss of binding sites; the degradation of the internalised hormone is inhibited by methylamine; 3. EGF release from the cells after binding is slower in the presence of methylamine than in its absence; 4. EGF receptors aggregate on the cell surface and amines inhibit this process; 5. EGF induces ornithine decarboxylase activity within 2-4 hr; this induction is transient and diminishes after 6 hr. These results indicate that EGF receptors in

neuroblastoma cells behave qualitatively similar to those in other cell systems. Experiments to test the physiological significance of EGF action in this system are in progress.

¹ Shechter, Y. *et al.* – 1978, Proc. Natl. Acad. Sci. USA 75, 2135-2139

² Schlessinger, J. *et al.* – 1978, Proc. Natl. Acad. Sci. USA 75, 2659-2663

³ Schlessinger, J. *et al.* – 1978, Proc. Natl. Acad. Sci. USA 75, 5353-5357

1i. Differentiation of pheochromocytoma cells treated with nerve growth factor

In 1976 Greene and Tischler¹ established a permanent cell line from a transplantable rat pheochromocytoma which expressed the differentiated properties of adrenal chromaffin cells *in vitro*, and discovered that these cells responded to treatment with nerve growth factor (NGF) by extending long, branching neuron-like processes and synthesising and storing catecholamines. (NGF is a naturally occurring peptide hormone that induces differentiation of sympathetic ganglia *in vivo* by interaction with a plasma membrane receptor.) The unique behaviour of these cells in response to NGF makes them very suitable for a study of the short-term and long-term effects of this defined inducer of differentiation. Preliminary experiments were performed to study the rapid effects of NGF on ⁴²K⁺ permeability. It was found that within 5-10 min the active (ouabain-sensitive) component of the K⁺ influx is stimulated by NGF. This stimulation reaches a maximum after 20 min at about 150-200% of the original value. No effects were seen on the passive influx or on the efflux of K⁺. Further experiments are in progress to determine the exact nature of the observed increase in the active K⁺ influx.

¹ Greene, L.A. and Tischler, A.S. – 1976, Proc. Natl. Acad. Sci. USA 73, 2424-2428

1j. Isozyme patterns of pyruvate kinase in neuroblastoma cells

A new project was started in collaboration with the Department of Haematology of the State University Hospital in Utrecht (Dr. G. Rijksen). The degree of malignancy of a brain tumour can be determined on the basis of the isozyme pattern of pyruvate kinase (ATP: pyruvate phosphotransferase)¹. There are at least three mammalian isozymes of pyruvate kinase, K, L and M, each of which consists of four identical subunits. The dominant fetal isozyme is type K. During development the two other isozymes become expressed. The three isozymes differ not only in their electrophoretic pattern but also in their kinetic properties. Type M is not inhibited by alanine, whereas type

K is strongly inhibited. By using these properties it is relatively simple to discriminate between types M and K. The normal developmental pattern of pyruvate kinase isozymes is reversed in tumours. While in adult brain type M is predominant, it was found by the above group that poorly differentiated brain tumours mainly contain type K, as does fetal brain.

We studied the pyruvate kinase isozyme patterns in exponentially growing and differentiated neuroblastoma cells both by alanine inhibition and by electrophoresis. Neuroblastoma N1E-115 cells induced to differentiate by serum withdrawal showed a clear shift

from type K to type M isozyme, whereas cells induced to differentiate by the addition of dimethyl sulfoxide did not. Neuroblastoma cells of the Neuro-2A type, induced to differentiate with dibutyl-c-AMP and c-AMP-elevating agents, showed the K to M shift to a much smaller extent. These results suggest that under certain conditions alanine inhibition of pyruvate kinase may provide a simple means of assessing the degree of differentiation of these cells.

¹ Veelen, C.W.M. van *et al.* – 1978, *Cancer Research* 38, 4681-4687

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, Indonesia)

The joint project started last year (see previous report, sect. I.A.III.3) was continued during a stay of P.D.N. of several months at the Institut Teknologi Bandung (Indonesia). The sea turtle material collected last year had been sectioned and proved to be very suitable, particularly for studying gastrulation and neurulation. However, older stages were required for the analysis of primordial germ cell origin. This material was collected on the sea shore. At the same time a study was made of the age distribution of eggs laid at one time by one female. It was found that eggs of different post-fertilisation ages become intermingled during storage in the uterus, so that a more or less arbitrary age assortment occurs in successive portions of each batch.

2. The difference in origin of the primordial germ cells in anurans and urodeles (P. Michael)

This work involves xenoplastic recombinations of the animal cap of a urodelan blastula with the vegetative yolk mass of an anuran blastula or *vice versa*, as well as deletions from urodelan and anuran gastrulae of the parts normally furnishing the primordial germ cells (see previous report, sect. I.A.III.1). This year the programme of definitive experiments drawn up last year was carried out. The material obtained will be analysed in India and the results incorporated in a Ph.D. thesis to be presented there.

3. The effect of UV-irradiation on the anuran germ cell lineage: cinematographic observations on cleavage in irradiated zygotes (Xenopus laevis) (K. Hara, P. Tydeman; K.E. Dixon, Flinders Univ., Australia)

It has been established that UV-irradiation of the vegetal hemisphere of anuran eggs leads to sterility of the embryo. Previous work (see report for 1977, sect. I.A.iv.4) suggested that UV-irradiation primarily disturbs early morphogenetic processes such as cleavage and gastrulation, and only secondarily leads to sterility. Further analysis of time-lapse films of cleaving irradiated zygotes revealed 1. that the 3rd ('horizontal') cleavage plane cuts through the four blastomeres at a higher level than normal, so that the size differences between the four smaller animal and the four larger vegetal blastomeres are accentuated; 2. that cleavage delay starts to appear in the vegetal hemisphere from the 7th cleavage onwards, but from the 11th cleavage onwards in the animal hemisphere. The significance of these morphogenetic disturbances in relation to sterility is still unknown.

4. Intercellular communication in early molluscan development (Patella vulgata, Limnaea palustris) (J.A.M. van den Biggelaar, A.W.C. Dorresteyn, Zoological Laboratory, State University of Utrecht; L.G.J. Tertoolen, W.J. Hage, S.W. de Laat, J.G. Bluemink)

Cellular interactions between the animal micromeres and the vegetal macromeres seem to be essential for the diversification of the developmental pathways of the four cell lines A, B, C and D; these derive from the four quadrants into which the egg is subdivided at second cleavage. One of the four macromeres attains a central position in the embryo. It is this macromere that gives rise to mesoderm. Evidence is available that its central position, and later its exclusive contacts with the overlying micromeres, determine this macromere to become the mesoderm stem cell^{1,2}. This

raises the question whether specialised membrane contacts and intercellular transfer of molecules could be involved in this determinative process.

We therefore decided to analyse intercellular communication from the 2- to the 32-cell stage of *Patella* by measuring electrotonic cell coupling and by iontophoresis of fluorescent dyes. So far the experiments indicate that between the 4th and the 5th cleavage a specific increase in electrotonic coupling between the micro- and macromeres occurs.

Limnaea and *Patella* embryos were also analysed for the presence of gap junctions by freeze-fracture electron microscopy, using a special device for fracturing spherical eggs (see previous report, sect. IV.1). Gap junctions were found at contact sites between the micromeres, the macromeres, and the micro- and macromeres. So far no evidence was found that gap junctions occur between specific cell couples only.

¹ Biggelaar, J.A.M. van den – 1977, *J. Morphol.* 154, 157-186

² Biggelaar, J.A.M. van den and Guerrier, P. – 1979, *Devel. Biol.* 68, 462-471

5. *Intercellular communication in hydroids (Hydra attenuata)* (C.J.P. Grimmelikhuijzen, European Molecular Biology Laboratory, Heidelberg; L.G.J. Tertoolen, S.W. de Laat)

In *Hydra* gap junctions have been identified as membrane specialisations¹. Morphogenetic substances have also been identified which according to their molecular weight should be able to pass through gap junctions; gradients of these morphogens have been implicated in the control of morphogenesis². However, so far no report has been published showing that cell-to-cell transfer of ions or small molecules is possible between epithelial cells in *Hydra*. We therefore decided to investigate the communicative properties of the junctional membranes by electrophysiological methods and by fluorescent dye iontophoresis. So far all experiments indicate that neither electrotonic coupling nor fluorescein transfer is detectable between epithelial cells 1. in intact animals, 2. in animals regenerating the head, and 3. in cell reagggregates. We hope to confirm these results in the near future by iontophoresis of lucifer-yellow, which is a more suitable probe.

¹ Wood, R.L. – 1977, *J. Ultrastr. Res.* 58, 229-315

² Grimmelikhuijzen, C.J.P. and Schaller, H.C. – 1977, *Cell Diff.* 6, 297-305

6. *Physico-chemical properties of amniotic fluid phospholipids as an indicator of fetal lung maturity (Homo sapiens)* (D.O.E. Gebhardt, H. Egberts, Dept. of Obstetrics and Gynaecology, University Hospital Leiden; S.W. de Laat)

During gestation the development of the pulmonary surfactant system leads to changes in the phospholipid composition of the amniotic fluid. This has been used as a diagnostic tool to determine fetal lung maturity, which is important to prevent premature birth and thereby the risk of respiratory distress syndrome. One frequently uses the lecithin/sphingomyelin ratio in amniotic fluid¹. Recently the determination of the apparent viscosity of the phospholipids by fluorescence polarisation has been suggested as a rapid alternative^{2,3}. We have initiated a study to investigate in more detail the applicability of this method and the biochemical and physico-chemical basis of the observed developmental changes in the apparent viscosity of the phospholipids.

¹ Gebhardt, D.O.E. *et al.* – 1975, *Clin. Chim. Acta* 64, 133-142

² Shinitzky, M. *et al.* – 1976, *Brit. J. Obstet. Gynaecol.* 83, 838-844

³ Blumenfeld, T.A. *et al.* – 1978, *Am. J. Obstet. Gynaecol.* 130, 782-787

7. *Research carried out in the amphibian facility* (R. Verhoeff-de Fremery)

7a. *The regulation of reproduction and spawning in anurans (Bombina orientalis, Rana lessonae, Discoglossus pictus)*

The procurement of newly fertilised eggs of *Bombina orientalis* at any desired time during the day is now possible. By means of injection of a suspension made of dried pituitaries of *Rana catesbeiana* the time of oviposition can be regulated to within about 2 hr. A similar method is being elaborated for *Rana lessonae* and *Discoglossus pictus*. We are also testing suspensions of dried pituitaries of *Xenopus laevis*. A more natural hibernation procedure for *Rana lessonae* has been elaborated and is being improved.

7b. *Permanent marking of adult amphibians (Xenopus laevis)*

To find an alternative for freeze-branding (see previous report, sect. IV.6b) we have tried homografting small pieces of white belly skin to wounds in the dark back skin. The grafts were fixed in place by means of 'Histoacrylblau' (butyl-cyanocrylate). The homografts healed in well; they have now persisted for seven months and remain well recognisable.

7c. *Prevention of rickets in anurans (Discoglossus pictus)*

Rickets is a very common disease in frogs reared in the laboratory. It was successfully prevented in newly metamorphosed and juvenile *Discoglossus* by exposing them for several hours daily to UV-irradiation (40 W PT, colour temp. 5500°K, colour index 91, 2400 lumen).

7d. Failure of fertilisation in *Xenopus laevis*

To find the possible causes of occasional failures of fertilisation (see previous report, sect. IV.6d) the testes of several males were examined histologically. No abnormalities were found.

7e. Tumours and other diseases

A spleen lymphosarcoma of *Xenopus laevis* and a

bacterial liver granuloma of axolotl were registered with the Registry of Tumors in Lower Animals, National Museum of Natural History, Washington D.C. An unknown contagious disease of axolotl is being investigated in cooperation with the Veterinary Faculty, State University of Utrecht.

VI. Projects carried out in cooperation with the Technical Workshop

1. A device for ultrarapid freezing of biological specimens

(J.G. Bluemink, W. Hage, W. Leurink, J. Beeker, H. Krielen; C. de Groot, Jan Swammerdam Institute, Amsterdam; R. van Venetië, Dept. of Molecular Cell Biology, State Univ. of Utrecht)

Conventional freezing for freeze-fracture electron microscopy is performed by quenching biological material in sludged nitrogen. Using this method an ultimate cooling rate of 200°C/sec can be obtained. Spray freezing is more rapid (ca. 10,000°C/sec) but is only effective for tiny objects such as cell organelles. Larger objects can be frozen adequately with an alternative quick-freezing method: propane-jet freezing¹. A propane-jet freezer was constructed which is equipped with a temperature-controlled specimen holder to maintain the specimen at a set temperature (e.g. 37°C) until the time of freezing. In addition the specimen holder is fitted with a switch connected to the valve for the release of nitrogen-cooled propane, in order to secure that the propane jet occurs at the very moment the specimen is slid in between the nozzles. The efficiency of the system was demonstrated using artificially prepared lipid mixtures showing a hexagonal array above and a lamellar array below the transition temperature. Freezing by dipping into sludged nitrogen was known to be inadequate for preserving the hexagonal phase of lipids of transition temperatures above 10°C. Mr. R. van Venetië, M. Sc. used the freezer for freeze-fracture electron microscopy of such artificially prepared lipid mixtures. They revealed hex-

agonal arrays after propane-jet freezing (figs. 1 and 2), indicating that a very fast temperature drop can indeed be obtained in this way.

¹ Müller, M., Meister, N. and Moor, H. – 1980, *Mikroskopie* 36, 1-7

2. An assembly to measure lateral mobilities of plasma membrane components and to visualise their movements

(S.W. de Laat, E.J.J. van Zoelen; J. Beeker, W. Leurink, H. Krielen, H. Reitsma)

Lateral mobilities of membrane components can be measured by the fluorescence photobleaching recovery (FPR) method (see also section IV.G.1d). Particular membrane components are labelled *in situ* with a fluorescent dye, or a fluorescent membrane (lipid) probe is introduced. The method is based on the measurement of ultra-low fluorescence intensities evoked by a defined laser light source in a small membrane area (radius ~ 1µm) through a fluorescence epiluminescence microscope. The fluorescence in this area can be photobleached by a short pulse of intense laser light. From the kinetics of the recovery of fluorescence within the area, due to migration of unbleached fluorophores into it, the lateral mobility properties of these molecules can be inferred.

An on-line computer-controlled measuring system was developed by the electronic and mechanical workshops, incorporating an argon laser, a fluorescence microscope, a photomultiplier in a cooled housing, and a mini computer, all of them commercially available. By combining this assembly with an electronic image intensifier and a time-lapse video recorder we are now able to analyse the movement, aggregation, internalisation, etc. of fluorescently labelled membrane components over long time periods.

VII. Miscellaneous

1. Prof. P.D. Nieuwkoop devoted the greater part of the year to writing and completing the manuscript of

the second monograph on primordial germ cells, to be published jointly with Dr. L.A. Sutasurya (see previous report, sect. I.A.III.2). Part of this was done with Dr. Sutasurya while he was in Indonesia (see V.1. above). The book will be entitled 'Primordial Germ

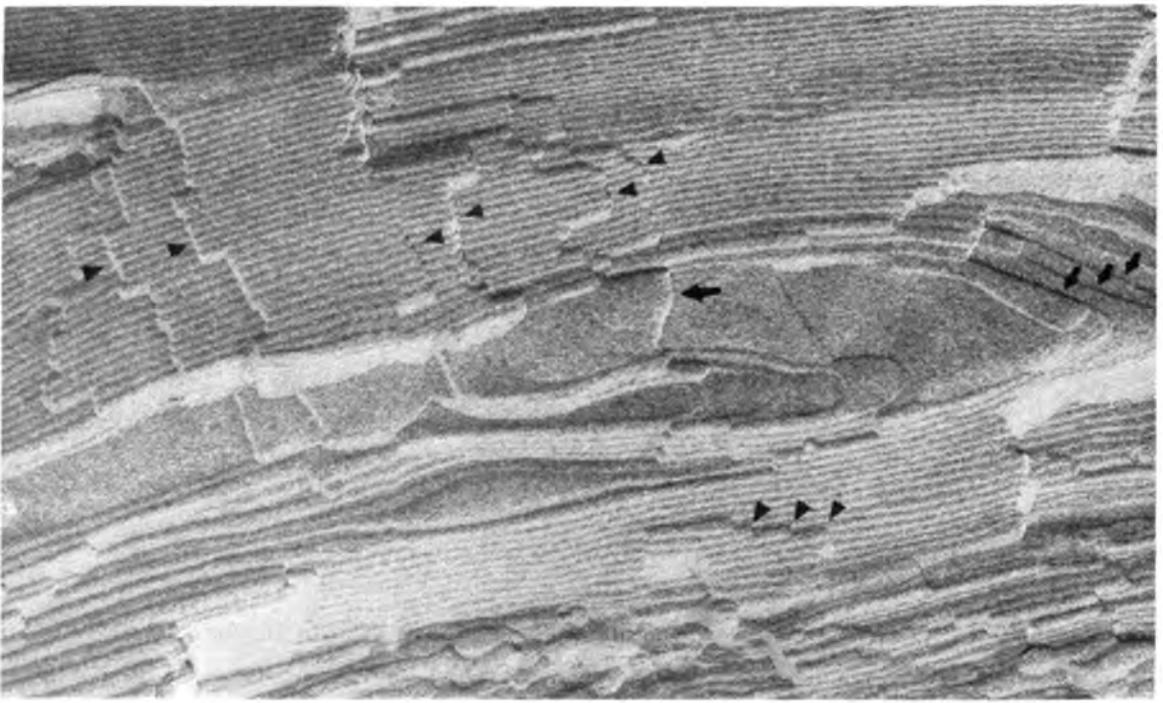


Fig. 1. Freeze-fracture replica of a mixture of di-oleyl phosphatidyl ethanolamine (DOPE)/di-oleyl phosphatidyl choline (DOPC)/cholesterol (transition temperature = 30°C), frozen from 60°C. Both hexagonal (arrow heads) and lamellar (arrows) arrays are visible. $\times 120,000$.

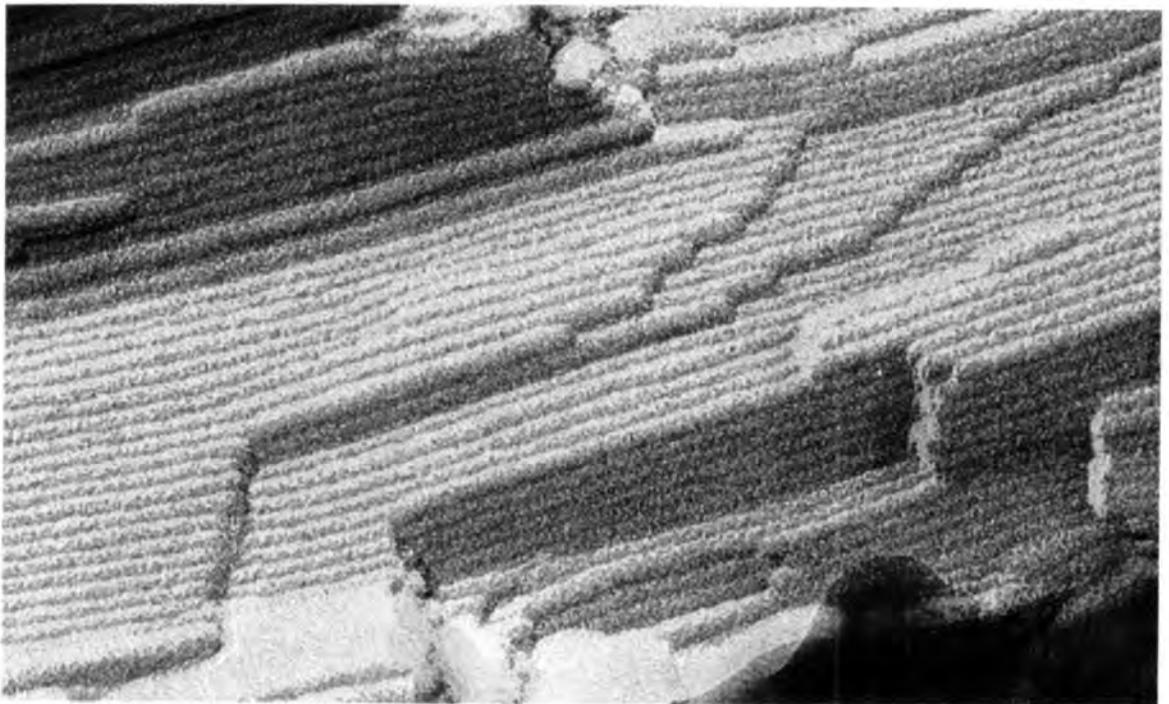


Fig. 2. Freeze-fracture replica of DOPE plus 4 per cent phosphatidic acid (transition temperature = 21°C), frozen from 60°C. Hexagonal array. $\times 331,200$. (Micrographs courtesy of R. van Venetië, M.Sc.)

Cells in the Invertebrates - from Epigenesis to Proliferation'.

2. Dr. W.P. Luckett worked in the Central Embryological Collection for two months. He studied the development of the fetal membranes in *Ctenodactylus* (Rodentia) and the early development of the permanent dentition in *Tarsius* (Primates); both subjects have a bearing on mammalian evolution.

3. Prof. R. Chandeboid used the library facilities for a period of two weeks.

4. Mr. P. Sura worked half-time in the amphibian facility for two months to learn methods of animal care and to exchange experience in that area.

VIII. Papers published and accepted for publication in 1979

Published

1. Durston, A.J. and F. Vork – A cinematographical study of the development of vitally stained *Dictyostelium discoideum*. *J. Cell Science* 36, 261-279 (1979).

2. Hara, K., P. Tydeman and C.W.A. Gorlee – A new method for local artificial insemination of *Xenopus laevis* eggs. *Mikroskopie* 35, 10-12 (1979).

3. Hara, K. and P. Tydeman – Cinematographic observation of an 'Activation wave' (AW) on the locally inseminated egg of *Xenopus laevis*. *Wilhelm Roux's Arch. Devl. Biol.* 186, 91-94 (1979).

4. Herkovits, J. and G.A. Ubbels – The ultrastructure of the dorsal yolk-free cytoplasm and the immediately surrounding cytoplasm in the symmetrized egg of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* 51, 155-164 (1979).

5. Kumar, K., W.J. Ouweneel and J. Faber – Differentiation capacities of the labial imaginal disc of *Drosophila melanogaster*. *Wilhelm Roux's Arch. Devl. Biol.* 186, 51-64 (1979).

6. Laat, S.W. de, P.T. van der Saag, E.L. Elson, J. Schlessinger – Lateral diffusion of membrane lipids and proteins is increased specifically in neurites of differentiating neuroblastoma cells. *Biochim. Biophys. Acta* 558, 247-250 (1979).

7. Matsukuma, S. and A.J. Durston – Chemotactic cell sorting in *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* 50, 243-251 (1979).

8. Moolenaar, W.H., S.W. de Laat and P.T. van der Saag – Serum triggers a sequence of rapid ionic conductance changes in quiescent neuroblastoma cells. *Nature* 279, 721-723 (1979).

9. Nieuwkoop, P.D. and L.A. Sutasurya – Primordial Germ Cells in the Chordates; Embryogenesis and Phylogenesis. *Developmental and Cell Biology Series*; Cambridge, Cambridge University Press; XII, 187 pp. (1979).

10. O'Day, D.H. and A.J. Durston – Evidence for chemotaxis during sexual development in *Dictyostelium discoideum*. *Can. J. Microbiol.* 25, 542-544 (1979).

11. O'Day, D.H. – Cell differentiation during fruiting body formation in *Polysphondylium pallidum*. *J. Cell Sci.* 35, 203-215 (1979).

12. Saag, P.T. van der and T.F.J. de Greeff – Ribosomal subunits from neonatal mouse brain highly active in polyphenylalanine synthesis. *J. Neurochem.* 32, 1423-1430 (1979).

13. Saag, P.T. van der and S. Bordin – A ribosome dissociation factor from neonatal mouse brain. *J. Neurochem.* 32, 1857-1859 (1979).

14. Ubbels, G.A., J.C. Gerhart, M.W. Kirschner and K. Hara – Determination of dorso/ventral polarity in the anuran egg: reversal experiments in *Xenopus laevis*. *Arch. Anat. Microscop. Morphol. Exp.* 68, 211 (1979).

Accepted for publication

15. Durston, A.J. and C.J. Weijer – *Dictyostelium discoideum*, een modelsysteem voor de embryonale ontwikkeling. *Vakbl. Biol.*

16. Grimmelikhuijzen, C.J.P., J. Lepault, A.W. McDowall, S.W. de Laat, L.G.J. Tertoolen and C.J. Weijer – Gap junctions in *Hydra* do not permit electrical and chemical transmission. In: *Proceedings of the IV Coelenterate Conference*; Ed. P. Tardent; Amsterdam, Elsevier (1980).

17. Hara, K., P. Tydeman and M.W. Kirschner – A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Nat. Acad. Sci. USA* 77, 462-466 (1980).

18. Kirschner, M.W., J.C. Gerhart, K. Hara and G.A. Ubbels – Initiation of the cell cycle and establishment of bilateral symmetry in *Xenopus* eggs. In: Proceedings of the 38th Symposium of the Society for Developmental Biology.
19. Kirschner, M.W. and K. Hara – A new method for local vital staining of amphibian embryos using Ficoll and 'crystals' of Nile red. *Mikroskopie* 36, 12-15.
20. Laat, S.W. de, P.T. van der Saag, E.L. Elson and J. Schlessinger – Lateral diffusion of membrane lipids and proteins during the cell cycle of neuroblastoma cells. *Proc. Nat. Acad. Sci. USA* 77, 1526-1528 (1980).
21. Moolenaar, W.H. – Functional aspects of plasma membrane studies: growth regulation by ionic fluxes. In: *Cell Biological Aspects of Disease: Plasma Membranes and Lysosomes*; eds. W.Th. Daems, E.H. Burger and B.A. Afzelius; 's-Gravenhage, Nijhoff.
22. O'Day, D.H. – Aggregation during sexual development of *Dictyostelium discoideum*. *Can. J. Microbiol.*

Centraalbureau voor Schimmelcultures

Progress Report 1979

Edited by G.S. de Hoog

Oosterstraat 1, 3742 SK Baarn,
the Netherlands

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

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History and function of the institute

The Centraalbureau voor Schimmelcultures was founded in 1904 by the 'Association Internationale des Botanistes'. Dr. Johanna Westerdijk was appointed as the first director in Amsterdam in 1907. After the dissolution of the AIB, the Bureau was supported by various Netherlands scientific institutions and associations, especially by the Royal Netherlands Academy of Arts and Sciences. In 1920 the institute moved to Baarn, and from 1922 the yeast collection was kept at the Laboratory of Microbiology, Technical University, Delft.

Prof. Westerdijk retired in 1959 and was succeeded as director by Miss A.L. van Beverwijk (1959–1963). In 1964 the CBS moved into a new building in Baarn

(Oosterstraat 1). Since 1968, the CBS has been an institute of the Royal Netherlands Academy of Arts and Sciences. The Centraalbureau voor Schimmelcultures maintains a collection of living fungi, yeasts and actinomycetes. In 1978 the total number of strains maintained was 24.300, including 4.000 yeasts. By supplying cultures, identifications and advice to workers in diverse fields of scientific and applied mycology, a service is rendered to all those interested in these organisms. Scientific research is mainly carried out on taxonomy of fungi. A separate division deals with human and animal mycology. Investigations into the chemistry of fungal metabolites are carried out in the biochemical department.

Facilities are available to students and guest workers who want to study a mycological subject. Each year courses are given on general and on human and animal mycology.

Scientific staff

(as from December 1st 1979)

Dr. J.A. von Arx, director (general mycology, Ascomycetes, Melanconiales)

Dr. G.A. de Vries (human and animal mycology, Actinomycetes)

Miss Dr. M.A.A. Schipper (Mucorales)

Mrs. Drs. E.J. Hermanides-Nijhof (Fusarium, Aureobasidium)

Mrs. Drs. A.J. van der Plaats-Niterink (Oomycetes)

Dr. H.A. van der Aa (Sphaeropsidales)

Dr. G.W. van Eijk (biochemistry)

Dr. W. Gams (Verticillium, Acremonium and allied genera, Mortierella)

Dr. R.A. Samson (Paecilomyces, Penicillium and related genera)

Dr. G.S. de Hoog (Dematiaceae)

Dr. J.A.J.M. Stalpers (Basidiomycetes)

Dr. A.C.M. Weijman (biochemistry, physiology)

Miss C.A.N. van Oorschot, M.Phil. (Chrysosporium and allied genera)

Drs. W. Windig (pyrolysis-mass spectrometry)

Yeast division

Drs. L. Rodrigues de Miranda (basidiomycetous yeasts)

Miss Drs. M. Th. Smith (ascomycetous yeasts)

D. Yarrow (Saccharomyces and related genera)

Introduction

A major part of the activities in 1979 was influenced by the 75th anniversary of the institute. An 'open day' was held to familiarize a wide range of the public with various fields of mycology. The jubilee was widely covered in the newspapers, on radio and TV, while many articles were printed in popular science magazines. The staff members produced an English booklet which emphasizes different aspects of the research and collection work.

Outside of the anniversary, several staff members worked on general text books dealing with topics on or

related to mycology. A semi-popular text on the fungal system in the form of analytical keys was written by Dr. W. Gams and published as 'Wetenschappelijke Mededelingen' No. 136 of the Koninklijke Nederlandse Natuurhistorische Vereniging. Miss M. Luykx wrote a chapter on medical mycological laboratory techniques in volume 4 of 'Paramedisch Handboek'. Dr. W. Gams was involved in the co-editing of the book 'Soil-borne Plant Pathogens', containing the papers presented at the 3rd International Congress of Plant Pathology, held in München in 1978. Dr. W. Gams also finished the work on the illustrations of a 'Compendium of Soil Fungi', a joint publication of K.H. Domsch, W. Gams and T.H. Anderson. The members of the yeast division in Delft

prepared different chapters for a new edition of the handbook 'The Yeasts'. Mr. D. Yarrow made a major contribution to the publication of the book 'A guide to identifying and classifying yeasts', which is entirely based on the strains maintained in Delft. Dr. J.A. von Arx devoted much of his time to a new edition of his book 'The genera of fungi sporulating in pure culture'. Drs. G.S. de Hoog, R.A. Samson, M.A.A. Schipper and G.A. de Vries were invited to contribute toward a medical mycological handbook, 'The fungi pathogenic for humans and animals: their biology, pathogenicity and detection'. Dr. R.A. Samson was co-author of the book 'Patterns of development in conidial fungi', the first compilation of different types of conidiogenesis in Hyphomycetes. Dr. J.A. Stalpers completed the manuscript for a joint publication en-

titled 'The resupinate non-poroid Aphyllophorales of the Northern Hemisphere', which will provide keys and condensed descriptions of all the respective species in that area.

The remaining time available was given to the current research. The tendency toward closer cooperation between colleagues was continued. A multidisciplinary research group has initiated a search for taxonomic yeast characters, and will be supported by the pyrolysis-mass spectrometry work done by Drs. W. Windig who joined us September 1st, 1979.

In general the CBS endeavours to cover the largest possible part of the fungal kingdom, both in research and collection work. This aim is complemented by a striving towards more in-depth research.

Mycological taxonomy

OOMYCETES

Pythium species are important plant parasites. Since Middleton's (Mem. Torrey bot. Club 20: 1-175. 1943) monograph and Waterhouse's (Mycol. Pap. 110: 1-71, 1968) compilation of diagnoses of species described in the genus, a great number of new species have been described. Some years ago Mrs. Drs. A.J. van der Plaats-Niterink started a revision of the genus *Pythium* with a study of the species incorporated in the CBS collection. In 1979 this study was continued and the necessary descriptions and drawings were almost completed. Much attention was paid to the synonymy of a number of species. In this light about 30 isolates of *Pythium aphanidermatum* and *P. butleri* were compared. According to the original description, *P. butleri* was somewhat more robust and had larger oogonia than *P. aphanidermatum*. In the studied isolates, however, all intermediate stages were present, so that *P. butleri* should be regarded as synonymous with *P. aphanidermatum*. *Pythium arrhenomanes* var. *canadense* proved to be synonymous with *P. aristosporum*. The manuscript of the monograph of the genus *Pythium* will be finished early 1980.

The taxonomic study of an undescribed species of *Phytophthora* isolated from water has been completed. This work was done in cooperation with Dr. M.J. Griffin (Leeds, U.K.) and Dr. J. Pittis (Manchester, U.K.). *Phytophthora citricola* was isolated as an etiologic agent from roots of diseased lilacs.

In cooperation with Mr. G. van Oosterbos (student of the State University of Utrecht) and at the request of the 'Zuiveringschap West-Overijssel', Mrs. van der Plaats-Niterink investigated water samples from the Overijssel river Vecht. Throughout the period that the

adjacent potato meal factories were in operation, the water was strongly polluted with organic material. During this time *Leptomitium lacteus* was present in great abundance in the water samples. At other times this fungus had apparently disappeared and *Pythium* and *Saprolegnia* species dominated.

Zygomycetes

The number of species described in the genus *Rhizopus* is considerable. Numerous species were isolated as active components in oriental foods or alcoholic beverages in Indonesia, China and especially Japan. Some supposedly new species have been based on very small differences, such as ability to assimilate or ferment a given compound or the abundance of sporulation. Part of the literature is not readily available. The CBS collection maintains numerous authentic isolates, approximately half of which date back to before 1935. In that year Zycha's (Krypt.-Fl. Mark Brandenburg VIa) revision of the Mucorineae, including the genus *Rhizopus*, was published. In recent years, particularly since the investigations of Scholer (Habilitationsschrift. 1970) and Scholer & Müller (lecture, 7th ann. Meeting Br. Soc. Mycopath. 1971) on pathogenic species of *Rhizopus*, it has become doubtful whether all Zycha's proposals concerning synonymy can be maintained.

All the strains of *Rhizopus* present in the CBS collection were therefore studied by Dr. M.A.A. Schipper applying the methods used previously in revising the genus *Mucor*. Mating experiments on beerwort agar at 30°C, yielded hitherto undescribed zygospores in *Rhizopus oryzae*. The zygospores are brown, up to 140 × 110 µm including stellate projections, and arise between unequal suspensors. They were obtained in CBS 112.07, type strain of *Rhizopus oryzae*; CBS 127.08, received as *Rh. nodosus*; CBS 110.17, type

strain of *Rh. maydis*; CBS 148.22, received as *Rh. tonkinensis*; CBS 257.28, *Rh. formosaensis*; CBS 264.28, type strain of *Rh. pseudochinensis*; CBS 266.30, type strain of *Rh. fusiformis*; CBS 329.47 = ATCC 10260; and in CBS 285.55 — all in matings with CBS 346.36, which was received as *Rh. norvegicus*. CBS 385.34, *Rh. achlamydosporus*, produced zygospores with CBS 127.08.

In cooperation with Dr. J.A. Stalpers, Dr. Schipper also investigated the zygospore wall ornamentation of a number of *Mucor* and *Backusella* species by means of the scanning electron microscope. The variation in zygospore ornamentation in *Mucor amphibiorum*, *M. inaequisporus*, *M. indicus*, *M. recurvus*, *M. variosporus*, *Backusella circina* and *B. lamprospora* was described and compared with azygospores formed in interspecific matings of *M. amphibiorum* CBS 764.74 (+) with various (-) strains of the other species studied. The development of the ornamentation of these azygospores normally ceased at an earlier stage than in the zygospores of the respective parents. Any similarity toward one of the partners always tended slightly toward the (-) parent.

These results revived interest in the literature on processes concerning the supposedly sexual reproduction in Mucorales. An attempt was made to reconsider the processes involved in azygospore production.

ASCOMYCETES AND THEIR CONIDIAL STATES

Phyllosticta is a Coelomycete genus in which about 3000 species have been described. Dr. H.A. van der Aa (Stud. Mycol. 5: 1-110, 1973) described the 46 species now accepted in *Phyllosticta* sensu stricto, and is continuing a study of all the remaining species which have to be excluded from *Phyllosticta*. In the course of 1979 rather extensive collections from the herbarium of Stockholm were revised. Subsequently a manuscript was started, which will contain a list of all the species described in *Phyllosticta* and revisions of the excluded species.

Several new data on the genus *Phyllosticta* sensu stricto were assembled. *Phyllosticta kigeliae* and *Ph. trigoniae* proved to be typical representatives of the genus, being close to *Ph. cyamopsidicola* and *Ph. hamamelidis* respectively. The holotype specimen of *Ph. trigoniae* also showed a well recognizable spermatial state of the form genus *Leptodothiorella*. *Phyllosticta manihoticola* proved to be a further synonym of *P. ghaesembillae*. The *Phyllosticta* conidial state of *Guignardia vaccinii*, well known from many locations in the U.S.A., was encountered for the first time in Europe: it had been described previously as *Phyllosticta oxycocci* on cranberries in the botanical garden of Berlin. A strain of *Phyllosticta*, isolated from *Yucca* and received from the Plantenziektenkun-

dige Dienst in Wageningen, proved to represent a new species closely resembling *P. citricarpa* but differing in certain morphological details of conidiomata and conidia.

In cooperation with Mr. H.A. van Kesteren (Plantenziektenkundige Dienst, Wageningen) Dr. van der Aa completed an extensive study of pycnidial fungi on *Chenopodiaceae*, mainly belonging to the genera *Ascochyta* and *Phoma*. A *Coniothyrium* species, isolated from soil in a maize field in Colombia by Mr. J. Veerkamp (student of the Technische Hochschule of Aachen), formed a *Didymosphaeria* ascigerous state under near-UV light. No other *Didymosphaeria* species is known to include a *Coniothyrium* conidial state in its life cycle. Descriptions of both states were prepared.

The carotenoid pigments of the Discomycete *Iodophanus carneus* were studied by Dr. G.W. van Eijk, in cooperation with Dr. L.R.G. Valadon (London, U.K.). At least 11 carotenoids were detected, of which the two major were the acidic carotenoid neurosporaxanthin (33%) and its methylester (47%). This is the first report of C₃₅ carotenoids in the Discomycetes. On the basis of its pigment characters, *I. carneus* should be placed in the Pyronemataceae as defined by Korf (In: Ainsworth et al. - The Fungi IV, pp. 249-319, 1973), and not in the Ascobolaceae.

In addition a comparative study of the carotenoids of *Aschersonia aleyrodis* and *Aspergillus giganteus* was initiated. The orange-red sporodochium of *A. aleyrodis*, a fungus shown to be an effective biological control agent of white fly population in citrus groves, contained 6 carotenes with β -carotene (87%) as the major pigment. It has also been shown to contain hopane derivatives. To date the only such component of fungal origin is dustanin, isolated from an *Aspergillus* species (Tsuda & Isobe - Tetrahedron Letters 3337, 1965).

Aspergillus giganteus produced 7 carotenoids, 5 of which are shared by *A. aleyrodis*. The above findings may indicate taxonomic relationships. However, on the basis of pigment patterns, *Aschersonia* and *Aspergillus* do not seem to be closely related. Presence of the pigment asperxanthin, detected in *A. giganteus* as well as *A. niger*, can possibly be regarded as a criterion by which to distinguish *Aspergillus* from other fungi.

The *niger* group of *Aspergillus* contains a number of species which are variable or little distinct. The group is being revised by Miss A. Al-Musallam, a guest worker from Kuwait. The results are partly in accordance with previous revisions, but the changes to be made in the aggregate of *A. niger* sensu stricto are considerable. The following subdivision of the group is now proposed.

1. Metulae absent: *Aspergillus japonicus* with its variety *A. japonicus* var. *vezoensis*, the latter formerly being known as *A. aculeatus*.

2. Metulae present: Five species show this character. A complex of species with conidia 6–10 µm in diameter, comprises *A. carbonarius*, *A. pulchellus* and the new species *A. helicothrix*. Strains with conidia smaller than 5 µm and with more discrete spines are classified in *A. ellipticus* and *A. heteromorphus*.

The remaining species were classified by means of numerical pattern detection, including iterative character weighting in cooperation with Dr. P. Hogeweg (Bioinformatics group, University of Utrecht, Utrecht). Two species were distinguished, viz. *A. foetidus* and *A. niger*. Within the latter, seven subspecific taxa are distinguished. A proposal is made to conserve the epithet *niger* against three older but less well known epithets.

Dr. R.A. Samson examined a number of *Penicillium* isolates from polluted soil in Canada. The strains included an undescribed species with darkly pigmented conidiophores: a relevant manuscript was prepared. Together with Miss Drs. A.C. Stolk (Baarn), Dr. Samson continued the revision of the genus *Eupenicillium*. Herbarium specimens of cleistothecial Ascomycetes were studied at the Botanical Department of the University of Toronto, Canada. From these specimens ascospores were prepared for further scanning electronmicroscopical examination. Together with Dr. D. Malloch (Toronto, Canada) a concept of a revised classification of the cleistothecial Ascomycetes was worked out.

Dr. W. Gams had previously initiated a study on anellidic conidiogenesis in *Monographella nivalis*. In cooperation with Prof. E. Müller (Zürich, Switzerland) a new genus, *Gerlachia*, is proposed for this fungus and also *Rhynchosporium oryzae*. Recent isolates of the latter species were received from Dr. V.O. Parkinson, Orsay. The work on *Niesslia* and connected *Monocillium* conidial states was taken up again with an examination of specimens collected by Dr. G.J. Samuels (Auckland, New Zealand) in addition to certain personal collections. A study of the type and other strains of various taxa placed in *Exophiala* was carried out jointly with Dr. R.A. Samson, and a modified key to the species resulted.

Rich collections of microfungi made in Burundi by Dr. J. Rammeloo (Brussels, Belgium) were studied by Dr. Gams in collaboration with Dr. Samson. A number of stilbellaceous fungi, particularly the *Stilbella* conidial state of *Nectria gracilipes* were isolated.

Dr. O. Constantinescu, a guest worker from the Institute of Biology, Bucharest, Rumania, worked at CBS for a period of 5 months. He continued a

literature study for an English updated version of his book 'Metode si tehnici în micologie'. Some Rumanian isolates of Deuteromycetes were studied at Baarn, among which an isolate of a *Triadelphia* species prompted a comparative study of the three known species; leading to the discovery of surprising pleomorphic conidiogenesis. These fungi were also studied by scanning electron microscopy in collaboration with Dr. Samson. A new isolate of *Pithoascus intermedius* and those from the CBS collection were found to scarcely produce a *Scopulariopsis* conidial state, contrary to the generic definition of *Pithoascus*.

Miss C.A.N. van Oorschot completed the revision of all species of *Chrysosporium* and allied genera. The major part of the year was devoted to the compilation of the manuscript which will appear in the series Studies in Mycology in 1980. The final monograph describes, illustrates and provides keys to *Chrysosporium* (22 species), *Myceliophthora* (8 species), *Emmonsia* (2 varieties), *Zymonema* (1 species), *Trichosporiella* (2 species) and *Geomyces* (1 species with 3 varieties). The genera were found to illustrate two types of conidiogenesis: thallic and blastic. All conidia are released rhexolytically. The known genera of associated ascigerous states belong to the Gymnoascaceae, Onygenaceae, Ascosphaeraceae and Sordariaceae. The distribution of the ascigerous states over these families does not correlate to the division of the genera of conidial states on the basis of conidium morphology.

YEASTS AND YEAST-LIKE FUNGI

The taxonomic research has seen dynamic changes in recent years. New characters have been introduced and others have been re-evaluated. The applicability of such characters has to be firmly established, particularly in those yeasts of which no perfect state is known, or is found with difficulty. Two parameters are available for the testing of characters. One is the variability, a character being usable when the ratio intrastrain/interstrain variation is low; a second is the correlation of mutually independent characters. In order to enable the evaluation of both parameters, groups of eight closely related strains, which may be mating partners, have been selected throughout the yeasts. They are studied in various aspects, starting with morphology and ultrastructure (Dr. G.S. de Hoog), physiology and G + C percentages (Miss Drs. M.Th. Smith), carbohydrates (Dr. A.C.M. Weijman) and other components (Dr. G.W. van Eijk). Confirmation of the above findings and possible new characters are being searched in the study of whole cell pyrolyzates (Drs. W. Windig) and volatiles (Mr. H.J. Roeymans).

strains and a number of type strains of other genera were tested. It was necessary to repeat their experiments and to extend them to all available strains of the genera *Candida*, *Cryptococcus*, *Rhodotorula*, *Phaffia*, *Bullera* and *Sporobolomyces*. Up till now the strains of the genera *Rhodotorula*, *Phaffia*, *Bullera*, *Cryptococcus* and *Torulopsis* (now known as a synonym of *Candida*) and several strains of the genus *Candida* have been tested. The results are different from those obtained by Golubev et al. All strains of *Rhodotorula lactosa*, *Rh. minuta*, *Rh. minuta* var. *texensis* (except one) and *Rh. pallida* were able to assimilate glucuronic acid. From the species *Rh. aurantiaca* three out of six strains were positive, the other three negative. From the two strains of *Rh. araucaria* one was positive, the other negative. All strains of *Phaffia rhodozyma* assimilate glucuronic acid weakly. A number of *Bullera* species assimilate glucuronic acid, but some species do not. The species of the genus *Cryptococcus* all assimilate glucuronic acid as well as inositol, except *Cr. melibiosum* which only assimilates inositol. In contrast to the statement of Golubev et al., all *Cryptococcus albidus* strains assimilate inositol.

In the genus *Torulopsis* there are a number of species which assimilate glucuronic acid and not inositol: *Torulopsis (Candida) cantarellii*, *T. auricularia*, *T. buchneri*, *T. fragariae*, *T. ingeniosa*, *T. pustulata*, *T. pilatii* and about half of the available strains of *Torulopsis candida* (now *Candida famata*).

Some of these species have an ascomycetous nature, for example *Candida cantarellii*, *C. famata* and *C. pustula*. The cell wall of *Candida pustula* was investigated by transmission electron microscopy and proved to have the structure of an ascomycetous cell wall.

With a number of *Candida* species mating experiments were carried out. In *Candida citrea* and *Candida diversa* ascospores were observed after conjugation. A manuscript on these results is being prepared.

In a cooperative study of Dr. G.W. van Eijk and Dr. J.A. von Arx, steroids and carotenoids of *Taphrina* species were investigated. The research was extended to 31 strains. Most strains (26) produce brassicasterol as the major compound. Of these, 20 strains do not form ergosterol, whereas for 6 strains the presence of ergosterol could be detected. Five strains only produce ergosterol with some other unidentified sterols. The UV-visible spectra of the carotenoid fractions obtained from most strains resemble that of β -carotene. Only a few strains show spectra with a different pattern, e.g. lycopene-, neurosporene- or zeacarotene-like, indicating quantitative differences in carotenoid production within *Taphrina*.

The type species of *Moniliella* is characterized by the presence of hyphae producing acropetal chains of conidia. It bears a superficial resemblance to the hyphomycete genus *Hyalodendron*. Other strains of *Moniliella* show budding cells in addition to hyphae, and are similar to *Trichosporonoides*, generally classified in the yeasts. Some recently isolated strains of *Trichosporonoides* are practically devoid of hyphae. In order to establish the taxonomic position of this complex of yeast-like fungi, characters currently used in both the hyphomycetes and the yeasts should be studied. In view of this, Dr. G.S. de Hoog studied the morphology and conidiogenesis of the fungi in detail. Dr. A.T. Martínez, a guest worker from Spain, was mainly occupied with the ultrastructure of the fungi concerned, and found lamellar cell walls and dolipore septa. Dr. Martínez also studied the physiologic properties in cooperation with Drs. M.Th. Smith; *Hyalodendron* strains were found to be quite different from the *Moniliella* complex e.g. in their fermentation ability and their capacity to grow on trehalose, melibiose, L-rhamnose, erythritol and myoinositol. Dr. A.C.M. Weijman analyzed the carbohydrates of cell walls and found that the chromatograms of *Hyalodendron* were particularly characterized by the presence of xylose, whereas those of the studied *Moniliella* and *Trichosporonoides* strains all demonstrated a hitherto unidentified polyol peak. The results of an analysis of volatile metabolites performed by Mr. H.J. Roeymans and Dr. G.S. de Hoog more or less confirmed the pre-established taxonomic pattern.

In a subsequent study Dr. G.W. van Eijk undertook steroid and fatty acid analyses of 4 strains of *Moniliella suaveolens* and one strain of *Hyalodendron lignicola*. The gas-chromatograms of the steroid fractions revealed the presence of ergosterol as the major sterol for all strains. Several very small unidentified peaks were also present in the chromatogram but these were not suitable for taxonomic purposes. However, the segregation of *H. lignicola* from the *Moniliella* complex was again confirmed by evaluating the gas-chromatograms of the fatty acid methylesters. The total amount of fatty acids was very low (ca. 1 mg/gr. dry weight) and compared with the *Moniliella* strains C14:0, C14:1, C18:3 and C20:1 fatty acids were absent. Comparing the *Moniliella* strains with each other, it was striking that *M. suaveolens* var. *suaveolens* produces a rather large amount of fatty acid (28 mg/gr. dry weight) with a high percentage of unidentified acidic material, probably hydroxy fatty acids.

In a literature review of fungal groups related to *Moniliella* and similar genera, Dr. G.S. de Hoog evaluated the supposedly 'important' characters shared with the studied fungi. Characters of

ultrastructure, such as the structure of cell walls and septa, are particularly significant since they have been studied in a variety of fungi but have been shown to be different in fungi of ascomycete and basidiomycete affinity. The presence of xylose is a useful criterion to recognize yeasts of basidiomycete affinity, but it has also been incidentally reported in higher Ascomycetes. Dr. A.C.M. Weijman studied the distribution of xylose in representatives of the Basidiomycetes in cooperation with Dr. J.A. Stalpers, in order to estimate the reliability of this component as a more general indicator of basidiomycete affinity.

BASIDIOMYCETES

The book on resupinate, non-poroid Aphylophorales, written by Drs. J.A. Stalpers and W. Jülich (Rijksherbarium, Leiden) was completed and offered to the Royal Netherlands Academy of Sciences for publication. In the book the Aphylophorales are considered in a broad sense, including genera often considered to be Heterobasidiomycetes, for example *Ceratobasidium*, *Cerinomyces* and *Thanatephorus*. Most of the effused-reflexed species as well as those cyphelloid and poroid species belonging to typically resupinate non-poroid genera are treated. The book covers 128 genera with about 800 recognized species. The area concerned covers the non-tropical region of the nor-

thern hemisphere, viz. Europe, the USSR and North America.

The book incorporates a scheme in which supposed generic relationships are indicated. At the same time it illustrates the problems of separating families. Classical families such as the Coniophoraceae and the Stereaceae cannot be maintained. The Coniophoraceae are connected with the main part of the Corticiaceae by *Leucogyrophana* and *Phanerochaete*, while the Stereaceae are connected with the Corticiaceae by the similarity of *Lopharia*-*Peniophora* as well as by that of *Aleurodiscus*-*Xylobolus*. The genus *Gloeocystidiellum* shows a connection to *Stereum* and *Xylobolus* on the one hand and to the Auriscalpiaceae (incl. Hericiaceae) on the other. Several other possible lines to other families are also indicated, e.g. to the Gomphaceae, Hymenochaetaceae, several groups of Polyporaceae, Thelephoraceae and some heterobasidiomycetous genera.

Together with Drs. W. Loerakker (Plantenziektenkundige Dienst, Wageningen) Dr. Stalpers made a survey of the causal agent of the red thread disease on grasses. Two species are involved, viz. *Laetisaria fuciformis* and a species often confused with *L. fuciformis*. Both species were studied in vitro and in vivo and much attention has been paid to the phytopathological symptoms.

Mycology at the service of other sciences

ECOLOGY

In connection with a revision of the thermophilic fungi, Dr. R.A. Samson spent much time investigating the identity of two isolates sent by Mr. D.H. Ellis (North Adelaide, Australia) and Dr. M.R. Tansey (Bloomington, U.S.A.). Both strains produce sclerotia: however, in the Ellis isolate pycnidia were formed and in the isolate from Dr. Tansey *Coprinus*-like fruitbodies developed.

A number of isolates from mosquito larvae were obtained from Mr. G. Soares (Berkeley, U.S.A.) and subsequently identified as *Tolypocladium cylindrosporium*. This hyphomycete was previously known as soil-borne and the discovery on mosquito larvae is the first entomopathogenic record. In cooperation with Dr. H.C. Evans (Belem, Brazil) the entomogenous fungal flora of Ghana and South America was investigated further. The study on the *Stilbella* and *Polycephalomyces* species from insects in Ghana was completed together with Mr. G. van de Klashorst (Amersfoort). Several hundreds of specimens, col-

lected by Dr. Evans in Columbia and Brazil were examined. In addition, special attention was paid to the material which was collected on the Galapagos Islands.

During a visit to the Agricultural Research and Education Center at Lake Alfred, Florida, U.S.A., Dr. R.A. Samson studied isolates of the mite pathogen *Hirsutella thompsonii*. This fungus will be applied as a biological insecticide against the citrus rust mite. In cooperation with Dr. C.W. McCoy (Lake Alfred, U.S.A.) the entomopathogenic fungal flora in citrus grooves were examined with special emphasis on the genus *Aschersonia* on white fly. Pure cultures of *Aschersonia aleyrodis* and other species were made and the processes of infection and conidiogenesis were studied by light and scanning electron microscopy.

Dr. G.A. de Vries continued the investigations into the development of the keratinophilic mycoflora of the soil of the new Dutch polders, E. and S. Flevoland with the collection of 30 soil samples. The following fungi have been isolated from these samples: *Arthroderma uncinatum*, *Chryso sporium keratinophilum*, a *Myceliophthora* species and *Nannizzia cajetani*.

MEDICAL AND VETERINARY MYCOLOGY

In connection with the study of mycoses of Cetaceae,

Dr. G.A. de Vries obtained a scraping from a skin lesion of unexplained etiology on a dolphin (*Tursiops truncatus*) at Harderwijk. Although direct microscopical examination did not reveal any fungal cell, 3 colonies were cultured of *Sarcinomyces crustaceus* = *Cladosporium werneckii*, a species known to cause tinea nigra in man. An attempt to obtain more material for direct examination failed owing

to the fact that the lesion had already healed spontaneously. A fungus from another dolphin (*Phocaena phocaena*), which had been studied since September 1977, was sent for identification purposes to Dr. A. Ulken (Bremerhaven, FRG.). Unfortunately Dr. Ulken could not identify the fungus as it did not sporulate. The study of this fungus is being continued.

Publications 1979

Aa, H.A. van der - Aandacht voor Christianseniagallen op *Collybia dryophila*. *Coolia* 22, 42-44 (1979).

Aa, H.A. van der - Een afwijkende vorm van *Leucoagaricus naucinus*. *Coolia* 22, 114-115 (1979).

Aa, H.A. van der and Kesteren, H.A. van - Some pycnidial fungi occurring on *Atriplex* and *Chenopodium*. *Persoonia* 10, 267-276 (1979).

Arx, J.A. von - Propagation in the yeasts and yeast-like fungi. *The Whole Fungus* pp. 555-572 (1979).

Arx, J.A. von - Ascomycetes as fungi imperfecti. *The Whole Fungus* pp. 201-214 (1979).

Barnett, J.A., Payne, R.W. and Yarrow, D. - A guide to identifying and classifying yeasts. Cambridge University Press (1979).

Cole, G.T. and Samson, R.A. - Patterns of development in conidial fungi. Pitman Publ. Ltd. (1979).

Eijk, G.W. van - Biosynthese van Schimmelmetabolieten II. *Coolia* 22, 45-48 (1979).

Eijk, G.W. van, Mummery, R.A., Roeymans, H.J. and Valadon, L.R.G. - A comparative study of carotenoids of *Aschersonia aleyroidis* and *Aspergillus giganteus*. *Antonie van Leeuwenhoek* 43, 417-422 (1979).

Gams, W. - Het systeem van de fungi. Tabel voor de hoofdgroepen en verwijzing naar determinatie-werken. *Wet. Meded. KNNV* 136 (1979).

Hoog, G.S. de - Deductive classification — worked examples using anamorph and teleomorph data in the Ascomycetes. *The Whole Fungus* pp. 215-239 (1979).

Hoog, G.S. de - Nomenclatural notes on some black yeast-like Hyphomycetes. *Taxon* 28, 347-348 (1979).

Hoog, G.S. de - Taxonomic review of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 1-36 (1979).

Hoog, G.S. de - The taxonomic position of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 81-90 (1979).

Hoog, G.S. de and Roeymans, H.J. - Volatile organic compounds produced by *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 69-75 (1979).

Martínez, A.T. - Ultrastructure of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 50-57 (1979).

Martínez, A.T. and Hoog, G.S. de - Development and conidiogenesis of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 37-49 (1979).

Martínez, A.T., Hoog, G.S. de and Smith, M. Th. - Physiological characteristics of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 58-68 (1979).

Polak-Vogelzang, A.A., Samson, R.A. and Leeuw, G.T.N. de - Scanning electronmicroscopy of *Acholeplasma* colonies of agar. *Can. J. Microbiol.*, 1373-1380. (1979).

Polonelli, L., Morace, L., Delle Monache, F., and Samson, R.A. - Studies of the PR toxin of *Penicillium roqueforti*. *Mycopathologia* 66, 99-104 (1978).

Rodrigues de Miranda, L. - *Clavispora*, a new yeast genus of the Saccharomycetales. *Antonie van Leeuwenhoek* 45, 479-483 (1979).

Samson, R.A. - Compilation of the *Aspergilli* described since 1965. *Stud. Mycol.* 18, 1-38 (1979).

Samson, R.A., Ramakers, P.J.N. and Oswald, T. - A new fungal pathogen of *Thrips tabaci*, *Entomophthora thripidum*. *Can. J. Bot.* 57, 1317-1323 (1979).

Samson, R.A., Stalpers, J.A. and Verkerke, W. - A simplified technique to prepare fungal specimens for scanning electron microscopy. *Cytobios* 24, 7-11 (1979).

Schipper, M.A.A. - *Thermomucor* (Mucorales). *Antonie van Leeuwenhoek* 45, 275-280 (1979).

Schippers, B. and Gams, W. (eds) - Soil-borne plant pathogens. Academic Press, London, 686 pp. (1979).

Stalpers, J.A. - *Heterobasidion* (*Fomes*) *annosum* and the *Bondarzewiaceae*. *Taxon* 28, 414-417 (1979).

Weijman, A.C.M. - Carbohydrate patterns of *Moniliella*, *Trichosporonoides* and *Hyalodendron*. *Stud. Mycol.* 19, 76–80 (1979).

Weijman, A.C.M. - Carbohydrate composition and taxonomy of *Geotrichum*, *Trichosporon* and allied genera. *Antonie van Leeuwenhoek* 45, 119–127 (1979).

Weijman, A.C.M. - Carbohydrate composition and taxonomy of fungi. Thesis, State University of Utrecht (1979).

Weijman, A.C.M. and Meuzelaar, H.L.C. - Pyrolysis mass spectrometry as an aid in the characterization and classification of 'problematic fungi', Abstr. 4th Int. Symp. anal. appl. Pyrolysis, Budapest (1979).

Weymann, A.C.M. and Meuzelaar, H.L.C. - Biochemical Contributions to the taxonomic status of the Endogonaceae. *Can. J. Bot.* 57, 284–291 (1979).

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Progress Report 1979

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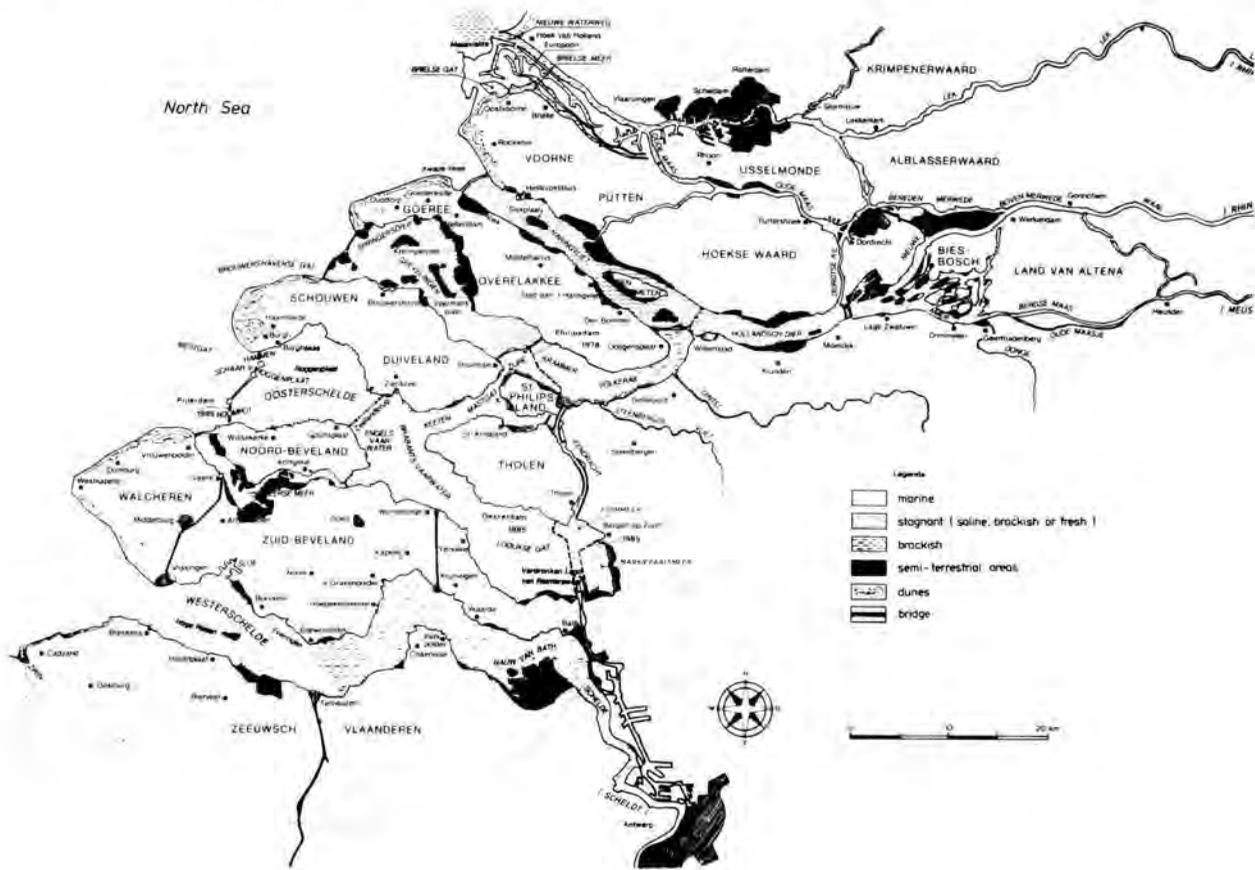
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Erratum: In Table 18 of the 1978 Progress Report ppm should be ppb for Cadmium and Mercury (Duursma,

E.K., 1979, Progress Report DIHO 1978, Verh. KNAW, Nat. 2e reeks 73, p. 109).

Fig. 1A. Delta of the rivers Rhine, Meuse and Scheldt



I. 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 southwest Netherlands, with the aim of studying the biological 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 fresh-water 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 Rhine, Meuse and Scheldt, 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 a ecological point of view, research was initiated to elucidate the causal background of the changes observed.

The institute is located at Yerseke on the Eastern Scheldt, the sea-arm to be semi-closed in the last stage of the s.c. 'Delta plan'. The exploitation of the institute is financed by means of funds allotted to the Academy, by the Ministry of Education and Science.

The institute had in 1979 a permanent staff of personnel of 57 including 13 scientists and 1 member paid by the Administrative Service Centre of Zeeland (Table 1A). Additionally short-term contract scientists, assistants, guestworkers, students and trainees took part in the programme of the Institute (Table 1B).

Thanks are due to Mrs. E.S. Nieuwenhuize-de Bruijn, who skilfully typed the manuscript, and to Mr. A.A. Bolsius, Mr. J. v.d. Ende and Mr. R.H.G. Kleingeld for the preparation of figures and photographs.

II. Introduction (E.K. Duursma)

By 1985 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 (Eastern Scheldt) (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 Eastern Scheldt and the Lake Grevelingen are still considered as well-developed, rather undisturbed ecological systems. In particular the Eastern Scheldt has a great value for the development of young marine species and as a winter refuge for migrating European and Northwestasian birds.

The hydrobiological studies of the institute have been started in 1957 with 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 1979 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 1A). The working-group themes are (i) the element cycling and food chains in the Grevelingen with future extension in the Eastern Scheldt, (ii) structure, functioning and dynamics of ecosystems in brackish waters and (iii) ecosystem studies on salt marshes. In preparation is a working group on mineralisation studies which at present is still part of the first mentioned working group.

The work is grouped into projects, each of them bearing a code number. G and K are codes for the energy flow studies, M for mineralisation research, B

Table 1A. Scheme of personnel (1 June 1980)

WORKING GROUPS

Elements cycling and food changes (G + K)	Microbiological Research: Drs. J.G.C.M. Goossens Dr. G.J. Lindeboom R.S. Minnaar H.A.J. de Klerk- v.d. Driessche	Planktonbiology: Ir. J.W. Rijstenbil L. de Wolf	Biomass budget: Ir. P. Verdonshot
Botany: Dr. P.H. Nienhuis (W.G. leader) B.H.H. de Bree J.M. Verschuure	Fishery: Drs. G. Doornbos F. Twisk	Entomology: B.P.M. Krebs	Short-term Project (3 yr): (VEGIN-R.W.S.) Drs. A.M. Groenendijk M.A. Lievaart
Zoology: Drs. R.H.D. Lambeck E.G.J. Wessel A.J.J. Sandee	Bottom-exchange: Drs. P. Keiderman A.M. van de Repe	Zoology: Drs. C.H. Borghouts R.H. Bogaards J.W. Francke	Miscellaneous (A-subjects) Dr. E.K. Duursma (coordinator)*
Plankton: Drs. C. Bakker J.C.M. Rijk P. van Rijswijk	Seston Research: Drs. J.H.B.W. Elgershuizen A.P.A. de Booy	Salt-Marsh Ecosystems (S)	Ecological Research Zoobenthos R.W.S. (ZACHTSUB) (3 yr-project) Drs. J. Coosen A. van den Dool
Primary Production: Drs. F. Vegter P.R.M. de Visscher	Brackish waters (B)	Communities: Dr. Ir. W.G. Beeftink* (W.G. leader) M.C. Daane B.P. Koutstaal W. de Munck	Pollution: Dr. E.K. Duursma* Dr. Ir. W.G. Beeftink* A.G.A. Merks* J. Nieuwenhuize*
Short-term projects (3 yr) (ZOWEC-R.W.S.)	Microbiology: Dr. A.B.J. Sepers (W.G. leader) F.W. Melissen	Populations: Drs. A.W. Stienstra Dr. A.H.L. Huiskes M.M. Markusse J. v. Soelen	Litoral-water exchange Drs. L.A. v. Geldermalsen (1979-1982)
			Residence times: DIHO - Shell Drs. M. Smies

GENERAL DEPARTMENTS

Science Information: Dr. E.K. Duursma* Drs. R. Peelen R.H.G. Kleingeld* P.J. van Boven	Biomathematics: Dr. A.G. Viasbiom J.J. Gueranc	Administration: L.J. Goud* M.A. Manneke J.C. Ruissen E.F. v.d. Plasje (guestworker)	Director: Dr. E.K. Duursma* Manager: L.J. Goud*
Library: M.A. Fronk	Sedimentology: J. Nieuwenhuize* J.M. van Liere C.H. Vos	Reception: M.N. van der Leer	
Photography: R.H.G. Kleingeld*	Chemistry: A.G.A. Merks* J.J. Sinke J.O. v.d. Zande	Workshop: C. Almekinders J.P. Hoekman	
Design and off-set: J.A. v.d. Ende A.A. Bolsius	Aquarium: P.J. van Boven*	Household Service: K.C. Zweedijk W.J. Baggelaar J.J. Braam J.A. Goedhart	
Research Vessels: W.J.L. Robër C.M. de Rooy J.A. v. Sprundel P. de Koeyer			* = double mentioned

Table 1B. Guestworkers, students and trainees

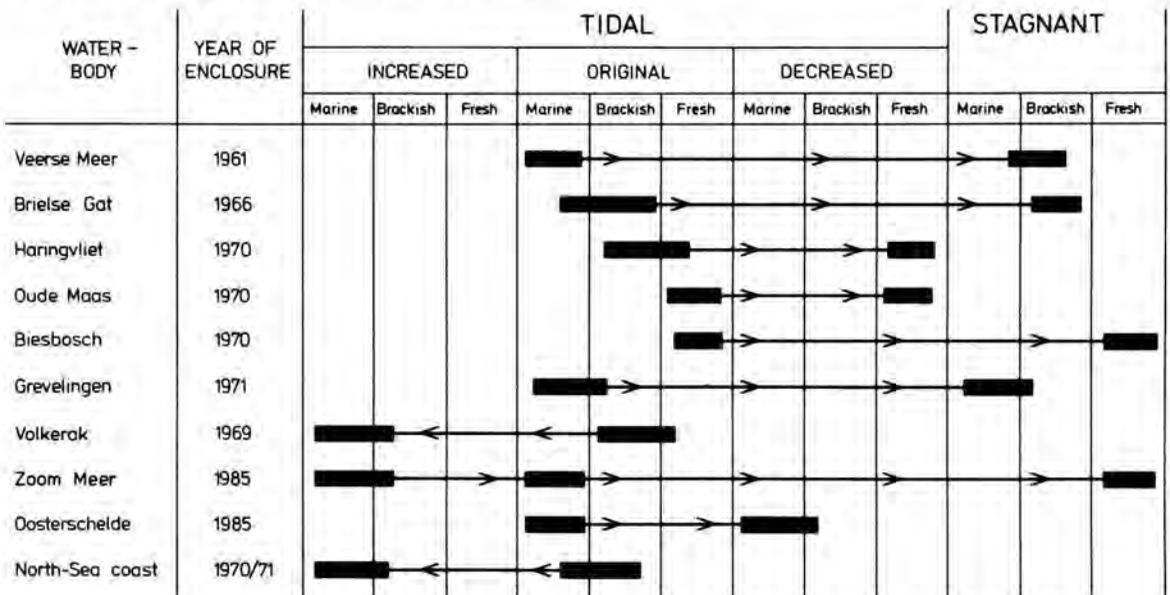
Guestworkers (1 Febr. 1980):

Drs. G.J.C. Buth (W.G. Salt-Marsh Ecosystems)
Drs. E.A.M.J. Daemen and Drs. A.W. Fortuin
(W.G. Elements cycling and food chains)
A.S.C. Lobbezoo (Photography)
S. Brik and E.S. Nieuwenhuize (Administration)
M.T.T. Vereecken and C.S. Vroonland
(Technical Assistants)
Dr. A.H. Pickaver, Trinity College, Dublin
($\frac{1}{2}$ year guestworker)

Students and Trainees (1979):

A.J. Hoek v. Dijke, M.A. Lievaart, P.A. Maas,
A.J.W. Phernambucq, B.M. Viasbiom, N.J.M. de Visscher,
M. Westveer, H. Beunder, F. Boelè, H. v. Dam, C.H. v. Duyl,
D.J. v.d. Haar, M. Ham, P.A. Marwitz, E. Miedema,
W. Smit and J. Sybesma

Fig. 1B. Hydrological changes due to the 'Delta Plan'



for the brackish water investigations, S for the salt-marsh studies while A denotes miscellaneous subjects, among which are the pollution studies. An overlapping code is used for the Eastern Scheldt projects (0) in which all working groups participate.

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 in-

dividually carried-out projects for a long period of years. Reference is made to the working plan 1980-1990 of the institute (Anonymous, 1980).

Reference

Anonymous - 1980: Werkplan 1980-1990. Delta Institute for Hydrobiological Research, Yerseke, WP-04, 74 pp.

III. General ecological conditions in 1979 (R. Peelen)

Temperature

The total period until September of 1979 was characterized by lower than normal temperatures. In particular January and February were extreme months. Only in October and November the temperatures were somewhat above normal (Table 2).

Solar radiation

At Flushing the representative station for the south-west Netherlands, the solar radiation quantities of 1979 were higher than the normal values given for The Bilt (central Netherlands), except for February, March and April (Fig. 2). Over the first 11 months The Bilt recorded 337,136 Joules cm⁻¹ and Flushing 359,656 Joules cm⁻¹ (6.26% higher).

Rainfall

In comparison with the normal rainfall pattern at Flushing the following characteristics can be noted: wetter than normal were the months March, April, May, June, October and November, leaving only January as normal and the rest dryer than normal (Table 2).

Windspeed

The windspeeds recorded in 1/2 m s⁻¹ were for all months except March close to the mean values. March was rather windy with 2 1/2 m s⁻¹ more than normal (Table 2).

River discharges and North Sea water exchange

1. The average discharges of the three rivers Rhine, Meuse and Scheldt are respectively 2200 m³s⁻¹, 330 m³s⁻¹ and 95 m³s⁻¹ to which the daily discharges given in Table 2 are compared. For the Scheldt no dai-

Table 2. Climate and river-discharge conditions in 1979.

	Temperature °C			Rainfall mm month ⁻¹	
	measur.	normal	deviation	measur.	normal
January	- 1.0	2.9	- 3.9	63.4	61.9
February	0.0	3.0	- 3.0	39.7	44.8
March	4.7	5.2	- 0.5	102.5	39.1
April	7.8	8.4	- 0.6	52.6	41.9
May	11.2	12.1	- 0.9	98.8	44.1
June	14.4	15.3	- 0.9	69.0	48.9
July	19.7	20.5	- 0.8	32.2	69.4
August	16.4	17.4	- 1.0	59.6	66.1
September	15.2	15.5	- 0.3	21.6	72.8
October	12.5	11.5	+ 1.0	124.0	70.2
November	7.5	7.3	+ 0.2	98.7	75.2

	Windspeed $\frac{1}{2}$ m s ⁻¹		river discharge x normal	
	measur.	normal	Rhine	Meuse
January	13	14	2½ - 2/3	3 - ½
February	11	13	3 - 1	3 - 1
March	16	11	1 - 3	1 - 4
April	11	12	1½ - 1	3 - 1
May	11	10	1½ - 1	1½ - 2/3
June	9	11	1½ - 1	2/3 - ½
July	10	11	1 - 2/3	1/3 - 1/7
August	11	11	½ - 2/3	1/6 - 2/3
September	11	11	2/3 - ½	1/3 - 1/10
October	10	11	½	½ - 1/10
November	13	12	½ - 1½	1/6 - 2
December			2½ - 1½	1 - 4

ly recordings were available, while those of the Rhine and Meuse are representing the discharges at Lobith (near German border) and Lith (last stow in Meuse), respectively.

The discharge of the river Rhine fluctuated from ½ to 3 times the normal discharge except at the end of March when the discharge was about 6700 m³s⁻¹. It is a mixed gletsjer and rain-fed river. The Meuse is a true rain-fed river which fluctuates from 1/10 till 4 times of its normal discharge. The managing of the sluices in the mouth of the Haringvliet is strongly related to the discharges of both the Rhine, the Meuse, the isohaline pattern in the Mouth of the Rotterdam Waterway and that of the mouth of the Haringvliet.

2. The sluices in the Haringvliet were nearly all opened only on the 18th of February and from the 17th until the 20th of March.

In order to stop salt intrusion through the Volkerak locks into the Hollands Diep, fresh water was introduced into the Volkerak at an amount of 50 m³s⁻¹. On 19th of November this amount was increased until 100 m³s⁻¹ in order to study the freshening velocity of the Keeten-Krammer-Volkerak-region. This experiment is carried out in order to evaluate the possible reaction on the future Eastern Scheldt system from the

Philips-dam locks with regards to the flora and fauna. 3. In order to increase the salinity of the Grevelingen water the sluice of the Brouwersdam was opened on 4 December 1978 and remained in operation during 1979. Only during a period of a few days (24, 25 February and 27 March) the sluice was closed because of too low salinity of the coastal North Sea water. From 1 to 10 May the sluice was closed for safety reasons because of high recreational activities around the sluice. During the beginning of October the sluice was closed in order to stop the migration to sea of eels.

Technical works by Rijkswaterstaat in the Delta area

1. The construction of the Storm-surge barrier in the Eastern Scheldt is in full operation. In the building pit of the created island Neeltje Jans, 19 pillars are under construction for later placement at the sluice-gate sites.

2. Concerning the secondary dams in the Eastern Scheldt, the Philipsdam and the Oysterdam, the constructions are in progress. The locks in the Philipsdam for the commercial shippingline Rotterdam-Antwerp and Ghent are constructed in an artificial island. The lower basins and part of the sea water - freshwater recycling system is completed.

For the Oysterdam a dam in the Markiezzaat is completed between the Molenplaat and the coast.

3. For purpose of water exchange between the Grevelingen and the Eastern Scheldt and Volkerak a sluice is in preparation which can be used to supply water in both directions. Thus saline water additions will be possible in future from a saline Grevelingen to the Eastern Scheldt, or the sluice may serve as fresh-water inlet for the Grevelingen in case it will be decided to convert this lake later into a fresh-water basin.

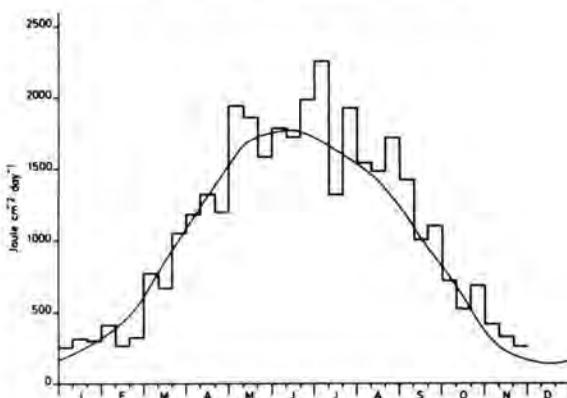


Fig. 2. Solar irradiation at Station 'Vlissingen' (block diagramme) as compared to 'normal' irradiation (smooth curve)

4. The refreshing sluices for lake Brielle, in the Bernisse, are completed.
5. The raising of the sea dykes around the Eastern Scheldt is for 80% ready and will be completed in 1980. Those of the Western Scheldt will be finished by 1985, and are completed for 75%.

References (sources)

Maandelijks overzicht der weersgesteldheid KNMI 76e jaargang. Rijkswaterstaat dagelijkse afvoeren in m^3s^{-1} van Rijn en Maas. Personal communications of Rijkswaterstaat (Department of Roads and Waterways).

IV. Working group: Elements cycling and food chains (Code G + K)

(formerly Carbon cycle in the Grevelingen)

IV.1. INTRODUCTION (P.H. Nienhuis)

The working group 'Carbon cycle in the Grevelingen' will terminate its integrated ecosystem study in the saline-brackish Lake Grevelingen in 1983 and will turn subsequently its attention to the Eastern Scheldt estuary. This estuary will be closed by a perforated dam in 1985, allowing the tides to enter in a diminished way. In the course of 1980-1983 the investigators, still working in the Grevelingen, will become more and more involved in the ecosystem study in the Eastern Scheldt. Anticipating on future efforts localized in the Eastern Scheldt a new research plan has been developed, grafted upon the results of the Grevelingen study, and dealing with the production and transformation of organic matter. Details about the research plan will be given in the progress report 1980 in connection with the first results.

Consequently, the working group has two main projects under study: a. Carbon cycle in the Grevelingen and b. Food chains and production of organic matter in the Eastern Scheldt. The central question behind both main projects is: in which way and to which extent will estuarine element cycles and food chains be influenced by an extinction (Grevelingen) or reduction (Eastern Scheldt) of the tidal movements.

The Grevelingen project will be terminated in 1983. A final effort, called ZOWEC, has been launched in September 1979 as a joint project between Rijkswaterstaat Deltadienst and DIHO, and financed by Rijkswaterstaat. The project runs to September 1982.

ZOWEC 1 and 2 deal with the mineralization of organic matter in water and sediment (G 10). ZOWEC 3 deals with the role of fishes in the carbon cycle (G 9). ZOWEC 4 studies aspects of sediment-water exchange (G 2) and ZOWEC 5 is looking after techniques for separation and quantification of seston (G 1).

IV. 2. CARBON CYCLE IN THE GREVELINGEN (CHANGES IN THE ECOSYSTEM IN 1979) (P.H. Nienhuis)

The Grevelingen project deals with the aquatic

ecosystem, with emphasis on the production and decomposition of organic material and the flux of organic material through the various foodchains. Lake Grevelingen originated in 1971 after the closure of the Grevelingen estuary; it is a large (108 km^2), shallow (5.3 m on average) brackish-saline lake. Almost half of the area is shallower than 2.5 m. About 40 km^2 is covered in summer with eelgrass fields. Up to December 1978 Lake Grevelingen was a closed system with only minor influence from polder and sluice water. The organic matter produced in the lake was the only source of energy available for consumers. In December a new sluice connecting the North Sea with Lake Grevelingen was opened. By this sluice, with a capacity of $100 \text{ m}^3\text{s}^{-1}$ on average, the residence time of the water was diminished to a few months.

The Grevelingen water quality was influenced in 1979 to a large extent (Stokman, 1980). During the period 1971-1978 chlorinity concentrations of the water had gradually decreased. In December 1978 values of 13‰ Cl^- were measured, increasing already in January 1979 to 16‰ and remaining during the rest of the year at a level of $16\text{--}17\text{‰ Cl}^-$.

The relatively high DOC concentrations in the lake in 1978 ($5\text{--}6 \text{ mg l}^{-1}$ in the surface water) decreased after the opening of the sluice to 4.5 mg l^{-1} in January and varied between 2.5 and 3.5 mg l^{-1} during the rest of the year.

SiO_2 concentrations showed only minor difference, comparing the 1978 situation (0.8 mg l^{-1} in winter and 0.2 mg l^{-1} in summer) with that of 1979 (0.7 mg l^{-1} in winter and 0.1 to 0.2 mg l^{-1} in summer). The same holds for NH_4^+ ; 1978: $0.05\text{--}0.1 \text{ mg l}^{-1}$ and 1979: 0.05 to 0.2 mg l^{-1} . NO_3^- increased from a maximum level of 0.1 mg l^{-1} in 1978 to 0.3 to 0.4 mg l^{-1} in 1979. PO_4^{3-} levels, extremely high in 1978 and preceding years, viz. 0.3 mg l^{-1} in April and 0.7 mg l^{-1} in September and October, dropped drastically in 1979 to 0.1 to 0.2 mg l^{-1} in spring and increased again to 0.4 mg l^{-1} in September and October.

Owing to the opening of the Brouwerssluice, Lake Grevelingen became a stagnant part of the North Sea again, leading to a biological enrichment. Marine species, that have disappeared after the closure in 1971, entered the lake again viz. the jellyfishes, *Aurelia aurita*, *Rhizostoma octopus*, *Chrysaora hysoscella* and *Cyanea capillata* and the crabs

Macropipus holsatus, *Hyas araneus* and *Macropodia rostrata*. To which extent the sluice affected the POC concentrations and the plankton populations will be dealt with in sections IV.4 and IV.5. In a separate section (IV.18) will be reported on fishes.

The Brouwerssluis, opened in December 1978, was in action in 1979 the whole year through. The most striking effect of this management regime was a salinity stratification in the lake during the period June to August, emphasized by an increase in watertemperature of the epilimnion. This led to consequent oxygen deficiencies (concentrations below 2 mg l^{-1}), mainly in the middle and eastern part of the lake. Below a waterdepth of 7-12 m an area of about 800 ha was affected by these oxygen shortages and mass mortality of benthic animal populations (molluscs, worms, sponges, sea anemones etc.) occurred. Quick recovery of the benthic fauna, however, took place in autumn after a natural break-up of the salinity and temperature stratification by a complete mixing of the watermasses.

In order to avoid stratification and consequent mass mortality in the future, the DIHO advised the Lake Grevelingen management to close the sluice in February and to reopen it again only in late autumn. This advice has been taken into account with regard to the sluice management regime for 1980.

Reference

Stokman, G.N.M. - 1980. Gegevensinwinning in het Grevelingenmeer. Report Deltadienst Hoofdafd. Milieu en Inrichting, 25 pp.

IV.3. AN INTERSTITIAL WATER INVESTIGATION OF LAKE GREVELINGEN SEDIMENT (G2). (P. Kelderman)

With the aim of gaining a better insight into the lake

Grevelingen sediment/water interaction, an investigation in interstitial water of the sediment was made during the first six months of 1979. Median-fine sand is the dominant sediment type in lake Grevelingen. For each of five representative permanent stations, which were visited fortnightly, sediment cores were taken by Scuba divers or with a manual sediment corer from a ship. Sediment cores were cut into sections: 0-1, 1-3, 3-5, 5-10, 10-20, 20-30 cm, thus emphasizing the sediment layer which plays a role in sediment/water interaction. Local variations in interstitial water nutrient content have been estimated once, by examining six separate cores, taken within an area of 100 m^2 at each station.

Interstitial water was obtained by N_2 -pressure filtration, under ambient water temperatures (Fanning and Pilson, 1971). Care was taken in preventing oxygen contamination of the sediment samples according to Bray et al (1973). Interstitial water was analysed for pH, Cl^- , PO_4^{3-} -P, P-tot, reactive Si, NH_3 -N, ($\text{NO}_2^- + \text{NO}_3^-$) and SO_4^{2-} , all by standard DIHO-methods.

Except for one station with a muddy type of sediment, all sediments showed irregular nutrient patterns (i.e. profiles). Significant correlations (95% confidence limit) between the various nutrients were scarce. Differences between the individual stations in interstitial water nutrient content were overshadowed by local variations. A comparison has been made between March and May values for the sediment upper 5 cm (Table 3). Average values for interstitial water nutrient contents have been listed, including 95% confidence limits (which were derived from local variation analysis). An identical picture holds for the other months, and for the deeper sediment layers.

No seasonal fluctuations in interstitial water nutrient content can be concluded from the data in

Table 3. Nutrient contents of interstitial and upper water in the Grevelingen in March and May 1979. Data are averages over 5 stations visited fortnightly, including 95% confidence limits.

		PO_4 -P ($\mu\text{g l}^{-1}$)	P-tot ($\mu\text{g l}^{-1}$)	Si ($\mu\text{g l}^{-1}$)	NH_3 -N ($\mu\text{g l}^{-1}$)	pH
MARCH	Upper water	195	220	225	120	8.05
	Interstitial water	394 \pm 84	665 \pm 57	2781 \pm 198	1160 \pm 230	8.05 \pm 0.05
MAY	Upper water	207		406	80	8.41
	Interstitial water	436 \pm 67	651 \pm 47	3138 \pm 227	1350 \pm 230	8.14 \pm 0.02

Table 3. In lake Grevelingen, seasonal patterns in sediment/water interaction do occur (Kelderman, 1979). Apparently interstitial water researches described here offer little insight into lake Grevelingen sediment/water interaction. In future experiments a process-oriented approach will be taken, including investigations of sediment/water interactions in laboratory and field experiments under various environmental conditions (light, temperature, etc.).

References

Bray, J.T., O.P. Bricker and B.N. Troup - 1973. Phosphate in interstitial waters of anoxic sediments: oxidations effects during sampling procedure. *Science* 180, 1362-1364.

Fanning, K.A. and M.E. Pilson - 1971. Interstitial silica and pH in marine sediments: some effects of sampling procedures. *Science* 173, 1228-1231.

Kelderman, P. - 1979. De fosfaatbalans van het Grevelingenmeer in de periode mei 1971-december 1977. *DIHO Rapp. en Versl.* 1979-9, 49 pp.

IV.4. PARTICULATE ORGANIC CARBON (POC) IN RELATION TO PLANKTON CONCENTRATIONS OF LAKE GREVELINGEN DURING FLUSHING WITH NORTH SEA WATER IN 1979 (G1, G3) (C. Bakker and J. Nieuwenhuize)

Calculations in relation to the POC-loading from the North Sea into Lake Grevelingen (Stokman, 1980) resulted in an estimated import in the order of magnitude of 10 g POC m^{-2} during the entire flushing period of one year, with peak values of more than 2 g C m^{-2} in December 1978 and April 1979. The average concentration of POC in surface water layers increased from ca. 0.5 mg l^{-1} in 1978 to 0.7 mg l^{-1} in 1979 (Stokman, 1980). The absolute quantities of this import may be considered of minor importance compared with the enlarged primary production of the lake. Its quality as food, on the other hand, may be emphasized, because particle diversity of the imported POC of the North Sea covers a broader spectrum than the autochthonous POC of the lake. Therefore favourable effects in relation to the food supply of several animals, planktonic as well as benthic, could be expected.

Table 4 gives weighted means calculated for the water column of the central lake station G11 during 1978 and 1979 in two series of samples: 1 l for PM (particulate matter) samples and 100 l samples filtered through plankton nets of $63 \mu\text{m}$ mesh width. The former series contained mainly small phytoplankton, the latter mainly zooplankton and larger phytoplankton.

The average POC-concentrations of the 1 l samples

of 1978 are similar, approaching ca. 0.5 mg l^{-1} , in spring as well as in summer. Seasonal differences were absent. In general higher POC values were found during the growing season, synchronously with and/or after chlorophyll peaks. When higher POC concentrations occurred during winter these values were nearly always due to resuspension of bottom material. In January 1979 a decrease of total PM and POC was paralleled by an increase of the living component, chlorophyll, represented by the flagellate *Cryptomonas* and the diatom *Skeletonema*. At the same time the percentage composition of POC, in relation to total PM content, increased. Wind-induced turbulence may effect Lake Grevelingen throughout the year and this phenomenon explains the large fluctuations in both absolute and relative POC-contents in winter and sometimes in summer too (see also de Korte, 1980).

Depth-time isopleths demonstrated clearly succession and fate of phytoplankton blooms. At the end of March small surface concentrations (0.4 mg l^{-1}) were accompanied by far higher amounts (0.8 to 1.4 mg l^{-1}) below a depth of 10 meters, probably a consequence of the entrance of North Sea water with higher POC loadings. POC concentrations reached a maximum of more than 1 mg l^{-1} at the end of April when chlorophyll contents decreased after a peak of $12\text{-}13 \text{ mg m}^{-3}$. Between a depth of 10 and 15 meters a large maximum of 2 mg l^{-1} POC was demonstrated, indicating a combined effect of settling or organic material produced in the surface layers and of POC transported from the sea into the lake. During summer, POC peaks of 0.8 to 1 mg l^{-1} were measured in the top 10 meters (at the same time, again, as or after chlorophyll maxima), but increased amounts of POC below 10 meters were not found. Both sedimentation and flushing effects could not be detected during this period, probably owing to high mineralization rates in summer.

The discrepancy between the POC values of the 1 l samples (similar in 1978 and 1979) and chlorophyll content of these samples (higher concentrations in 1979: see Bakker and Rijk, this report), cannot yet sufficiently be explained. The difference between these two sets of data suggests systematically lower conversion factors from chlorophyll to organic carbon in 1979, perhaps due to changed nutrient conditions for the phytoplankton. The ratio's of chlorophyll to carbon and of phytoplankton biomass to carbon will be studied in detail using selected samples.

Average amounts of POC in particles exceeding $63 \mu\text{m}$, however, are larger in 1979 (Table 4), due to significantly ($P < 0.05$) larger summer values. Note the clear differences between spring and summer values of the two years: in spring zooplankton development is limited to a few groups (mainly rotifers), in summer all

Table 4. Weighted means of POC-concentrations in the water column of station G11 (Lake Grevelingen). A: samples of 1 l. B: samples of 100 l filtered through 63 µm plankton nets.

A	mg POC l ⁻¹ (month)	1978	1979
	annual mean	0.46	0.47
	spring " (III-IV)	0.54	0.51
	summer " (VI-VIII)	0.49	0.53
B	mg POC 100 l ⁻¹		
	annual mean	2.41	3.25
	spring " (III-V)	1.96	1.89
	summer " (VI-VIII)	4.37	6.82

zooplankton groups, notably copepods, are present in numbers. Moreover, in spring larger phytoplankton is lacking, but in summer clogging of the nets may occur, caused by numerous *Chaetoceros*-threads (diatoms), contributing substantially to total POC-content.

The higher POC- contents of the 63 µm fraction in 1979 can be influenced by 3 factors:

1. Import of POC from the North Sea is improbable, because in this case higher POC values in the 1 l sedimentation samples might have been expected too.
2. More wind-induced water turbulence in the lake, resulting in enlarged resuspension of organic material from the bottom is unlikely, as there were no indications that such effects occurred more frequently during 1979.
3. Higher primary and secondary production values. In 1979 not only denser phytoplankton stocks were estimated but also larger zooplankton stocks compared with 1978 (see Bakker *et al.*, this report) were found to occur. Especially this component might have accounted for the higher POC values in the 63 µm summer samples.

References

- Korte, D. de - 1980. Gesuspendeerd organisch materiaal in de Grevelingen. DIHO-Student Report D3-1980, 100 pp.
- Stokman, G.N.M. - 1980. Gegevens-inwinning in het Grevelingenmeer voor het project ZOUVER in 1979. Report Delta Dienst Hoofdafd. Milieu en Inrichting, 25 pp.
- IV.5. PHYTOPLANKTON COMPOSITION AND -ABUNDANCE LEVELS OF LAKE GREVELINGEN FLUSHED WITH NORTH SEA WATER DURING 1979 (G3) (C. Bakker and J.C.M. Rijk)
- The phytoplankton populations of the lake were sampled at station G11, depths of 2.5, 7.5, 12.5, 17.5 metres and at a nearly shallow area of ca. 3 metres.

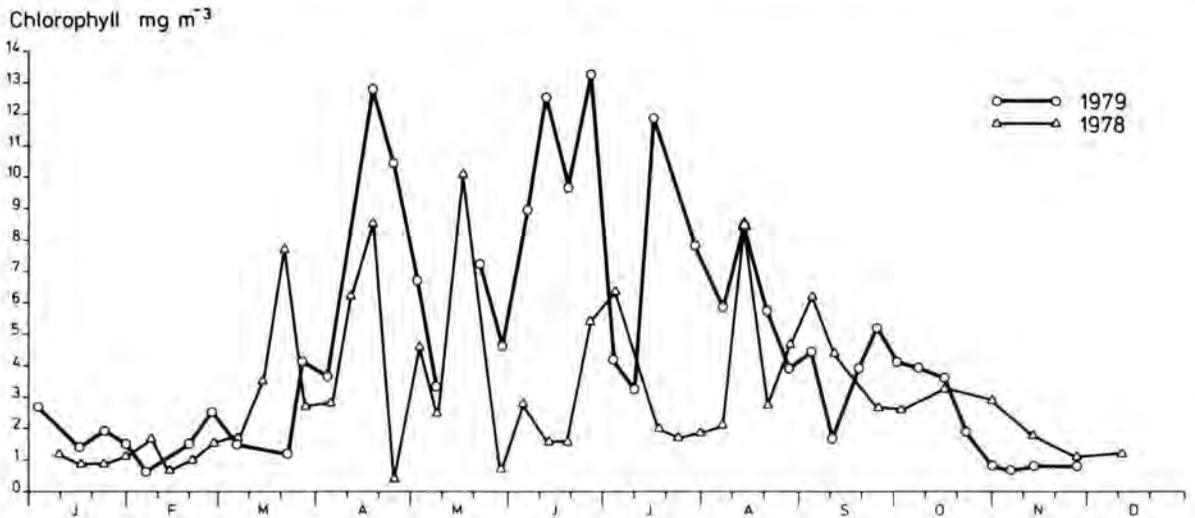
Sedimentation samples of 1 litre as well as samples of 100 litres filtered through 63 µm mesh gauze were taken to study the smaller phytoplankton resp. the larger phytoplankton and the zooplankton.

Diversity of the larger diatom species increased immediately after flushing with coastal North Sea water started. Several species, common in the former tidal Grevelingen estuary and disappearing when the estuary was closed, were observed, again, e.g. *Paralia sulcata*, *Stephanopixis turris*, *Coscinodiscus granii*, *Chaetoceros densum*, *Bellerochea malleus*, *Bacteriastrum hyalinum*. Other species, still occurring in the lake in small numbers, were demonstrated more abundantly during 1979: *Actinopterychus senarius*, *Actinocyclus octonarius*, *Thalassionema nitzschioides*, *Streptothecha thamesis*, *Biddulphia* spp., *Guinardia flaccida*, *Rhizosolenia stolterfothii*, *Schroederella schoederi*. Also some armoured dinoflagellates as *Ceratium fisis* and *Dinophysis* sp. were noticed. We have to mention especially the diatom *Rhizosolenia robusta* Norman, a species of warmer waters, penetrating the North Sea via the English Channel and observed for the first time in the Dutch Delta area (January-February 1979).

Table 5. Average algal densities during the summer season and average chlorophyll concentrations in spring and summer of stagnant (1978) and flushed (1979) Lake Grevelingen (station G11, depth 2.5 meters).

Algal species	1978 cells ml ⁻¹	1979 cells ml ⁻¹
<u>non-diatoms</u>		
<i>Cryptomonas</i> spp.	560	770
<i>Eubreviella</i> spp.	40	700
<i>Katodinium rotundatum</i>	200	390
flagellates 4 µm diam.	490	390
coccioid µ-cells	28000	64000
<u>diatoms</u>		
<i>Nitzschia sertata</i>	50	72
<i>Rhizosolenia delicatula</i>	70	10
" <i>setigera</i>		30
" <i>hebetata</i>	1	
<i>Chaetoceros</i> spp.	1120	3450
<i>Ditylum brightwellii</i>	14	39
<u>Chlorophyll</u>		
overall value	mg m ⁻³	mg m ⁻³
spring " (III-IV)	3.2	4.5
summer " (V-VIII)	4.2	5.8
	3.9	6.9

Fig. 3. Annual variations of chlorophyll concentrations in Lake Grevelingen 1978-79 (Station G11, depth 2.5 m)



Medium-sized and smaller phytoplankton, of basic importance for most of the secondary producer species in the lake, showed marked differences with 1978 in relation to cell densities. In Table 5 the average cell concentrations from 10 dominating species (or species groups) during the summer season (May to September) are listed. Several species reached considerably higher numbers during 1979, especially euglenophycean flagellates (*Eutreptiella* spp.), a naked dinoflagellate (*Katodinium rotundatum*), coccoid μ -cells, and the diatoms *Rhizosolenia hebetata* + *setigera*, *Chaetoceros* spp. (predominantly small forms) and *Ditylum brightwellii*. Only the rounded flagellates of 4 μ m diameter and the diatom *Rhizosolenia delicatula* were more numerous in 1978. The higher standing stocks of phytoplankton were clearly reflected in higher chlorophyll values too, notably during summer (Fig. 3, Table 5).

Average biomass of phytoplankton, therefore, was significantly larger in 1979 as a consequence of the flushing with North Sea water. This picture corresponds well to the far higher primary production figures, especially in summer, as measured by Vegter and de Visscher. Import of $N-NO_3^-$ from the North Sea, amounting to more than $0.2g\ m^{-2}\ month^{-1}$ in spring and approaching $0.1\ g\ m^{-2}\ month^{-1}$ in summer (Stokman, 1980), will have stimulated phytoplankton strongly.

Also the spring phytoplankton development was quite different in 1979 as compared with 1978, although the levels of daily production were approximately similar. It was the species composition of the blooms that strongly differed as cryptomonad

flagellates did not dominate so excessively in 1979 as during the preceding years 1976-78. The flagellates were accompanied now by the diatoms *Thalassiosira nordenskiöldii*, *Detonula confervacea* and *Skeletonema costatum*, giving the assemblage in the lake a much more marine character. Both *Thalassiosira* and *Detonula* were, besides *Cryptomonas*, photosynthetically very active (cf. van Iwaarden, 1979). The tendency therefore is that dominance shifted from diatoms to cryptomonad flagellates notably in spring, a characteristic phenomenon in the brackish lakes in the S.W.-Netherlands (Bakker and Vegter, 1979; Bakker and Rijk, 1979), has changed in the flushed salt water lake as diatoms regained importance and flagellates receded.

Abundance levels of cryptomonad flagellates contrasted with those in 1976-78, not only in spring but also in summer. In 1979 the lower spring peaks were followed by higher summer values both compared with 1978. This resulted in a clear levelling of the usual seasonal fluctuations. The flushing regime of Lake Grevelingen seemed, thus, to exert a stabilizing influence on the strong fluctuations of *Cryptomonas* in the course of the year. This phenomenon of 'levelled' seasonal periodicity could be discovered in other non-diatom species too.

Some species changed their period of dominance. *Nitzschia seriata* e.g., in preceding years blooming not earlier than in September, in 1979 reached its maximum during July-August. *Skeletonema costatum* came to dominance in spring, a phenomenon not observed since 1976. Remarkably one species, *Gymnodinium*

splendens, common during the period of stagnancy, was not found at all during the flushing period of 1979.

References

Bakker, C. and J.C.M. Rijk - 1979. Phytoplankton standing stocks in Lake Grevelingen. In: Duursma, E.K. (ed.). Progress Report DIHO 1978, Verh. KNAW, Nat. 2e reeks 73, 23-26.

Bakker, C. and F. Vegter - 1979. General tendencies of phyto- and zooplankton development in two closed estuaries (Lake Veere and Lake Grevelingen) in relation to an open estuary (Eastern Scheldt), S.W.-Netherlands. Hydrobiol. Bull. 12:226-245.

Iwaarden, A.J.W. van - 1979. Micro-autoradiografische bepaling van de primaire productie van verschillende phytoplanktonsoorten tijdens de voorjaarsbloei (1979) in het Grevelingenmeer. DIHO-Student Report D9-1979, 85 pp.

IV. 6. THE SPATIAL DISTRIBUTION OF CHLOROPHYLL IN LAKE GREVELINGEN (G1, G3) (A.G.A. Merks and J.O. v.d. Zande)

In 1973 three sampling programmes were carried out in Lake Grevelingen in which, among some other parameters, chlorophyll was determined as a biomass parameter. The intention of these programmes was to select a sampling station which would be representative for the lake. Based on the results of these sampling programmes station G11 was chosen as the representative station (Vegter, pers. comm.).

Since the analytical techniques have become more sensitive, and furthermore new parameters as DOC and POC could also be analyzed, the sampling station G11 does not appear to be fully representative. On 4 april 1979 samples from the water surface were taken at 85 stations in Lake Grevelingen. In these samples were carried out determinations of chloride, DOC, POC, ortho-phosphate and chlorophyll. The results of this one-day sampling programme are to be

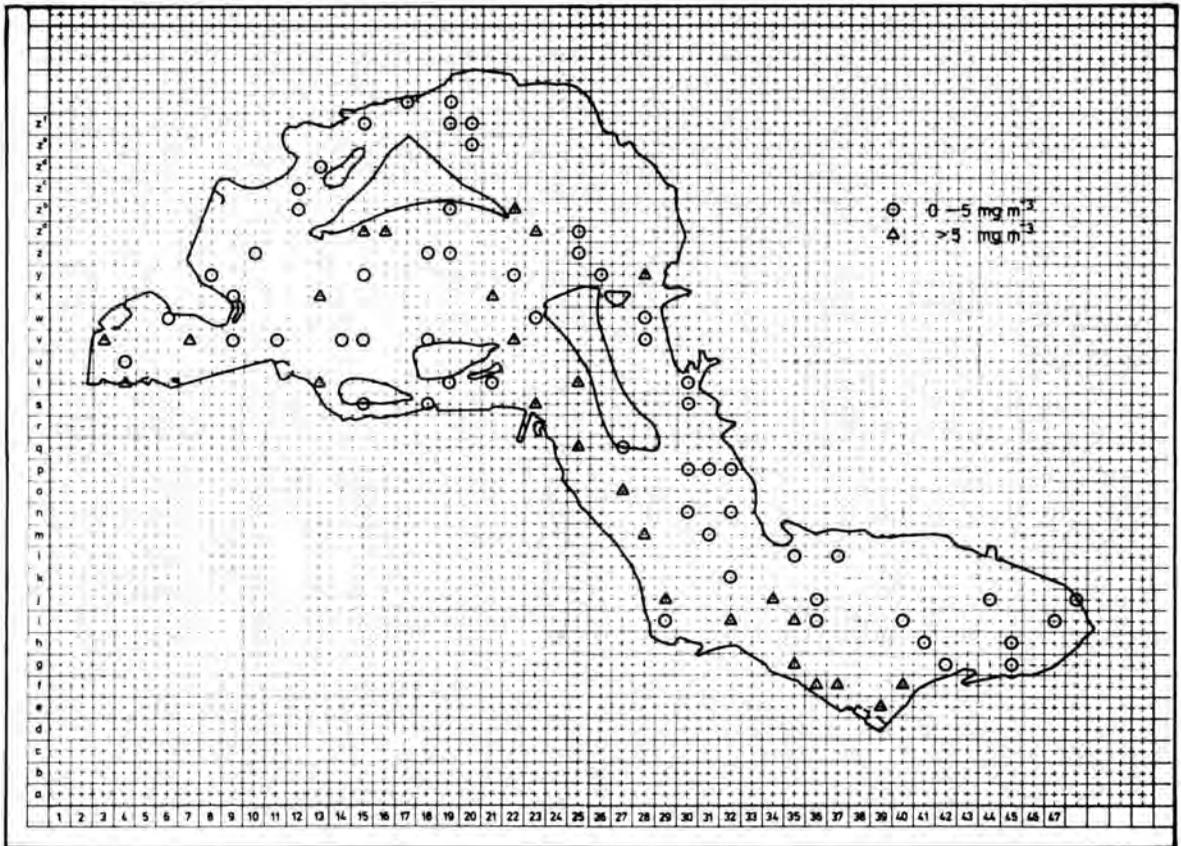


Fig. 4. Chlorophyll concentration calculated in mg m⁻³ in Lake Grevelingen

reported in full by Merks *et al.* (1980). In this contribution the results of the chlorophyll analysis are reported and discussed.

Chlorophyll samples were filtered on board of the research vessel 'Jan Verwey' over glassfibre and deep-frozen afterwards. In the laboratory a sample was destructed in a mill and then extracted with acetone 90%. The measurement took place in a fluorometer (Turner 111) and the chlorophyll concentration was calculated according to Merks (1976).

The values for this important biomass parameter lie between 1.5 and 16 mg m⁻³ (Fig. 4). Dividing the results into two classes we find that the South Eastern part of the lake has higher concentrations than the rest of it. As shown in Fig. 4 the concentration of chlorophyll is not homogeneously distributed throughout Lake Grevelingen. Other parameters (Merks *et al.*, 1980) also show that the concentrations strongly depend on the sampling location. Therefore, we conclude that station G11 is hardly representative for Lake Grevelingen as a whole. The study of biomass and other parameters to monitor biological processes in Lake Grevelingen needs to be done more purposefully. A sampling programme as carried out at station G11 only tells us something about the prevailing circumstances.

References

- Merks, A.G.A. - 1976. De meting van chlorofyl en feofitine in water en in de bodem. DIHO Rapp. and Versl. 1976-4, 30 pp.
- Merks, A.G.A., J.J. Sinke and J.O. v.d. Zande -1980. De ruimtelijke verdeling van enkele chemische milieu-parameters in het Grevelingenmeer in april 1979. DIHO Rapp. en Versl. In press.

IV.7. THE USEFULNESS OF A LIGHT INCUBATOR FOR PRIMARY PRODUCTION MEASUREMENTS (G3) (P.R.M. de Visscher and F. Vegter)

Since the light incubator, designed and built in 1978 (De Bree, in press), has been routinely used for phytoplankton primary production studies from May 1979 onwards it became necessary to know whether the simulation results are comparable with those obtained *in situ*. For that purpose a few experiments were carried out almost simultaneously at the sampling site (G11, Grevelingen) and in the laboratory in the incubator.

Primary production measurements *in situ* as well as in the incubator were carried out following our standardized working procedure (De Bree *et al.*, 1979). Our first interest went to the comparability of *in situ* and 'incubator' particulate assimilation, but excretion

rates were also determined. The measurements were carried out on April 23rd, September 10th, and on October 10th. Using the measured underwater light attenuation (Kahlsico Underwater Lightmeter) and solar irradiation (measured by the Delta Service at Bommenede with a Kipp-solarimeter), hourly irradiation intensities (J cm⁻² hr⁻¹) at sampling depths (surface, ½, 1, 2, 3, 5, 7, 10 metres) were calculated for the hours during which the *in situ* measurement was carried out. In this way it is possible to determine the daily production from incubator measurements and diurnal light intensities. Taken from the different sampling depths pairs of subsamples (50 ml) were incubated in Duran 50 tubes at ambient temperature in the incubator compartments, having light intensities of respectively 50.4 - 34.14 - 33.66 - 15.91 - 10.86 - 7.76 - 5.59 - 4.19 - 2.96 - 2.21 - 1.63 J cm⁻²hr⁻¹. These values were calculated from quanta measurements done with a Macam Photometer with mini-sensor. After an incubation-period (mostly 5 hours) the samples were treated as earlier described, and after scintillation counting and computing, eight photosynthesis - light curves were obtained: one for each sampling depth, in order to trace disturbances in the vertical distribution caused by an inhomogeneous plankton partition.

Sometimes, especially in the surface and the near-surface samples, the *in situ* light intensity reaches inhibiting levels which we are not able to simulate in our incubator. Nevertheless, when fitting our experimental data to the production - light model as described in Smith's equation (Patten, 1968; Crill, 1977) it is possible to obtain reasonable values for the photo-inhibition light levels. Data fitting and calculations are being computerised in our mathematical department using the photo-inhibition model of Vollenweider (Vollenweider, 1965). Results are shown in Table 6.

In general, when the incubation is done the same day as the sampling we find comparable productions. Samples that were incubated one day after sampling data and after storage at ambient temperature in complete darkness and quietness gave an integral decrease in production of 35.5% on 24th April and an increase of 74.8% on 11th September. Based upon *in situ* measurements and laboratory incubations and a few calculations with a chlorophyll-production conversion factor we found a particulate production of 249 gC m⁻² and an excretion percentage of 3.41% in 1980. One summer peak reached over 3 gC m⁻² per day, which is very high compared with the years before (highest summerpeak 1976-1978 about 2 to 2.5 gC m⁻²).

Table 6. Comparison of integral vertical *in situ* productions with incubator production.

Date	<i>in situ</i> production (mg C m ⁻² 5 hr ⁻¹)	incubator production (mg C m ⁻² 5 hr ⁻¹)	% of <i>in situ</i>
April 23	257.7	248.1 a.	96.3
	358.0	422.0 b.	117.8
September 10	405.7	395.9	97.6
October 10	667.1	671.8	103.3

a. Incubation period from 9.00 - 14.00 h

b. Incubation period from 10.00 - 15.00 h

References

Bree, B.H.H. de - in press. Een nieuwe incubator met lichtgradiënt voor experimenten met plankton en benthos. DIHO Rapp. en Versl.

Bree, B.H.H. de, A.G.A. Merks, F. Vegter and P.R.M. de Visscher - 1979. Primary Production of Phytoplankton. In: E.K. Duursma (ed.). Progress Report DIHO 1978. Verh. KNAW, Nat. 2e reeks, 73, 19-22.

Crill, P.J. - 1977. The photosynthesis - light curve: a simple analog model. J. Theor. Biol. 64, 503-516.

Patten, B.C. - 1968. Mathematical Models of Plankton Production, Int. Revue ges. Hydrobiol. 53 (3), 357-408.

Vollenweider, R.A. - 1965. Calculation models of photosynthesis curves and some implications regarding day rate estimates in primary production estimates. Mem. Ist. Ital. Idrobiol. 18 (Suppl.), 425-457.

IV.8. ZOOPLANKTON COMPOSITION AND -ABUNDANCE LEVELS OF LAKE GREVELINGEN FLUSHED WITH NORTH SEA WATER DURING 1979 (G4). (C. Bakker, W.J. Phaff and J.C.M. Rijk)

Zooplankton populations in the lake were sampled at 17 stations, situated both in the channels (covering the entire water column) and the shallows. The water layers of the depth of 0 to 5, 5 to 10... etc. meters were sampled in such a way that the total number of samples within each layer was proportional to the total volume of that layer. The samples were pooled in 3 categories, an eastern, central and western lake section, then counted and finally pooled to one integrated sample, representative for the entire lake. Using this procedure weighted means could be obtained of all abundant zooplankton species. Samples of the central-

ly located station G11, however, always were counted separately before pooling, in order to study vertical distribution and to integrate all parameters estimated on this locality (physico-chemical, phytoplankton, chlorophyll and primary production figures), with zooplankton standing stock data.

The flushing of the lake with coastal North Sea water resulted, just as for the phytoplankton, in an increase of species diversity. A series of copepod species, lacking (or found sparsely) during the stagnant period 1976-78, was demonstrated (more frequently) in 1979: *Temora longicornis*, *Centropages hamatus*, *Pseudocalanus minutus elongatus*, and *Acartia clausi* (see Table 7). Sometimes *Calanus helgolandicus* was observed. When North Sea water was influenced by large fresh water loadings of the Rhine via the Haringvliet sluices, this could be discovered by the occurrence of adult specimens of the estuarine copepod *Eurytemora affinis* in numbers (January 1979). In February numerous specimens of *Pleurobrachia pileus* (Ctenophora) were observed. In summer, besides *Oikopleura dioica*, till 1979 the only appendicularian occurring in the lake, also *Fritillaria borealis acuta* was found, the latter being even more abundant than the former. The chaetognath *Sagitta setosa* entered the lake again. *Noctiluca* was noticed more frequently.

In view of the strongly changed salinity relations (16-17‰ Cl instead of 12-13‰) and the more diversified phytoplankton assemblages (Bakker and Rijk, this report), shifts in abundance and periodicity of zooplankton species could be expected. Table 7 gives a survey of the main zooplankton groups and species during 1977-79. The species will be discussed following the same sequence as in this table. During early spring (February-March) a few zooplankton groups, notably rotifers and tintinnids, used to develop strongly in the stagnant brackish lake. From the last category *Tintin-*

Table 7. Average abundance levels and/or peak occurrences of the main zooplankton species of Lake Grevelingen (mainly station G11) from 1977-79. Numbers of animals are given per 100 litres of water.

Zooplankton group	Species and stage	Period and abundance		1979	1978	1977	
Tintinnida	<i>Tintinnopsis beroidea</i>	Jan-Febr	mean	500	150	<100	
		April-May	peak	-	50000	>80000	
	<i>T. cylindrica</i>	May-June	peaks	400	4000	4300	
		Aug-Sept	peaks	3000	5-10000*	6-10000*	
	<i>Favella ehrenbergii</i>	June-½ Aug	mean	6200	1500	2700	
	<i>T. campanula</i>	½ Aug-Oct	mean	900	1700	300	
		June-July	peak	9000	-	200	
Rotifera	<i>Synchaeta cf. vorax</i>	March	peak	1000	15000	30000	
		April-May	peak	15000	60000	100000	
		May-June	peak	60-20000	500	1800	
Polychaeta	Nectochaete larvae	April-Sept	mean G11	2600	140	880	
			mean G tot.	560	180	860	
Lamellibrachia	Veliger larvae	½ May-July	mean G11	9200	1340	1800	
		July-Sept	mean G11	1330	1400	2400	
		½ May-July	mean G tot.	5680	1390	860	
		July-Sept	mean G tot.	2260	1730	1800	
Cirripedia	<i>Balanus</i> Nauplii	Febr-May	mean	810	350	250	
Cladocera	<i>Podon polyphemoides</i>	May-Sept	mean	740	330	370	
Copepoda	<i>Eurytemora</i> spp. Nauplii	May	peak	1900	3100	900	
		May	peak	420	900	140	
	<i>Acartia clausi</i> Nauplii	Jan-Febr	mean	>40	incidental		
		Jan-Febr	mean	10	"		
	<i>Acartia tonsa</i> Nauplii	March-Nov	mean	940	1020	1940	
		April-Nov	mean	580	640	860	
	<i>Temora longicornis</i> Nauplii	May	peak	2000	incidental		
		May	peak	500	"		
	<i>Centrop. hamatus</i> Nauplii	May-June	max.	260	"		
		May-June	max.	130	"		
	<i>Pseudoc. m. elong</i> Nauplii	July	max.	170	"		
		June	max.	150	"		
		June	max.	150	"		
	Appendicularia	<i>Oikopleura dioica</i>	July-Sept	peak		3-800*	1-3000*
		<i>Idem+Fritillaria borealis acuta</i>	June-Oct	peak	2-3000*		

* = 5 - 10000 = 5000 to 10000

nopsis beroidea maintained a relatively high winter level (at temperature below 0°C), but, in spite of this favourable starting position, disappeared completely during and after the phytoplankton spring blooms in April and May, an unexpected event because adequate food (μ -algae) was abundant at that time. *T. cylindrica* too played a less important role both during early and late summer. *Favella ehrenbergii* however reached higher values than before. The come back of *T. campanula* with a distinct summer peak is considered a consequence of the flushing with sea water. The species, characteristic for the marine tidal Eastern Scheldt, was not found in Lake Grevelingen in 1978 and in very low numbers in 1977.

In 1977 development of rotifers deviated strongly from preceding years: *Synchaeta* failed to produce its usual March peak, which might be explained by the far lower densities of cryptomonad flagellates, representing the main food algae, during that period. The first maximum was developed not before the end of April, followed by strong outbursts in May and June when *Cryptomonas* was most numerous. We may conclude that the early spring development of rotifers, highly characteristic for the stagnant lake, was retarded 1½ month due to the renewed connection with the North Sea in 1979.

In relation to polychaete larvae Table 7 gives averages not only for the station G11, but also for the entire lake (Gtot.). In 1977-78 these values were approximately similar, but in 1979 they differed distinctly. The lower value of G11 has been influenced by decreasing oxygen contents (less than 2 mg l⁻¹) due to salinity stratification starting in June and effecting the bottom area below a depth of 8-10 metres (Nienhuis, 1980; this report). Development of polychaete larvae in these strata was suppressed, resulting in a lower total average for G11. The value of the entire lake, on the other hand, includes all shallows, not influenced by oxygen shortages: conditions for larval development remained favourable here and, consequently, lead to an average more than twice as large as that of G11. Table 7 shows that larval densities were very low in 1978, due to the low salinities (12.2-12.7‰ Cl in summer). Abundance in 1979 was distinctly higher, especially the integrated value, reflecting more favourable conditions based on the increased salinities.

Lamellibranchia, starting in May with similar concentrations of veliger larvae as in 1977-78, reached much higher numbers in the course of May-June. During the remaining summer months, however, average numbers dropped sharply, whereas concentrations in the preceding years were similar (1978) or more comparable (1977) throughout the summer. The summer decrease of 1979 may be explained in the same way as for the Polychaetes, as weighted means for the entire

lake demonstrate. Naupliar stages of *Balanus* developed a retarded spring peak (ca. 3 to 4 weeks) in March-April, probably due to the low temperature (less than 0°C) during January-February 1979. Average numbers however were much larger than those of the preceding years, a phenomenon which might be correlated with salinity and especially with more favourable food conditions.

The cladoceran *Podon polyphemoides* reached averages twice as large as in the preceding years, based on higher peaks in June and August-September. Moreover the animal started its development earlier (April instead of May) and finished later (October instead of September). In 1979 the copepod *Acartia tonsa* reached similar average population densities, both of naupliar and of copepodid + adult stages, as in 1978. A small winter population (January-February) was found, consisting mainly of *A. clausi*, transported into the lake from the sea. About the other copepod species mentioned in Table 7, some remarks were already made.

We already noted the introduction into the lake of the appendicularian *Fritillaria borealis acuta*. Together with *Oikopleura dioica* far higher abundance levels of these animals were reached than in 1978. Evidently, the strong decrease of population densities of *Oikopleura* in 1978, forcing the species to marginal existence, was caused by the reduced salinity in the stagnant brackish lake (12.5‰ Cl, see Bakker *et al.*, 1979). In 1977 maximum densities comparable with those of 1979 were demonstrated, but during a much shorter (2 months) time. Both the more extended seasonal range and the high abundance levels of 1979 can be explained adequately by the increased salinity.

Biomass figures of the zooplankton assemblage of 1979 have not yet been calculated. Data of Table 7 however indicate that most groups (total copepods, lamellibranchs, polychaetes, cirripedes and cladocerans) increased in numbers. Only rotifers and total tintinnids remained rather constant. In conclusion: total average zooplankton biomass rose as a consequence of the flushing regime, including both increase of salinity and improvement of food conditions.

References

Bakker, C., W.J. Phaff and J.C.M. Rijk - 1979. Abundance of dominant zooplankton species in Lake Grevelingen. In: E.K. Duursma (ed.) Progress Report 1978 DIHO, Verh. KNAW, Nat. 2e reeks, 73, 26-29.

IV. 9. INVENTORY OF BENTHIC DIATOMS IN LAKE GREVELINGEN (G5) (E.A.M.J. Daemen)

Pigment measurements in the sediment of Lake Grevelingen showed that considerable amounts of chloro-

phyll and phaeophytine are present (De Bree and Verschuure, 1978) and measurements of the primary production by the ^{14}C method made clear that the microphytobenthos is an important chain in the carbon cycle of Lake Grevelingen.

Because hardly any data are available about the species composition of the microphytobenthos in Lake Grevelingen an inventory of the benthic diatoms was made (Daemen, 1980). 35 Samples were taken at 16 different sampling stations during the period January-July 1979. In these samples 135 pennate taxa (belonging to 33 genera) and 13 centric taxa (13 genera) were identified. Among the detected species 14 had not been described earlier for the Netherlands. The results of the study were compared with those of Prof. H. Pankow (University of Rostock, D.D.R.) and Mr. van der Werff (Neth.) who both have examined some samples of Lake Grevelingen. When their lists of species were compared with our checklist it was found out that in Lake Grevelingen 197 pennate and 27 centric diatom taxa were present.

The study showed that hardly any species is dominating in Lake Grevelingen. Only 11 pennate species were both common all over the lake, as well as present in large numbers.

The benthic diatomflora in the present Lake Grevelingen consists of three main groups:

1. Marine and estuarine species, already present before the closing.
2. Marine species, reintroduced after the opening of the sluice in the Brouwersdam in 1978.
3. Ampotixene species; these are species that cannot survive in a tidal area proper, because the environmental changes (e.g. salinity, temperature, light) are too rigorous. After the closing the ecological conditions in the lake became much more stable, enabling these species to survive.

References

Bree, B.H.H. de and J.M. Verschuure - 1978. Pigment en particuliere organische koolstof in de bodem van het Grevelingenmeer in 1976 en 1977. DIHO Rapp. en Versl. 1978-11, 51 pp.

Daemen, E.A.M.J. - 1980. Inventarisatie van de benthische diatomeeën in het Grevelingenmeer in het voorjaar van 1979. DIHO Rapp. en Versl. 1980-3, 117 pp.

IV.10. PRIMARY PRODUCTION OF MICROPHYTOBENTHOS (G5) (P.H. Nienhuis, B.H.H. de Bree and J.M. Verschuure)

The construction of a large indoor incubator for primary productivity measurements was finished. The

incubator is characterized by a light and temperature regime, simulating the water column in Lake Grevelingen. At 4 localities, bi-weekly, 10 sediment samples were taken from the lake. These samples were incubated in the laboratory after adding a ^{14}C source. Samples were burnt after 5 hours of incubation in a carbon oxydyzer, and the activity was measured in a liquid scintillation counter. Both particulate and extracellular production of organic material were established.

Concomitant, at the same localities in Lake Grevelingen 10 sediment samples were taken for chlorophyll and particulate organic carbon analysis.

The results show an extremely heterogeneous spatial productivity pattern and large fluctuations in the course of time. The chlorophyll pattern in the top sediment is also very heterogeneous. Relatively high ^{14}C uptake values were measured in the dark, suggesting intensive bacterial activity. Interpretation of the 1979 results is in progress. It is our intention to continue these measurements during 1980, and to publish the full results in 1981.

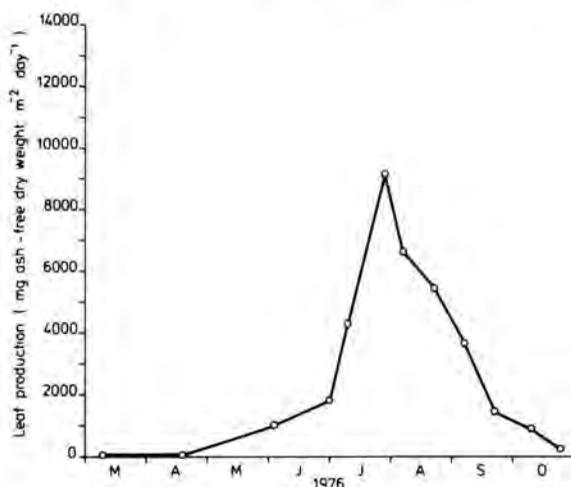
IV.11. RE-ESTIMATION OF THE PRIMARY PRODUCTION OF EELGRAS, ZOSTERA MARINA (G6) (P.H. Nienhuis, B.H.H. de Bree and J.M. Verschuure)

During summer Lake Grevelingen harbours large growths of eelgrass that die back each winter. In fact it is the largest *Zostera marina* population along the southern North Sea. Although the eelgrass ecosystems are among the most productive marine systems in the world, accurate data about their quantitative role in productivity are rather scarce (Mc Roy and Mc Millan, 1977). Production studies of eelgrass are mainly based on indirect measurements, viz. short term changes in standing stock. It should be realized however, that sequential harvesting will give a minimum estimate of net primary production.

In 1976 we applied the leaf marking technique, in order to estimate overground production and rhizome production as accurate as possible. The elaborate method is based on the measurement of biomass increment per leaf-surface area per unit of time. Fig. 5 shows some results. Rhizome production can also be estimated: each leaf corresponds to one node, which means that rhizome segments will be produced at the same rate as leaves.

We found out that overground net production in 1976 was 6.4 times the net increase of biomass. In 1975 we still accepted the assumption, originally made by Petersen (1913) and confirmed by Mann (1972) and Phillips (1974), that net yearly overground production is twice the maximum standing crop (Nienhuis and De Bree, 1977). Maximum standing crop in Lake Greve-

Fig. 5. Average leaf production of eelgrass in the permanent sample plot near Herkingen, Lake Grevelingen, in 1976



lingen corresponds approximately to net biomass increase during the growing season.

Our data show that the often applied hypothesis of Petersen (1913) should be rejected, at least for Lake Grevelingen. Sand-Jensen (1975) who worked in Danish eelgrass fields and used the leaf-marking technique, reached the same conclusion. He estimated a ratio between net production and net biomass increase in an eelgrass population in Denmark of 5.1 for leaves, of 2.0 for rhizomes and of 3.8 for total plant material. These data show a good agreement with our data (Nienhuis and De Bree, in press). A P/B ratio of approximately 6 for overground parts, and a P/B ratio of approximately 5 for overground and underground parts together, seem reasonable good average values for non-tidal *Zostera marina* populations in NW Europe.

In Table 8 the biomass data of 1973 and 1975, originally published by Nienhuis and De Bree (1977), have been recalculated on the basis of the conversion factors mentioned. The data for 1978 have been added. Table 8 shows the enormous increase in biomass and production of eelgrass in Lake Grevelingen after the closure in 1971.

Bittaker and Iverson (1976) and Capone *et al.* (1979) compared the leaf marking technique and the ¹⁴C technique in studies on the seagrass *Thalassia testudinum*. Bittaker and Iverson (1976) showed that differences in the results contained between the two methods were insignificant. Capone *et al.* (1979) demonstrated that production estimates with the leaf marking technique yielded lower (but nevertheless very useful) values, than the ¹⁴C uptake assays.

References

- Bittaker, H.F. and R.C. Iverson - 1976. *Thalassia testudinum* productivity: a field comparison of measurement methods. Mar. Biol. 37:39-46.
- Capone, D.G., P.A. Penhale, R.S. Oremland and B.F. Taylor - 1979. Relationship between productivity and N₂(C₂H₂) fixation in a *Thalassia testudinum* community. Limnol. Oceanogr. 24, 117-125.
- Mann, K.H. - 1972. Macrophyte production and detritus food chains in coastal waters. Mem. Ist. Ital. Idrobiol. 29. Suppl., 353-383.
- Mc Roy, C.P. and C. Mc Millan - 1977. Production ecology and physiology of seagrasses. In: C.P. Mc Roy and C. Helfferich (eds.). Seagrass ecosystems, a scientific perspective. M. Dekker, New York 2, 52-87.
- Nienhuis, P.H. and B.H.H. de Bree - 1977. Production and ecology of eelgrass (*Zostera marina* L.) in the Grevelingen estuary. The Netherlands, before and after the closure. Hydrobiologia 52, 55-66.
- Nienhuis, P.H. and B.H.H. de Bree - in press. Production and growth dynamics of eelgrass (*Zostera marina* L.) in brackish Lake Grevelingen (The Netherlands). Neth. J. Sea Res.
- Petersen, C.J.G. - 1913. Om baedeltangens (*Zostera marina*) aarsproduktion i de danske Farvande. In: H.P.E. Jungersen and E. Warming (eds.). Mindeskr. Japetus Steenstrups Føds. 9, 1-20. G.E.C. Gad, København.
- Phillips, R.C. - 1974. Temperate grass flats. In: H.T. Odum, B.J. Copeland, E.A. Mc Mahan (eds.). Coastal ecological systems of the United States II C-7A, 244-299. Conserv. Found. Washington.
- Sand-Jensen, K. - 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. Ophelia 14, 185-201.
- IV.12. STANDING ROCK AND PRODUCTION OF MACROALGAE (G6) (F.E. Boelé, P.H. Nienhuis and J. Sybesma)
- Up to 1978 it was supposed that macroalgae in Lake Grevelingen formed a minor source of organic carbon. Previous estimates, based on standing stock surveys, indicated a production of roughly 10 to 15 g C m⁻² yr⁻¹ over the entire lake and approximately 30 g C m⁻² yr⁻¹ in the shallow areas.
- Red algae (especially *Polysiphonia violacea*) and brown algae (especially *Ectocarpus confervoides*) showed large variations in distribution and biomass over the years. Far out the most conspicuous group is formed by green algae *viz.*: *Chaetomorpha* spp., *Ulva*

Table 8. Average overground standing stock and production of *Zostera marina* in Lake Grevelingen. The 1973 and 1975 data, derived from Nienhuis & de Bree (1977) have been recalculated on the basis of the results with the leaf-marking technique.

	1973	1975	1978
Area of eelgrass beds (km ²)	15.85	27.64	44.42
Percentage Grevelingen covered	15	26	41
Standing crop in eelgrass beds (g m ⁻² ash fr. dr. wt.)	130	98	95
Production in eelgrass beds (g C m ⁻² yr ⁻¹)	296	223	220
Production in Grevelingen (g C m ⁻² yr ⁻¹)	44	58	91

spp., *Cladophora* spp., and *Enteromorpha* spp.

Distribution patterns and standing stocks vary over the years, and it is difficult to discover a tendency of increase or decrease in the course of time related to the closure of the Grevelingen estuary in 1971.

Among the green algae *Chaetomorpha* spp. are far outstanding. In summer 1978 it was estimated that *Chaetomorpha* standing stock amounted to approximately 60% of the total macro-algal standing stock. The species covered an area of 20 km²; their total organic carbon weight amounted to 166 tons, i.e. 1.5 g C m⁻² over entire Lake Grevelingen.

In order to get more insight into the share of macroalgae in the overall production pattern some preliminary experiments were performed in 1979 with a number of green algal species by means of the ¹⁴C

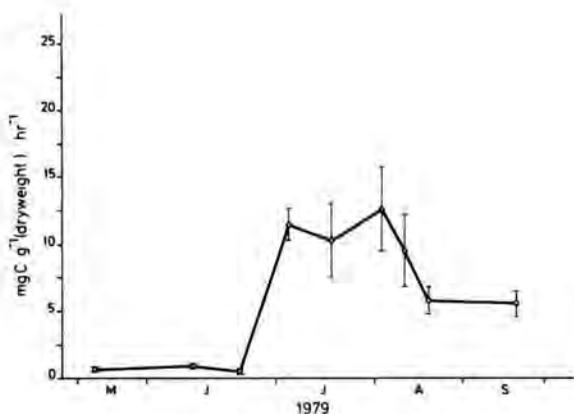


Fig. 6. Primary production of *Chaetomorpha* spp. from Lake Grevelingen, estimated in a laboratory incubator at 421 μE m⁻²s⁻¹. Vertical lines: standard deviation

technique. Periodically samples of dominant green algae were collected in Lake Grevelingen. Four hours of incubation at different lightlevels, dry combustion and counting in a liquid scintillation apparatus were used to determine the amount of ¹⁴C incorporated in the algae. From the radioactivity of the algal material the carbon fixation was calculated. A maximum production rate for the greenalgae was found at a lightlevel of 421 μE m⁻²s⁻¹. A higher light intensity (630 μE m⁻²s⁻¹) caused the algae to reduce their production rate. Lower light intensities (305 and 100 μE m⁻²s⁻¹) yielded lower amounts of radioactive carbon fixed into the algae.

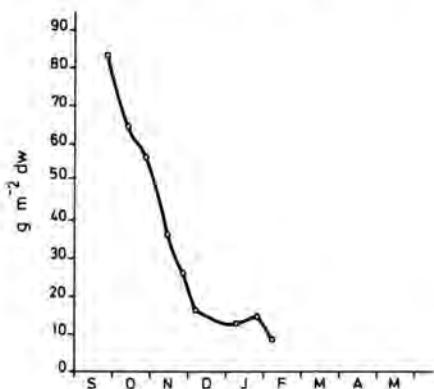
Chaetomorpha spp. showed an increase of production in late spring, with a highest level in summer (average production 7 mg C g⁻¹ dry weight hr⁻¹) and a decrease in autumn (Fig. 6). An estimation of the total amount of carbon fixed by *Chaetomorpha*, taking the standing stock into account, leads to figures several times higher than previous data.

IV.13. ACCUMULATION AND DECOMPOSITION OF EELGRASS (*Zostera marina*) IN LAKE GREVELINGEN AFTER THE GROWING SEASON (G6) (A.M. Groenendijk and H. Beunder)

Results of sampling in an eelgrass meadow in Lake Grevelingen show a fast decline in biomass after the growing season. From September 1978 until December 1978 the eelgrass biomass in a permanent sample plot dropped from 84.3 g m⁻² dry weight to 16.0 g m⁻² dry weight. This is mainly caused by transport of dead organic matter (detritus). In February 1979 approximately 10% of the original amount of eelgrass detritus was still present (Fig. 7).

Observations of large eelgrass transports, during September and October, from the eelgrass meadows into the deeper parts of the lake, and the formation of

Fig. 7. Average eelgrass detritus biomass (dry weight) in an eelgrass meadow in Lake Grevelingen, from September 1978 till February 1979



a salt peat-swamp in the eastern part of the lake showed the necessity to obtain more quantitative and qualitative data on transport and accumulation (refractory components) of eelgrass detritus.

In March and April 1979, just before the beginning of a new vegetation season, eelgrass detritus has been collected all over Lake Grevelingen and special attention has been given to the gullies. Due to the prevailing south-western winds accumulation occurred mainly in the eastern part of the lake. Locally the gullies obtained enormous amounts of eelgrass detritus. This detritus appeared to be in an increased state of decay, the fragments found were not larger than a few centimeters. In April 1979 the eelgrass detritus biomass was roughly estimated at 7% of the total net primary production of eelgrass during the forgoing season.

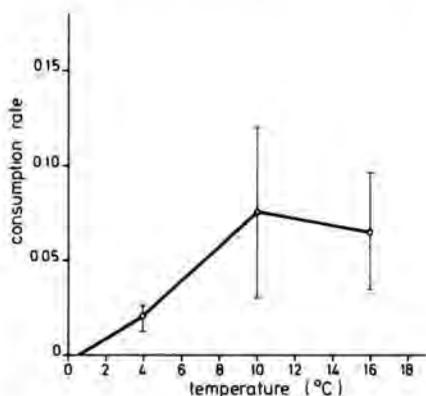


Fig. 8. Consumption rate (g eelgrass dry weight · g⁻¹ *Idotea* dry weight · day⁻¹) of eelgrass by *Idotea chelipes* under different temperature regimes

The role of epifauna invertebrates on the decomposition of eelgrass detritus after the growing season has also been studied. In experiments the consumption of the isopod *Idotea chelipes*, by far the most abundant epifaunal invertebrate, was estimated. Average consumption of one *Idotea* individual (dry weight 2.5 mg) amounted to 0.2 mg day⁻¹ dry weight. There is little evidence that consumption is affected by the state of decay of the eelgrass material.

Consumption experiments with *Idotea* in seawater tanks under different temperature regimes show no consumption of eelgrass between 1°C and 4°C; maximum consumption took place between 10°C and 18°C (Fig. 8). For the period between September 1978 and February 1979 total *Idotea* consumption in an eelgrass meadow was estimated on 2.8 g m⁻² dry weight.

Consumption experiments with another epifaunal invertebrate, *Gammarus locusta*, a species that became co-dominant with *Idotea* during late autumn 1978, showed that this amphipod also fed on eelgrass-detritus. However, consumption was rather low compared with that of *Idotea*, while the abundance of *Gammarus* came into a fast decline after December.

This altogether seems to justify the conclusion that consumption and transformation by *Idotea chelipes*, and other epifaunal invertebrates as well, play a minor role in the decomposition of eelgrass (see also Boelè *et al.* 1979; Nienhuis and van Ierland, 1978). Evidently other decomposition processes as leaching, autolysis, physical fragmentation and microbial decay are of more importance. Up to now there are hardly any quantitative data available on this subject.

References

- Boelè, F., E.A.M.J. Daemen, B.H.H. de Bree, A.M. Groenendijk, G.C. Pellikaan, P.H. Nienhuis and J.M. Verschuure - 1979. Production, consumption and decomposition of macrophytes. In: E.K. Duursma (ed.). Progress Report DIHO 1978. Verhand. KNAW, Nat., Tweede Reeks 73, 161-163.
- Nienhuis, P.H. and E.I. van Ierland - 1978. Consumption of eelgrass, *Zostera marina* by birds and invertebrates during the growing season in Lake Grevelingen (S.W. Netherlands). *Neth. J. Sea Res.* 12(2), 180-194.
- IV.14. A MACROZOOBENTHOS-BIOMASS SURVEY IN THE SHALLOW WATERS (0.8-2.1 m) OF LAKE GREVELINGEN IN JUNE 1979 (G7) (R.H.D. Lambeck, J.W.P. van Dam, C.S. Vroonland and E.G.J. Wessel)

Most of the bottom-fauna sampling in Lake Grevelingen has been done with a Van Veen grab. The disadvantage of this very handy type of grab is the in-

sufficient penetration depth in coarser sediments, often only a few centimeters (Ankar 1977). Deep-burrowing species as the bivalve *Mya arenaria* and the polychaete *Arenicola marina* are seriously underestimated: adult specimens have never been caught.

For this reason a survey has been carried out with a flushing-sampler (Van Arkel and Mulder, 1975), borrowed from the BOEDE-group, Texel. The operation range of this sampler is restricted, from about 0.7-2.3 m waterdepth. On the other side, the bottom fauna of this particular depth-range is badly known, because it is in between the operation possibilities of the research vessels and that of people wading in the water with a bottom-corer. So, the survey held was also valuable in getting information on the whole macrofauna.

Along 16 transects, spread over the lake, 5 localities with water depths of 0.8, 1.1, 1.5, 1.8 and 2.1 meters were sampled. Per locality 5 samples of 200 cm² each, and with a penetration depth of about 35 cm were taken. Samples were preserved by deep-freezing. At the laboratory only the larger animals were sorted out, excluding e.g. the very common mud snail *Hydrobia ulvae* and most of the polychaetes, implying biomass-underestimates (probably in the range of 0-10 g m⁻² ash-free dry weight (AFDW)). AFDW of the different species were determined by incinerating the whole animals (including the shells) at 580°C during two hours.

Results are presented in Table 9. Within the given limitations the mean biomass in this depth range for all transects amounted to 56.9 g AFDW m⁻², at a total sampled surface of 7.8 m². Per sampling-point biomass values ranged from about 4-237 g AFDW m⁻². Biomass is dominated by the cockle *Cerastoderma edule* with a share of about 50%. Other important molluscs were *Cerastoderma glaucum*, *Mya arenaria* and to a lesser extent *Mytilus edulis* and the snail *Nassarius reticulatus* (see also Table 9).

Of the larger polychaetes only *Arenicola marina* was of importance with a mean biomass of 5.7 g AFDW m⁻². The impact of sea anemones and larger crustaceans as *Idotea chelipes* and *Gammarus locusta* was negligible. The later species, however, may escape easily from the sampler.

Although the maximum biomass found at 0.8 m is considerably lower than at the other depths (due mainly to *Cerastoderma edule*), no clear correlation between mean total biomass and waterdepth could be detected. At the species level *Mytilus edulis* is considerably scarcer at 0.8 and 1.1 m (resp. 0.7 and 1.9 g m⁻²) than at the deeper points (about 4 g m⁻²). Also *Scrobicularia plana* has a higher biomass with increasing depth. Reversely, top biomasses of *Mya arenaria*

and *Arenicola marina* were found at 0.8 m, although differences with the other water-depths were not impressive.

Interesting are the very clear differences in biomass between the transects in the eastern and western part of Lake Grevelingen, as presented in Table 9. In the east mean values over all 9 transects amounted to 20.7 g AFDW m⁻², versus 105.5 in the west (7 transects). Especially for *Cerastoderma edule* the distribution difference is striking. Also *C. glaucum* is more common in the western part, although differences are less extreme. The same can be said of *Mya arenaria* (with the exception of 1.8 m), *Arenicola marina* and, although of minor importance, *Angulus tenuis*. Only the snail *Littorina littorea* and *Mytilus edulis* are more common in the east.

The western part of Lake Grevelingen, and especially its centre, is an open area with a sandy bottom without a seagrass (*Zostera marina*) vegetation. Wind-driven water currents are much stronger than in the more sheltered eastern part, probably favouring filterfeeders as the cockle. The distribution of the filter-feeding *Mytilus edulis* seems to contradict this explanation. However, the large musselbeds do occur in the western part. Obviously, as regards mussels, the settling conditions in the shallow areas are better in the east, which may be correlated with the occurrence of seagrass.

Seagrass may have an important influence on the macrozoobenthos, at least the distribution of *Littorina littorea* depends almost fully of this vegetation type. Illustrative in this respect are three additional 2.1 m samples taken at the Slikken van Flakkee, a very large shallow area, formerly investigated up to 1.5 m by Koniuszek (1979), covered by the only large seagrass population in the western part of Lake Grevelingen. Here biomasses were in the same range as in the east. One of the samples was even devoid of macrobenthic life, due to an amount of decaying seagrass remains.

In 1980 the relation seagrass-macrozoobenthos will be investigated in more detail.

References

- Ankar, S. - 1977. Digging profile and penetration of the Van Veen grab in different sediment types. Contr. Askö Lab. Univ. Stockholm no. 16, 22 pp.
- 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.
- Koniuszek, J.W.J. - 1979. Biomassa en produktie van macrozoobenthos op de Slikken van Flakkee. DIHO-Student Rep. D7-1979, 48 pp.

Table 9. Results of a biomass survey in June 1979 of large macrozoobenthic species in the shallow parts (0.8 to 2.1 m) of Lake Grevelingen. Data have been split up according to water depth and geographic position of the sampled transects in the lake (east and west of the island Veermansplaat). Biomass figures in $g\ m^{-2}$ ash-free dry weights. "Other molluscs" are *Buccinum undatum*, *Lepidochitona cinerea* and *Petricola pholadiformis*. "Other polychaetes" are *Nereis virens*, *N. succinea*, *Harmothoe imbricata* and *Pectinaria koreni*.

n = number of samples, each of $0.1\ m^2$

E = eastern part of lake and

W = western one.

species	0.8 m		1.1 m		1.5 m		1.8 m		2.1 m	
	E n=9	W n=6	E n=9	W n=7	E n=9	W n=7	E n=9	W n=7	E n=9	W n=6
<i>Littorina littorea</i>	1.27	0	3.46	0.01	2.34	0.42	1.73	0.34	2.91	0.09
<i>Nassarius reticulatus</i>	1.69	5.33	2.41	2.75	5.59	2.24	4.45	3.82	3.14	5.20
<i>Mytilus edulis</i>	1.13	0	2.71	0.79	7.49	0	7.65	0.19	5.04	0.54
<i>Cerastoderma edule</i>	1.54	47.54	0.87	66.55	0	68.06	1.18	79.16	1.04	67.16
<i>Cerastoderma glaucum</i>	4.08	7.56	2.75	13.01	1.23	12.92	0.60	16.16	0	13.23
<i>Scrobicularia plana</i>	0.41	0.53	0.16	0	0.16	1.45	0.58	1.51	0.53	2.05
<i>Macoma balthica</i>	0	0.31	0.09	0.50	0.15	0	0.27	0.01	0.14	0
<i>Angulus tenuis</i>	0	0.28	0	0.19	0	0.19	0.01	0.05	0	0.03
<i>Mya arenaria</i>	3.02	19.14	2.62	8.33	1.73	15.64	8.07	3.38	2.19	4.15
Other Molluscs	0	0	0.01	0	0.35	0	2.31	0	0.02	3.51
<i>Nephtys hombergii</i>	0	0.09	0.03	0.38	0.14	0.09	0.21	0.31	0.23	0.19
<i>Arenicola marina</i>	4.03	10.73	1.36	10.98	1.60	11.83	3.22	6.81	3.14	8.62
Other Polychaetes	0	0.26	0	0	0	0.19	0.03	0.02	0.08	0
Total values	17.2	91.8	16.5	103.5	20.8	113.0	30.3	111.8	18.5	104.8
Mean total biomass for the whole lake	46.9		54.7		61.2		66.0		53.0	

IV.15. NET-EFFICIENCY ESTIMATES FOR BROWN SHRIMPS (*Crangon crangon L.*) (G7) (A.W. Fortuin)

In behalf of the investigations on epi- and hyperbenthic macrocrustaceans in Lake Grevelingen the efficiency of the used bottom skimming planktonnet (Borghouts & Deelder, 1973), was tested on the catch

of the brown shrimp in different parts of the lake. For this purpose an area of about 25×15 metres (depth 80 cm) was enclosed with a standing net (enclosure) and fished out. To facilitate fishing out the enclosure, its surface was usually reduced in the beginning, and again after a number of hauls. The efficiency

Table 10. Net-efficiency at different densities of Brown shrimp.

station	date	mean	efficiency	eelgrass
		number m ⁻²		density
Herkingen	12- 9-79	0.03	167%	70/90%
Bommenede	23- 8-79	5.1	50%	<5%
Archipel	17-10-79	18.6	23%	0%
Archipel	2-10-79	21	23%	0%

measured will be overestimated, due to escape of shrimps out of the enclosure. The extent of this loss is not yet investigated, however, estimates of loss of various kinds of fish out of such enclosures ranged from 25 to 75 percent (Doornbos pers. comm.).

The estimates of efficiency (based on a 100% catch out of the enclosure) show a great difference between various parts of the lake (Table 10). This difference seems to be closely bound up with the density of shrimps, and probably also with the density of the eelgrass, *Zostera marina*. Two measurements, carried out in the same area with approximately identical densities, gave the same results. Notice the efficiency of more than 100% at Herkingen, probably due to the very low density of shrimp in the area. Estimates of efficiency of 38 and 52% (as given by Platenkamp, 1979), which were carried out in a slightly different way, correspond with estimates found in this investigation.

References

- Borghouts, C.H. and C.L. Deelder - 1973. A device to catch bottomdwelling macro-plankton. *Aquaculture* 1, 435-442.
- Platenkamp, G.A.J. - 1979. Distribution, biomass and growth of epi- and hyperbenthic macrocrustaceans in

Lake Grevelingen (G7). In: E.K. Duursma (ed.). *Progress Report DIHO, 1978*. Verh. KNAW, Nat. 2e reeks, 73, 167-168.

IV.16. THE ROLE OF MEIOZOOBENTHOS IN THE CARBON CYCLE OF LAKE GREVELINGEN (G8) (K. Willems and A.J.J. Sandee)

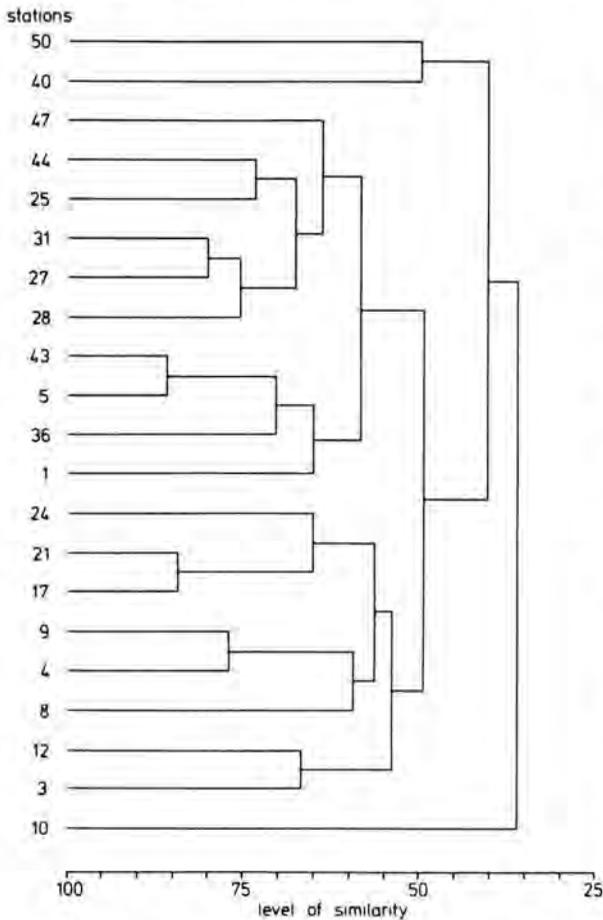
A field survey on Lake Grevelingen was ended with the investigation of wintersamples from 21 stations. Data on meiofaunal densities and biomasses are now available on a year basis (Table 11). Harpacticoid fauna was studied in detail for Lake Grevelingen, Eastern Scheldt and Eems Dollard. The harpacticoids of the Western Scheldt were investigated by Van Damme (Heip *et al.*, 1979). The harpacticoid fauna of the dutch estuaries is composed of 56 species. In order to investigate harpacticoid associations, cluster analysis is used on either binary and quantitative data. Different indices (Sørensen, Czekanowski) and sorting strategies are used.

Preliminary results indicate 2 main station groups in Lake Grevelingen according to sediment type: one with clean sandy sediments and the other with mixed and muddy sediments (Fig. 9). Data analysis of all Dutch estuaries, using Sørensen index combined with groupaverage sorting, demonstrates that cluster Lake Grevelingen is a very distinct group of stations

Table 11. Mean seasonal and annual densities, biomasses and standard errors of nematoda and harpacticoida from Lake Grevelingen.

		DENSITY (N 10 cm ⁻²)				BIOMASS (µg 10 cm ⁻²)			
		spring	summer	winter	year	spring	summer	winter	year
SAND	Nematoda	525 _± 121	826 _± 112	668 _± 101	708 _± 69	747 _± 210	466 _± 67	343 _± 50	495 _± 61
	Harpacticoida	41 _± 8	248 _± 32	105 _± 16	157 _± 19	119 _± 30	441 _± 67	258 _± 45	310 _± 37
MIXED	Nematoda	690 _± 114	2854 _± 181	1938 _± 239	1848 _± 156	661 _± 108	1277 _± 113	852 _± 123	921 _± 74
	Harpacticoida	41 _± 5	233 _± 24	145 _± 16	136 _± 14	124 _± 23	327 _± 199	229 _± 28	227 _± 22
GREV.	Nematoda	675 _± 89	1488 _± 163	1272 _± 158	1193 _± 91	700 _± 114	731 _± 77	585 _± 75	676 _± 50
	Harpacticoida	41 _± 5	243 _± 22	124 _± 12	146 _± 10	121 _± 19	403 _± 48	244 _± 27	275 _± 24

Fig. 9. Harpacticoid fauna: dendrogram sequence of Q-analysis; SØRENSEN, Group average



with very high similarities. This could be explained by the environmental stability throughout Lake Grevelingen resulting in a specific faunal composition.

F. Volkaert, a student, joined the program for a 9 month period. He studied the meiobenthic polychaetes. Distribution and abundance was investigated in 21 stations for a spring and summer period. One station, Archipel, being sampled over a year period.

For all stations 25 species were found. Dominant species are *Streptosyllis websteri*, *Tharyx mariori*, *Exogone naidina*, *Capitella capitata* and *Pygospio elegans*. The interstitial polychaetes consisted of 4 species: *Microphthalmus listensis*, *M. aberrans*, *Streptosyllis websteri* and *Protodrilus adherens f. gracilis*. A relation between sediment and diversity was not found. Polychaet associations were studied using Sørensen and Czekanowski indices combined with group average sorting and principal component analysis.

No sediment preference was clearly demonstrated; however, some interstitial polychaetes seem to prefer sandy sediments. As for harpacticoids almost the same main station groups were found. Archipel was characterised by a low diversity, limited seasonal fluctuations and absolute dominance of *Streptosyllis websteri* throughout the year. Mean density was 43 ind. 10 cm^{-2} . High abundance of juveniles were found in Jan.-Febr.-March and Sept.-Oct.

References

Heip, C., R. Herman, G. Bisschop, J.C.R. Govaere, M.D. Holvoet, D. van Damme, C. Vanosmael, K.A. Willems and L.A.P. De Coninck - 1979. Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries.

Volkaert, F. - 1979. Oecologische studie van de meiobenthische polychaetenfauna van de Grevelingen (Nederland). Lic. Thesis R.U.G., 96 pp.

IV. 17. A BOTTOM MAP OF THE SEDIMENTS OF LAKE GREVELINGEN IN 1979 (G1, G5, G6, G7, G8) (J. Nieuwenhuize, J.V. van Liere and A.G. Vlasblom)

By means of 430 sediment samples from the bottom of Lake Grevelingen, taken in April 1979, detailed maps were made, showing the grain-size distribution, silt content, POC, Chlorophyll and CaCO_3 content.

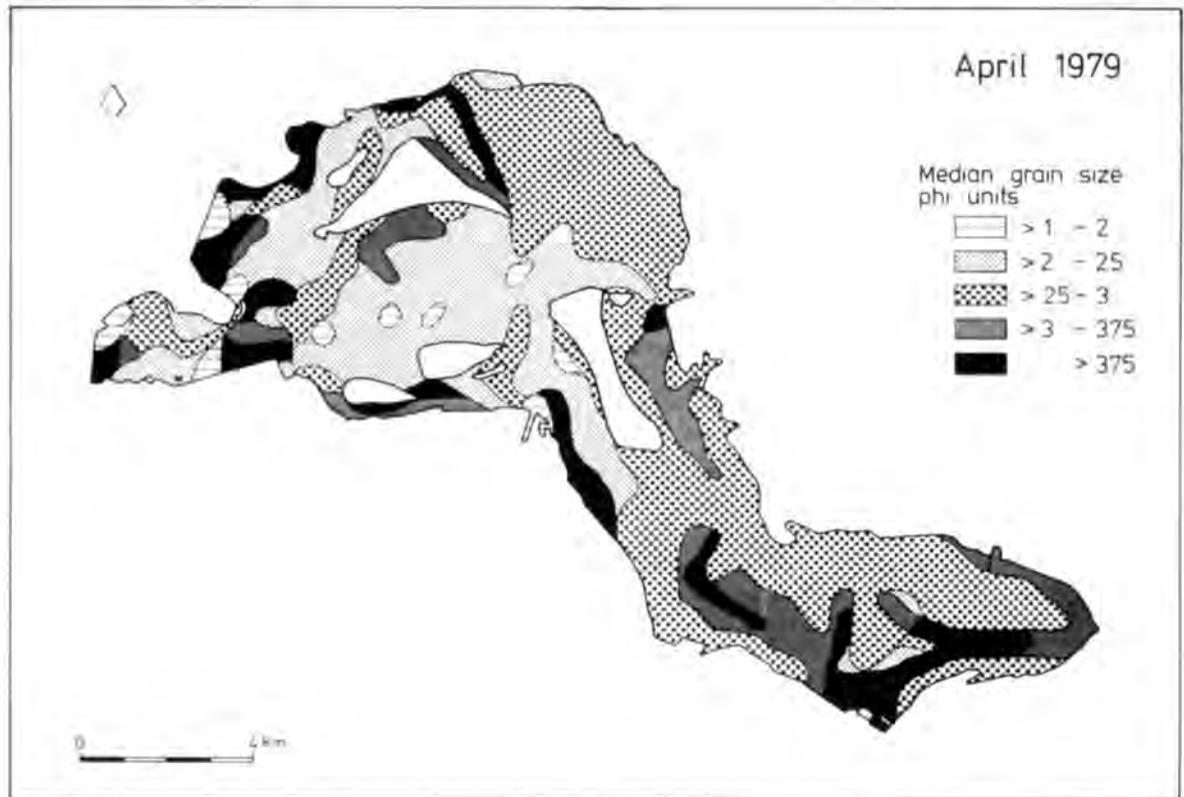
The bottom of Lake Grevelingen is mainly composed of fine-grained sand (Archipel, 175 to 250 μm) and very fine-grained sand (the former mudflats, 125 to 175 μm) and silt (the gullies, smaller than 75 μm) (Fig. 10). The sorting of the sediment varies between very good (smaller than 0.35 phi-units) to less good (0.50 to 2.00 phi units). The highest silt content is found in the gullies, whereas on the Slikken van Flakkee, near Herkingen and on Archipel the silt content amounts to 0-3%. The same is true for POC and CaCO_3 content. Chlorophyll shows an irregular pattern, varying in the bottom of the gullies from 80 to 200 mg m^{-2} and peaks up to 2800 mg m^{-2} . A number of correlations between the parameters were calculated and a comparison was made between five different areas of the lake.

Reference

Nieuwenhuize, J.M. van Liere and A.G. Vlasblom - 1980. Een bodemkaart van het Grevelingenmeer in 1979. DIHO Rapp. en Versl. in press.

IV. 18. CHANGES IN THE FISH FAUNA OF LAKE GREVELINGEN SINCE THE OPENING OF THE SLUICE IN THE BROUWERSDAM IN 1979 (G9) (G. DOORNBOS AND F. TWISK)

Fig. 10. Median grainsize of the sediments in Lake Grevelingen



From December 1978 through February 1980 the sluice in the Brouwersdam has been in near full-time operation. Through this sluice which has an average capacity of $100 \text{ m}^3 \text{ s}^{-1}$, the connection of Lake Grevelingen with the North Sea was reopened (Nienhuis, 1979).

The qualitative changes in the composition of the fish fauna of Lake Grevelingen as a result of the opening of the sluice, have been studied by comparing the catches out of the deeper parts ($> 2 \text{ m}$) of the lake from 1979 with those of previous years.

Fishing has been done with a 3 m beam trawl, equipped with a shrimp net. Although a beam trawl has been developed for the catching of demersal fishes, almost all pelagic species are also caught (Vaas, 1978).

The number of species (26) caught in 1979 has, in comparison to previous years, increased with about 30% (Fig. 11).

The number of individuals of the newly found species in the lake (Table 12) are generally small in autumn. They may have been more common during summer. For example, we found many more small Whiting in the catches in August than later on in the

season. In addition, schools of small Horse Mackerel and Garfish were only seen in August. It is likely that as the water became colder, they pulled out (Wheeler, 1969).

In 1979, for the first time ever since 1972, young individuals ($< 15 \text{ cm}$) of Flounder, Plaice, Dab and Sole were caught. Therefore, they must have immigrated through the sluice. On the other hand, there is some indication that the older individuals of Plaice, ripe of spawn, have been gone since December.

The Sand-smelt, *Atherina mochon*, seems to have disappeared from the lake. However, it is unlikely that the opening of the sluice is the cause, for during the previous years this species was already becoming rare. Its relative, *A. presbyter*, is still very common.

A new species for Lake Grevelingen is the Painted Goby (*Pomatoschistus pictus*). This species has been regularly found in the catches, ever since October. The first records of the Painted Goby from Dutch coastal waters came from the Wadden Sea in 1961 (Fonds, 1964). From the Delta area, there are only a few records, viz. the North Sea, just outside the Haringvliet (28-9-'72), the Eastern Scheldt (30-11-'73) and

Lake Veere (19-12-'74) (pers. comm. P. de Koeyer). As the Painted Goby seems to prefer areas with eelgrass, *Zostera marina*, (Wheeler, 1969) it is possible that in the future, this species will settle more permanently in Lake Grevelingen.

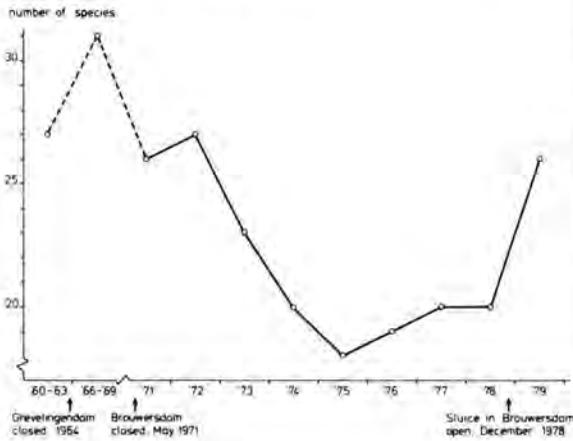
Except those species recorded in Table 12 in 1979 some others were observed during fishing surveys in shallow water (<2 m). These are the following: 1 × Bass (*Dicentrarchus labrax*), 1 × Thick-lipped Mullet (*Mugil labrosus*), 2 × Tadpole-fish (*Raniceps raninus*)

Table 12. Fish species caught with a 3 m beamtrawl in the deeper parts of Lake Grevelingen in the period 1960-1979 (+).

Also indicated are species which have reproduced themselves in the lake (x).

Period	1960		1966									Reprod.
	1963	1969	1971	1972	1973	1974	1975	1976	1977	1978	1979	
Number of fishing surveys	7	10	5	12	13	10	8	8	5	20	6	
Number of hauls	92	102	63	136	142	103	92	98	39	236	79	
Number of species	27	31	26	27	23	20	18	19	20	20	26	
<i>Agonus cataphractus</i> (Hook-nose)	+	+	+	+	+							
<i>Alosa fallax</i> (Twaite Shad)		+										
<i>Ammodytes lanceus</i> (Sand Eel)	+	+										
<i>Anguilla anguilla</i> (Eel)	+	+	+	+	+	+	+	+	+	+	+	
<i>Aphla minuta</i> (Transparent Goby)			+	+		+				+	+	
<i>Atherina mochon</i> (Sand-smelt)					+	+	+	+	+	+	+	x
<i>Atherina presbyter</i> (Sand-smelt)		+	+	+	+	+	+	+	+	+	+	x
<i>Belone belone</i> (Garfish)	+	+							+		+	
<i>Buglossidium luteum</i> (Solenette)		+										
<i>Callionymus lyra</i> (Dragonet)	+	+										
<i>Ciliata mustela</i> (Five-bearded Rockling)	+	+	+	+	+							
<i>Clupea harengus</i> (Herring)	+	+	+	+	+	+	+	+	+	+	+	
<i>Dicentrarchus labrax</i> (Bass)				+								
<i>Engraulis encrasicolus</i> (Anchovy)			+					+				
<i>Entelurus aequoreus</i> (Snake Pipefish)	+											
<i>Gadus merlangus</i> (Whiting)	+	+	+	+	+	+	+		+		+	
<i>Gadus morhua</i> (Cod)	+	+	+	+					+			
<i>Gasterosteus aculeatus</i> (Three-spined Stickleback)		+	+	+	+	+	+	+	+	+	+	x
<i>Gobius niger</i> (Black Goby)								+	+	+	+	x
<i>Limanda limanda</i> (Dab)	+	+	+	+	+	+	+	+	+	+	+	
<i>Liparis liparis</i> (Sea snail)	+	+	+								+	
<i>Microstomus kitt</i> (Lemon Sole)	+	+				+						
<i>Mugil labrosus</i> (Thick-lipped Mullet)			+									
<i>Mugil ramada</i> (Thin-lipped Mullet)											+	
<i>Myoxocephalus scorpius</i> (Bull Rout)	+	+	+	+	+	+	+	+	+	+	+	x
<i>Osmerus eperlanus</i> (Smeit)	+	+	+	+								
<i>Pholis gunnellus</i> (Butter fish)	+	+	+	+	+	+	+				+	x
<i>Platichthys flesus</i> (Flounder)	+	+	+	+	+	+	+	+	+	+	+	
<i>Pleuronectes platessa</i> (Plaice)	+	+	+	+	+	+	+	+	+	+	+	
<i>Pomatoschistus microps</i> (Common Goby)	+	+	+	+	+	+	+	+	+	+	+	x
<i>Pomatoschistus minutus</i> (Sand Goby)	+	+	+	+	+	+	+	+	+	+	+	x
<i>Pomatoschistus pictus</i> (Painted Goby)											+	
<i>Raniceps raninus</i> (Tadpole-fish)							+	+				
<i>Scophthalmus maximus</i> (Turbot)				+	+	+	+	+	+	+	+	
<i>Scophthalmus rhombus</i> (Brill)			+	+	+	+	+	+	+	+	+	
<i>Solea solea</i> (Sole)	+	+	+	+	+					+	+	
<i>Sprattus sprattus</i> (Sprat)	+	+	+	+	+	+	+	+	+	+	+	
<i>Syngnathus acus</i> (Great Pipefish)	+	+	+	+						+	+	
<i>Syngnathus rostellatus</i> (Lesser Pipefish)	+	+	+	+	+	+	+	+		+	+	x
<i>Trachurus trachurus</i> (Horse Mackerel)	+	+		+	+						+	
<i>Trigla lucerna</i> (Tub Gurnard)		+										
<i>Trisopterus luscus</i> (Bib)	+	+	+	+	+		+	+				
<i>Zoarces viviparus</i> (Eelpout)	+	+	+	+	+	+	+	+	+	+	+	x

Fig. 11. Number of fish species caught with a 3 m beamtrawl in the deeper parts of Lake Grevelingen. After data from Vaas (1978)



and 2 × Dragonet (*Callionymus lyra*).

We have found that ten of the most abundant fish species in Lake Grevelingen reproduce in the lake (Table 12).

References

Fonds, M. - 1964. The occurrence of *Gobius pictus* Malm and *Onos septentrionalis* Collett in the Dutch Wadden Sea. *Neth. J. Sea Res.* 2(2), 250-257.

Nienhuis, P.H. - 1979. Working group Carbon cycle in the Grevelingen: Introduction, In: E.K. Duursma (ed.). *Progress Report DIHO, 1978.* Verh. KNAW, Nat. 2e reeks, 73, p. 140.

Vaas, K.F. - 1978. Veranderingen in de visfauna van de Grevelingen tussen de jaren 1960 en 1976. *DIHO Rapp. en Versl.* 1978-4, 26 pp.

Wheeler, A. - 1969. The fishes of the British Isles and North-West Europe. MacMillan and Co. Ltd, London, 613 pp.

IV.19. SIMULTANEOUS PRODUCTION AND MINERALIZATION MEASUREMENTS IN A WATER-COLUMN AND THE UNDERLYING BOTTOM (G3, G5, G10) (A.B.J. Sepers, B.H.H. de Bree and P.R.M. de Visscher).

In the frame of a cooperation project between the Delta Institute for Hydrobiological Research and the Centre de Recherches de Sédimentologie Marine in Perpignan investigations were carried out to determine the production and mineralization processes in the water and bottom of the mesotrophic saline Lake Grevelingen (The Netherlands). Also the relation bet-

ween the pelagic zone and the bottom had been taken into consideration with reference to these processes. For this purpose a number of geochemical parameters was measured, as well as the activity of the micro-phytobenthos, the phytoplankton and the heterotrophic bacterial population. The experiments were carried out in June 1979, September 1979 and January 1980. The last series of experiments will be performed in April 1980. For a comparison of the production and mineralization processes between a mesotrophic and oligotrophic environment it is important to continue the existing cooperation by an analysis of the same processes in the oligotrophic Etang de Bages - Sigean near Perpignan.

To give an example of the experiments some results are presented in Table 13 of the activity measurements in June 1979. The chemical parameters were omitted from these tables but will be included in the final report. The primary production of the phytoplankton and the benthos, and the mineralization rate (oxygen consumption method) were measured on three consecutive days. The activity of the heterotrophic bacterial population was also determined with three different ¹⁴C-labeled substrates (pyruvic acid, glycolic acid and a mixture of amino acids).

A comparison between the mineralization rate (as determined by the oxygen consumption method) and the primary production indicates that at least for G11-channel the mineralization is greater than the primary production. As far as the measurements in June are concerned this is quite surprising for this period of the year.

The data demonstrate also a remarkable difference between the mineralization rate as determined with ¹⁴C-labeled compounds and the mineralization as quantified by the oxygen consumption method.

IV.20. MINERALISATION OF ORGANIC MATTER IN THE WATER COLUMN AND IN THE BOTTOM SEDIMENTS (G10) (J.G.C.M. Goossens and H.J. Lindeboom)

After preliminary measurements by Sepers (1979) a new project started on a larger scale in September 1979. Both in the water column and in the sediments mineralisation of organic matter will be measured frequently by estimating the oxygen consumption rate. Bacterial biomass will be estimated by epifluorescence microscopy. In the water column mineralisation experiments will be restricted to aerobic environmental conditions. In the bottom sediments anaerobic mineralisation must be taken into account. Methane production in piled-up eelgrass litter was demonstrated, which means that anaerobic pathways should not be underestimated. Preliminary methodological investigations were carried out. Diver-operated perspex

Table 13. Primary production and mineralisation in Lake Grevelingen (June 1979, 3 subsequent days of measurements).

sampling site	G 11 - shallow total depth \pm 2 m	G 11 - channel total depth \pm 20 m
primary production	88 mg C m ⁻² day ⁻¹	2537 mg C ⁻² day ⁻¹
phytoplankton	219 "	1540 "
	407 "	1786 "
primary production	365 mg C m ⁻² day ⁻¹	
benthos	235 "	
	265 "	
mineralisation (water)		
activity		
- ¹⁴ C-amino acid mixture	9.1 mg C m ⁻² day	115.3 mg C m ⁻² day ⁻¹
- ¹⁴ C-pyruvic acid	19.9 "	101.1 "
- ¹⁴ C-glycolic acid	6.7 "	62.5 "
-oxygen consumption	0.61 g C m ⁻² day ⁻¹	4.50 g C m ⁻² day ⁻¹
method	0.49 "	3.34 "
	0.32 "	2.84 "

incubators (rigid jars) will be used to measure oxygen consumption rated in the sediments.

Reference

Sepers, A.B.J. - 1979. De aerobe mineralisatie van aminozuren in natuurlijke aquatische milieus. Proefschrift Groningen DIHO, Yerseke, 101 pp.

IV.21. FOOD CHAINS AND PRODUCTION OF ORGANIC MATTER IN THE EASTERN SCHELDT (CODE O)

1. Measurement on organic matter transport in the Eastern Scheldt (Code O)

(J.H.B.W. Elgershuizen and P.B.M. Stortelder (Delta Service of the State Dept. of Waterworks))

The pelagic and benthic biota of the Eastern Scheldt are sustained by a food base of suspended and deposited organic matter. This food base represents the energy input into the ecosystem, including solar energy trapped in organic molecules by in situ photosynthesis, and energy contained in organic matter that is imported into the ecosystem from external sources.

In 1985 the construction of a storm-surge barrier across the mouth of the Eastern Scheldt will be finished. This barrier can be closed at times of extremely

high water in the southern North Sea. At other times, the gates will be open to permit water to flow between the Eastern Scheldt and the North Sea. The area through which water will flow, however, will be small enough to reduce the tide and thus possibly alter the circulation pattern within the Eastern Scheldt. This altered circulation might modify the exchange of organic matter with the North Sea, which might, in turn, affect the estuarine biota that depend on this exchange for food.

Estuarine transport of organic matter is at present poorly understood. Generally accepted principles or procedures (i.e. relating to topography, currents, depth, concentrations of organic and inorganic materials etc.) by which we can estimate the importance of imported organic matter from available data are non-existent. Furthermore, direct complete measurements of organic matter transport during e.g. one tidal cycle in the Eastern Scheldt were not available. Reasons, why on 23th of April 1979 an orientating measurement on the transport of organic matter through a transect in the Eastern Scheldt was done as a joint effort of the Delta Institute for Hydrobiological Research at Yerseke and the Delta Service of the State Department of Waterworks at Middelburg and Zierikzee (Elgershuizen and Stortelder, 1979). In this measurement primary production

data were also collected there was uncertainty of the relative importance of the transport of organic matter through a transect as compared with in situ biological production at the same time. Eleven ships were involved in this measurement of transport of organic matter on the traject Wemeldinge-Tholen during one tidal cycle (16 hours). The current velocities and directions were vertically registered each 15 min at 9 stations; POC-, DOC-, seston-, and chlorophyll concentrations were measured each 3/4 h at 5 stations and plankton-concentration each 3 h at 2 stations. Samples for primary production measurements were collected at 2 stations each 1½ h and brought to the Isotope Lab. at Yerseke. Profiles of daylight transmission through the watercolumn were measured at the same frequency and stations as when and where the primary production samples were taken.

Preliminary interpretation of the collected data gave already some insight in the transport of particulate organic matter through the transect Wemeldinge-Tholen at 20th April 1979. Through the southern part of the transect a nett import and through the Northern part an nett export resulted in an overall nett import into the Eastern part of the Eastern Scheldt. Primary production during the time of the measurement was of the same order as the overall import of particulate organic matter. Plankton studies made already clear the differentiated directional transport of different plankton algae: some are transported into, some are transported out of the eastern part of the Eastern Scheldt, while other ones may have no obvious transport direction.

Furthermore Groen's (1967) model of transport of suspended matter in an estuary was studied with the collected data. Future application of this model needs a statistical development/transformation of the model.

V. Working group: Brackish Waters (Code B)

V.1. INTRODUCTION (A.B.J. Sepers)

In the Delta area of the Netherlands there is a great number of large and small waterbodies with widely divergent characteristics. Beside the great differences in size there are also considerable differences regarding the influence of the bottom and the littoral zone on the metabolism of the aquatic system as a whole. The mean chlorinity varies from near zero to about 18‰ Cl^- ; also the mean nutrient concentrations show striking differences, whereby the variation in the value of the abiotic environmental parameters (stability in the

References

Elgershuizen, J.H.B.W. and P.B.M. Stortelder - 1979. Achtergronden en opzet van een meting aan het transport van seston en organische stof in de Oosterschelde. Delta Inst. Hydrobiol. Ond., Yerseke en RWS Deltadienst, afd. Milieuond., Middelburg. Nota DDMI IMPEX 79-12. 43 pp.

Groen, P. - 1967. On the residual transport of suspended matter by an alternating tidal current. Neth. J. Sea Res., 3, 564-574.

2. *Biological inventories of the Eastern Scheldt proper.* (C. Bakker, R.H. Bogaards, E.A.M.J. Daemen, J.H.B.W. Elgershuizen, P. de Koeyer, P.H. Nienhuis and A.J.J. Sandee)

Biological inventory studies of the Eastern Scheldt are a prerequisite in order to interpret future changes in the environment adequately. A number of these studies was finished in the course of 1979: a. quantitative distribution of sublittoral macrofauna species (see V.2); b. inventory of aquatic plants and animals (Elgershuizen, Bakker and Nienhuis, 1979); c. distribution and biomass of eelgrass species *Zostera marina* and *Z. noltii* (Daemen, 1979). Abstracts of the two last mentioned papers are given in section IX.

References

Daemen, E.A.M.J. - 1979. Verspreiding en biomassa van *Zostera marina* en *Zostera noltii* Hornem. in de Oosterschelde. DIHO-Stud. Versl. D6-1979, 44 pp.

Elgershuizen, J.H.B.W., C. Bakker and P.H. Nienhuis - 1979. Inventarisatie van aquatische planten en dieren in de Oosterschelde. DIHO Rapp. en Versl. 1979-3, 105 pp.

meaning of constancy of the environmental parameters) demonstrates important differences too. 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, originated 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 space and time, 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 inland waters of the Delta region are marked by strong fluctuations in environmental conditions. The development of the community in these waters 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.

The code B is used for the projects. Additional abstracts are presented in the section of published papers.

V.2. A ROUGH INVENTARISATION OF THE SUBLITTORAL MACROFAUNA IN THE EASTERN-SCHELDT DURING THE SUMMER OF 1979 (B 1/A 15) (A.J.J. Sandee, P. de Koeper and R.H. Bogaards)

In the near future hydrobiological changes can be expected in the Eastern-Scheldt due to the construction of the storm surge barrier and other related embankments. Quantitative data describing the macrofauna communities on the slopes of dikes and gullies situated below mean low water level are scarce (Bogaards *et al.*, 1972).

During the summer of 1979 a semiquantitative inventarisation was made of the macrofauna on 17 sampling stations by the diving team of the institute. At each station an inventarisation was made at various depths below the mean low water level. As an example of these inventarisations the occurrence of *Metridium senile* L. is presented in Fig. 12. A total number of 79 species was found whereof 15 species contributed significantly to the differences in the macrofauna communities on the considered sampling locations. The sampling stations were compared by means of a cluster analysis as described by Orloci (1967, 1975). At the 80% level the 17 sampling stations are split up into three groups. One group covers the stations west of

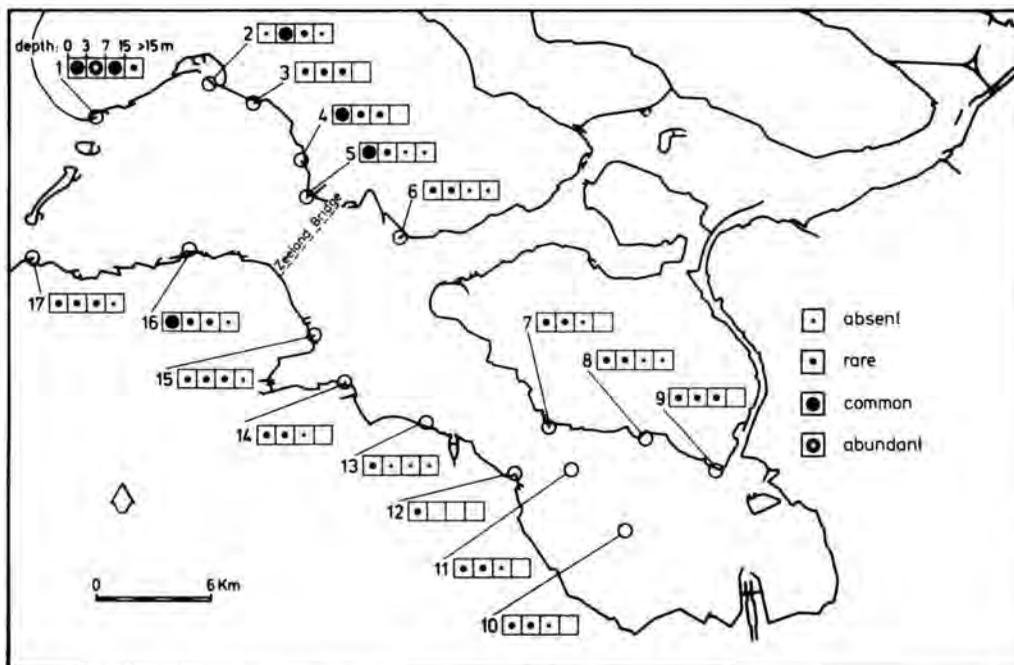
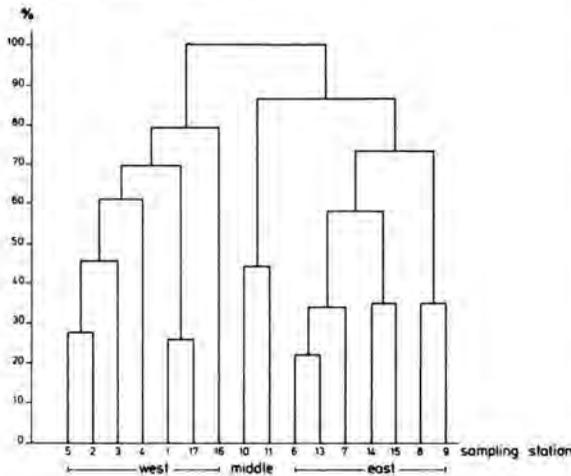


Fig. 12. The distribution of *Metridium senile* L. in the Eastern Scheldt

Fig. 13. Similarity of the sampling stations as determined by a cluster analysis



the Zeeland-bridge, the second group consists of the stations east of the Zeeland-bridge and the third group is made up by the two sampling stations (10 and 11) in the central part of the Eastern-Scheldt (Fig. 13).

References

Bogaards, R.H., de Koeyer, P., Sandee, A.J.J., Borghouts, C.H. and Wolff, W.J. - 1972. Inventarisatie van enkele macrobenthossoorten in het Oosterscheldegebied. DIHO Rapp. en Versl. 1973-7, 22 pp.

Orloci, L. - 1967. An agglomerative method for classification of plant communities. *J. Ecol.* 55, 193-206.

Orloci, L. - 1975. *Multivariate analysis in vegetation research.* Junk, Den Haag.

V.3. IMPACTS OF SALINE SEEPAGE AND PHOTOSYNTHESIS ON THE OXYGEN DYNAMICS OF A BRACKISH DITCH (B1) (J.W. Rijstenbil, A.G.A. Merks and C.L. van Duijl)

In a main ditch on Noord-Beveland vertical chlorinity gradients are present, which enhance vertical oxygen gradients (Parma and Krebs, 1977; Rijstenbil, 1978). This ditch (120 cm deep and 12 m wide) is connected with Lake Veere by an intermittent functioning pumping-station. During summer the water level of Lake Veere is higher than the water level in the ditch. The resultant saline seepage appeared to build up the chlorinity gradients, especially in summer. A seepage of 6-10 mm hr⁻¹ caused a permanent anoxic layer above the bottom (Fig. 14). At the aerobic-anaerobic interface 1.2-2.0 g O₂ m⁻² hr⁻¹ was consumed for the oxidation of reducing matter in the seepage water.

This seepage oxygen demand should be compensated by the photoautotrophic system (Fig. 15). During summer dense populations of light-sensitive flagellates show a vertical motion (Wall and Briand, 1979), which enables them to remain at their optimal light conditions. Together with centric diatoms these flagellates produced up to 34 mg O₂ l⁻¹ in the surface water. In this hypertrophic ecosystem light is the limiting growth factor. The oxygen supersaturation during the day disappeared overnight due to a large oxygen demand (Uhlmann, 1966); surface oxygen values decreased to about 10 mg O₂ l⁻¹ in the early morning as a result of algal respiration, heterotrophic consumption and seepage oxygen demand.

During winter the water level of Lake Veere is lower than the water level in the ditch, except for short periods of polder water discharge. During these periods a weak chlorinity gradient was restored by a seepage which was always lower than 4.4 mm hr⁻¹. When the levels of both the ditch and Lake Veere were equal, the gradient was maintained by the diffusion of chloride from the bottom. At very low temperatures the oxygen in the water column was supplied only by benthic diatoms (Fig. 16). These diatoms produced a bottom oxygen maximum in the afternoon and a bottom oxygen minimum just before the daybreak.

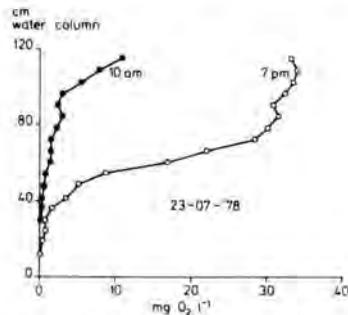


Fig. 14. Diurnal maximum and minimum of oxygen in summer in the Adriaan Polder ditch (Noord-Beveland)

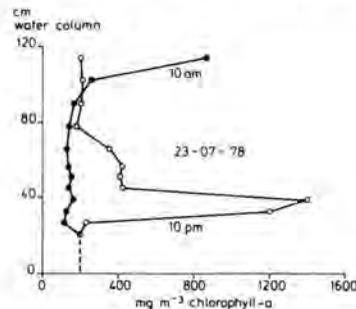
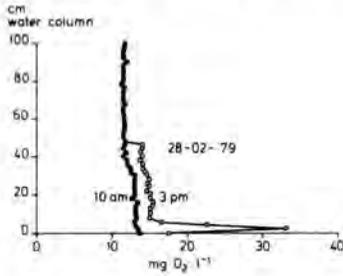


Fig. 15. Vertical migration of flagellates along the water column of the Adriaan Polder ditch (Noord-Beveland)

Fig. 16. Oxygen changes during a 5 hour period under an ice cover illustrating benthic photosynthesis (Adriaan Polder ditch, Noord-Beveland)



References

Parma, S. and Krebs, B.P.M. - 1977. The distribution of Chironomid larvae in relation to chloride concentration in a brackish water region of the Netherlands. *Hydrobiologia* 52, 117-126.

Rijstenbil, J.W. - 1978. De zuurstofhuishouding van brakke sloten. *DIHO Rapp. en Versl.* 1978-2, 67 pp.

Uhlmann, D. - 1966. Produktion und Atmung im hypertrophen Teich. *Verh. Internat. Verein. Limnol.* 16, 934-941.

Wall, D. and Briand, F. - 1979. Response of lake phytoplankton communities to in situ manipulations of light intensity and colour. *J. Plankton Res.* 1, 103-112.

V.4. SHORT NOTE ABOUT THE DISTRIBUTION AND ECOLOGY OF *Sigara selecta* IN THE DELTA-REGION OF THE NETHERLANDS (B1) (B.P.M. Krebs)

A study was made of the distribution and ecology of *Sigara selecta* in brackish waters in the Delta area of The Netherlands. *S. selecta* was found, usually accompanied by *S. stagnalis*, in about 20 for the greater part polyhaline, inland waters (Fig. 17). In waters with only a poor vegetation *S. stagnalis* dominated *S. selecta*; in waters with a dense vegetation *S. selecta* was the dominant species. This finding is in accordance with the data of Howes (1939), describing the occurrence of only *S. selecta* in a brackish creek with a dense vegetation. The conclusion may be that in mesohaline and polyhaline waters an increase in the density of the vegetation is accompanied by a replacement of *S. stagnalis* by *S. selecta*. It is remarkable that these species could not be found in the large stagnant polyhaline lakes, like Lake Grevelingen and Lake Veere, even when there is a dense vegetation of *Zostera spec.* About the occurrence of *S. selecta* in saltmarshes a sampling program is in preparation.

References

Howes, H.N. - 1939. The ecology of a saline lagoon in south-east Essex. *J. Linn. Soc. Zool.* 40, 383-445.

V.5. OLIGOCHAETA IN BRACKISH INLAND WATERS (B3) (P.F.M. Verdonshot)

At research dealing with the structure and function of ecosystems it is necessary to gather information about the various elements (species) within this system. Information about the distribution and ecology of oligochaetes is very scanty and especially in The Netherlands little is known about the ecological importance of these worms. Oligochaetes were often omitted from macrofauna research because of taxonomical difficulties. An introductory investigation was started to obtain some data about the distribution and ecology of oligochaetes in instable, brackish waters. Other macrofauna species, which could be important in relation to the community structure, were also considered. It was not the intention to get a 'complete' list of species. In 37 inland waters the bottom was quantitatively sampled with a corer with a diameter of 6.5 cm; the aquatic vegetation consisting of higher plants and algae on branches and stones, was sampled qualitatively. Moreover, a number of chemical parameters was determined in the bottom and the

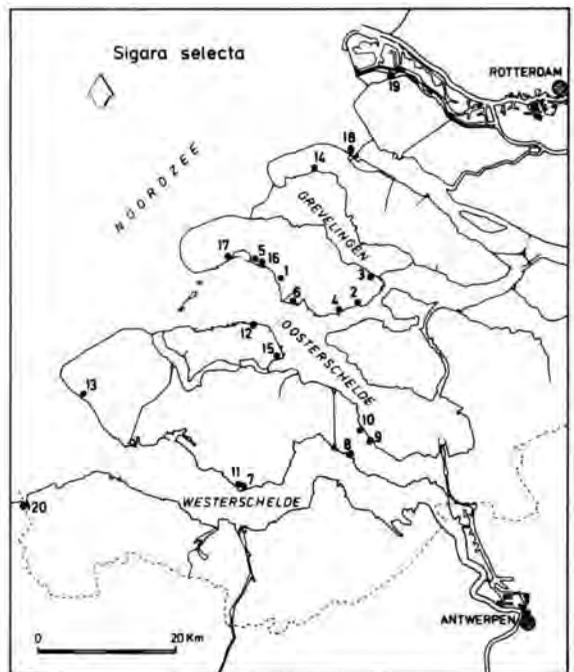
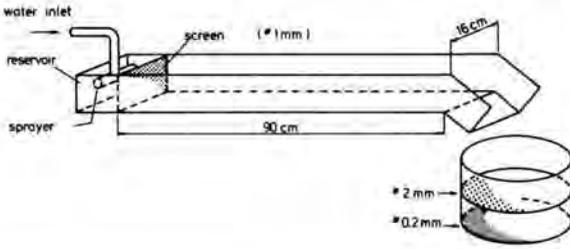


Fig. 17. Distribution of *Sigara selecta* in the Delta area of The Netherlands

Fig. 18. Gutter for the separation of oligochaete worms



water. The sampled inland waters are limnetic or brackish, eutrophic or hypertrophic, calciferous, alkaline and to a varying extent enriched with organic matter. There were considerable differences in the organic matter content of the bottom and the grainsize distribution.

The samples were sorted out in the laboratory using a large gutter (Fig. 18). For the identification the worms were cleared up with a mixture of benzylbenzoate and 2-propanol. The distribution of the animal species was related to the environmental parameters applying a clusteranalysis as described by Orloci (1967). The distribution of the oligochaetes appeared to be correlated with the salinity; the other environmental parameters did not influence significantly the distribution pattern of the collected oligochaetes (Table 14).

Not only the oligochaetes, but all the macrofaunal species from these instable brackish waters appeared to have a large abiotic tolerance. Nevertheless they do not occur in stable environments. The organisms which are characteristic for brackish, instable waters, may be characterized by a r-life strategy (MacArthur and Wilson, 1967), indicating a high resistance against rapid changes in the abiotic environmental parameters, a high reproduction capacity and a high mortality. These species characterize the pioneer stage of an ecosystem. It is possible that these species, which are specialized to inhabit these instable environments, have a specialized genetic structure in order to obtain a high abiotic potential and as a consequence of the limitations of the genetic scheme a low biotic potential. From the cluster analysis some trophic relations between the common macrofaunal species can be derived (Fig. 19). The vertical distribution of oligochaetes is correlated with the redox-potential (Fig. 20). The Naididae are restricted to the top 2 cm of the sediment. The Tubificidae require less oxygen and are more or less independent of the oxygen content of the bottom. The Enchytraeidae live mainly interstitially. About 90% of the total population of oligochaetes was found in the top 2 cm of the bottom.

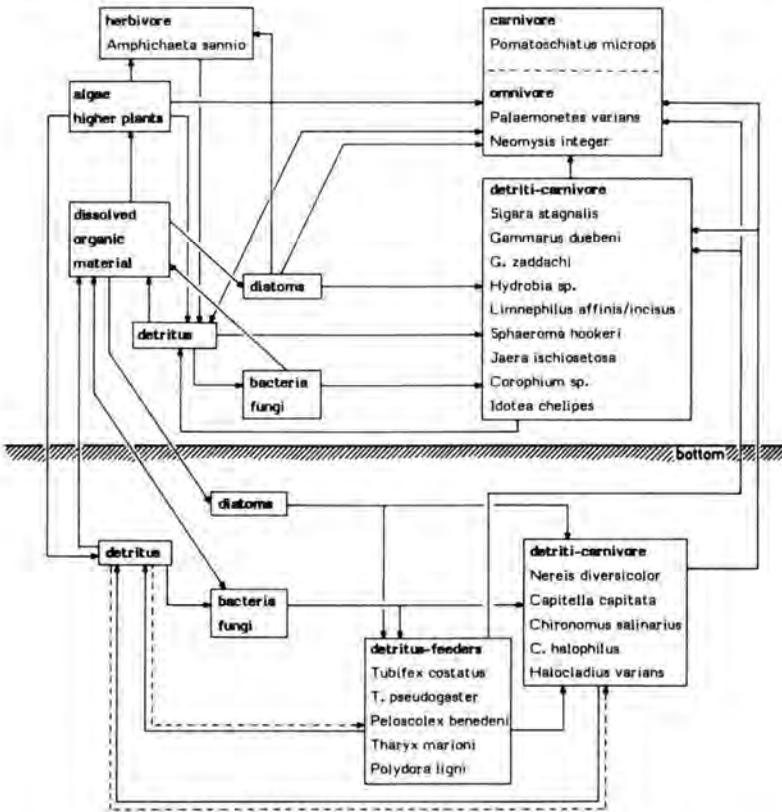
References

MacArthur, R.H. and Wilson, E.O. - 1967. The theory of island biogeography. Monographs in

Table 14. Distribution of some Oligochaeta in relation to fluctuations in chlorinity and median chlorinity.

Species	Chloride ‰															to
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
<i>Limnodrilus claparedianus</i>	—															
<i>L. hoffmeisteri</i>	—															
<i>L. profundicola</i>	—															
<i>L. udekemianus</i>	—															
<i>Peloscolex benedeni</i>	—															25
<i>Potamothenix bavaricus</i>	—															
<i>P. hammoniensis</i>	—															
<i>Tubifex costatus</i>	—															39
<i>T. tubifex</i>	—															
<i>T. pseudogaster</i>	—															25
<i>Amphichaeta sarusio</i>	—															19,5
<i>Chaetosaster diaphanus</i>	—															
<i>C. diastrophus</i>	—															
<i>Nais barbata</i>	—															
<i>N. communis</i>	—															
<i>N. ellingsi</i>	—															19,5
<i>N. variabilis</i>	—															
<i>N. sp.</i>	—															
<i>Paranais littoralis</i>	—															39
<i>Pristina bilobata</i>	—															
<i>Stylaria lacustris</i>	—															
<i>Uncinaxis uncinata</i>	—															
<i>Eiseniella tetraedra</i>	—															
<i>Lumbriculus variegatus</i>	—															

Fig. 19. Some trophic relations between the community components in a brackish water biotope



Population Biology, Vol. 1. Princeton University Press, Princeton.

Orloci, L. - 1967. An agglomerative method for the classification of plant communities. J. Ecol. 55, 193-206.

V. 6. OLIGOCHAETA FROM ESTUARINE ENVIRONMENTS (B3) (P.F.M. Verdonshot)

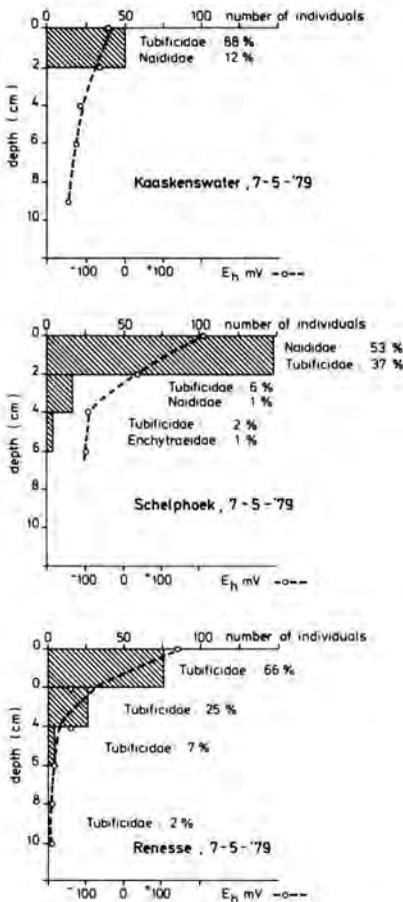
Wolff (1973) presented a nearly complete survey of the soft-bottom macrofauna in the estuaries of the Delta area. Due to taxonomic difficulties the oligochaetes were omitted from this survey. However, preserved samples were stored in the laboratory. These samples, which were collected from 1959 to 1970, were used now for a description of the distribution of oligochaetes in the Delta area.

The majority of the oligochaete species appeared to be restricted to the fresh or slightly brackish regions of the estuary. In the brackish and marine parts the next species were found: *Tubifex costatus*, *Tubifex pseudogaster*, *Peloscolex benedeni* and *Monopylephorus rubroniveus*. *P. benedeni* appeared to be eury-mesohaline with a preference for clay bottoms. This

preference is most probably related with the tolerance of this species for the low oxygen concentrations and the presence of hydrogen-sulphide in this bottom-type.

T. costatus is an oligohaline-marine species and is restricted to the littoral zone of the estuary. The other identified species were euryhaline-limnetic organisms. The distribution of these species was positively correlated with the organic matter content of the bottom, which might be explained by the utilization of microorganisms as their food. A positive correlation between the grainsize distribution and the occurrence of oligochaete worms was found for *Limnodrilus hoffmeisteri* (sandy clay) and *L. udeke-mianus*. After the completion of the Haringvliet sluices (1970), by which the Haringvliet was cut off from the influences of the North Sea, an investigation was carried out from 1971 to 1977 to study the development of the macrofaunal community in the fresh Hollands Diep – Haringvliet basin. After the closure there was a mass development of Tubificidae, which was related to the high organic matter content of the bottom and the high colonization and reproduction capacity of these worms. After two years the total amount of worms decreased and stabilized at a lower

Fig. 20. Vertical distribution of Tubificidae, Naididae en Enchytraeidae in relation to the redox-potential



level, which was however still higher than before the closure of the Haringvliet. During the research period only small changes in the species composition of the oligochaete fauna could be detected. *Tubifex tubifex* disappeared slowly due to the decrease of the oxygen concentration in the bottom. The species distribution was irregular and habitat preferences of single species could not be demonstrated. A positive correlation was found between the organic matter content of the bottom and the occurrence of oligochaetes. For the other environmental parameters it was impossible to demonstrate a correlation between the value of these parameters and the observed species distribution. It appeared that several species of tubificid worms coexisted for several years in the same habitat. The relative abundance of the species at a particular sampling station was remarkably constant, whilst between the sampling stations differences in the species distribution were found (Fig. 21). The indicated constancy in distribution might be

explained by the microbiological nature of the food resources of these worms. Between the tubificid worms there exist differences in food requirements, resulting in a niche delimitation, which is determined by the species-specific food preferences.

References

Wolff, W.J. - 1973. The estuary as a habitat. An analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse and Scheldt. Zool. Verh. Leiden 126, 3-242.

V.7. CHIRONOMIDS IN THE INLAND WATERS OF THE DELTA REGION (B3) (B.P.M. Krebs)

In the Delta region a variety of waterbodies can be found, ranging in salinity from limnetic to polyhaline. Therefore this area is a very interesting research object for zoological studies. Chironomids are very common in these inland waters. During several years a study was made of the distribution of these midges. Initially a selected series of mesohaline and polyhaline waters, like ditches and creeks was sampled. Thereupon also oligohaline and limnetic waters were studied. The chironomid larvae were sampled and grown in the laboratory at 15-20°C under a constant photoperiod of 14 hours (fluorescent light). The cultures were

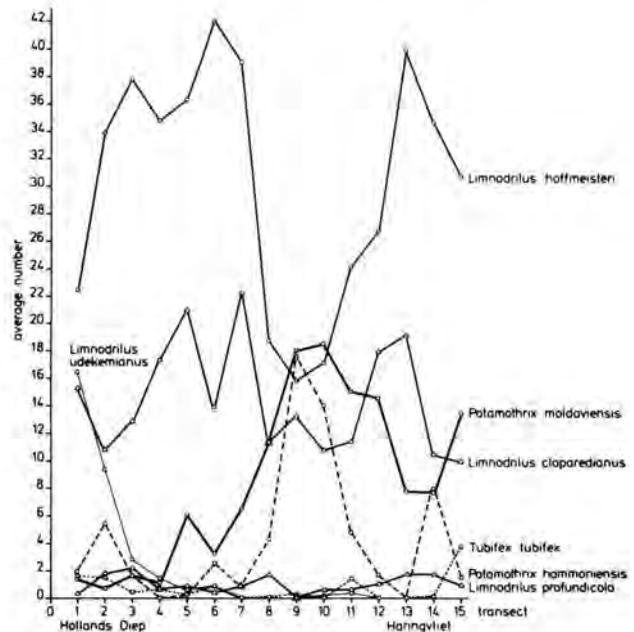


Fig. 21. Average numbers of oligochaete worms per transect per year for the sampling stations in the Hollands Diep - Haringvliet basin

aerated and the larvae fed on spinach. Regarding the difficulties of a species identification at the larval stage, adult midges were used for this purpose. A disadvantage of this procedure is that during the cultivation a selection between the larvae can occur, resulting in an incomplete species survey.

Sixty chironomid species were found, whereof the majority was found in limnetic waters. The characteristic, most abundant species were: *Chironomus annularius*, *Ch. plumosus*, *Ch. piger*, *Ch. luridus*, *Camptochironomus pallidivittatus*, *Glyptotendipes barbipes*, *Gl. pallens*, *Cryptochironomus supplicans*, *Parachironomus arcuatus*, *Polypedilum nebeculosum*, *Psectrotanyptus varius*, *Procladius* spec., *Tanyptus punctipennis*, *Xenopelopia nigricans*, *Cricotopus intersectus*, *Cr. sylvestris*, *Acricotopus lucens*, *Corynoneura* spec. and *Metricnemus hirticollis*.

At increasing salinities in the oligohalinicum a number of limnetic species disappeared, whereas some other limnetic species, like *Ch. annularius*, *Ch. piger*, *Glyptotendipes barbipes*, *Cricotopus sylvestris* and *Metricnemus hirticollis*, can maintain themselves in these waters. Further a number of species was found with a preference for (slightly) brackish waters, like *Microchironomus deribae*, *Dicretendipes pallidicornis* and *Cricotopus ornatus*. *Cr. ornatus* was also found in

fresh waters, indicating that this species is limnetic-tolerant. Another group in the oligohaline waters consists of species with their optimum in mesohaline and polyhaline waters, like *Ch. halophilus* and *Ch. salinarius*.

In the mesohalinicum some species disappeared, like *Cr. sylvestris*, whilst *Ch. halophilus* and *Ch. salinarius* are dominant in the chironomid population as a whole.

In the polyhalinicum *Ch. salinarius* meets its optimum, accompanied by *Halocladus varians*. *Ch. halophilus* disappeared already in the more saline part of the polyhalinicum.

It is conspicuous that in laboratory experiments *Ch. halophilus* and *Ch. salinarius* are both tolerant for oligo-, meso- and polyhaline waters. In the natural environment however, *Ch. halophilus* seems to be most appropriately adapted to oligohaline and β -mesohaline waters and is able to exclude *Ch. salinarius* from these environments. In the mesohalinicum and polyhalinicum the reversed situation is found. Similarly *Ch. annularius* can replace *Ch. halophilus* in slightly oligohaline waters. Research into the genetic potentialities of *Ch. halophilus* and *Ch. salinarius*, regarding the adaptation to salinity and to changes in salinity, is in preparation.

Table 15. Uptake of several organic compounds by HIS 53 in relation to growth rate.

	max. uptake ($\mu\text{mole l}^{-1}\text{hr}^{-1}$)	rate V_{max}^+
specific growth rate (hr^{-1})	0.003	0.01
aspartate	18.7	62.0
aspartate uptake rate in the culture	4.0	12.0
alanine	14.0	13.9
serine	23.3	28.3
glutamate	12.8	42.8
asparagine	24.6	62.9
proline	2.9	4.0
histidine	5.6	3.3
arginine	2.4	2.2

⁺ The presented V_{max} values indicate the maximum uptake rate of the substrate utilized for respiration purposes only. The oxygen consumption rate was converted to an uptake rate for the substrate in question assuming a full respiration of the substrate.

V.8. UPTAKE CAPACITY OF HETEROTROPHIC BACTERIA FOR ORGANIC COMPOUNDS AT LOW SPECIFIC GROWTH RATES (B9) (A.B.J. Sepers and F.W. Melissen)

In natural aquatic environments the specific growth rates of micro-organisms are very low, as is the case with the concentrations of the organic compounds permitting heterotrophic growth. In continuation of our studies as described in Sepers and Cappendijk (1979) the uptake capacity for a range of organic compounds was determined using the bacterial strain HIS 53. HIS 53 was grown in a chemostat with aspartic acid as the growth limiting substrate at specific growth rates of 0.003 and 0.01 hr⁻¹. The maximum specific growth rate of HIS 53 at aspartate limited growth is 0.32 hr⁻¹ and the growth constant K_s is lower than 50 μM aspartate. Samples taken from the chemostat were used to determine the uptake kinetics for a variety of organic compounds. The uptake kinetics were determined by determination of the oxygen consumption rate. The oxygen consumption rate measured at saturating substrate concentrations is proportional to the concentration of the uptake systems for the considered substrate.

Some indicative results are presented in Table 15. An increase of the growth rate is accompanied by an increase of the maximum uptake rate as far as the growth limiting substrate (aspartate) is concerned. This increase is explained by the adjustment of the

physiology of the organism to the increase of the growth rate at cultivation in an aspartate-limited chemostat. The maximum uptake rates of glutamate and asparagine were greater at the higher growth rate and varied nearly proportional with the V_{max}-values determined for aspartate. These findings may be explained by a common transport system for these substrates and the direct involvement of the substrate in the citric acid cycle.

For the other substrates a clear relation between the maximum uptake rate and the growth rate could not be demonstrated. The results differ from the data of Matin *et al.* (1976) describing for a fresh water *Spirillum spec.* and *Pseudomonas spec.* cultured at a variety of growth limitations, an increase of the concentration of a number of enzyme systems at a decrease of the specific growth rate.

References

- Matin, A., Grootjans, A. and Hogenhuis, H. - 1976. Influence of dilution rate on enzymes of intermediary metabolism in two fresh water bacteria grown in continuous culture. *J. Gen. Microbiol.* 94, 323-332.
- Sepers, A.B.J. and Cappendijk, M.P.G. - 1979. Uptake capacity of heterotrophic bacteria for organic compounds at low specific growth rates. In: E.K. Duursma (ed.) Progress Report DIHO, 1978, Verh. KNAW, Nat. 2e reeks 73, 67-69.

VI. Working group: Salt-marsh Ecosystems (Code S)

VI.1. INTRODUCTION (W.G. Beefink)

The objective of this working group is to investigate the structure and functioning of salt-marsh ecosystems. In whole Europe, but also outside it, many parts of the coastal zone undergo changes by technical and industrial activities and by recreation. Consequently, many coastal ecosystems, including salt marshes, are under stress of degradation, or even deterioration.

The problem is to which extent such systems can exert resistance and resilience against anthropogenically added dynamics in water, nutrient relations and pollution, and in which manner such stresses may lead to the development of other biosystems. The working group aims at gathering more information on these questions.

The Delta Works are very useful for research on the influence of such forms of stress. They cause large-scale changes in estuarine conditions towards non-tidal

and non-saline environments. Studies on salt-marsh ecosystems during these works, together with those in estuarine and coastal water-bodies, are of outstanding interest for getting more insight in the ecological side-effects of coastal engineering.

The group is working on the community and population levels. To these lines of research a third one may perhaps be added, viz. production, decomposition and accumulation of materials; cycles of nutrients and toxic materials.

The plant-population associations are considered to rank a central place for understanding phenomena of order and disturbance in ecosystem processes as well as in their arrangements. Plant and animal populations are selected as key or indicator species, or species groups.

The working group continues to encourage external co-operation with university departments and institutes, national and abroad, to arrange research projects of mutual interest. One new joint project was arranged this year. From 1980 onwards the group will participate in an ecophysiological and population-biological study of plants growing on the strand line, which has been started by the Ecology Department of

the Free University at Amsterdam (Prof. Dr. W.H.O. Ernst, Dr. J. Rozema). In the same year also a contract research for three years, awarded by the Delta Service of the State Authority for Roads and Waterways, will start. In this mainly experimental study the influence of diminished tidal difference and of stagnant water on the growth and development of salt-marsh plants will be examined. The running projects, viz. numerical analysis of large ecological data sets, halophyte-soil fungi relationships, and heavy-metal distribution in salt-marsh soils and plants were continued and have already partly resulted joint publications.

The activities of the group are rearranged in five research projects, covering the nine projects of former years. In the following lines the 1979 results are reported, framed in these new projects which have been numbered with the code S, and stressed with the abstracts of published articles and reports. For information on the localities mentioned in this section the reader is referred to the map in Fig. 1A.

The field work on the community level has been done by M.C. Daane, B.P. Koutstaal, W. de Munck and W.G. Beeftink, assisted by J. Beijersbergen en G.J.C. Buth, and the students W. Luytjes and P.J. Waeijen. On the population level field work as well as experimental work under lab conditions has been executed by A.H.L. Huiskes, B.P. Koutstaal, M.M. Markusse and J. van Soelen. They were assisted by the students D.J. van der Haar, M. Ham, E. Miedema and M. Westveer. A.M. Groenendijk deputized for A.W. Stienstra, and studied production and decomposition processes in the marsh with the assistance of M. Lievaart. A.G. Vlasblom and J.J. Guerand advised in statistics and executed some calculations. J. Nieuwenhuize was involved in the preparation of programmes for the analysis of soil samples and plant materials which was performed by M. van Esbroek, J.M. van Liere and C. Vos.

VI.2. COMMUNITIES (S1) (W.G. Beeftink)

The methods used for studying vegetation dynamics in salt marshes, as a consequence of changing environmental factors, are hitherto (1) repeated vegetation mapping of large areas, and (2) repeated analysing of the vegetation in permanent plots selected on credential grounds. The data thus obtained can serve to construct dynamic vegetation models which, in their turn, may serve for developing hypotheses to be tested experimentally. B.P. Koutstaal, W. de Munck and W.G. Beeftink continued their examinations on the about 500 permanent sample plots. About half of the 100 permanent plots in the Haringvliet-Spui estuary which were analysed for the last time in 1975, were reexamined. In some plots halophytes are still present, viz. *Glaux maritima* and *Triglochin maritima*.

Besides these methods a new one has been applied, viz. an integrated grid system of plots with different dimensions. This system has been laid in the nature reserve De Middelpaten of Lake Veere and was analysed through three years by C. van Schaik (State University, Utrecht). With these data he carried out pattern detection analyses according to the BIOPAT program system, under the supervision of Dr. P. Hogeweg. The results showed that the dimensions of the plots used for data collecting determine whether convergence or divergence phenomena turn up. The evidence of these phenomena is thus scale-dependent. He also found that spatial differences in the floristic composition appear to be more correlated with the winter values of carbonate and nutrient contents in the soil than with the summer ones.

P. Bruinsma (State University, Utrecht) is processing the data of the repeated vegetation mapping in the Springersgors near Ouddorp, as well as those of the grid-system established in a non-tidal salt marsh near Kattendijke. The analyses are carried out with the BIOPAT program under supervision of Dr. P. Hogeweg.

As a consequence of the construction of a storm-surge barrier in 1985 in the mouth of the Eastern Scheldt the tidal difference in this water-body will decrease to about $\frac{3}{4}$ of the present one, and seepage of seawater underneath the dikes into the polder will diminish. Serving as a reference for a research on this effect upon the vegetation W. Luytjes and P.J. Waeijen (State University, Utrecht) started a study on the present ecology of the non-tidal salt marshes behind the dikes bordering the Eastern Scheldt. The study is sponsored by the Delta Service of the State Authority for Roads and Waterways, and Dr. P. Hogeweg supervises the processing of vegetational and environmental data.

The research program, carried out by M.C. Daane and W. de Munck, on the influence of cultural practices of man on the vegetation of the salt marsh and on that of former tidal flats is continued. In the latter, established 1973-74 in the nature reserve De Middelpaten, dominant *Calamagrostis epigejos* has nearly totally disappeared after N-fertilization, introducing turbulent changes in floristic composition. Grazing with sheep and cows did suppress this species as well, but to a lesser extent. Phosphor fertilization resulted in only slight changes in the vegetation which recovered or showed an increase in *Trifolium repens*. On untreated but grazed plots *Trifolium* increased to, as well as *Holcus lanatus*, at the cost of *Calamagrostis*.

J. Beijersbergen, appointed under the Job Creation Scheme of the Department of Social Services, determined over 300 bryophyte samples of the sample plots in the nature reserve De Middelpaten, collected in the period 1962-1978. He found 39 species under which

some rare ones. G.J.C. Buth, appointed under the same conditions, is making an inventory of insects and arachnids living in the marsh. His activities are preliminary reported below.

1. Insect and arachnid fauna of salt marshes (S1) (G.J. Buth)

Insects can have a significant function in the salt-marsh ecosystem (Foster and Treherne, 1976). They also form an important object for ecological analyses (Heydemann, 1962). Therefore, in May an investigation of the insect and arachnid fauna of tidal marshes in the S.W.-Netherlands has been started.

Special attention is given to those species that require the salt-marsh environment for one or more stages of their life cycle. Among them the herbivorous animals are of special interest. Because of time limits, less attention could be given to the animals living under ground and to the animals living in some special subhabitats, such as salt-pans and plant debris washed ashore.

Investigations were carried out in several tidal marshes, but most frequently in the salt marsh near Oosterland. Several methods for trapping this group of fauna have been tried out and their trapping capacities were compared. For advices and identification of the systematic groups contact has been made with several amateur and professional entomologists. Literature and insect-collections were studied for previous faunistic and autoecological data.

A start with a habitat collection of insects and arachnids has been made. Tables with seasonal periodicity were made for the halophilic species of some insect orders. From the more than 3800 Coleoptera species in the Netherlands (Brakman, 1966), 84 can be considered halophilic. The Staphylinidae (mostly detritus eaters) with 31 species, and the Carabidae (mostly carnivores), with 21 species, are the largest families. Thirteen Coleoptera species, most Curculionidae, and at least 22 Lepidoptera species, have a salt-marsh plant as host.

References

- Brakman, P.J. - 1966. Lijst van Coleoptera uit Nederland en het omliggende gebied. Monograph. Ned. Entom. Ver. 2, 1-219.
- Foster, W.A. and Treherne, J.E. - 1976. Insects of marine salt marshes: problems and adaptations. In: L. Cheng (ed.), Marine insects. North-Holland, Amsterdam, p. 5-42.
- Heydemann, B. - 1962. Die biozönotische Entwicklung vom Vorland zum Koog. Akad. Wissensch. Liter. Mainz. Abh. math.-naturwiss. Kl. Nr. 11, 768-964.

VI.3. POPULATIONS (S2) (A.H.L. Huiskes and W.G. Beeftink)

On the population level the working group is beginning to round off its studies on the population dynamics in *Salicornia europaea* agg. A field experiment on germination of *S. brachystachya* and *S. stricta* carried out by B.P. Koutstaal resulted in higher germination rates of the first species. In both seed populations germination elapsed in lower salt-marsh zones more slowly than in higher ones.

In autumn B.P. Koutstaal started three field studies, viz. 1) on the time which passes before *Salicornia* seeds are free from the plants, 2) on the transport of these seeds over the marsh, and 3) on the germination behaviour of these seeds during winter time. Running water promotes strongly the release of seed. In all three processes there appeared to be an evident gradient with the height of the marsh.

A.H.L. Huiskes and M.M. Markusse continued lab experiments on the germination behaviour of the two *Salicornia* species. They examined the influence of different soil-moisture contents as well as of different fluctuations in environmental salinity. B.P. Koutstaal studied the effect of seed dimorphism in *Salicornia stricta* on germination, growth and development of the plants. Of both investigations some preliminary results are reported below.

J. van Soelen set up an experiment with the tide-simulator developed by him and F.W. Melissen (see Progress Report 1978, p. 185). Plants of *Salicornia stricta* were submitted to different tidal inundation periods, as long as and even more than the outermost pioneer plants in the marsh undergo. Plant growth was reduced considerably when submitted to longer inundations causing lower light conditions at the same time.

W. de Munck continued his studies on plant-animal relationships in *Salicornia* on the Bergen op Zoom salt marsh, in relation to seed production. Of these examinations some results are given below.

For an ecological interpretation of the dynamics of vegetation and plant populations A.H.L. Huiskes, F.W. Melissen and J. van Soelen developed and elaborated a program for continuous and integrated measurements of the salt-marsh environment. A demontable platform is placed in the marsh, and provided with recording instruments. Electric supply is realized by batteries. For more details and some preliminary results is referred to the report below. In 1980 a series of 13, 25 or more hours-measurements will be started with intervals of 2-4 weeks combined with demographic studies in *Salicornia stricta*.

Together with the rounding-off of its studies on *Salicornia* the working group started with examinations in the population ecology of other salt-marsh

species. In the following years the annuals *Atriplex hastata* and *A. littoralis* as well as the short-lived perennial *Aster tripolium* will be the main study-objects. Besides these species some observations will be made on *Halimione portulacoides*.

In 1979 A.H.L. Huiskes studied the fate of *Halimione portulacoides* seedlings growing on plots where this shrub died on account of heavy winter frost in the preceding winter. He gathered some data on the death risk of these seedlings, and its causes, and will follow this new subpopulation in the coming years (see preliminary report below).

M. de Jong (Agricultural University, Wageningen) examined the state of growth and development of *Limonium vulgare* populations growing in 7 tidal and 3 non-tidal salt marshes. He concluded that the species grows optimally under conditions of a relatively high accretion with silt. Large and thick stem internodes which especially develop under these conditions, promote the formation of leaves and inflorescences.

The start of a population study on *Aster tripolium* in various habitats over several years has been given by the work of M. Ham and E. Miedema (Agricultural University, Wageningen). These students examined the population dynamics of *Aster* in a salt and a brackish marsh along the Western Scheldt. For preliminary results see below.

The study on *Atriplex* populations has been started with a greenhouse experiment carried out by D.J. van der Haar (State University, Utrecht). The aim of this experiment was to separate the effects of soil salinity and nitrogen supply on the growth and development of both species. Preliminary results are reported below. Next year, demographic examinations on these species will start, in combination with similar and ecophysiological ones carried out by Dr. J. Rozema (Free University, Amsterdam) who co-operates in this project.

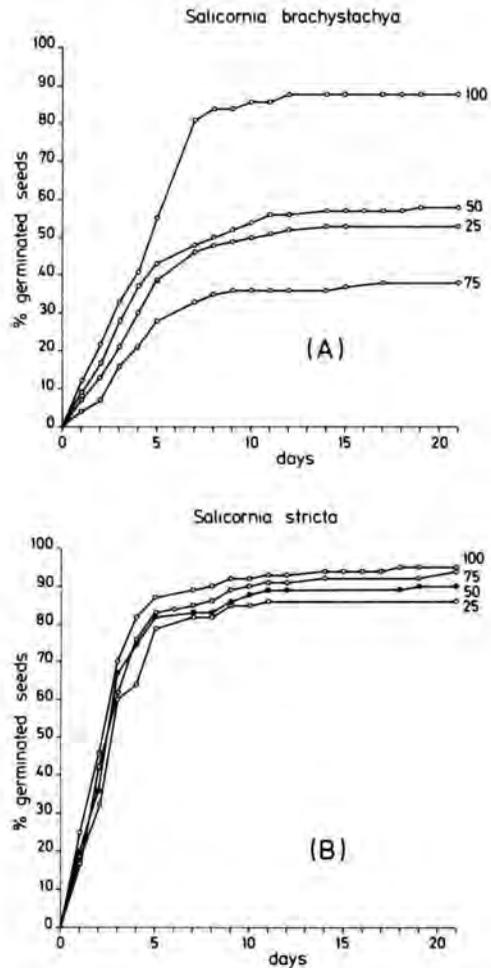
Finally W.J. Drok (Agricultural University, Wageningen) worked out his data on the taxonomy and ecology of *Spartina townsendii* s.l. Some of the results are summarized in his contribution below.

1. Some germination experiments with *Salicornia* species (S2) (A.H.L. Huiskes and M.M. Markusse)

The investigations on germination behaviour with respect to soil moisture and salinity in *Salicornia stricta* and *S. brachystachya* of Stienstra and Van Soelen (1978) were continued in 1979. The experiments were carried out at a temperature of 25°C ($\pm 1^\circ\text{C}$), giving optimal germination results. The seeds received light only when handled.

To test the influence of soil moisture on the germination of both species, petri dishes filled with the same amount of dried sieved sand, were moistened

Fig. 22. The influence of different soil moisture on the germination of *Salicornia brachystachya* (A) and *Salicornia stricta* (B). The numbers at the end of the graphs show the level of saturation with demineralised water of the layer of coarse sand on which the seeds were placed



with 0.25, 0.5, 0.75 or 1 times the amount of demineralised water needed to saturate the sand. For each species 100 seeds were placed in each petri dish; the experiment was carried out in duplo. The moisture level was checked every day by weighing. The results are shown in Fig. 22. A lower moisture level has little effect on the germination of *S. stricta*, while it has a much greater effect on the germination of *S. brachystachya*.

Another experiment was set up to test the influence of fluctuations in salinity on the germination of both *Salicornia* species. 100 seeds were placed in a petri dish

on filter paper, that was moistened with NaCl solution of known strength. After three days the seeds were changed to another petri dish of which the filter paper was moistened with a solution of different strength. After another two days the seeds were changed back. The experiment was carried out in duplo at 25°C. The seeds received light only when handled.

Fig. 23 shows some of the results. Only a small percentage of the seeds that were placed in the higher concentration first germinated as compared with the seeds that were placed in the lower concentration first. After the change to the lower concentration, considerable germination was observed, while the germination of the seeds that were changed again to the higher concentration was strongly diminished. Subsequent changes did have only little effect on the germination. It seemed that a high NaCl concentration following a period of lower salinity induced dormancy.

The results agree with the hypothesis of Ungar (1977, 1978), who supposes that a temporary decrease in soil salinity, as occurs mainly in spring, promotes germination while a subsequent increase, as can occur in summer, induces seed dormancy. There are indications that a cold treatment can overcome this dormancy. This could mean that in this respect *Salicornia* is better adapted to arid than to atlantic conditions: in

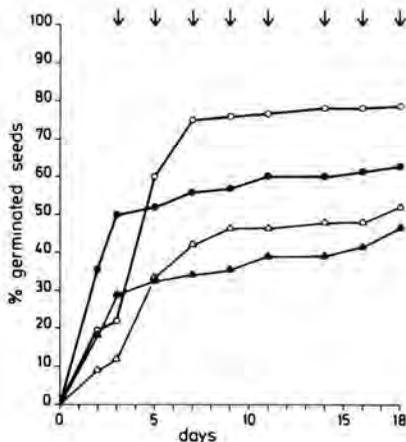


Fig. 23. The influence of fluctuating salinity on the germination of *Salicornia brachystachya* (\blacktriangle and \triangle) and *Salicornia stricta* (\circ and \bullet). The seeds were changed from a solution of NaCl with a concentration of 16‰ Cl^- to a solution of NaCl with a concentration of 8‰ Cl^- and vice versa at the days indicated by the arrows. Open symbols show results whereby the higher concentration was applied first; closed symbols show results whereby the lower concentration was applied first

summer no seeds are 'wasted' by germination, when seedling have little chance to survive due to high salinities or drought, while in winter on mild days numerous seedlings appear, which do not survive. *S. stricta* shows better germination in high salinities than *S. brachystachya*. This, together with the fact that the species is less sensitive for low soil-moisture levels, could explain why *S. stricta* acts in nature more as a pioneer species than *S. brachystachya*.

References

Stienstra, A.W. and J. van Soelen - 1978. A germination experiment with two annual *Salicornia* sp. In: E.K. Duursma (ed.). Progress Report DIHO, 1977, Verh. KNAW, Nat. 2e reeks 71, 123-125.

Ungar, I.A. - 1977. Salinity, temperature, and growth regulator effects on seed germination of *Salicornia europaea* L. Aquat. Bot. 3, 329-335.

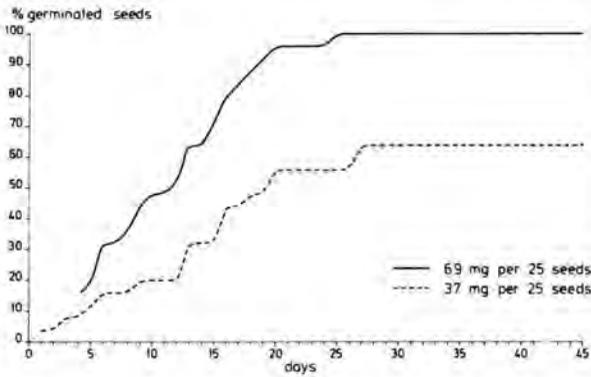
Ungar, I.A. - 1978. Halophyte seed germination. Bot. Rev. 44, 233-264.

2. A germination experiment with apical and lateral seeds of *Salicornia stricta* (S2) (B.P. Koutstaal)

The aim of this experiment was to investigate the ecological implications of seed dimorphism in *Salicornia stricta*, and of seed size. In December 1978, apical and lateral seeds were harvested from four plants separately. These plants were collected from the Bergen op Zoom salt marsh in the middle of October. In the two months before harvesting, the plants with seeds ripened in an outdoor cage. Before starting the experiment (26th May) the seeds have been kept in a refrigerator at 4°C. The lengths of each seed were measured; at the same time the total weight of 25 seeds were determined. The seeds were laid to germinate in petri dishes, 25 seeds per petri dish, and kept in an incubator at 25°C.

Some results are shown in Fig. 24. It appears that there are considerable differences in germination rate as well as in the final results. In the first days of the experiment, the differences in germination between the four parent plants increased, later on they decreased. The apical seeds germinated better than the lateral ones, but in the long run, the lateral seeds made up. Big seeds germinated better than smaller ones during the first 20 days of the experiment. Later on, this difference was smoothed away. The difference in germination rate between big and small seeds of apical origin decreased already after the first 4 days of the experiment. With seeds of lateral origin this difference decreased only after 10 days. The arrears in germination rate of the small seeds is thus made up earlier in the apicals than in the laterals.

Fig. 24. Germination rates of 25 lateral seeds of two size classes collected from one *Salicornia stricta* plant



3. Herbivory of invertebrates and seed production in *Salicornia* (S2) (W.G. Beeftink and W. de Munck)

As in former years invertebrate herbivory has been studied in *Salicornia europaea*, to establish its influence on seed production. The examinations were carried out on the Bergen op Zoom salt marsh where locations from the *Spartina* to the *Limonium* marsh and in a salt meadow behind these zones have been sampled at intervals of about 3 weeks. At each time 50 plants per location have been sampled, as far as possible, and examined on tracks of herbivory (Table 16).

Hydrobia ulvae (Mollusca) was actively grazing to about mid-summer. After that period the number of grazed plants gradually decreased, because the stems

were beginning to lignify and the grazing pattern made in early summer was less detectable. Apparently, the snail is limited in its grazing pattern to the lower (vegetative) parts of the plant which is perhaps related to a lower relative humidity at low tides of the higher air layers in the vegetation (compare Huiskes *et al.*, this Progress Report).

Larvae of *Baldratia salicorniae* (Diptera) and of *Coleophora salicorniae* (Lepidoptera) were found in smaller numbers than in 1978. Hence the influence of the latter species on seed production was much less: The net seed consumption of *Coleophora* was only about 3% in both *Salicornia* species, and was limited to the plants in the higher parts of the marsh. Seed formation varied from 38 to 73% in *S. stricta*, and from 59 to 71% in *S. brachystachya*. *Lita salicorniae* (Lepidoptera) was found in the marsh this year, especially in the salt meadow.

4. Measurements of abiotic environmental factors in salt-marsh vegetation (S2) (A.H.L. Huiskes, B.P. Koutstaal, M.M. Markusse and J. van Soelen)

For the interpretation of the dynamics of vegetation and plant populations it is necessary to have some knowledge of their abiotic environment. Climatological factors as well as physical and chemical soil factors have strong influence, especially in the early stages of succession, where pioneer plants play a major role and where the influence of abiotic factors is more important than interactions between the plants themselves, one of the major factors influencing the

Table 16. Percentages of *Salicornia* plants grazed by *Hydrobia ulvae* (H), *Baldratia salicorniae* (B), *Lita salicorniae* (L), and *Coleophora salicorniae* (C). Number of plants 4 x 25, random sampled from 5 April to 11 October, 1979. Area of investigation: Bergen op Zoom salt-marsh. Zones: 3: closed *Spartina anglica* vegetation; 4: *Puccinella maritima* community; 5: *Limonium vulgare* community, and 6: salt pan in an *Amerion maritima* meadow. *stricta* = *Salicornia stricta*; *brach.* = *Salicornia brachystachya*. n.c. = not collected.

Date	zone 3	zone 4	zone 4	zone 5	zone 5	zone 6	zone 6
		<i>stricta</i>	<i>brach.</i>	<i>stricta</i>	<i>brach.</i>	<i>stricta</i>	<i>brach.</i>
5 April	0	0	n.c.	0	0	0	0
26 April	0	0	n.c.	0	1(H)	0	0
16 May	0	4(B)2(H)	n.c.	0	0	0	0
18 June	18(H)	10(H)	8(H)	23(H)	22(H)	32(H)	7(H)
28 June	28(H)	35(H)1(L?)	39(H)	46(H)	47(H)	43(H)	32(H)
16 July	46(H)	39(H)	41(H)	47(H)	42(H)	n.c.	n.c.
8 August	33(H)7(C)	21(H)5(C)	n.c.	25(H)4(C)	16(H)3(C)	29(H)	6(H)3(C)1(L)
28 August	26(H)	18(H)1(C)1(L)	n.c.	31(H)1(C)	2(H)2(C)	31(H)	3(H)8(C)3(L)
18 September	8(H)1(C)	12(H)	n.c.	5(H)4(C)	2(C)1(L)	10(C)14(L)	2(H)4(C)
11 October	1(C)	1(H)3(C)	n.c.	19(C)	1(H)1(C)	5(C)	15(C)

population size in later successional stages (Southwood, 1976).

In salt marshes the microclimate and soil parameters are influenced by the tidal rhythm. Little work has been done to study these factors and especially their changes due to the tides. Therefore measurements at one point in time are of little significance, continuous measurements over one or two tidal cycles will give more useful information.

A research project was devised for such measurement. Equipment was chosen so that it did not require recalibration during the measurements. Some in-

struments were built or adapted by F.W. Melissen (Melissen, 1980). In 1979 three 24 hour-measurements in summer and one 12 hour-measurement in December were performed.

In summer measurements were made in the *Salicornia* vegetation at the lower part of the Bergen op Zoom salt marsh, whereby the recording equipment and personnel were housed on a platform, 5 m high and covered with a tent, made of scaffolding materials and designed by SSH b.v., Helmond. In December the measurement took place in the *Limonium* vegetation on the higher parts of the marsh from a minibus. As at this measurement more parameters could be measured than in summer, these results are reported here and shown in Fig. 25. During the measurement the area was not flooded.

Temperatures were measured with NTC resistance sensors with a range of -5 to $+45^{\circ}\text{C}$; relative humidity with a humidity sensor (Humicap HMP 14 U by Vaisala Ltd); light with an underwater quantum sensor (LI-192S by Lambda Instruments), which is sensitive for light with wavelengths between 400 and 700 nm.

The highest fluctuations in temperature were found in the vegetation, 15 cm above ground level. This agrees with the findings of Liddle and Moore (1974) who measured temperature profiles in dune vegetation. The relative humidity was 100% early in the morning and in the beginning of the evening due to fog. That the relative humidity can be comparatively low at certain moments could affirm the ideas of Ranwell (1972) on the possible drought effects in the higher parts of the salt marsh. The measurements will continue for some years and the factors to be measured will be extended.

References

- Liddle, M.J. and K.G. Moore - 1974. The microclimate of sand dune tracks. The relative contribution of vegetation removal and soil compression. *J. Appl. Ecol.* 11, 1057-1068.
- Melissen, F.W. - 1980. Electronic equipment for a micrometeorological station. This Progress Report, p. 61.
- Ranwell, D.S. - 1972. Ecology of salt marshes and sand dunes. Chapman and Hall. London, 258 pp.
- Southwood, T.R.E. - 1976. Bionomic strategies and population parameters. In: R.M. May (ed.), *Theoretical Ecology*. Blackwell, Oxford, 26-48.

5. Seedling survival of *Halimione portulacoides* (S2) (A.H.L. Huiskes)

Halimione portulacoides, a dwarf shrub, may grow in the salt marsh in very dense stands, which exclude any

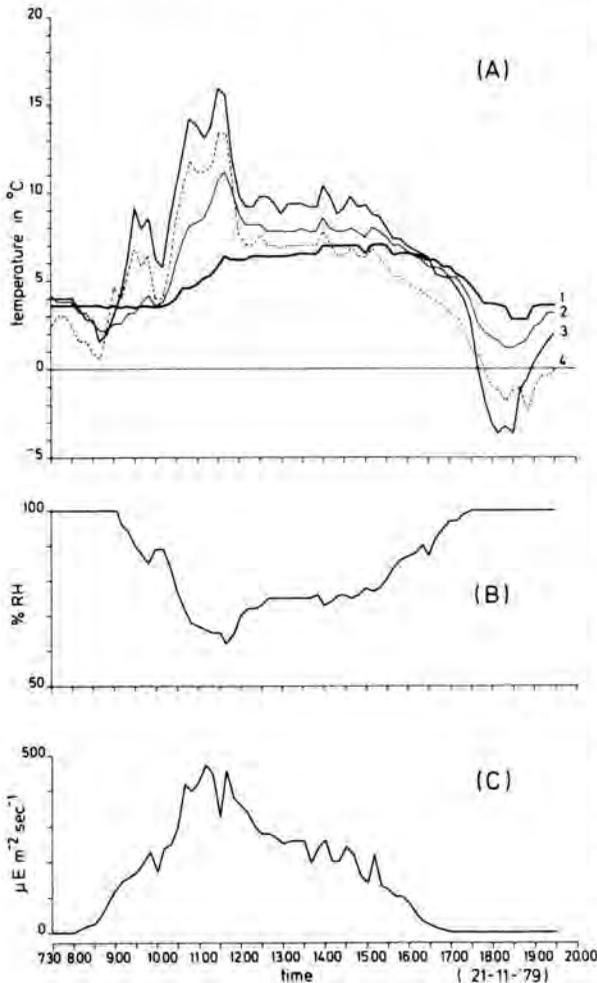
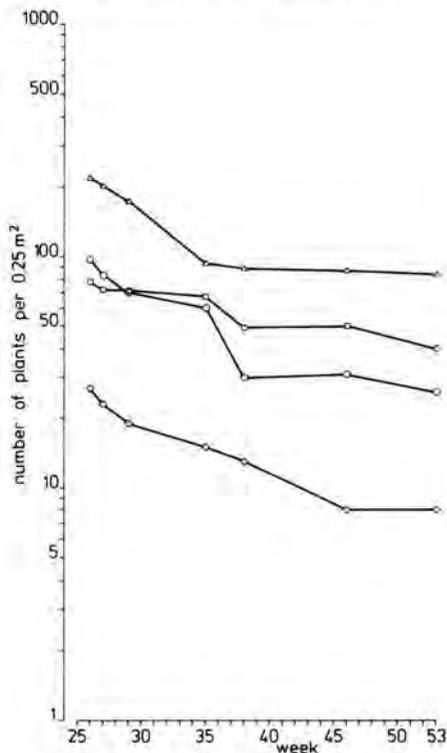


Fig. 25. The fluctuation of temperature (A), relative humidity (B) and light (C) in a *Limonium* vegetation on the Bergen op Zoom salt marsh, during a period of 12 hours on November 21st 1979. 1 = temperature at 15 cm below the ground surface; 2 = temperature in the vegetation, ± 15 cm above the soil surface; 4 = air temperature ± 2 cm above the soil surface

Fig. 26. Survivorship curves of seedlings of *Halimione portulacoides* in four plots of 0.25 m² of the Ellewoutsdijk salt marsh • = Quadrat I; ○ = Quadrat II; ◇ = Quadrat III; □ = Quadrat IV



other species because of light interception. Seedling survival of *H. portulacoides* is rare for the same reason (Schat, 1978).

During the severe winter of 1979 large numbers of adult plants died; it was observed that losses were particularly heavy in areas not covered with snow or ice. Between and under the remains of these plants in spring numerous seedlings emerged.

In the Ellewoutsdijk salt marsh along the Western Scheldt, a number of plots was established in those areas to study the fate of these seedlings. The size of the plots was 0.25 m². The seedling were mapped and when necessary labeled. Three quadrats (I, II and IV) had a comparatively open cover of *Aster tripolium* and dead *Halimione* shrubs; quadrat III had a dense cover of dead *Halimione* only. In quadrats III and IV some regrowth of the adult plants was observed in the course of the year. Fig. 26 shows the fate of the seedlings in the second half of 1979. Presumably because of the dense cover with dead *Halimione* in quadrat III, the number of seedlings was much lower as compared with those in the other quadrats.

To compare the death rate of seedlings in the four quadrats the following formula was used:

$$r = \frac{7}{d} cN$$

in which

r = the death rate of seedlings per week;

d = the number of days between two subsequent recordings

N = the number of seedlings lost between two subsequent recordings

c = the ratio between the initial number of seedlings and a standard population of 100 seedlings, used to make the results comparable (Huiskes and Harper, 1979).

The results shown in Table 17 show a high death risk in the first life stages of the seedlings. The main death cause was burial with sediment during flooding. In older stages plants seem to be less vulnerable to burial. The cause for the high death rates in September in quadrats II and IV is not known.

Table 17. Death rates per week of seedlings of *Halimione portulacoides* in four quadrats on the Ellewoutsdijk salt marsh.

quadrat	Date 1979/1978					
	3 July	17 July	28 August	19 Sept.	14 Oct.	3 Jan.
I	9	6	6	1	0	0
II	17	7	2	10	0	1
III	17	7	2	2	2	0
IV	8	3	1	8	1	1

References

Huiskes, A.H.L. and J.L. Harper - 1979. The demography of leaves and tillers of *Ammophila arenaria* in a dune area. *Oecol. Plant.* 14, 435-446.

Schat, H. - 1978. *Populatiebiologie van Salicornia stricta en Salicornia brachystachya, en van enkele andere soorten op de schorren ten zuiden van Bergen op Zoom.* DIHO-Student Report D-3, 218 pp.

6. Some results of a demographic study in *Aster tripolium* (S2) (M. Ham and E. Miedema)

On the brackish Waarde marsh and the more marine Ellewoutsdijk salt marsh, both situated in the Western Scheldt estuary, 4 vegetational zones were distinguished. In each zone the *Aster* population was studied in 10 selected sample plots. In both marshes considerable differences have been found in shoot and seedling densities between the zones. The higher seedling densities occurred in other zones than the higher shoot densities.

The number of leaves per vegetative shoot appeared to be very constant, both in time and in the different zones. In some sample plots the mean mortality of the leaves per shoot exceeded mean natality during the

study period. The number of flowering shoots varied from 13-48% of all shoots present. In September new shoots developed from the older rhizome columns, varying from 0.5 to 3.1 shoots per column per m².

Plants flowered simultaneously in the different zones, but varied considerably in the number of flowering heads per shoot. Seed development per flowering head was rather equable in the different zones, just as mean seed weight. Seed consumption by insect larvae was considerable in the Waarde marsh (in 53-94% of all flowering heads), but few in the Ellewoutsdijk marsh (less than 20%).

Seedling survival was very low except in the *Spartina* zone of the Waarde marsh. In the populations examined vegetative propagation seems to be much more important for population survival than reproduction by means of seed.

7. Comparative ecophysiological research on *Atriplex hastata* and *Atriplex littoralis* (S2) (D.J. van der Haar)

This study has been carried out to understand more about the ecological background of the differences in habitat of both *Atriplex* species: *A. littoralis* is dominant on plant debris thrown up against the dike faces, while *A. hastata* mainly grows in the marsh itself and under brackish estuarine conditions.

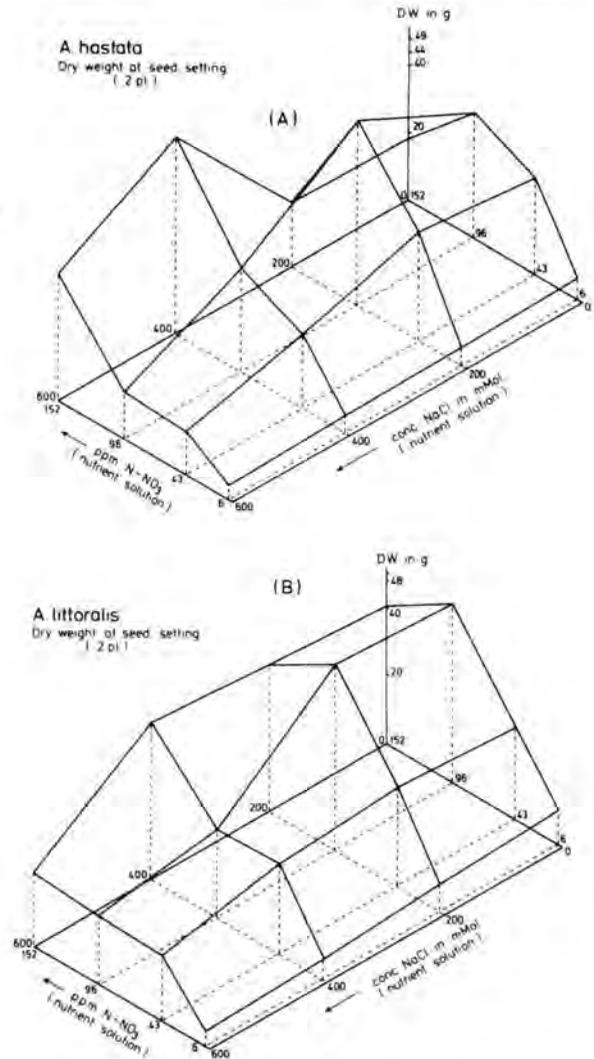
It is hypothesized that salinity and nutrient level should be major factors governing growth and development of these plants. Germination experiments were set up, and a greenhouse experiment with plants on a 0.5 N Hoagland water culture with various contents of sodium chloride (0-200-400-600 mMol) and NO₃-N (6-43-96-152 ppm) was carried out.

Germination rate appeared to be dependent on seed size. Large seeds germinated considerably better. Germination was delayed more in *A. littoralis* than in *A. hastata* by higher salinities of the medium. Seeds enclosed by bracts germinated much less, probably because of high salinity contents of the bracts (Waisel, 1972).

In the greenhouse experiment measurements were carried out on total plant length, fresh weight, dry weight, relative growth rate, and shoot-root ratio. Especially in the beginning of the experiment growth of *A. littoralis* was much faster than that of *A. hastata*, but the latter continued longer. The plants grown up in water cultures with low salinities yielded maximal biomass production under intermediate NO₃-N concentrations (Fig. 27). In cultures with high salinities maximal production shifted to the highest nitrogen concentration in the experiment. In *A. hastata* growth suppression by high salinities seems to be more compensated by a high nutritive condition than in *A. littoralis*.

Water economy of the species was related to

Fig. 27. Production of biomass in *Atriplex hastata* (A) and *A. littoralis* (B) (g dry weight per plant) at time of seed setting in relation to different concentrations of NaCl and NO₃-N added to a 0.5 n Hoagland solution



stomatal resistance in the leaves, and to the water-use per day. Succulence (fresh weight/dry weight ratio) was higher in *A. littoralis* than in *A. hastata*. Both species accumulated considerable amounts of sodium and chloride, but *A. hastata* more than *A. littoralis*. These species also accumulated more sodium in proportion to the availability of nitrogen in the water solution. This could be related to a higher production of tolerance-stimulating amino-acids (Rozema, 1978). For nearly all solutions seed production of *A. littoralis* was considerably higher than that of *A. hastata*. The former species, however, produced mainly small seeds.

References

Rozema, J. - 1978. On the ecology of some halophytes from a beach plain in the Netherlands. Thesis Free University, Amsterdam, 191 pp.

Waisel, Y. - 1972. Biology of halophytes. New York, Acad. Press, 395 pp.

8. Studies on the taxonomy and ecology of *Spartina townsendii* s.l. in the S.W.-part of the Netherlands (S2) (W.J. Drok)

The aim of the present investigation was to find out how far the hybrid *Spartina* × *townsendii* H. et J. Groves and the amphipolyploid *S. anglica* Hubbard occur in the S.W.-Netherlands, and if so, how they can be distinguished and under which environmental conditions they live. For that purpose, 75 plant samples were taken from 20 locations ranging from tidal to non-tidal, and from marine to brackish habitats.

The main results of the investigation are:

1. Only rarely plants of *S. × townsendii* were found. They occur at the same places as *S. anglica*, but never in the lowest zone of the tidal salt marsh.
2. Because only quantitative characteristics can be considered to distinguish the taxa, their range of variation within the taxa is decisive. In contrast to what is suggested in British publications (e.g. Hubbard, 1968, Goodman *et al.*, 1969), it appeared in this investigation that all characteristics overlap (Fig. 28). This in fact means that it is impossible to be sure of sterile specimen of *S. × townsendii* without assessing the chromosome number. So Dutch florists are wrong in accepting the view of Hubbard (1968) for the Dutch situation, as they have done recently (Heukels and Van Oostroom, 1977, Adema and Mennema, 1979).
3. There is a large genotypic variation in *S. anglica*, in-

cluding the occurrence of slender, sterile forms. This harmonizes with the conclusions of Boyle and Kavanagh (1961), examining *Spartina* in Ireland. Probably this variation has ecological significance, like Silander (1979) proved in *Spartina patens*.

4. An interesting correlation was found between fruiting success and habitat in *S. anglica*. Totally sterile plants can be found, but also plants with good pollen that nevertheless produce no good fruits. The contrary, viable seed in the absence of good pollen, was never observed. Below MHW most plants are female-sterile; the three exceptions found are plants from sheltered places without much wave action. In all other zones sterile plants are in the minority. In the Spartinetum of the lower salt marsh the fertile plants usually produce 5-20% viable seed, in the general salt marsh 5-50%. Percentages higher than 50% were only observed on the highest parts of the tidal marsh and in non-tidal habitats.

References

Adema, F. and J. Mennema - 1979. De Nederlandse Slijkgrassen. *Gorteria* 9, 330-334.

Boyle, P.J. and Kavanagh, J.A. - 1961. A Spartinetum at Baldoye in Ireland. *Nature* 192, 81-82.

Goodman, P.F. and C.J. Marchant - 1969. Biological flora of the British Isles: *Spartina*. *J. Ecol.* 57, 285-313.

Hubbard, C.E. - 1968. Grasses, Penguin Books, Harmondsworth, 463 pp.

Heukels and Van Oostroom - 1977. Flora van Nederland, 19th Ed. Groningen, 925 pp.

Silander, J.A. - 1979. Microevolution and clone structure in *Spartina patens*. *Science* 203, 658-660.

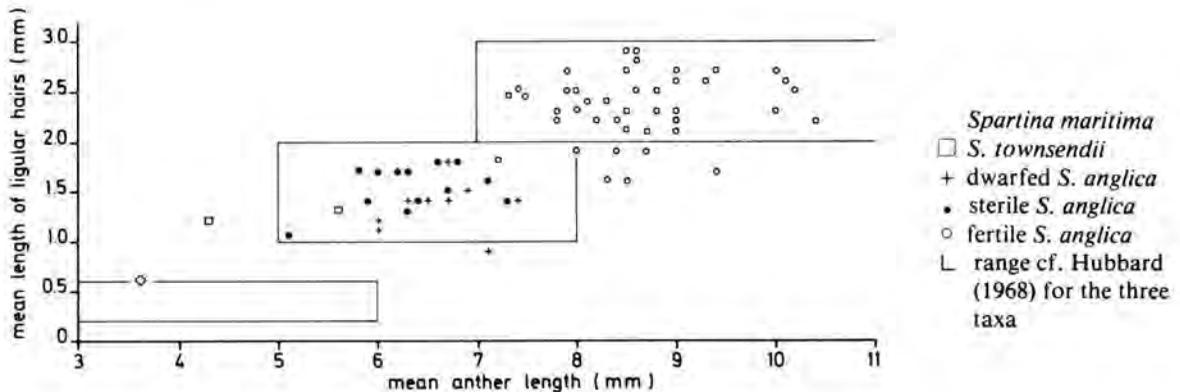


Fig. 28. Observed range of two of the most important diagnostic characters in *Spartina*. Notice that it is impossible to distinguish sterile forms of *S. anglica* from *S. × townsendii*

VI.4. MATERIALS (S3) (W.G. Beeftink and A.M. Groenendijk)

The study of production, transformation and decomposition of organic materials can account for much of the energetic basis on which the salt-marsh ecosystem is functioning. There are two main aspects in this approach: 1. the transport and turnover of organic matter and nutrients in the marsh itself, and 2. the interaction of organic matter and nutrients between the marsh and the adjacent tidal water-body. Microbial and animal relations play an important role in the transformation and decomposition processes, while leaching could also have an essential share in these semi-terrestrial habitats.

In Europe only incidental investigations have been carried out in this field of salt-marsh ecosystem research, in contrast with along the Atlantic and Gulf coasts of North America, where ecologists already started about 20 years ago. At our Institute E. Lamens (Catholic University, Nijmegen) and M. van Eeden (Agricultural University, Wageningen) carried out a pilot study on the interaction of particulate organic matter between a single marsh and the adjacent water-body and on budgetting the organic matter relations. The results of this study will be published soon. From further investigations it appeared however that problems can arise in such salt-marsh studies resulting in a considerable underestimation of production if: (1) below-ground production, (2) leaching, detaching and transport of plant materials in summertime, and (3) plant growth in autumn and wintertime are insufficiently taken into account. Although these aspects of the study of salt-marsh ecosystems are not included in the actual program of the working group, A.M. Groenendijk, who deputized for A.W. Stienstra, started, with the assistance of M. Lievaart, a pilot study on the methodology for estimates of above-ground production and below-ground biomass of halophytes in the Stroodorpepolder salt marsh (Eastern Scheldt). In the following lines preliminary results are given.

1. Above-ground production in an Eastern Scheldt salt marsh (S3) (A.M. Groenendijk)

In North America net aerial primary production (NAPP) is often used as a measure of the growth potential of salt-marsh plants and their subsequent contribution to the detritus-based food webs of the adjacent estuary (Teal, 1962, Hopkinson *et al.*, 1978, Eilers, 1979). In Europe however production data of salt marshes are very few (Tyler, 1971, Ketner, 1972, Wolff *et al.*, 1980). Differences in geographical range and in floristic and animal composition between American and European marshes make it hard to app-

ly the American results to European conditions. This lack of information stimulated to continue with some further work on NAPP of the most important salt-marsh macrophytes before the construction of the storm-surge barrier in the Eastern Scheldt in 1985.

The harvest methods developed are widely considered to underestimate primary production on salt marshes because they do not adequately account for transport of dead plant tissues between harvests. Therefore, in the Stroodorpepolder salt marsh two of the most common methods are compared, viz. the method of Wiegert-Evans and that of Smalley. The Wiegert-Evans method is based on the production of living and dead plant tissue, and on the disappearance of dead plant tissue; the Smalley's method is based on the production of living and dead plant tissue (this method was modified to prevent import and export of algae and dead plant tissue). Four stands (three monospecific ones with *Spartina anglica*, *Halimione portulacoides* and *Elytrigia pungens* resp., and one multispecific with *Triglochin maritima*, *Limonium vulgare* and *Puccinellia maritima*) have been harvested every two weeks according to Smalley's method from March onward. The method of Wiegert-Evans which is very time-consuming is only used once a month in the *Spartina* and *Elytrigia* stands.

Preliminary results show no distinct differences between the production figures obtained with the two methods. The NAPP of *Spartina anglica* was 1400-1500 g m⁻²yr⁻¹ (dry weight) for both methods. This similarity of results is mainly caused by the relatively small transport of dead plant tissues during the growing season of 1979. Outside the growing season considerable transport took place but that did not influence the production figures.

Considering this result it seems at the moment that – mainly for practical reasons – Smalley's method (modified) is suitable for routine measurements in the Stroodorpepolder salt marsh.

References

- Eilers, H.P. - 1979. Production ecology in an Oregon coastal salt marsh. *Estuar. Coastal Marine Sc.* 8, 339-410.
- Hopkinson, C.S., J.G. Gosselink, R.I. Parrondo - 1978. Aboveground production at seven marsh plant species in coastal Louisiana. *Ecology* 59, 760-769.
- Ketner, P. - 1972. Primary production of salt-marsh communities on the island of Terschelling. Thesis Catholic University, Nijmegen, 184 pp.
- Linthurst, R.A. and R.J. Reinold - 1978. An evaluation of methods for estimating the net areal primary productivity of estuarine angiosperms. *J. Appl. Ecol.* 15, 919-931.

Teal, J.M. - 1962. Energy flow in the salt marsh ecosystem of Georgia. *Ecology* 43, 614-624.

Tyler, G. - 1971. Distribution and turnover of organic matter and minerals in a shore meadow ecosystem. *Oikos* 22, 265-291.

Wolff, W.J., M.J. van Eeden and Lammens, E. - 1980. Primary production and import of particulate organic matter on a salt marsh in the Netherlands. *Neth. J. Sea Res.* 13, 242-255.

2. Estimation of below- and above-ground biomass in some halophytes (S3) (A.M. Groenendijk and M. Lievaart)

Estimates of biomass and primary production of salt-marsh plants mostly concern data of shoots only, even though root biomass may account for large percentages of the total amount. For that reason, biomass and production can be underestimated considerably (Ketner, 1972, Smith *et al.*, 1979).

In June 1979 a monthly sampling program was started in four vegetation stands (see A.M. Groenendijk, this Progress Report, p. 49). For soil sampling a cylindrical auger (length 110 cm, diameter 7 cm) is used having a removable rubber plug at its top side. This auger has been constructed at the Institute by C. Almekinders and J.P. Hoekman, and proved to be very useful for taking in situ samples from wet soils. The soil samples were treated according to Schuurman and Goedewaagen (1971). Roots were separated from soil particles using a 0.106 mm sieve. To distinguish dead from living organic matter subsamples have been taken and observed microscopically. Dead roots and humus particles proved to be in a minority with respect to the living biomass.

Preliminary results showed that high amounts of below-ground organic matter occur in all the vegeta-

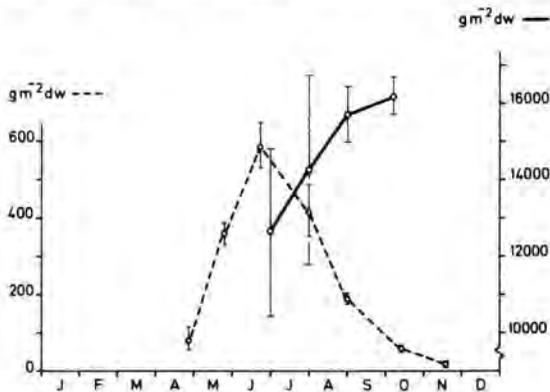


Fig. 29. Quantities of living above-ground (• - - - •), and total below-ground biomass (• — •) in a *Triglochin maritima* stand

tion stand examined (Fig. 29). Root/shoot ratios, calculated from the total organic matter below-ground and the corresponding amount of living organic matter above-ground, were more than 10 for all vegetation stand. Thus, more than 90% of the plant materials in these stand is found below-ground. Especially the *Triglochin* stand showed extremely high root/shoot ratios (20 to more than 100). These results are very similar to those of vegetation types in xeric environments (Bray, 1963, Valiela *et al.*, 1979), suggesting ecological similarities (physiological drought) in spite of the wet conditions in the salt marsh. The sampling program will be continued in 1980 in order to have a year-long data series.

References

Bray, J.R. - 1963. Root production and the estimation of net productivity. *Canad. J. Bot.* 41, 65-71.

Ketner, P. - 1972. Primary production of salt marsh communities on the island of Terschelling. Thesis Catholic University, Nijmegen, 184 pp.

Schuurman, J.J. and M.A.J. Goedewaagen - 1971. Methods for the examination of root systems and roots. Pudoc Wageningen, 86 pp.

Smith, K.K., R.E. Good and N.F. Good - 1979. Production dynamics for above and below ground component of a New Jersey *Spartina alterniflora* tidal marsh. *Estuar. Coastal Marine Sci.* 9, 189-201.

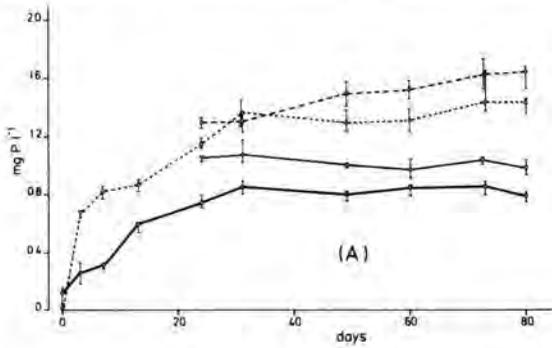
3. Decomposition experiments with *Spartina anglica* (S3) (L.A. van Geldermalsen, M.T.T. Vereecken and A.M. Groenendijk)

In the last few years much research has been done on the role of salt-marsh angiosperms in the nutrient and carbon budget of estuarine waters (Hopkinson *et al.*, 1978). Most of the work has been concentrated on the primary production and decomposition of dominant angiosperms viz. *Zostera marina* and *Spartina alterniflora* (Kirby and Gosselink, 1976).

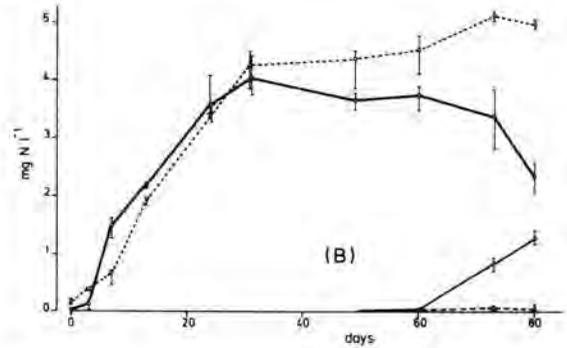
In studies on decay of salt-marsh vegetation most interest has been given to data such as loss of dry weight, changes in nutritional value of leaf material, changes in C/N ratio and chemical composition. These data indicate the contribution of decomposition to the environment (Nichols and Keeney, 1973).

Up till now there is not much structural information available about the processes involved in decomposition, which take place in the aquatic phase, and include: a. 'mechanical' fragmentation due to grinding, b. 'chemical' fragmentation due to biochemical action, such as cellysis mediated by plant-, bacterial- and fungal enzymes and autolysis mediated by plant enzymes, c. 'biological' fragmentation due to partial

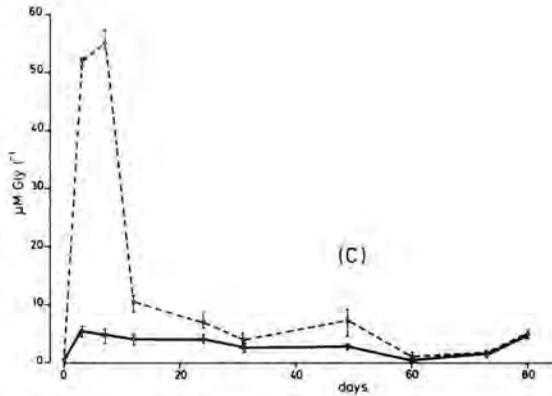
Fig. 30. Leaching experiments with *Spartina anglica* in tanks with artificial seawater in the presence and absence of 0.3‰ sodiumazide and 0.1‰ chloramphenicol



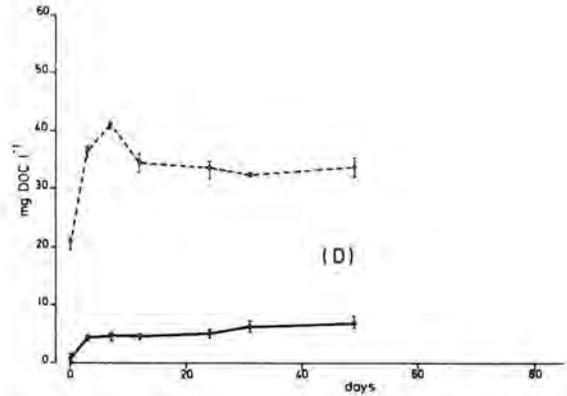
A. Orthophosphate, as $\text{mg P-PO}_4^{3-} \text{ l}^{-1}$, ----, and total phosphate, ----, in the presence of 0.3‰ NaN_3 and 0.01‰ chloramphenicol. Orthophosphate, —, and total phosphate, —, in natural seawater



B. Ammonia, as $\text{mg N-NH}_4^+ \text{ l}^{-1}$ in the presence, ----, and absence — of 0.3‰ NaN_3 and 0.1‰ chloramphenicol. Nitrate, as N-NO_3^- , in the presence, ----, and absence, —, of 0.3‰ NaN_3 and 0.1‰ chloramphenicol



C. Total free amino acids (TFAA), as $\mu\text{M Glycine l}^{-1}$ in the presence, ----, and absence, —, of 0.3‰ NaN_3 and 0.1‰ chloramphenicol



D. Dissolved organic carbon (D.O.C.) in the presence, ----, and absence, —, of 0.3‰ NaN_3 and 0.1‰ chloramphenicol

digestion by micro- and macrofauna and d. 'leaching' which is the removal of soluble compounds, partially produced by the other processes. More knowledge of these processes and their interactions will give us a better insight in the ecology of the communities in the Eastern Scheldt.

Experiments were performed to show the influence of microbial action on the autolysis and leaching of *Spartina anglica*, one of the most dominant angiosperms in the salt marshes along the Eastern Scheldt. The experiments consisted of incubations of *Spartina anglica* leaves in tanks with aerated artificial

seawater. Under laboratory conditions two different series are used: 1. an 'unsterile' series to which a natural microbial population has been added and allowed to develop. 2. a 'sterile' series to which chloramphenicol and sodium azide has been added to inhibit microbial and fungal growth. The appearance of several compounds in the water phase was followed during some months (Fig. 30). Together with these experiments an in situ experiment has been carried out to compare total loss of dry weight and loss of organic and nitrogen compounds of plant material with the laboratory results.

At the moment the experiments are still carrying on. Preliminary results show that the liberation of nutrients is not affected by microbial fungal growth and that a large part of dissolved organic compounds is consumed by bacteria and fungi.

References

- Hopkinson, C.S., J.G. Gosselink and R.T. Parrondo -1978. Above-ground production of seven marsh plant species in coastal Louisiana. *Ecology* 59, 760-769.
- Kirby, C.J. and J.G. Gosselink - 1976. Primary production in a Louisiana Gulf coast *Spartina alterniflora* marsh. *Ecology* 57, 1052-1059.
- Nichols, D.S. and D.R. Keeney - 1973. Nitrogen and phosphorus release from decaying water millfoil. *Hydrobiologia* 42, 509-525.

VI.5. ECOTOXIC EFFECTS OF POLLUTANTS (S4) (W.G. Beeftink)

The study on possible effects of heavy metal contamination in the salt marsh, in collaboration with Dr.

M. Stoeppler (Institute for Applied Physical Chemistry, Nuclear Research Centre, Jülich, B.R.D.) has been continued with the analysis of samples mainly from the Ellewoutsdijk salt marsh. Heavy metal contents in this marsh proved to be generally less than those in the marshes nearer to the industrial sites of Antwerp.

In the whole dataset obtained so far plant samples from lower parts of the marsh have often heavy metal contents higher than those collected from higher parts. A relation to the frequency of inundation with the estuarine water is obvious. In the higher marsh, however, many samples from localities where plant debris is usually washed ashore show relatively very high contents, indicating that accumulation in organic materials is an essential aspect in the transfer and distribution of heavy metals in the salt-marsh ecosystem.

VII. A-subjects (miscellaneous)

VII.1. THE CARBON-NITROGEN RATIO OF SEVERAL WATERS IN THE DELTA AREA OF THE NETHERLANDS (A6) (A.G.A. Merks)

As part of a research programme on the determination of dissolved carbon and nitrogen in surface waters of the Delta area a first investigation was made on the levels of possible concentrations in the different brackish and saline waters of the Southwest part of The Netherlands. A sampling programme was carried out in June 1979 for 17 stations all over the Delta area (Fig. 31). The stations were chosen in the Western Scheldt, Eastern Scheldt and Haringvliet (Rhine), and furthermore in several inland waters as Lake Grevelingen, Lake Veere and others. Also some water from the North Sea was collected.

The parameters which were analysed were: chloride, sulphate, orthophosphate, ammonia, nitrate + nitrite, silicate, DOC and organic nitrogen. Most of these analyses are from the standard programme of the Delta Institute (Merks, 1975), but for DOC and organic nitrogen special methods were handled. DOC is analyzed in a Phasesep Tocsin 2B Analyser, in which the sample is washed with acid to remove inorganic carbon and where the organic carbon is oxidised to CO₂, dried and reduced to CH₄, which is detected in a flame-ionisation detector (Merks, 1978). Organic

nitrogen is determined in a modified version of an instrument based on Duursma (1961). The sample is brought into a silvertube and at 500°C hydrogen is

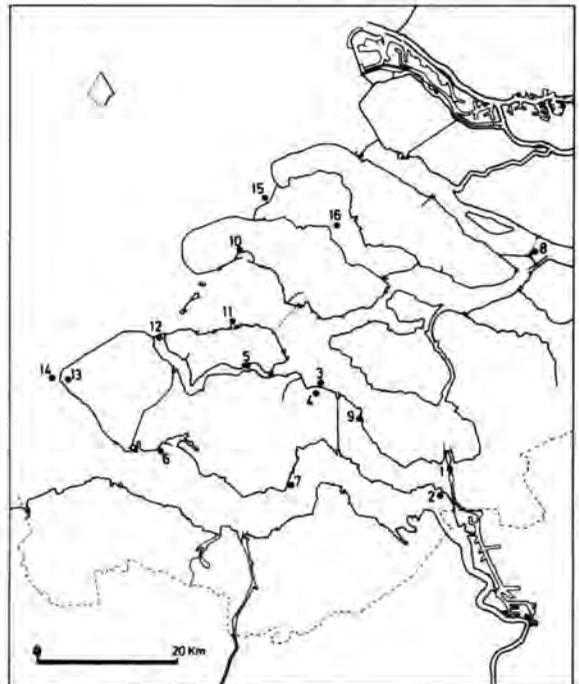


Fig. 31. Map of the Delta Area of the Netherlands with sampling stations

Table 18: Chemical parameters of various Deltawaters with specific attention to SO_4/Cl^- and DOC/DON ratios.

Station	No.	ortho PO_4^{3-} mg l^{-1}	SiO_2 mg l^{-1}	NH_3 mg l^{-1}	NO_2^- + NO_3^- mg l^{-1}	SO_4^{2-} o/oo	Cl^- o/oo	Ratio $\text{SO}_4^{2-}/\text{Cl}^-$	DOC mg l^{-1}	DON mg l^{-1}	Ratio C/N
Western Scheldt:											
Bath	2	0.407	3.147	2.11	3.11	0.826	4.39	0.188	5.5	1.20	4.6
Hoedekenskerke	7	0.193	0.756	0.13	1.58	2.444	14.57	0.168	1.9	0.91	2.1
Rithem	6	0.147	0.332	0.22	1.22	1.960	11.79	0.166	1.8	0.52	3.5
Eastern Scheldt:											
Kattendijke	3	0.058	0.079	0.28	0.39	2.461	14.81	0.166	2.5	1.81	1.4
Colijnsplaat	11	0.040	0.081	0.16	0.05	2.761	16.85	0.164	4.6	0.50	9.2
Lake Veere:											
Kortgene	5	0.419	1.783	0.32	0.29	1.550	8.30	0.187	7.1	1.15	6.2
Veerse Gat Dam	12	0.303	1.430	0.19	0.49	1.538	8.78	0.175	3.6	0.33	10.9
North Sea:											
Walcheren	14	0.54	0.120	0.13	0.07	2.856	16.50	0.173	2.7	0.82	3.3
Brouwersdam	15	0.269	0.166	0.11	0.03	2.693	14.32	0.188	1.7	0.30	5.7
Inland Waters:											
Schelde-Rijn Canal	1	0.153	5.837	0.48	3.36	0.904	4.08	0.222	6.1	0.45	13.6
Kattendijke	4	1.346	5.837	2.03	0.07	0.679	4.12	0.165	6.7	0.75	8.9
Haringvliet Bridge	8	0.231	1.672	0.38	2.50	0.088	0.00		3.6	0.78	4.6
Schelphoekkreek	10	1.018	4.297	0.13	0.05	1.810	12.46	0.145	6.8	0.47	14.5
Westkapellekreek	13	3.363	9.533	0.15	0.58	1.04	5.80	0.173	9.9	0.44	22.5
Lake Grevelingen											
G 11	16	0.352	0.118	0.05	0.01	2.509	14.25	0.176	2.6	0.17	15.3
Aquarium	9	0.082	0.183	0.23	0.71	2.437	13.39	0.182	1.9	0.69	2.8
Glass house	17	6.760	7.545	0.30	78.48	0.310	6.55	0.047	3.8	78.00	0.05

lead through the solution. Organic nitrogen is reduced into ammonia, which is distilled into acid, forming NH_4Cl . In this resulting solution ammonia is analyzed following the normal procedure for the analysis of ammonia of the Technicon AA II. Fig. 32 shows the reduction oven as constructed in the Delta Institute.

The results of this analysis programme are given in Table 18; Because of the large environmental differences of the stations there are no implicite connections between the parameters. They are mainly given as an identification of the stations. Especially chloride can be used for this purpose. Culkin (1965) found the sulphate/chlorinity ratio for ocean water to be stable at 0.14. We calculated this ratio based on the value's we found in the samples. At present it is not possible to give more information, as more research will be required so far.

The dissolved carbon/nitrogen ratios of these samples are given in Table 18 on weight basis. As part of a larger sampling programme, starting in 1980, among others more detailed analyses are planned to study the C/N ratio.

References

- Culkin, F. - 1965. The major constituents of sea water. In: J.P. Riley and G. Skirrow (Editors). Chemical Oceanography. Acad. Press, London. 1st edn. vol. 1, 121-161.
- Duursma, E.K. - 1961. Dissolved organic carbon, nitrogen and phosphorus in the sea. *Neth. J. Sea Res.* 1, 1-148.
- Merks, A.G.A. - 1975. Analysemethoden voor water. DIHO Rapp. en Versl., 4 pp.
- Merks, A.G.A. - 1979. Measurement of dissolved organic carbon. In: E.K. Duursma (ed.) Progress Report DIHO 1978, Verh. KNAW, Nat. 2e reeks 73, 73-77.

VII.2. ORGANOCHLORINE COMPOUNDS IN MUSSELS (*Mytilus edulis*) OF THE DELTA AREA (A9) (J. Nieuwenhuize, J.M. van Liere and E.K. Duursma)

From November 1979 on, mussels have been sampled from various locations of the Delta area for analysis

on organochlorine compounds. The mussel is used as indicator organism for the local aquatic system so far the accumulation of pollutants concerns (Fig. 33).

A spectrum of PCB and organochlorine pesticides have been detected with PCB's in the range of 254 to 1859 ng g⁻¹. The influence of the Rhine is detectable at stations 8 (1859 ng g⁻¹) while the lake Grevelingen 5 (254 ng g⁻¹) and Eastern Scheldt 4 (389 ng g⁻¹) show lower values. In the Western Scheldt the concentrations are about 3 × those of the Eastern Scheldt (all PCB-values on dry weight basis).

Additional to PCB's are found hexachloorbenzene and pentachloorbenzene with comparable gradients of concentration. Lindane (gamma HCH) is high at all stations while dieldrin in the Grevelingen is, although low, 4 × higher than in the Eastern Scheldt.

VII.3. DETERMINING THE RATE OF BIOTURBATION IN SEDIMENTS WITH COLOURED SAND GRAINS (A10)

(M. Smies and J.W. Francke)

In order to estimate bioturbative activity in sediments we used coloured sand grains (Nieuwenhuize and Sips, 1978) as a sediment tracer. The coloured sand was dry-sieved to obtain the same grain-size distribution as that of the sediment. Bioturbation experiments were carried out in cores in standard PVC water pipes (diameter 6.7 cm). For analysis these were subsampled by coring with polypropylene test tubes (diameter 1.3 cm). The test tube cores were sectioned at 0.5 cm intervals and the sections were thoroughly mixed. From each section three slides were prepared for microscopic examination. The number of coloured fields per 400 squares was determined for each slide using a stereo-

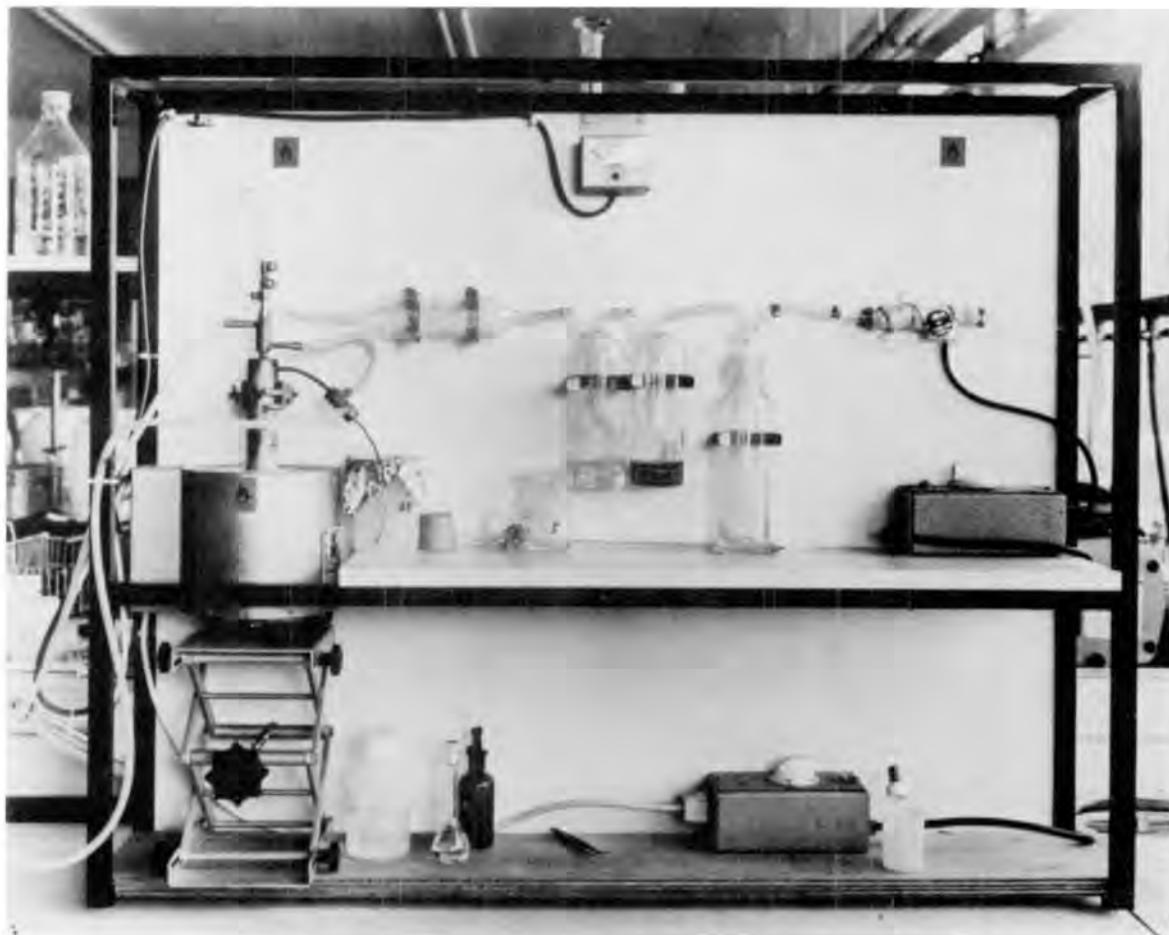
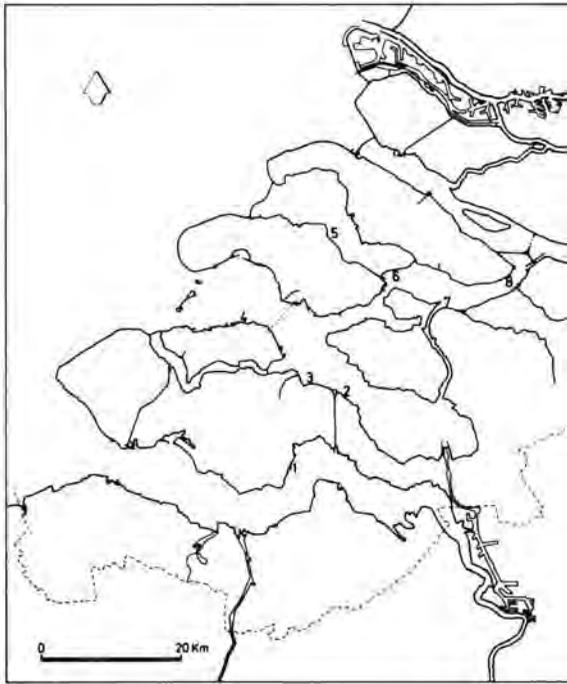


Fig. 32. Picture of the reduction apparatus used for determining organic nitrogen

Fig. 33. Sampling stations of mussels for organo-chlorine studies



microscope (Wild M5) at $25\times$ magnification with a 20×20 squares grid ($400\times 0.25\text{ mm}^2$; Wild) in the $10\times$ wide-angle eye piece.

The depth of the centre of the distribution of coloured grains was determined as follows.

Let r_1, r_2, r_3, \dots be the numbers of coloured grains in sections 1, 2, 3, ... and $\Sigma r_i = r$. At the start of the experiment ($t = 0$) a layer of coloured grains is placed on top of the core. The thickness of the layer (d) in the test tube cores is

$$d = \frac{r}{1200} \times 0.5 \text{ cm}$$

in the top section. At the end of the experiment ($t = t$) the thickness of the layer of coloured grains in section i ,

$$d_i = \frac{r_i}{1200} \times 0.5 \text{ cm.}$$

Let m be the section with largest r_i . If $m = 1$ then $X = 0.5 - d_1 + d/2$ cm is the depth of the centre of the layer of coloured grains. If $m > 1$ and $r_{m-1} = 0$ then $X = 0.5 \times m - d_i + d/2$ cm. Assuming that the coloured grains are distributed normally around the centre their relative distribution declines approximately exponentially. Therefore, if $m > 1$ and $0 < r_{m-1} > r_{m+1} > 0$, then

$$X = \left\{ \frac{\log r_{m-1}}{\log (r_{m-1} \times r_{m+1})} + m - 1 \right\} \times 0.5 + 0.25 \text{ cm}$$

and if $m > 1$ and $0 < r_{m-1} < r_{m+1}$ then

$$X = \left\{ \frac{\log r_{m+1}}{\log (r_{m+1} \times r_{m-1})} + m - 1 \right\} \times 0.5 + 0.25 \text{ cm}$$

The advective rate of bioturbation $V = X/t$ cm s^{-1} . Dispersion was calculated using the model

$$\frac{\partial C}{\partial t} = -V \frac{\partial C}{\partial x} + D \frac{\partial^2 C}{\partial x^2}$$

with C the concentration, V the advective component of bioturbation, D the coefficient of the dispersive component, X the depth and t the time. If C is expressed as the number of coloured grains per unit of area of the core's cross-section times the thickness of the section, then at $X = Vt$

$$C(X, t) = \frac{C_0}{4 \sqrt{(\pi Dt)}}$$

with C_0 the initial concentration of coloured grains. Taking $C(X, t) = r_m$ and $C_0 = r$ then

$$D = \frac{1}{\pi t} \left(\frac{r}{4 r_m} \right)^2 \text{ cm}^2 \text{ s}^{-1}$$

A computerprogram to perform the necessary calculations was written.

References

Nieuwenhuize, J. and J.J. Sips, 1978. The preparation of coloured sand tracers. In: E.K. Duursma (ed.). Progress Report 1977. DIHO: Verhand. KNAW, Nat., 2e reeks 71: 155-157.

VII.4. BIOTURBATION IN SEDIMENT CORES FROM A MUDFLAT IN THE EASTERN SCHELDT (A10) (J.W. Francke and M. Smies)

We collected sediment cores from an intertidal mudflat in the Eastern Scheldt. For coring standard hard PVC water pipes (internal diameter 6.7 cm, length 12 cm) were used. Cores were transferred to the laboratory and kept at ambient seawater temperature. Bioturbative activity of benthic organisms in the cores was studied by covering the sediment surface with a layer of coloured sand grains of the same grain size-distribution. Analysis followed the method detailed by Smies and Francke (this report)

A number of cores was collected on 5 April 1979 and samples were taken for a period of nine weeks, by taking randomly spaced replicate subsamples by coring

with polypropylene test tubes. Meaningful estimates of the advective rate of bioturbation, caused by burial of the sediment surface by (pseudo) faeces, and the coefficient of dispersion could only be made after 25 days. Advective rates varied strongly between cores and less between subsamples. Variation in dispersion coefficients was smaller. At ambient seawater temperatures of 12°C (5 April) to 16°C (8 June) in the laboratory the geometric mean advection rate was 1.5×10^{-7} cm s⁻¹ and the geometric mean dispersion coefficient 2×10^{-8} cm² s⁻¹. The thickness of the layer of intensive bioturbation was 2-3 cm. In additional experiments in July and August (seawater temperature in aquarium 20°C) we found advection rates of 7.5×10^{-7} cm s⁻¹ and dispersion coefficients of 5×10^{-8} cm² s⁻¹.

VII.5. THE MEASUREMENT OF TOTAL FREE AMINO ACIDS (TFAA) IN NATURAL WATERS (A11) (L.A. van Geldermalsen)

An automated technique to measure primary amino acids was developed according to the indications of Dr. R. Dawson (Kiel, a Scientific Market agreement) (Fig. 34). The detection is based on the measurement of the highly fluorescent substituted iso indoles formed by the reaction of o-phthalaldehyde and 2-mercapto ethanol with primary amines (Fig. 35). This reaction, which proceeds at room temperature in an alkaline medium is very rapid and the fluorescent products can be quantitatively measured within 2 minutes. The procedure can be used to measure TFAA in natural waters as well as to measure distinct amino acids in

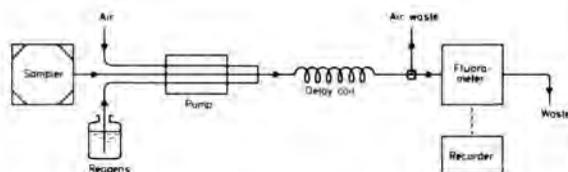


Fig. 34. Scheme of the total amino acid analyzer. The reagent is a 0.4 mole borate buffer, pH 10.5, with 0.5% β-mercaptoethanol, 0.1% o-phthalaldehyde and 1% ethanol. The ethanol is used to dissolve the o-phthalaldehyde

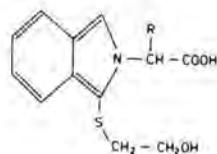
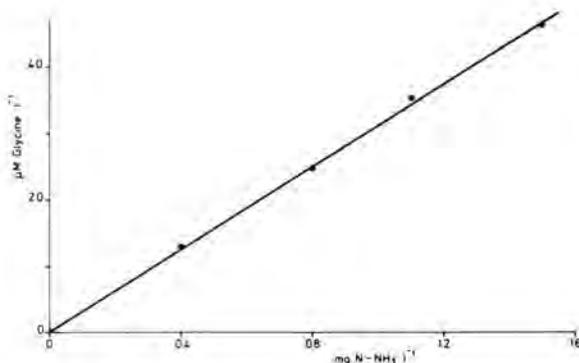


Fig. 35. The fluorescent reaction product of a primary amino acid with o-phthalaldehyde and β-mercaptoethanol. R denotes the amino acid rest group

Fig. 36. The correlation curve of the relative fluorescence intensities of ammonia and glycine as measured on the 0.1 scale of the Farrand A-4 fluorometer



laboratory setups. The fluorescence intensities of the various samples of natural waters have to be related to a glycine calibration curve (Fig. 36) to find the TFAA content expressed as micromoles Glycine per liter ($\mu\text{mole Gly l}^{-1}$).

A complication in natural waters, however, is the contamination with ammonia which also forms a fluorescent product. Hence correction for the ammonia content of natural waters must be made.

If the equipment is used in order to measure distinct amino acids a correction to the value found as $\mu\text{mole Gly l}^{-1}$ has to be made according to the relative intensities of the different amino acids, see Table 19.

It is well known that TFAA in natural waters decay on storage so that direct measurement is necessary. The apparatus is made suitable for ship board operation.

Table 19: Relative fluorescence of 20.0- μmole amino acid solutions expressed in glycine units.

Amino Acid	Rel. Fl.	Amino Acid	Rel. Fl.
Alanine	0.87	Lysine	0.25
Arginine	0.80	Methionine	0.99
Asparagine	0.81	Norleucine	1.03
Aspartic acid	0.75	Ornithine	0.24
Cysteine	0.06	Phenylalanine	0.82
Glutamine	0.87	Proline	-
Glutamic acid	0.85	Serine	0.98
Glycine	1.00	Threonine	0.78
Histidine	1.11	Tryptophan	0.90
Isoleucine	0.99	Valine	0.99
Leucine	0.84	NH_4^+	0.002

VII.6. TIDAL EXCHANGE OF NUTRIENTS IN THE 'KRABBENKREEK' (A11) (L.A. van Geldermalsen)

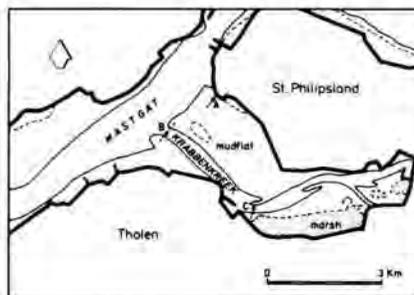
In the Eastern Scheldt tidal mud flats and salt marshes cover about 60% of the total area. These flats and marshes are sites of intense biological and geochemical activity and it has always been thought that in conjunction with tidal flushing this will lead to exchange of nutrients and nutrition substances between these sites and the aquatic environment.

So far no research has been carried out on this role of exchange between the flats and the aquatic environment in the dynamics of nutrients and nutrition substances in the Eastern Scheldt.

When the Storm Surge Barrier will be completed in 1985, by which the area of marshes and flats will be diminished to 30% of the total Eastern Scheldt area due to the reduced tide, a more profound knowledge of this phenomenon is needed to better explain the changing ecology of the Eastern Scheldt.

Vertical exchanges of materials of tidal water with marshes and mud flats will be represented in a concentration change in the tidal water flushing on and off the flats. By sampling the horizontal tide of an enclosed sea arm this vertical exchange should be retrieved. In order to measure this and to indicate the seasonal fluctuations in the overall import-export pattern of a specific area the 'Krabbenkreek', bimonthly measurements were planned. The 'Krabbenkreek' (Fig. 1A en Fig. 37) between Tholen and St. Philipsland was chosen because of its representivity for the Eastern Scheldt; 60% of the area is covered by mud flats and 15% by marshes. It drains via two creeks. A large creek near Tholen transports $(13 \text{ to } 23) \times 10^6 \text{ m}^3$ water per tide and a small one near St. Philipsland transports $(1 \text{ to } 2) \times 10^6 \text{ m}^3$ water per tide depending

Fig. 37. Krabbenkreek area



on the vertical tide. So with a few sampling stations (A, B and C, Fig. 37) the whole enclosed area is covered. The bimonthly measurements last two tidal cycles i.e. 27 hours. During this 27 hours, waterflow velocity, temperature and water depth is continuously recorded at stations A and B. Sampling of water is done every half hour. At station C the sampling is done hourly. Samples are analysed in the laboratory for NO_2^- , NO_3^- , NH_4^+ , ortho PO_4^{3-} , total P, dissolved organic N, particulate N, total free amino acids, silicate, chloride, particulate organic C, dissolved organic C, chlorophyll a and natural fluorescence. Fluxes of material can be calculated by multiplying concentrations with the exchanged masses of water at that moment and to summate this values over a tide period (Table 20). Due to the release of river water at the Volkerak sluices mixing of fresh water and seawater occurs in the 'Keeten-Krammer-Volkerak', this mixing is to trace in the fluctuation of the specific substances in the tidal water (Fig. 38a and b and 39).

The bimonthly measurements are to be continued for at least one year.

Table 20. Tidal fluxes of materials at Station B in the Krabbenkreek on 10 and October, 1979.

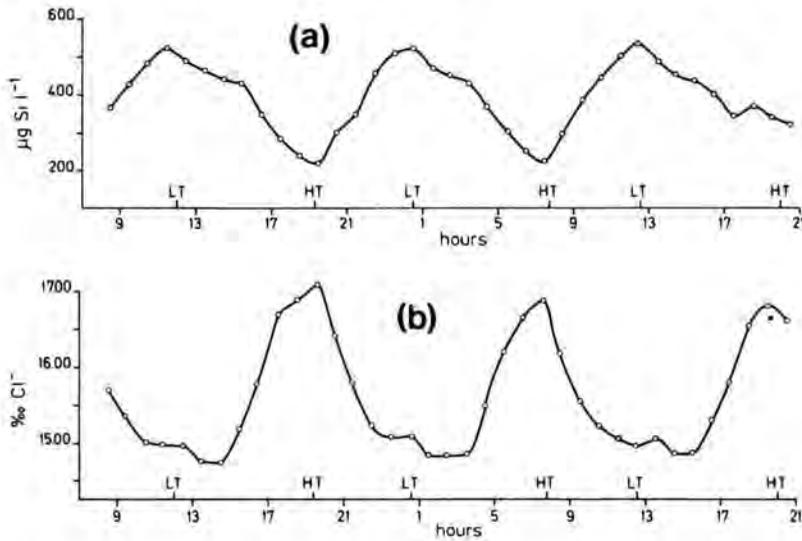
	Import I	Export I	Import II	Export II
Cl^- (10^9 g)	296.47	290.28	290.70	249.53
DOC (10^6 g)	44.08	47.17	44.72	37.35
POC (10^6 g)	14.24	11.04	10.24	8.18
P_{tot} (10^6 g)	3.49	2.88	3.21	2.75
ortho PO_4^{3-} (10^6 g)	2.17	2.06	2.31	2.03
Silicate (10^6 g)	6.60	6.56	6.75	6.22
N-NH_3 (10^6 g)	4.70	4.66	4.57	3.67
$\text{N-NO}_{2/3}$ (10^6 g)	7.91	8.00	8.81	7.40
Chlorophyll a (10^7 g)	40.83	37.49	40.08	24.44
Volume (10^6 m^3)	18.78	18.31	18.66	16.02

VII.7. HOMOGENISATION TESTS ON TWO PREPARED INTER-CALIBRATION SEDIMENT SAMPLES (A12) (J. Nieuwenhuize, J.M. van Liere and E.K. Duursma)

Within the programme 1979-1980, subsidized for 30% by the Commission of European Communities, sediment and salt-marsh plant samples have been prepared for plutonium analysis by the ITAL-Wageningen and the Laboratoire Géologique-Paris. Where the plutonium concentrations vary between 1 to 38 fCi g^{-1} (dry basis) of 239 , ^{240}Pu and 0.3 to 4.4 fCi g^{-1} ^{238}Pu , it is essential to control the applied techniques with an intercalibration test.

Two sediment samples, one organic rich SD-N-1 (Western Scheldt) and one organic poor SDN-2 (Eastern Scheldt) were prepared. A quantity of 150 kg each was dried at 80°C and grinded in a Beco grinding mill. The homogenisation was carried out in a conventional concrete mixer for 25 hours.

Fig. 38. a. Silicate and b. chloride at station B on 10 and 11 October 1979. HT: High Tide, LT: Low Tide



On 12 subsamples, taken at random, various parameters have been determined; Table 21. Additionally ^{137}Cs and ^{60}Co measurements on 6 subsamples were carried out by Dr. J.M. Martin (Paris).

The results show that for the major components like CaCO_3 the homogeneity is for both samples within a standard deviation of 2%. For other components it depends on the concentration.

VII.8. THE BOOK 'CHANGING DELTA WATERS' (A15) (R. Peelen)

The work on the book 'Changing Delta Waters' is in progress. Various meetings of authors were held, also mutual of the chapters or pieces of it. The manuscripts

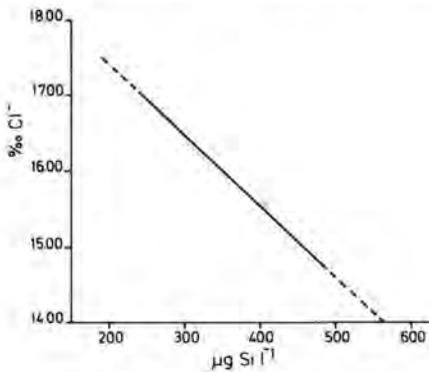


Fig. 39. Correlation of chloride and silicate at station B on 10 and 11 October 1979, 36 observations,
 $X = -107.8 Y + 2075$
 $r = -0.907$ $tr = 12.77$
 $P < 0.001$

of the first chapter are completed. The photographer is very busy to collect and prepare the illustrations of the book.

VII.9. AN EXPLOITED POPULATION OF THE ANNELID, *Nereis virens*, IN THE EASTERN SCHELDT: REPRODUCTIVE BIOLOGY AND ARTIFICIAL BREEDING (M. Lehmann and A.E. Fischer, University of Cologne, Germany)

Problems

Nereis virens is a particularly large annelid with weights sometimes exceeding 30 g. It is therefore an excellent subject for biochemical research on this phylum (projects in work at the University of Cologne, supported by Deutsche Forschungsgemeinschaft) and suited for fisherman's bait. A very dense population is exploited as a source of additional income by local inhabitants in the Eastern Scheldt close to Yerseke. Density of polychaete infauna in Wadden Sea biotopes is known to be controlled mainly by predation (Reise 1978). The reasons for the unusually high density of *Nereis virens* in the Eastern Scheldt are unknown like other facets of the life cycle of the species. Knowledge of unknowns such as habitat of newly settled worms, longevity, commercial importance and possibilities for effective aquaculture are therefore very desirable. This is particularly true due to the impending closure of the Eastern Scheldt and the remodeling of the biocenosis which is expected. With our experience on nereid cultivation as a prerequisite, we have successfully bred this important species in 1978 and 1979 at Yerseke in close cooperation with the Delta Institute, the help of which is appreciated.

Table 21. Homogeneity test of two intercalibration samples SD-N-1 and SD-N-2 (now with Dr. R. Fukai, IAEA, Monaco). K, ^{137}Cs and ^{60}Co determined by Dr. J.M. Martin, Paris.

No samples	Determined compounds	SD-N-1 (Western Scheldt)			SD-N-2 (Eastern Scheldt)		
		average	st. dev.	% dev.	average	st. dev.	% dev.
12	organic carbon (%)	3.50	0.068	1.9	0.098	0.007	7.1
"	organic nitrogen (%)	0.0207	0.0037	1.9	detec. limit		
"	CaCO_3 (%)	13.03	0.25	1.9	1.84	0.030	1.6
"	humidity (%)	0.89	0.067	7.5	0.12	0.016	13.3
8	total alpha (p Ci g $^{-1}$)	21.9	1.2	5.7	1.6	0.492	30.8
6	K (%)	1.71	0.09	5.3	-	-	-
"	^{137}Cs (p Ci g $^{-1}$)	0.35	0.05	14.3	-	-	-
"	^{60}Co (p Ci g $^{-1}$)	0.44	0.05	11.4	-	-	-

Results

We have estimated that a total of 1.2 tons of *Nereis virens* were collected as bait worms during one year at Yerseke. Spawning in both years occurred during the third week of April exclusively, with only the males leaving their burrows and swarming. Temperature and day length were the same at both occasions, however, the lunar phases were different. Starting with sexually mature worms, dug at their habitat, artificial insemination, culture of embryos and maintenance of larvae were performed in a manner similar to that described for a different nereid (Hauenschild and Fischer, 1969). Embryos and larvae, though exposed to temperatures as low as 8°C in the natural habitat, did survive nicely at 19°C. After two weeks of development at 19°C, the larvae settled and did so without conceivable preference for particular substrate, but preferred furrows or ridges to spin a small web as a home. Beginning at a stage of three body segments, the tiny worms fed on benthic phytoflagellates and commercial aquarium fish food for two months. Finally, the worms were allowed to live in two types of sediments with running seawater from the sea. Both groups, those living in sand and gravel as well as those living in plastic granulate, started growth. The worms were fed during an artificial low tide in order to avoid floating and loss of food particles. The worms readily fed on a variety of food, including seaweed, mussels, commercial fish food and diatom films covering the sediment. However, the worms stopped feeding when exposed to winter water temperatures. Under these conditions, one group of worms attained weights of 2-14 g (average 7.4 g) in 19 months, with no cannibalism occurring in spite of large size differences. This result gives an idea of the time needed for breeding *Nereis virens* at natural seawater temperatures. The length of the life cycle is still unknown. However, since we know that, after one

year of gametogenesis, the life of *Nereis virens* ends with the first spawning, and since at present no gametocytes were detected in the 19 months old worms, life span at least exceeds two years (cf. Bass and Brafield 1972, and Snow and Marsden 1974).

Conclusions

Nereis virens larvae may develop at a considerably wide range of temperatures and will settle on a wide variety of substrates. The life cycle is now better understood, but still we cannot explain what parameter(s) allows the particularly high densities of *Nereis virens* in the Eastern Scheldt. Young worms will grow under laboratory conditions with a variety of foods and without loss by cannibalism. Several parameters of the culture methods employed so far are considered to be open for improvement. Artificial breeding of *Nereis virens* for commercial purposes seems worth considering.

References

- Bass, N.R. and Brafield, A.E. - 1972. The life-cycle of the polychaete *Nereis virens*. J. mar. biol. Ass. U.K. 52, 701-726.
- Hauenschild, C. and Fischer, A. - 1969. *Platynereis dumerilii*. Mikroskopische Anatomie, Fortpflanzung, Entwicklung. Großes Zool. Prakt. Heft 10 b, G. Fischer Verlag.
- Inamori, Y. and Kurihara, Y. - 1979. Analysis of the environmental factors affecting the life of the brackish water polychaete *Neanthes japonica*. Part 1-5. Bull. mar. biol. station Asamushi 16(3), 87-132.
- Reise, K. - 1978. Experiments on epibenthic predation in the Wadden Sea. Helg. wiss. Meeresunters. 31, 55-101.

Snow, D.R. and Marsden, J.R. - 1974. Life cycle, weight and possible age distribution in a population of *Nereis virens* (Sars) from New Brunswick. *J. nat. hist.* 8, 513-527.

Wolff, W.J. - 1973. The estuary as a habitat. *Zool. Verh. Leiden* nr. 126.

VII.10. A CHEMICAL METHOD TO PREVENT HEAVY SETTLEMENT OF MUSSELS IN A COOLING WATER SYSTEM (J.W. Rijstenbil and L. de Wolf)

The production processes of Hoechst Holland NV at Vlissingen are conditioned by marine cooling water from the Western Scheldt. In former years a heavy settlement of barnacles and mussels *Mytilus edulis* occurred in the cooling system. The applicability of acroleine as a precaution against mussel settlement in thick agglomerates, was investigated in the laboratory. Several concentrations of this biocide were tested in running and standing sea water. In particular sublethal doses of acroleine on small mussels (1 mm to 2.5 mm) were used. These small mussels originated from the seaweeds (Bayne, 1964), i.e. the growth stage before final settlement. It was observed that an 8 h dosage of 0.6 ppm acroleine, followed by a resting period of 16 h, gave the best results; 60 to 70 percent of the mussels detached from their substrate, being alive though. Extended exposition to this dosage may kill the mussels. In a 24 h period a LD⁵⁰ of 0.5 ppm acroleine was observed for nauplii of brine shrimps *Artemia salina*. In the summer of 1979 a monitoring system for mussel control was constructed in the factory. Five iron tubes with diameters ranging from 50 mm to 150 mm, were connected, carrying 0.6 ppm acroleine 8 h a day with velocities of 1.6-0.7-0.4 and 0.2 m s⁻¹, respectively, into an 80 l vessel with con-

crete walls. Mussel settlement reached a maximum in summer. No growth was observed in the 50 mm and 80 mm tubes. In the 100 mm tube 0.45 percent of the inner area was covered with mussels after 6 months of growth. During the same period 1.09 percent of the inner area of the 80 l container was covered with mussels. Therefore mussel agglomerates may be expected in quiet parts of the cooling system (Fraser Ross, 1965). Although heavy settlement can be suppressed using acrolein (Walko, 1971), mechanical cleaning will still be necessary after some time. Acroleine breaks down rather fast after discharge into sea; a concentration halftime of 19 h was measured.

References

Bayne, B.L. - 1964. Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). *J. Anim. Ecol.* 33, 513-523.

Fraser Ross, F. - 1965. The control of mussels in sea water cooling systems. *Trav. Cent. Rech. Etud. Océanogr.* VI, 437-439.

Walko, J.F. - 1971. Biological control in cooling systems, new developments and pollution considerations. *Proc. 32nd Int. Water Conf.*, 47-53.

VII.11. A TIDE SIMULATION APPARATUS (F.W. Melissen)

To study the influence of water movement, for instance on salt-marsh plants (van Soelen and Melissen, 1979), an electronic-mechanical system has been developed to simulate natural and artificial tide fluctuations. With the tide fluctuations time, high and low 'tide' and water in- and output are controlled by an electronic unit.

The equipment (Fig. 40) is made in a manner that

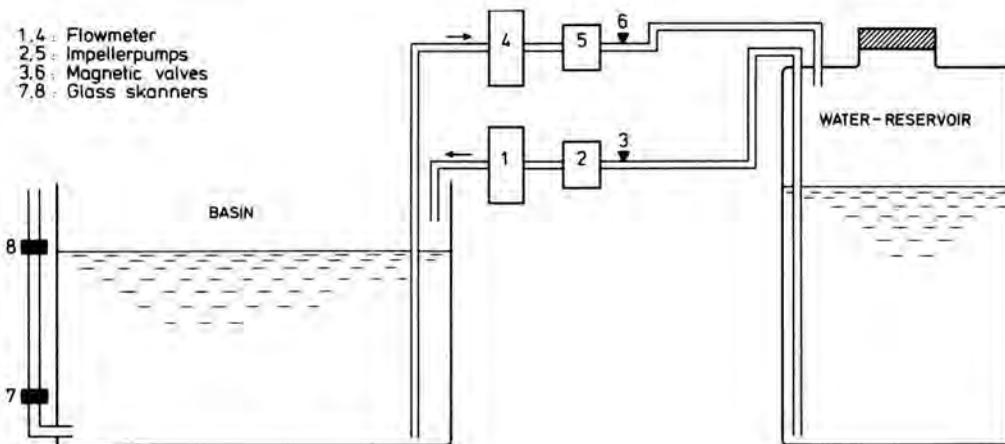


Fig. 40. Scheme of pilot plant tide basin

the speed regulated Flotec impellerpumps transfer the water from the reservoir into the basin to a maximum level (flood), in a programmed time. After a programmable stagnant period the water is pumped out to a minimum level (ebb). When the control-unit was developed (Fig. 41) only a few time-switch micro-processors, the TMS 1000 from Velleman and the National MM57160, with a minimum of possibilities were available. An electronic-mechanical time switch with a day and week cycle had better results and was placed in the unit. When the maximum or minimum level is reached, photoelectric sensors give a signal to the Skan-a-matic T40300 amplifiers that switch off the pumps.

An alarm unit (Birkhoff, 1974) switches off, when set-high level is reached, to prevent overflow and any subsequent damage.

References

Birkhoff, W. - 1974. R.B. vochtindikator. Radio Bulletin 43, 339-441.

Soelen, J. van and F.W. Melissen - 1979. A tide simulation apparatus. In: E.K. Duursma (ed.) Progress Report DIHO 1978, Verh. KNAW, Nat., 2e reeks 73, 185-186.

VII.12. ELECTRONIC EQUIPMENT FOR A MICROMETEOROLOGICAL STATION (F.W. Melissen)

For micrometeorological measurements in a salt-marsh vegetation, electronic equipment has been developed with a minimum of accessories. The station (5 m high), standing on the mudflats near Bergen op Zoom, can

withstand floods up to 2.5 meters. A power supply in the neighbourhood of the station is not available, so it has its own supply consisting of 12V rechargeable batteries. The equipment was so constructed that a minimum of power is consumed.

The blockscheme and wiring diagram of the most important instruments are shown in Fig. 42. The instruments built are: 4 temperature meters, accuracy better than 99.5%, a frequency-voltage-converter (Dirksen, 1972) for an Ott water-velocitymeter, a power supply precision 2 mV (Anon, 1977) for a Vaisala humidity probe, switch boxes for Thies wind-direction and windspeed instruments and for a LiCor and a Macam quantum photometer.

It is quite difficult to develop a depth measuring instrument with an accuracy of 1 cm, unless the maximum depth of 2 meters of water is reached. Nevertheless in 1980 an instrument will be realised with a National pressure transducer which gives a signal of 1000 mV over 1 meter of water.

All the signals of the total equipment are recorded on a 12 channel Linseis recorder which will be replaced by a Solarton data logger. The total power consumption is less than 40VA. The possibility of recharging the batteries near the station with solar cells or a wind generator is studied.

References

Anonymus - 1977. Zener voeding. Electuur 17, 55 only.

Dirksen - 1977. Een frequentiemeter voor zelfbouw. Meetinstrumenten voor zelfbouw, Muiderkring, Bussum, 152 pp.

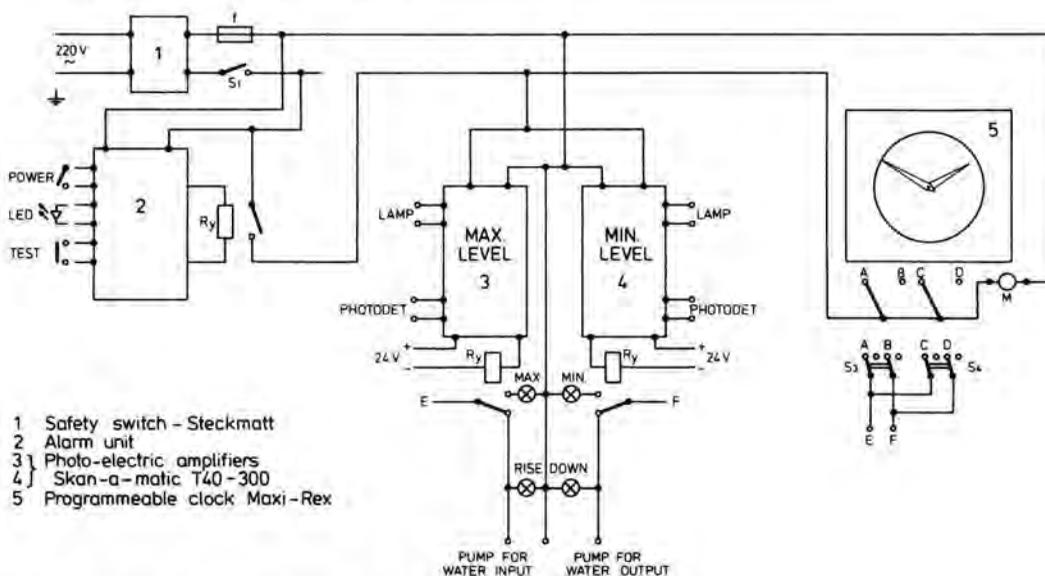
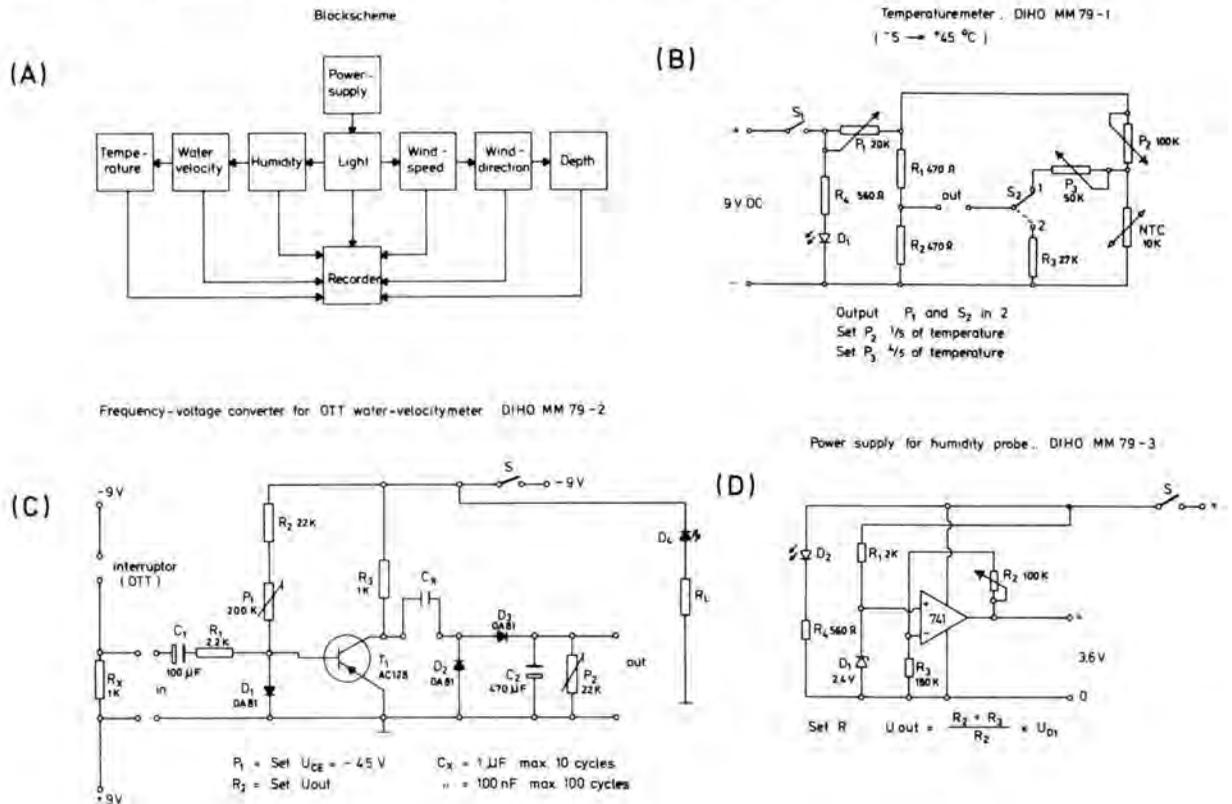


Fig. 41. Blockscheme of control-unit of pilot plant tide basin

Fig. 42. (A) Blockscheme, (B) Temperature meter, (C) frequency-voltage converter and (D) power supply of electronic equipment for a micrometeorological station



VIII. Articles submitted for publication or in press

(Δ -181 = publication number of the Delta Institute)

Beeftink, W.G. - Types of natural and man-made disturbances in salt-marsh communities. Proc. Symp. Working Groups for Data-Processing and Succession Research, Montpellier, September 1980. (Δ -193).

Beeftink, W.G., M.C. Daane, W. de Munck and J. Nieuwenhuize - Vegetation dynamics in salt marshes as a consequence of some traditional cultural practices of man. *Phytocoenosis* (Warschau). (Δ -165).

Daemen, E.A.M.J. - De rijke diatomeeënflora van het Grevelingenmeer. *Natura*.

Duursma, E.K. - Remarks on river inputs and other pathways of organic materials in the coastal zone, SCOR Workshop Rome.

Duursma, E.K. - The biogeochemical cycle of lead in the oceans. In 'The Health of the Oceans' GESAMP Working group (1978) Copenhagen.

Duursma, E.K. - Techniques and methods of interfacial transfer and transport processes in the sediment-water layer. CEC-IAEA Joint Techn. Rep., Ispra/Vienna.

Duursma, E.K. and R. Dawson (eds.) - Organic Chemistry of Sea Water. Elsevier Sc. Publ. Co., Amsterdam.

Duursma, E.K. and M. Smies - 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. (Δ -188).

Huiskes, A.H.L. - The effects of habitat perturbation on populations of *Ammophila arenaria*. *Acta Bot. Neerl.* (Δ -186).

Huiskes, A.H.L., W. de Munck, P. Paalvast and H.

Schat - Some aspects of the population dynamics of *Salicornia* spp. Acta Bot. Neerl.

Kortekaas, W.M., D. Lausi, W.G. Beeftink and E. van der Maarel - Survey of salt-marsh relevés included in the data bank of the working group for Data-processing. In: E. van der Maarel, L. Orloci and S. Pignatti (eds.). Data-processing in phytosociology. Vegetatio, 195.

Krebs, B.P.M. - *Microchironomus deribae* (Freeman, 1957) (Diptera, Chironomidae) in the Delta region of the Netherlands. Hydrobiol. Bull. 13 (Δ-182).

Lindeboom, H. - Nitrogen fixation SA)AR (in press). Nitrogen fixation in algal mats on Marion Island (sub-Antarctic).

Lindeboom, H. - Uric acid degradation SA)AR (1980). The chemistry and microbiology of the aerobic degradation of uric acid in a penguin rookery and the influence there of the Marion Island ecosystem.

Lindeboom, H. - N-cycle in a penguin rookery (in prep.). The nitrogen pathway in a penguin rookery on Marion Island (sub-Antarctic).

Merks, A.G.A. - Application of an automated method for dissolved sulphate analysis. Mar. Chem. (Δ-200).

Merks, A.G.A. and J.W. Rijstenbil - Saline seepage and vertical distribution of oxygen in a brackish ditch. Estuar. Coast. Mar. Sc.

Nienhuis, P.H. - The eelgrass (*Zostera marina*.) subsystem in brackish Lake Grevelingen: production and decomposition of organic matter. Ophelia.

Nienhuis, P.H. - The epilithic algal vegetation of the SW-Netherlands. Nova Hedwigia 32 (1980).

Nienhuis, P.H. - Organic matter in living marine organisms. In: E.K. Duursma en R. Dawson (eds.). Organic chemistry of sea water. Elsevier Publ. Comp. Amsterdam.

Nienhuis, P.H. - Production and growth dynamics of eelgrass (*Zostera marina* L.) in brackish Lake Grevelingen (The Netherlands). Neth. J. Sea Res.

Nieuwenhuize, J., J.M. van Liere and A.G. Vlasblom - Een bodemkaart van de Grevelingen. DIHO Rapp. en Versl.

Pugh, G.J.F. and W.G. Beeftink - Fungi in coastal and inland salt marshes. Bot. Marina.

Rijstenbil, J.W. and A.G.A. Merks - Micro algae and oxygen in a brackish ditch. Estuar. Coast. Mar. Sci.

Smies, M. - Environmental impact of tsetse control/eradication operations. Trop. Pest Manage.

Smies, M. - Aquatic micro-ecosystems in environmental toxicology: a critical appraisal. Ecotoxicol. Environ. Safety.

Smies, M., R.H.J. Evers, F.H.M. Peijnenburg and J.H. Koeman - Environmental aspects of field trials with pyrethroids to eradicate tsetse fly in Nigeria. Ecotoxicol. Environ. Safety.

Smies, M. and J.H. Koeman - The effects of tsetse fly control measures on birds in West Africa. Proc. XVII Congr. Intern. Ornithol.

Wolff, W.J., M.J. van Eeden and E. Lammens - Primary production and transport of particulate organic matter between a salt marsh and the adjacent estuary in the Netherlands. Neth. J. Sea Res.

IX. Published articles and reports in 1979

(Δ-176 = publication number of the Delta Institute)

IX.1. WORKING GROUP 'ELEMENTS CYCLING AND FOOD CHAINS'

Bakker, C. - 1979. Water en plankton van de Oosterschelde. Zeeuw Nieuws. Natuur, Landschap en Milieu 5:34-38. The article provides a short survey of the rich plankton community of the Eastern Scheldt. A schematic food web is given of primary, secondary and tertiary producers. The necessity is emphasized of complete conservation of the great natural values of this large marine tidal area.

Bakker, C. and F. Vegter - 1978. General tendencies

of phyto- and zooplankton development in two closed estuaries (Lake Veere and Lake Grevelingen) in relation to an open estuary (Eastern Scheldt). S.W. Netherlands. Hydrobiol. Bull. 12, 226-245 (Δ-176).

The main lines of plankton development in the closed estuaries Lake Veere and Lake Grevelingen (S.W. Netherlands) are discussed in comparison with the original marine tidal system.

Strong salinity fluctuations are characteristic for Lake Veere, including an instable environment with high primary and secondary productivity levels of a few adapted species. In spite of slowly declining salinities (still within the polyhaline range) environment of Lake Grevelingen has been far more stable than Lake Veere. Species numbers were also reduced but remained larger than in Lake Veere.

In early spring a shift of dominance could be demonstrated in both lakes from diatoms to crypto-

monad flagellates, coupled with mass development of rotifers of the genus *Synchaeta*. Primary production starts earlier in the lakes than in the tidal estuary as a consequence of increased transparencies.

Average annual primary production of Lake Grevelingen amounts to approx. 170 g C m^{-2} , reaching the same value as the former estuary. Annual primary production of Lake Veere increased, compared with the tidal estuary, varying from $250\text{-}360 \text{ g C m}^{-2}$ during the years 1969-71.

In relation to zooplankton biomass the dominance of a number of copepod species in the tidal estuary was taken over by rotifers, polychaete larvae and one copepod in the brackish lakes. Zooplankton biomass reached highest levels in Lake Veere; biomass values of Lake Grevelingen were generally higher than in the tidal estuary.

The pelagic zone of the brackish lakes show a simplification of the original foodweb structure.

An important goal of the environmental accompaniment of the Delta projects is to reach or to maintain relatively stable ecosystems. When in a closed sea arm salinity can be maintained as high as possible without strong fluctuations, it appears that species richness may remain rather high and productivity levels are not forced to increase strongly. Lake Grevelingen represents a good example of such a development, Lake Veere does not.

Daemen, E.A.M.J. - 1979. Verspreiding en biomassa van *Zostera marina* L. en *Zostera noltii* Hornem. in de Oosterschelde. DIHO Stud. Rep. D6-1979, 44 pp.

Between June and September 1977 standing crop and production of seagrasses were studied in the Oosterschelde, a branch of the large Rhine-Meuse estuarine system in the SW-Netherlands.

The results were compared with data obtained from the Grevelingen, another estuarine branch closed off from the North Sea in 1971 and being stagnant since. Such a comparison is valuable because the situation in the Grevelingen before the closure resembles the actual situation in the Oosterschelde, so the latter can serve as a model for the Grevelingen before 1971.

Since there are data available about standing crop and production of eelgrass in Lake Grevelingen in 1973 and 1975 it is possible to compare primary production and standing crop of seagrasses in a salt water lake and a tidal environment.

Annual production was calculated by doubling the maximum standing crop, though it was realised that this procedure can only result into a rough approximation. For *Zostera marina* the total production of both above- and underground parts was $0.29 \text{ g C m}^{-2} \text{ yr}^{-1}$. This value was calculated for the part of the Oosterschelde east of the Zeelandbrug (Fig. 1).

Nienhuis and De Bree (1977) calculated for *Zostera marina* in the Grevelingen $4.3 \text{ g C m}^{-2} \text{ yr}^{-1}$ in 1968,

$17.8 \text{ g C m}^{-2} \text{ yr}^{-1}$ in 1973 and $23.4 \text{ g C m}^{-2} \text{ yr}^{-1}$ in 1975. This means that after the closure the production of *Zostera marina* has increased considerably.

For *Zostera noltii* the total production in the Oosterschelde was $0.98 \text{ g C m}^{-2} \text{ yr}^{-1}$ (in 1977).

There are hardly any data available on *Zostera noltii* in the Grevelingen; it almost disappeared within a few years after the closure. Apparently *Zostera noltii* cannot live in a permanently submerged environment.

Plans are in progress for building a perforated dam in the mouth of the Oosterschelde, that can be closed during storms. It is obvious that this dam will influence the tidal range and therefore the seagrass community. Therefore it is advocated to survey the *Zostera* beds again, a few years after completion of this dam, to ascertain its influence on this vegetation.

Doornbos, G. - 1979. Winter food habits of Smew (*Mergus albellus* L.) on Lake Yssel, the Netherlands: species and size selection in relation to fish stocks. Ardea 67, 42-48.

From January through March 1977 a study was made of the numbers, activity, food habits and condition of Smew wintering in the southwest part of Lake Yssel. In this survey the results will be given of the stomach and gullet analyses of 21 Smew shot in this period.

The food of Smew in Lake Yssel is composed almost entirely of fish. Only the crustacean *Asellus aquaticus* may be collected actively during food-seeking by some Smew. The fish remains were identified from study of otoliths and pharyngeal-bones (the latter being useful aids in the Cyprinidae). By making correlation diagrams between length of otolith (resp. pharyngeal-bone) and length and weight of the most frequent fish species of Lake Yssel it was possible to assess the species taken, and the lengths and weights of the fish. The composition of the fish taken by Smew was compared with the supply in Lake Yssel, estimated by surveys by the Rijksinstituut voor Visserijonderzoek. With these data it was also possible to investigate size preference. In Lake Yssel (inclusive Oostvaardersdijep and Markermeer) Smew eat almost entirely smelt (*Osmerus eperlanus*), with some perch (*Perca fluviatilis*). Remains of pike-perch (*Lucioperca lucioperca*) were found too. Neither smelt nor pike-perch were previously known as food species for Smew. Ruffe (*Acerina cernua*), the most frequent species (on a weight basis) in Lake Yssel is avoided. In areas with little or no smelt, perch is most often eaten and other species in the sequence ruffe, pike-perch, roach (*Rutilus rutilus*) and pike (*Esox lucius*). Of the fish taken, 67% belong to the length-classes 5 and 6 cm. Fishes longer than 11 cm are rarely taken. However, remains of two pike-perch of 17 and 18 cm were found. There does not appear to be an active selection for size with regard to smelt and ruffe, the

size distribution of the fish taken being similar to the population composition in Lake Yssel, but of perch only the smallest individuals (yearclass 0) are eaten. The equation of Nilsson and Nilsson is used to estimate the total fish consumption by Smew in Lake Yssel. From January through March 1977 Smew will have eaten about 37 tons of fish, including 28 (76%) tons of smelt. In the entire winter period 1976/1977 they may have consumed about 45-50 tons of fish (including 34-38 tons of smelt) from Lake Yssel.

The total smelt consumption by Smew will be less than 1% of the winter population of smelt in Lake Yssel. The Smew accounts for 17 and 11%, respectively, of the numbers and biomass of the major fish-eating birds on Lake Yssel in January 1977.

Because of the relative ease with which fish stocks can be followed and the accuracy attained in the bird census programme, Lake Yssel presents an extremely favourable opportunity for a complete assessment of the impact of the bird community on the food base. I see my work as a pilot study and hope a more comprehensive programme will be forthcoming.

Elgershuizen, J.H.B.W., C. Bakker and P.H. Nienhuis - 1979. Inventarisatie van aquatische planten en dieren in de Oosterschelde. DIHO Rapp. en Versl. 1979-3.

Within the Eastern Scheldt Research being done by the Delta Institute for Hydrobiological Research a survey was made of aquatic plant-animal species found in the Eastern Scheldt and all the literature about them. 417 Plant species divided into 14 groups and 1046 divided into 53 groups were found in the water resp. on or in the underwater substrate of the Eastern Scheldt during the last 60 years.

Most of the research done about these plants and animals in the Eastern Scheldt had a qualitative and a few a quantitative character. The total number of relevant publications is some more than 400 and are put in this report.

Graaf, U.H. de - 1979. Enkele vissoorten in de ondiepe delen van het Grevelingenmeer: een schatting van hun aantal, biomassa en productie. DIHO Rapp. en Versl. 1979-1, 49 pp.

Abundance, growth, biomass and production were estimated for *Pomatoschistus microps*, *Pomatoschistus minutus* and *Gasterosteus aculeatus*. Estimates were derived from a sampling program carried out in the shallow waters of Lake Grevelingen from May until the end of November 1977. Besides the species mentioned, four other small fish species were found but they did not occur frequently enough to include them in the investigation.

During the summer the numbers of *P. microps* and *P. minutus* increased until August/September. At the

end of July juvenile gobies appeared in the catches in great numbers. Low numbers of gobies were found in spring and again in autumn. *P. minutus* and probably also *P. microps* disappeared from the shallow waters and migrated to deeper parts of the lake when the water temperature dropped in October and November. On the whole *P. microps* was more abundant than *P. minutus*.

Sticklebacks, *G. aculeatus*, were numerous in the catches throughout the sampling months. In summer, however, their numbers were more evenly dispersed over the whole lake, while in autumn these fishes were almost exclusively found in the *Zostera*-fields. Their number was highest in June and again in October; however, biomass in October was only half the biomass in June, because in autumn almost all *G. aculeatus* in the catches were newly recruited ones. Production between May and November was for *P. microps* 1.2 g fresh weight m^{-2} , in the same period for *P. minutus* 0.18 g m^{-2} , and for *G. aculeatus* 1.84 g m^{-2} .

Iwaarden, A.J.W. van - 1979. Micro-autografische bepaling van de primaire productie van verschillende fytoplanktonsoorten tijdens de voorjaarsbloeï (1979) in het Grevelingenmeer. DIHO Stud. Rep. D9-1979, 84 pp.

In studying the production of phytoplankton, the ^{14}C -method is used generally to determine the total primary productivity. A disadvantage of this method is, that one cannot distinguish different primary production rates of individual phytoplankton species. A direct estimation of primary production rates of individual species is possible through micro-autoradiography of individual radioactive phytoplankton cells after incubation with ^{14}C (Brock and Brock, 1968, Knoechel and Kalff, 1976a).

During the period February-April 1979 the relative contribution of individual phytoplankton species to primary production of Lake Grevelingen was investigated autoradiographically. The results showed that only a few species contributed significantly to community primary production, while many other species were not productive at all. The major contributors to the primary production were the diatoms *Thalassiosira* spp., *Detonula* sp., *Skeletonema costatum* and *Nitzschia closterium* and some flagellates, mainly *Cryptomonas* sp. On the other hand diatom-species like *Navicula* spp., *Licmophora* sp., *Achnantes* spp., *Cocconeis* spp., *Diploneis* spp., *Chaetoceros* spp., *Nitzschia* spp. and *Coscinodiscus* spp. did not show any photosynthetic activity. During the periods of high primary productivity *Thalassiosira* spp. was one of the most important producers. This species was photosynthetically active during

the whole period February-April, as were the flagellates. By contrast, *Detonula* sp., *Skeletonema costatum* and *Nitzschia closterium* contributed only in February and the beginning of March.

Kelderman, P. - 1979. De fosfaatbalans van het Grevelingenmeer in de periode mei 1971-december 1977. DIHO Rapp. en Versl. 1979-9, 49 pp.

Phosphate-budgets are presented from May 1971 (closure) till December 1977 in lake Grevelingen. Year-budgets show, that P-increase from 1973-1977 can be attributed to P-mobilisation from the sediment for around 60%. Polder/waste water, rain etc. are responsible for the remaining 40%.

Month-budgets are useful in revealing seasonal trends. From May to August mobilisation of 130-180 tons P was found. Accumulation takes place during the rest of the year. Mobilisation shows significant correlation with water temperature.

Koniuszek, J.W.J. - 1979. Biomassa en productie van macrozoobenthos op de Slikken van Flakkee. DIHO Stud. Rep. D7-1979, 48 pp.

Shallow areas (less than 1.5 m deep) constitute a 30% of the surface of the marine-brackish Lake Grevelingen. Due to technical reasons hitherto few was known about the macrozoobenthos in these areas. This study carried out between March and December 1977 was meant to gather information about biomass and production of the dominant species in the largest shallow area of the lake, the Slikken van Flakkee, a former area of extensive intertidal flats (20 km²). For a biomass survey of the whole area along three transects 5 corersamples from approx. 30 cm deep and totalling 0.026 m² were taken every 150 m. The total number of sampling stations amounted to 47. A mean biomass of 26.5 g ash-free dry weight (ADW) m⁻² was found. Small polychaetes like the numerous *Pygospio elegans* and *Polydora* sp. have not been included. Molluscs, especially *Cerastoderma edule*, *Mytilus edulis* and to a lesser extent *Angulus tenuis* formed the most important group with 82.2% of the total biomass. Polychaetes, including as most important species *Arenicola marina*, *Nephtys hombergii* and *Scoloplos armiger* constitute 15.6% of the total biomass and some smaller crustaceans the remaining 2.2%. The mean biomass increased from the eastern (13 g ADW m⁻²) to the western transect (50 g), almost fully due to an increase of mollusc biomass. Total number of species was at least 27: some were determined only to the family level and some smaller polychaetes were not included. In water of less than 30 cm only crustaceans occurred. This group increased in biomass till 80 cm and decreased thereafter. Within the range of 0-1.5 m *Cerastoderma* increased signifi-

cantly with depth. No relation was found for *Arenicola* and *Angulus*.

Patterns of numbers and biomass were followed on three permanent stations with a mean waterdepth of 0.40 (A), 0.75 (B) and 1.05 m (C). They differed also in *Zostera marina* cover. Between April 24 and October 15 stations were sampled five times. On each date 35 times three cores of in total 0.01545 m² have been taken. Work was handicapped by the disappearance of the marking stake on station C after the second sampling-date.

Total number of species (with the same restrictions as on the transects) increased to 36. Fauna composition of the stations differed to a certain extent. Number and biomass patterns have been discussed for the most important species. Production calculations have only been possible for *Cerastoderma edule* and *Angulus tenuis*. Production in the research period amounted for *Cerastoderma* to 3.94 and 8.12 g ADW m⁻² on station A and B (P/B_{April} respectively 0.67 and 1.29) and for *Angulus* 0.72 and 2.54 g (P/B_{April} 0.23 and 1.41) on station B and C respectively. Under certain assumptions for *Arenicola marina* a production of near 2 g ADW m⁻² was calculated as a mean for the whole Slikken van Flakkee. Although mean biomass of *Mytilus edulis* was rather high, numbers were too small to permit production calculations.

If it is assumed that three stations in this area before the closure of the Grevelingen estuary in 1971 were representative for the intertidal Slikken van Flakkee, then the biomass of the most important species has decreased from 41 till 21 g ADW m⁻². Present biomass is in the same range as on the intertidal flats of the Dutch Wadden Sea. Production of the same species decreased from 40 to 12 g. Only *Angulus tenuis* has increased since the Grevelingen closure. Causes of these differences are discussed.

Nienhuis, P.H. - 1979. Ecology and production of eelgrass in the former Grevelingen estuary, an evolving saline lake in the SW Netherlands. In: Ecological processes in coastal environments; Edited by R.L. Jefferies and A.J. Davy. Oxford, Blackwell Scientific Publ., p. 650.

The Grevelingen estuary was cut off from the North Sea and from the influences of the river Rhine by a dam in 1971, and became a stagnant saltwater lake. The production and the ecology of *Zostera marina* L. were studied in 1968 and in 1973-76. Standing crop estimations were made and biomass changes in permanent quadrats recorded. Correlations of the distribution patterns of this species with ecological factors also were attempted. After the closure of the estuary the area occupied and the density of the eelgrass beds increased strongly.

Eelgrass annual above-ground production, based on doubled maximum standing crop values in July-August, was estimated at 50 g C m^{-2} in 1968, 121 g C m^{-2} in 1973 and 91 g C m^{-2} in 1975 in *Zostera* beds.

Nienhuis, P.H. - 1979. Unieke zeegrasvelden in het Grevelingenmeer. *Natura* 76, 189-193.

After the closure of the Grevelingen estuary in 1971 an enormous increase occurred in biomass and production of *Zostera marina*. The role of eelgrass in the brackish Lake Grevelingen ecosystem is discussed. Eelgrass provides food for birds and crustaceans and it gives shelter for a variety of invertebrates and fishes. The development of the eelgrass population in Lake Grevelingen has been compared with the situation in the Waddensea, where before 1930 large stretches of eelgrass occurred. The Waddensea population was struck by the wasting disease in the early thirties and it has never restored since then.

Nienhuis, P.H. - 1979. Landschap, flora en fauna van de zeedijken rond de Oosterschelde. *Zeeuws Nieuws Natuur, Landschap en Milieu* 5:42-44.

Man-made sea walls covered with stones form a very characteristic type of landscape along the Eastern Scheldt. On these sea walls a number of specific habitats can be recognized. On the landward side a vegetation of epilithic lichens, an extensive algal zonation and a number of littoral invertebrates is conspicuous. A popular description of these habitats, together with the species living there, is given.

Nienhuis, P.H. - 1978. Dynamics of benthic algal vegetation and environment in Dutch estuarine salt marshes, studied by means of permanent quadrats. *Vegetatio* 38, 103-112, (Δ -163).

Knowledge of the spatial structure of benthic algal vegetations in salt marshes, consisting of about 100 species of blue-green, green, brown and red algae, formed the basis of a detailed study into the dynamics of the algal mat.

The temporal changes in algal vegetations and concomitant processes in their environment were studied in 27 permanent quadrats (PQs), plotted in tidal salt marshes and along brackish inland waters in the SW Netherlands. Once a month vegetation and environment of the PQs were examined over the period March 1968-February 1971. From the Spearman rank-correlation between vegetational and environmental parameters it appeared that in semiterrestrial and terrestrial environments the average number of algal species in a relevé and the average similarity between the species composition of successive relevés obtained from one PQ have a significant positive correlation with the stability of the substrate, and hardly any correlation with fluctuations in soil-moisture content and salinity of the soil moisture and with the maximum

cover percentage of the phanerogams.

Stable substrates tend to bear stable algal mats, as appeared from quantitative data. Small and moderate fluctuations in soil-moisture content and salinity of the soil moisture have no limiting influence on the algal cover degree. During periods of extremely high salinity and concomitant extremely low soil-moisture contents that coincide with evaporation surpluses, the green algal mat bleaches and decreases in cover in semi-terrestrial and terrestrial environments. Below 20% the soil-moisture content is limiting for the expansion of a mat of green algae (*Rhizoclonium riparium*, *Percursaria percursa*, *Enteromorpha tortia*, *E. prolifera*) and *Vaucheria* species. The mat desiccates and bleaches but recovers after increase of the soil-moisture content above 20% within one month. Two weeks of severe frost (temperature down to -10°C) do not influence the cover degree of the algal mat in aquatic brackish PQs, but the cover degree in semi-terrestrial PQs decreases. Short lasting soil-moisture salinities of 130-190 ‰ Cl^- have no limiting effect on the expansion of the algal mat.

Nienhuis, P.H. - 1978. An ecosystem study in Lake Grevelingen, a former estuary in the SW-Netherlands. *Kieler Meeresforsch., Sonderheft* 4, 247-255, (Δ -175).

A working group of the Delta Institute at Yerseke, The Netherlands, studies the ecosystem of saline lake Grevelingen, a former estuary. The theme of the group is the cycling of organic matter in the two phases of the Grevelingen. The pool of particulate organic matter in the Grevelingen estuary ($385 \text{ g C m}^{-2} \text{ yr}^{-1}$) was fed from various sources. The amount of organic carbon from the North Sea, entering the estuary as detritus equalled the in situ primary production. After the closure of the estuary the import of organic matter from the North Sea was completely cut off. Overall yearly production of the phytoplankton was not notably influenced by the closure, notwithstanding the large changes in environmental conditions. The significance of the phyto-benthos production increased considerably. The total amount of organic matter available for consumers, decreased by roughly 40% to a level of $235 \text{ g C m}^{-2} \text{ yr}^{-1}$. In the estuary net production of macrozoobenthos was estimated at $28 \text{ g C m}^{-2} \text{ yr}^{-1}$. This production was almost divided by a factor two after the closure, just as the amount of food available. Changes in the feeding habits of birds may reflect the often less striking changes in the lower parts of the foodchains. Herbivore bird consumption has increased more than 20 fold after the closure. Consumption of zoobenthos showed a threefold decrease whereas the predation by fish-feeding birds increased about a 30 fold after the closure. The change from an estuarine into a stagnant saline ecosystem, bordering the North Sea, resulted in a sharp decrease

in the amount of organic matter available as food, and a shift in the relative significance of predominant species.

Nienhuis, P.H. - 1978. Lake Grevelingen: a case of ecosystem changes in a closed estuary. *Hydrobiol. Bull.* 12, 246-259, (Δ -175).

Grevelingen estuary, formerly in open connection with the rivers Rhine and Meuse and with the North Sea, was closed by a dam in May 1971, excluding influences both from the rivers and from the sea (tides dropped out); brackish Lake Grevelingen originated. A number of changes took place in the environment, on species level, on community level and on ecosystem level.

Changes on species level. The life cycles of certain species were blocked, because their food disappeared, the particle size spectrum of the food changed, or their migration routes were blocked. It was found that 1. species richness decreased; 2. shifts in numbers per species took place; 3. species with broad ecological tolerance remained; 4. brackish water species appeared.

Changes on community level. Structural aspects: a. habitats disappeared; b. pattern diversity decreased; c. habitat-bound species disappeared.

Functional aspects (trophic relations): a. the energy subsidy from the North Sea was blocked; b. the amount of organic matter decreased; c. the production of some primary consumer groups decreased; d. tertiary and some secondary consumers disappeared; e. the food chains shortened.

Changes on ecosystem level. The overall ecosystem simplified, both in structure and in functioning. The impact of the shallow macrophyte dominated subsystem increased.

Pellikaan, G.C. - 1979. Enige aspekten van de decompositie van zeegras. *DIHO-Stud. Rep. D 11-1979*, 91 pp.

It is assumed that the largest part of the yearproduction of eelgrass (*Zostera marina* L) in Lake Grevelingen enters detritus food chains. I define detritus as the non-living plantfragments and the associated micro-organisms. In July 1977 experiments with freshly cut eelgrass in litter bags and enclosures have been started to investigate some aspects of the decomposition. The decomposition rate is 4.2-6.7% of the initial weight per week, and fits in with literature data. After 16 weeks a residue of 3.2% fresh weight remains, being more resistant to break down. In the beginning the dry weight is 13.5% of fresh weight and later on it increases. The ashfree dry weight in the litter bags falls from 80% of dry weight in July to 55% in February. The organic material disappears gradually. In the two enclosures these values fluctuate bet-

ween 56 and 66%. The chlorophyll a and pheophitin content decreased a little after 7 months; it did not appear to be a useful parameter for the vitality of the plant cells of the eelgrass detritus. The POC content is 27-34% of dry weight; values decreased during the decomposition process. C/N and C/P ratios show the same general picture in time: in the beginning a rise could be observed and after 10 weeks a fall. This indicates probably an initial leaching of N- and P-rich compounds, and afterwards a detectable enrichment by micro-organisms which colonize the detritus. According to certain investigators the detritus would be acceptable food for macro-consumers if $C:N \leq 17:1$. In litter bags experiments started in July 1977, this lower limit has been passed in February 1978. The differences between fragmented and non-fragmentated eelgrass are very small. It is questionable whether the C/N ratio is a good criterion for detritus being acceptable food. The particle size can be an important datum too.

At three stations at the northeast shore of the Lake Grevelingen samples were taken from October till February from beached eelgrass detritus. Plants in various decomposition stages are concerned here, which are transported regularly by wave action. Consequently, the values for C, N, P and ashfree dry weight fluctuate in time. The C/N ratio tends to decrease somewhat, the C/P ratio fluctuates considerably per station. The quantity of beached eelgrass at the northeast shore has been estimated at 1086 tons wet weight, that is only 3.1% of the roughly estimated standing stock in summer. It is unknown where the rest has to be found. Eelgrass takes up nutrients from the bottom and acts as a 'pump' and 'temporal reservoir'. During decomposition of dead plants the nutrients are released. Among the beached eelgrass detritus high concentrations of NH_3 , P- PO_4 , P-total and Si have been measured. At short distance from the eelgrass accumulations the concentrations were rather normal. No distinct gradient has been measured for DOC. Among the eelgrass the NH_3 concentration was high, whereas the NO_2 - and NO_3 -concentrations were very low. This might indicate the importance of anaerobic decomposition. There was a considerable local enrichment of the water with nutrients. If mineralization of the standing stock in summer 1977 was complete, then 75.600 kg N and 25.988 kg P would be discharged into the water. However, many aspects of the eelgrass decomposition have to be elucidated in the future.

Sandee, A.J.J. - 1979. Bemonsterings- en bewerkingsmethodiek van Hapacticiden en Nematoden in de bodem van het Grevelingenmeer. *DIHO Rapp. en Versl.* 1979-6, 8 pp.

A method has been described to sample meiozoo-

benthos elements (Harpacticoids and Nematodes) in the underwater bottom of saline Lake Grevelingen, and to work up the material in the laboratory and to quantify the densities of the animals. Some results on the vertical distribution in the sediment are given.

Vaas, K.F. - 1979. Studies on the fish fauna of an estuary in the SW Netherlands, before and after its change into the stagnant, saline Lake Grevelingen. *Hydrobiol. Bull.* 13(2/3), 177-188.

The Grevelingen was fished with a beam-trawl during the years 1960-1963, when in open connection with the North Sea as well as with the river Rhine, during the years 1966-1969, when the connection with the river was blocked by a dyke, and from 1971-1976, when the connection with the sea was also blocked by a dyke and the stagnant, saline Lake Grevelingen was created.

The (mainly) demersal fishes captured were classified into 1. frequent species spawning within the area; 2. frequent species spawning outside the area, and 3. incidental visitors from the coastal sea.

After closure the incidental visitors either became much less frequent or dropped out entirely. Some of the larger spawners decreased in numbers. Their place was occupied by smaller fishes, such as sand smelts and the stickleback.

As piscivorous fishes almost completely dropped out, the above small species were able to increase and so were the two gobies present before the closure. The black goby (*Gobius niger*) invaded the lake and built a large, propagating population, in a similar way as it had done in Lake Veere, a neighbouring saline lake, created in a similar way. The turbot and the brill increased in numbers and there are indications that both spawn in the lake. These two species are the only large piscivorous fishes left at the moment and the nearly empty niche, created by the absence of other, incidental visiting, marine piscivores, is now filled by numbers of markedly increased grebes and cormorants.

The flatfishes, used to spawn at sea, were trapped inside the lake. They grew on, but, as no reproduction was possible and recruitment through the locks in the dyke on the land side was minimal, their population decreased, the more rapidly as many were captured by fishermen.

It is concluded that in the stagnant, saline lake, species diversity decreased and food chains became shorter, as compared with the more mature situation in the open estuary.

Volkaert, F., 1979. Oekologische studie van de meiobenthische polychaetenfauna van de Grevelingen (Nederland). Licentiaat Thesis, Rijksuniversiteit, Gent, 96 pp.

The distribution and abundance of meiobenthic polychaetes in a boreal salt lake (Grevelingen - The

Netherlands) was investigated at 21 stations in April and August 1976 with one station, Archipel being sampled 22 times in 1977. The sampled station had a depth of 0.2 m to 14.5 m and contained zones of mud, fine and medium sand with salinity fluctuating between 12.9‰ Cl⁻ and 14.5‰ Cl⁻. The polychaetous fauna of this area is composed of 25 species and characterised by a relatively homogenous distribution and a poor species richness. A relation between sediment and diversity was not found. Archipel has a low diversity and limited seasonal fluctuations.

SØRENSEN and CZEKANOWSKI-indices, in combination with group-average sorting and principle component analyses were used to define meiobenthic polychaetous communities. Most polychaetes have no specific sediment preference, although some of them (including interstitial polychaetes) prefer sandy bottoms. Topographically 2 station groups can be distinguished: one in the western part (sandy bottom) and the other in the south-eastern part (muddy and sandy bottom) of Lake Grevelingen. Limited seasonal variations have been shown, with a higher diversity and larger numbers in spring and summer. Deposit feeders are as well qualitatively as quantitatively the most important feeding-group. The autecology of 25 determined polychaetes has been studied concisely with special attention being given to *Streptosyllis websteri*. Finally a qualitative model of the food web between the polychaetes of the Grevelingen has been drawn up.

IX.2. WORKING GROUP 'BRACKISH WATERS'

Bogaards, R.H. - 1979. Autoecologie en levenshistorie van *Palaemonetes varians* (Leach, 1814) (Decapoda, Palaemonidae). DIHO Rapp. en Versl. 1979-10, 48 pp.

Palaemonetes varians (Leach, 1814) (Decapoda, Palaemonidae) is abundant in nearly all brackish inland waters of the SW part of the Netherlands. Life cycle and autoecology have been studied monthly from March-December 1978 in two localities with a chlorinity of resp. 10‰ Cl⁻ and 1‰ Cl⁻. The sex ratio is dominantly 1:1.

Dorsal rostrum teeth number is usually 5, although variations from 1 to 8 have been observed. Rostral indexes of both places hardly differ which does not support one of the existing theories on the salinity-index relationship. The mean size of the male is smaller than that of the females. Growth takes place during the summer months. Their maximum age is 2 years. Maximum rostrum carapax size is 20 mm corresponding with a total body length of 5 cm. Berried females (egg-carrying) have been observed from May up till August. Normally one brood is hatched per season. Maximum brood size is 500 and is correlated with the size of the

female. Relationship between brood size and female size is not the same for the two localities. Wet and dry weight are correlated with length.

Boogert, J.J. van den - 1979. Klassificatie van brakke binnenwateren in Zeeland op grond van hun macrofauna. DIHO Stud. Rep. D5-1979, 36 pp.

During the period April-July 1975 a qualitative sampling was carried out in 60 ponds and creeks in the SW-part of The Netherlands. By means of a multivariate analysis it proved to be possible to distinguish six groups of sampling locations, characterized by the species composition.

A similar analysis to various aspects of the chloride content showed that the mean chlorinity, calculated over the period 1968-1975, is the main discriminating factor for obtaining three distinct clusters. The maximal chlorinity fluctuation and the standard deviation of the mean are of limited importance for the discrimination between the three groups. A comparison of the clusters based on the chloride content, with the clusters based on the composition of the macrofaunal community, showed a strong correlation.

Borghouts, C.H. - 1978. Population structure and life-cycle of *Neomysis integer* (Leach) (Crustacea, Mysidacea) in two types of inland waters. Verh. Internat. Verein. Limnol. 20, 2561-2565, (Δ -180).

During 2½ years population structure and life-cycle of *Neomysis integer* were studied in two types of inland waters: a large brackish-water lake (Lake Veere) and a small waterbody (Den Inkel). The differences between the populations in Lake Veere and Den Inkel concerned mainly the length of the animals and the portion of the juveniles. The maximum length of *Neomysis integer* from Lake Veere was 19 mm and from Den Inkel 13 mm. The life-cycle of both populations does not seem to differ very much.

In Lake Veere as well as in Den Inkel the breeding females were larger in spring than in autumn. The size-classes of these breeding females were different in both waters. In spring these lengths were in Lake Veere 15 up to 19 mm, in Den Inkel 10 up to 13 mm. In autumn the lengths of the breeding females were 10 up to 15 mm, and 7 up to 9 mm, respectively. The brood-size varies with the body-length of the females. Therefore it is clear that the brood-size of Lake Veere animals is larger than of Den Inkel females. It is plausible that the difference in length between the animals of the two populations is due to slower growth in Den Inkel.

Goedbloed, J. - 1979. Substraat- en zoutpreferentie van *Chironomus halophilus* en *Chironomus salinarius* (Diptera, Chironomidae). DIHO Stud. R. p. D8-1979, 60 pp.

Two brackish water chironomid species *Chironomus halophilus* and *Chironomus salinarius* were cultured.

The experiments were carried out in indoor cages (150 × 240 × 65 cm) in rooms with a constant temperature (22.5°C; light-dark period, 14-10 hours) and in outdoor cages (200 × 200 × 200 cm).

Third and fourth instar larvae of *C. salinarius* showed a slight preference for a silt substrate in comparison with a sand substrate. A comparable preference for *C. halophilus* was not observed. Ovipositing females of *C. halophilus* had a strong preference for water basins with a dark bottom and white sides in comparison with completely white basins. A preference of the ovipositing females of both species for the water from the sampling site in a comparison with diluted sea water of the same chlorinity could not be demonstrated. Ovipositing females of *C. halophilus* preferred chlorinities lower than 8‰ Cl⁻. A comparable preference for *C. salinarius* could not be assessed. Ovipositing females of *C. salinarius* showed a preference for water in which larvae are present in a comparison with water without larvae.

In a 24-hour sampling period in the field no migration of larvae of *C. halophilus* could be demonstrated, not even of the very mobile first instar larvae.

Janssen, H. and R. Mooij - 1979. De kolonisatie van een brakwater biotoop. DIHO Stud. Rep. D1-1979, 45 pp.

The course of the colonization process by the macrofauna was followed in two recently cut, brackish ditches during the period March to August 1978. Species richness and population dynamics were determined and compared with the corresponding parameters of two adjacent older ditches. Quantitative samples of the zooplankton and of the benthic and pelagic macrofauna were taken at fortnightly intervals. Also a number of physico-chemical parameters was determined (temperature, chlorinity, oxygen concentration, nitrogen and chlorophyll). Significant differences in the physico-chemical conditions between the new and old ditches did not exist. The four ditches were of the mesohaline type. The irregular fluctuations of the abiotic parameters indicate a rather inconstant biotope. The macrofauna was composed of pioneer species. Three months after the start of the investigations the species composition in the new and old ditches were nearly the same. For the settlement of the benthic macrofauna the presence of a soft mud layer is of vital importance. A layer of 5-10 cm, already present 3-6 months after cutting of the ditch, was sufficient. For most of the free-swimming species the presence of macrophytes was very important. The rapid colonization of the new ditches was favoured by the presence of open connections with the old polder system.

With the present water quality and ditch management old ditches can not develop to biocoenoses with a com-

plex structure. The biological structure will be characterized by a very low maturity.

Krebs, B.P.M. - 1979. Waterbug research at the Delta Institute. In: J. Vangenechten and O. VanderBorgh (eds.) Biology and distribution of waterbugs (aquatic Hemiptera). Studiecentrum voor Kernenergie, Brussel. Rapport BLG 529, 21-24.

Since 1960 about 10 biologists have sampled the waterbugs of the Delta region. Most of these samples were qualitative, although a quantitative sampling is available on 500 spots in 1977-1978. These findings will be correlated with abiotic factors and with data of the vegetation. An atlas will illustrate the distribution for each species as it was collected during the sixties and the seventies. A description of the habitat will be given for each species. Up to now, about 35 species of waterbugs were collected. The distribution of *Sigara selecta* is discussed in more detail.

Maas, P. - 1979. De aquatische macrofauna van de Otheense Kreek. DIHO Stud. Rap. D4-1979, 29 pp.

The aquatic macrofauna of the Otheense Kreek was described by a one-off sampling at six stations. The chlorinity of the water was also determined. The occurrence of about 40 macrofauna species could be demonstrated.

Merks, A.G.A. - 1979. A microgradient sampler for shallow waters. Hydrobiol. Bull. 13, 61-67, (Δ -179).

A cheap, light and simple device is described for the sampling of physico-chemical microgradients in the free-water zone of shallow water bodies. The sampler is commercialized by ATS, Mariastraat 42, The Hague, The Netherlands.

The sampler was tested for gradients of oxygen and chlorinity and proved to be very appropriate for research in shallow waterbodies. Also other parameters can be analysed, e.g. plankton, pigments, nutrients, etc. Due to the short distance between two successive syringes, the sample volume is a limiting factor for chemical or biological analyses. In that case, some adjacent samples might be put together. Consequently a smaller number of waterlayers is analysed and the gradient is demonstrated less detailed.

Schmidt-van Dorp, A.D. - 1979. Literatuuronderzoek naar de soortenrijkdom van het makrozoobenthos in relatie tot het zoutgehalte. DIHO Rapp. en Versl. 1979-5, 94 pp.

The literature concerning the species richness of benthic macrofauna in relation to salinity was reviewed. A numerical relationship between salinity and species richness was set up for seven groups of benthic macrofauna both in the Zuiderzee-area (Netherlands), in which after the enclosure by a dam salinity has gradually and irreversibly decreased, and in the tidal

estuaries of the Delta region of the rivers Rhine, Meuse and Scheldt (Netherlands). These relationships were compared to those found in the Baltic Sea – an inland sea without tidal water movement – and those found in small inland waters in Zeeland (Netherlands). The investigation and description of the seven fauna-groups have not been equally intensive in each of these areas. Especially the species richness of insects is hardly known in several of the areas. Taking into account this factor of uncertainty, the relationship of salinity to species richness of benthic makrofauna yet seems to be of a different shape and to reach the minimum number of species at a different level of salinity in the distinct areas.

The relationship between salinity and species richness differs for groups with exclusively or mainly organisms from those with representatives in both sea and fresh water.

Knowledge about the influence of salinity fluctuations on the species richness is very limited. The available information suggests, that the minimal salinity as well as the maximal salinity values may play a role.

Per area the species richness can be limited by other environmental factors than salinity. Temperature, the rate of watermovement, the nature of sediments and the oxygen concentration are considered as such. The influence of these factors in relation to the influence of salinity is not exactly known, mainly because very few data have been recorded inside and at the surface of the bottom-sediments, where the benthic macrofauna lives.

Sepers, A.B.J. - 1979. Gewenst voor de Oosterschelde: een stabiel milieubeheer. Zeeuws Nieuws 5(2), 68-69.

After completion the storm surge barrier in the mouth of the Eastern Scheldt have to be operated exclusively when there is in relation with the weather conditions a real chance of a dam-burst. Otherwise the biological important values of the Eastern Scheldt region will be considerably reduced.

The creation of new 'natural' areas cannot offer a serious compensation for the loss of areas which are very valuable from a biological point of view.

Sepers, A.B.J. - 1979. The aerobic mineralization of amino acids in natural aquatic environments. Doctorate's Thesis, University of Groningen, The Netherlands. In Dutch, 101 pp., (Δ -177).

This thesis describes research into the aerobic mineralization of amino acids in natural aquatic environments.

The uptake of dissolved organic matter by bacteria, phytoplankton and invertebrates is discussed with reference to the literature.

Many marine invertebrates are able to take up dissolved organic compounds from the ambient sea water. In fresh water the uptake of dissolved organic matter proceeds at a considerably lower rate or is com-

pletely absent. Dissolved organic compounds may have nutritional value as an additional nutrient source, especially during shortages of particulate food. Some phytoplankters can grow heterotrophically in the dark, utilizing dissolved organic substrates as a carbon and energy source. The range of compounds permitting heterotrophic growth is generally very limited. Many algae are able to take up organic compounds in the light. These substrates may serve as an additional carbon and energy source.

Heterotrophic bacteria possess uptake systems for dissolved organic matter which are adapted to very low substrate concentrations. Results of experiments using autoradiographic and differential filtration techniques show that the uptake of dissolved organic compounds in natural waters is primarily a bacterial process.

The primary purpose of the investigations described in this thesis, was to obtain information about the role of the micro-organisms, involved in the ammonification in the natural aquatic environment. By spreading samples from the Haringvliet, a freshwater lake, on agar media and by application of enrichment procedures in batch and continuous cultures, bacteria were isolated which are able to utilize a single amino acid as the carbon, nitrogen and energy source. A total number of 169 bacterial strains was obtained, which differ from each other in the morphology of the colonies, growing on agar surfaces.

The utilization of 41 organic compounds as the sole source of carbon and energy was determined for 68 isolated strains. Within this group of organisms there was no specificity for the substrate: 83% of the tested bacterial strains was able to utilize 50-83% of the applied organic substrates as the sole carbon and energy source. Some organisms showed a substrate preference, mostly not for amino acids but for other organic compounds. The bacterial population which is able to take up amino acids in natural aquatic environments, is composed of a variety of micro-organisms which only slightly differ in their ability to utilize a variety of substrates. Moreover, preliminary results of some counts indicate that the aerobic mineralization of amino acids is a very common property among the heterotrophic bacterial population.

The uptake of several amino acids and of a mixture of amino acids (an acid hydrolysate of *Chlorella* protein) was measured during a one year period in Lake Grevelingen and the Haringvliet, applying ^{14}C -labeled substrates. Aspartic acid was respired for 80%; the percentage of respired substrate for leucine, glycine and histidine were 24%, 66% and 66% respectively. A difference was found in the percentage of respired substrate of the amino acid mixture between Lake Grevelingen and the Haringvliet (43% versus 63%). From the maximum uptake rates, the potential yearly

uptake of the substrate in question can be calculated. These data indicate the relative importance of the several subpopulations in the carbon mineralization process as a whole. The highest values of the potential yearly uptake were obtained for the amino acid mixture; the comparable values for the uptake of aspartic acid were only slightly lower. The potential yearly uptake of glycine and leucine was much lower. The potential yearly substrate uptake in the Haringvliet, as measured with aspartic acid and the amino acid mixture, was high compared with the same data of Lake Grevelingen, corresponding with the higher nutrient levels in the Haringvliet.

The potential yearly uptake as determined with the ^{14}C -labeled amino acid mixture is only 3.7% (Haringvliet) and 2.8% (Lake Grevelingen) of the amount of mineralized carbon, as calculated from oxygen uptake experiments. These percentages are very low in view of the fact that 35-55% of the organic matter of living phytoplankton and zooplankton consists of protein and that the aerobic mineralization of amino acids is a very common property among the heterotrophic bacterial population.

The uptake experiments showed an uptake process characterized by a saturation constant in the nanomolar range. The saturation constant for the growth of bacteria in carbon limited cultures varies normally in the micromolar range; the same holds good for the affinity constant for the active transport of amino acids through the cell membrane.

The value of the applied activity measurements was investigated in order to obtain information about the relation between the uptake process as measured with ^{14}C -labeled substrates and the activity of the bacterial population in situ. For this purpose a bacterial strain (HIS 42) was grown in a histidine limited chemostat, because histidine appeared to be a suitable substrate for the growth of nearly all isolated ammonifying bacteria. The uptake of histidine was determined experimentally in samples from the chemostat using ^{14}C -labeled histidine and the oxygen consumption rate after histidine addition. The maximum growth rate of HIS 42 with histidine was 0.20 h^{-1} ; the growth constant K_s for histidine limited growth was $20 (\pm 6)\ \mu\text{M}$ histidine.

The uptake of ^{14}C -labeled histidine was characterized by a saturation constant of 12.8-78.6 nM histidine. At higher growth rates the measured maximum uptake rate of histidine was lower than the actual uptake rate in the culture. The percentage of respired substrate (76-93%) was about 30-40% higher than the comparable value for the culture.

The uptake of histidine as analyzed through the measurement of oxygen uptake rates, was characterized by a saturation constant of 1.7-10.5 μM histidine; the maximum uptake rate was always greater than the

actual histidine uptake rate in the culture. It appeared that the determination of the histidine uptake by measuring the oxygen consumption rate is a better reflection of the actual uptake process of histidine in the culture.

The results indicate also that the interpretation of the parameters obtained by application of ^{14}C -labeled compounds, so as to determine the heterotrophic activity in natural waters, is quite obscure. From the available data it is impossible to correlate this uptake process with the metabolism of the bacterial population.

Ammonifying bacteria are able to utilize many organic compounds as the sole carbon and energy source. This can be interpreted as an indication for the simultaneous uptake of several organic compounds by heterotrophic bacteria in the natural environment.

Some pilot experiments were performed to assess the possible relationship between the specific growth rate and the concentration of the enzyme systems, necessary for the transport of the organic compound and the subsequent utilization in respiratory and biosynthetic pathways. The capacity for the uptake of a variety of organic compounds by organisms grown on a single amino acid was also determined.

Bacterial strain HIS 42 was grown in a chemostat with histidine as the growth limiting substrate at dilution rates between 0.01 and 0.1 h^{-1} . Samples taken from the chemostat, were used to determine the uptake kinetics for glycine, leucine, aspartic acid, a mixture of amino acids, glucose and acetate with the application of ^{14}C -labeled substrates.

At the applied culture conditions the concentration of the uptake systems for these organic compounds is independent of the specific growth rate. Further research with other bacterial strains grown under a number of culture conditions, is needed to show whether these results represent a general phenomenon.

Over a wide range of specific growth rates (0.01-0.1 h^{-1}) the utilization of a number of organic compounds in respiratory and biosynthetic pathways could be demonstrated, indicating that a simultaneous uptake of several organic compounds under natural conditions is very likely.

Sepers, A.B.J. and F.B. van Es - 1979. Heterotrophic uptake experiments with ^{14}C -labeled histidine in a histidine limited chemostat. Appl. Environ. Microbiol. 37, 794-799, (Δ -171).

The histidine uptake by bacterial strain HIS 42 was determined with [U - ^{14}C]-histidine and through oxygen uptake experiments on samples taken from a histidine-limited chemostat. The uptake of [U - ^{14}C]-histidine was characterized by a saturation constant of 12.8 to 78.6 nM histidine. At higher growth rates, the measured maximum uptake rate of histidine was lower than the

actual uptake rate in the culture. The percentage of respired substrate (76 to 93%) was about 30 to 40% higher than the comparable value for the culture. The uptake of histidine as analyzed through the measurement of oxygen uptake rates was characterized by a saturation constant of 1.7 to 10.5 μM histidine; the maximum uptake rate was always greater than the actual histidine uptake rate in the culture. By the application of the two cited methods, set up to determine the histidine uptake kinetics, two different uptake processes were analyzed. It appeared that the determination of the histidine uptake through measurement of the oxygen uptake rate showed a better reflection of the actual uptake process of histidine in the culture. With the available data it was impossible to assess a correlation between the uptake of histidine, as determined with [U - ^{14}C]-histidine, and the actual metabolism of the bacterial population.

Tramper, N.M. - 1979. Veedrinkputten als instabiele aquatische oecosystemen. DIHO Stud. Rep. D2-1979, 74 pp.

During the period March-October 1976 31 isolated waterbodies (mainly cattle pools) were qualitatively sampled for macrofauna and environmental factors. The locations were situated on the island of South-Beveland and in Zeeuws-Vlaanderen.

Specimen of the next taxonomical groups were identified: Platyhelminthes, Annelida, Insecta, Crustacea, Mollusca, Bryozoa, Hydracarina, Amphibia and Pisces. Furthermore the morphometry and the chloride and kation content (fortnightly samplings) were determined. By means of a clusteranalysis a classification of watertypes was made. Shading, mean chlorinity and instability proved to be the most important factors. Concerning the instability the following factors were considered: occurrence of extreme situations (e.g. drying-up) and the number of increases and decreases in the concentrations of the chemical parameters. The following groups of locations with the corresponding indicative organisms were distinguished:

- I. Shaded. Indicative species: *Asellus aquaticus*, *Heperocorixa sahlbergi*, *Pionopsis lutescens*.
- II. Not shaded, relatively high chlorinity (2.5‰), stable. Indicative species: *Sphaeroma hookeri*, *Gammarus salinus*.
- III. Not shaded, relatively high chlorinity (3-14‰), instable. Indicative species: *Idotea chelipes*, *Gammarus zaddachi*, *Palaemonetes varians*, *Bidessus parallelogrammus*, *Enochrus bicolor*, *Aedes spec.*, *Chironomus salinarius*, *Hydrachna skorikowi*.
- IV. Not shaded, oligohaline, instable. Indicative species: *Callicorixa concinna*, *Palpomyia spec.*, *Ephydra spec.*
- V. Not shaded, oligohaline or fresh, stable. Indicative

species: *Polycelis* spec., *Glossiphonia complanata*, *Theromyzon tessulatum*, *Haliplus lineakocollis*, *Bidessus pusillus*, *Ochthebius minimus*, *Rana esculenta*.

Referring to the macrofaunal communities cattle pools are biologically important systems. It is recommended to preserve and manage them carefully.

Velde, L. van de - 1979. De zuurstofbalans en plankton van brak slootwater. DIHO Stud. Rep. D3-1979, 76 pp.

During two months some details were investigated of the oxygen budget of a brackish ditch. The most important items were the vertical plankton distribution and the relation between light intensity and oxygen production as measured near the water surface and near the mud layer. Also the relation between wind velocity and reaeration of the water was studied under laboratory conditions.

Phytoplankton was identified and its biomass was calculated. In autumn the diatoms *Achnanthes lanceolata* and *Nitzschia closterium* and the flagellate *Euglena viridis* were dominant. A relation between the vertical phytoplankton distribution and the wind velocity could be demonstrated. In the absence of light a slight downward increase of diatoms as well as an increase of the chlorophyll concentration occurred. This might be due to the migration of phytoplankton to water layers with optimal light conditions. Removal of the large plankton species by sieving resulted in a decrease of the biomass, but did not induce a lower oxygen production. By removing zooplankton less oxygen and less phytoplankton was consumed. Under these conditions the oxygen production was higher and was not necessarily related to the phytoplankton biomass.

An exponential relationship between the wind-velocity and the reaeration could be assessed. The mixing of absorbed oxygen was completed by convection.

Verdonschot, P.F.M. - 1979. Aquatische Oligochaeta; introductie. DIHO Rapp. en Versl. 1979-11, 45 pp.

The Oligochaeta have proved to be successful in evolution and they have spread all over the world. In spite of their simple structure they have a great number of specialized inner- en outer 'organs' (e.g. hairform, sexual organs, etc.). Reproduction may be sexual or asexual, dependent on taxon. It is very difficult to distinguish the different stages of life. Oligochaeta are in general very tolerant to different abiotic factors but water is more or less essential. Besides water only salt seems to be a main factor for distribution. Relations with other abiotic factors are less clear. Some taxa have a high reproduction potential. Some sample-techniques are described. New for macrofauna research is the use of a large gutter for the departure of individuals.

Weeber, I.J. - 1979. Typologie van een aantal Zeeuwse binnenwateren, voornamelijk sloten en watergangen, op grond van hun makrofauna. DIHO Rapp. en Versl. 1979-2, pp. 102.

In 1977 40 inland waters, mainly ditches of varying size, in the province of Zeeland (S.W. Netherlands) were qualitatively sampled twice for benthic and pelagic macrofauna. The median salinity of these waters varied from 0.04-11.3‰ chloride. From February to December 1977 samples were also taken fortnightly for the chemical analysis of these waters. In these samples temperature, chlorinity, pH, oxygen concentration, dissolved inorganic phosphate, ammonia, calcium, magnesium, sodium and potassium were measured. The dissolved organic content of the water and the particular organic carbon of the bottom were measured only once. Also other environmental factors were investigated, like depth and width of the ditch, the nature of the bottom, the amount of water plants, the presence or absence of currents and the influence of pesticides.

A cluster analysis based on the macrofaunal species composition was applied to make a typology of the investigated waters. A total of 280 taxa was found, of which the main groups were: 62 species of adult aquatic Coleoptera, 21 Coleoptera larvae, 25 aquatic and surface-living Heteroptera, 48 chironomids, 34 other Diptera-larvae, 14 Hydracarina, 21 Crustacea, 25 Mollusca and 12 Tricladida and Hirudinea. Compared with the macrofauna of the brackish parts of the estuarine areas in the Delta region, the sampled inland waters are characterized by a conspicuous richness in insect species.

The cluster analysis indicated two groups of locations, correlated with chlorinity and magnesium, sodium, potassium and calcium levels, the latter ions being strongly correlated with chlorinity. The splitting line between the two clusters could be positioned at a median chlorinity of 1.5-2‰ chloride. Supplementary research revealed that roughly 40 salt tolerating fresh water species, mainly aquatic Coleoptera and Heteroptera, did not occur at higher salinities. This group of species seemed particularly responsible for the bifurcation of the sampling stations into two main clusters.

It appeared that a waterbody of which the chlorinity increased to 4-5‰ Cl⁻ could still have a fresh water macrofauna association, i.e. an association belonging to the fresh water cluster, provided this higher chlorinity exists for only a short period in summer. Within the fresh water cluster a group of purely fresh waters could be distinguished, all situated in Zeeuws-Vlaanderen. These locations were richer in fresh water species than comparable fresh waters on Zuid-Beveland and Schouwen; 74 taxa were found only in Zeeuws-Vlaanderen. Within the brackish cluster two

groups could be distinguished, correlated with chlorinity. The splitting line could be positioned in this case at a median chlorinity of 7.5‰ Cl^- . A number of brackish water and marine species was found only beyond this line.

The impact of chlorinity fluctuations on the total number of species present at each location and on the numbers of fresh water, brackish water and marine species was investigated. This was done within narrow ranges of the median chlorinity, i.e. only waters with nearly the same median chlorinity were compared with each other. An effect could be demonstrated for the marine and brackish water species, an only slight effect for the fresh water species and no effect for the total number of species. The number of marine species decreased with increasing fluctuations, whereas the number of brackish water species increased.

IX.3. WORKING GROUP 'SALT-MARSH ECOSYSTEMS'

Beeftink, W.G. - 1979. The structure of salt marsh communities in relation to environmental disturbances. In: R.L. Jefferies and A.J. Davy (eds.). *Ecological Processes in Coastal Environments*; Oxford, Blackwell Scientific Publ., p. 77-93, (Δ -166).

The results of three types of disturbance on salt marsh vegetation in the south-west Netherlands are discussed. These disturbances are sudden and extreme changes in weather conditions (e.g. winter frost), and a sudden increase or a decrease in tidal influence (e.g. barrage schemes). Their effects on the vegetation of permanent plots were studied each year, using the 14-point scale estimation method.

Die-back in *Halimione portulacoides* is followed by sequential maxima in the densities of *Suaeda maritima*, *Aster tripolium* and *Puccinellia maritima*. Recovery of the original vegetation takes 4 to 15 years, and at least 10 to 50 years are required when the soil has been involved in the disturbance. An increase in the frequency of tidal immersion causes a regression in the development of the plant communities which is proportional to the increase in tidal height. Intermediate changes in the species composition of the vegetation similar to those mentioned above may occur. Sudden cessation of tidal cover results in the disappearance of halophytes within 1 to 7 years, and the colonization of the site by glycophytes. These are either tall herbs, or else pasture plants when the marsh is grazed. Spatial and temporal patterns in the decline of halophytes and increase in glycophytes are described. Vegetational changes in salt marshes as a consequence of disturbances can be predicted with some accuracy.

Beeftink, W.G. - 1979. Veranderingen in de vegetatie in relatie tot milieudynamiek. *Contactbl. voor Oecologen* 15 (2/3), 59-77 (Δ -184).

In this paper some considerations are given on

vegetation dynamics in relation to different types of environmental changes. After dealing with some postulates from which studies on vegetation dynamics should start and after enumerating the aims for which can be strived with studies in vegetation dynamics, some case-studies are described. Special attention is paid to the influence of different types of disturbances inducing retrogression or secondary succession in vegetation. Some results attained to examinations in the salt marsh are mentioned. Finally, some perspectives for integration of vegetation dynamics into other specialisms are dealt with.

Beeftink, W.G. - 1979. Vegetation dynamics in retrospect and prospect. Introduction to the proceedings of the second symposium of the working group on succession research on permanent plots. *Vegetatio* 40, 101-105, (Δ -185).

This paper serves as an introduction to the proceedings of the symposium mentioned in the title and held in 1975, at the Delta Institute, Yerseke. After some historical remarks about the activities of the Working Group the different approaches and object studies presented at this meeting are enumerated. Next, some reflexions are given on the study of vegetation dynamics from the view-point of historical and future development of vegetation science and ecosystem research. The reasons for an increase of interest among phytosociologists in research on vegetation dynamics are displayed. Finally, some ecological problems for which this approach will be valuable or even essential are touched.

Buth, G.J.C. and A.M. Groenendijk - 1979. Vegetatie en milieu op voormalige slikken en zandplaten in relatie tot de bedijkingsduur. *DIHO Stud. Rep.* D10-1979, 124 pp.

Investigations into the vegetation development on intertidal soils after embankment have been carried out for more than fifteen years by Dr. W.G. Beeftink and his collaborators. For this purpose, permanent sample plots were established in the mud soon after the barrage was built. In this report an attempt is made to detect temporal sequence in a spatial pattern of vegetational and environmental structures of creek-bank areas of different ages from embankment. Differences in management pressure have been excluded as much as possible (mowing and/or extensive sheep grazing). The age of embankment varies from five to about threehundred years. The soil texture extends from sandy silt to clay.

In each area a series of compound sample plots placed along a gradient from low to high soil surface, has been established. From each sample plot the vegetation has been analysed by making levees, and soil samples have been taken for analysing pH and clay, moisture, carbonate, humus, chloride and phosphate

contents. Vegetation and soil data were processed with cluster analysis using BIOPAT, Program System for Biological Pattern Analysis, and with diversity estimates using the Shannon formula.

Near the water's edge the vegetation is poor in species and the soil parameters show extreme and partly highly fluctuating values. Beyond the direct influence of the adjacent stagnant water-body the number of species increases strongly and the soil shows a remarkable decrease in its extreme and dynamic character (salinity, humidity and nutrient supply). In most areas the gradient character of the soil is apparent as appears from parameters running from wet to dry, from saline to fresh, and/or from relatively rich to poor in nutrients. Most gradient situations have in their vegetational aspects high values for species richness and diversity in about the middle of the gradient assuming a maximum in spatial environmental diversity and/or a minimum in temporal fluctuations. Next to the gradient zone, in the older areas the vegetation is formed by communities which are rich in species and characteristic for hayfield. Species in these communities are characteristic for environments with lower dynamics.

In order to find a relation between the spatial patterns and the age of embankment of the areas the data sets of all areas have been compared with each other using cluster analysis. It appears that in the first fourteen to twenty years after embankment big changes take place in the vegetation. Different stages follow each other with high frequency. These changes seem to be caused chiefly by maturation processes in the soil. After this period the floristic and structural differences appear to be mainly caused by the local situation and management. Especially the soil moisture content and its fluctuations seem to be important factors. Bryophytes play an important role in the development of the vegetation during the first fourteen years. After twenty years they play an ever lower part.

Groenendijk, A.M. - 1979. Dagvlinders in de Zak van Zuid-Beveland; inventarisatie en beheer van dijken. Zeeuws Nieuws. Natuur, Landschap en Milieu 5, 82-85.

For management derectives the innerdikes of the Zak van Zuid-Beveland have been subject to investigations on the impact of burning, grazing, mowing and no maintenance on both vegetation and entomofauna, especially the butterflies.

Most of the dikes in the area of study had been burned or neglected for many years. This has caused a serious decline of the dike vegetation and was in favour of a few grasses and blackberries. Total numbers and diversity of the butterfly populations were low. Highest diversities in vegetation and butterfly populations were found on dikes with a southern exposition

and a long lasting mowing regime. On these dikes the vegetation consisted for a large part of nectar supplying species.

Most of the grazed dikes were used intensively and showed low diversity in vegetation and butterfly populations.

Huiskes, A.H.L. - 1979. Schorren. Zeeuws Nieuws. Natuur, Landschap en Milieu 5, 59-61.

A description of the formation of salt marshes is given, in which the importance of the vegetation as building agent of these salt marshes is stressed. The salt-marsh areas along the Eastern Scheldt are described, especially their botanical and/or zoological importance. The changes that will occur on the salt marshes after the construction of the storm surge barrier are discussed.

Huiskes, A.H.L. - 1979. Biological flora of the British Isles. *Ammophila arenaria* (L.) Link. J. Ecology 67, 363-382.

The morphology, physiology and ecology of *Ammophila arenaria* are described. In the paper literature on these subjects is reviewed, but own research data are also included.

Huiskes, A.H.L. - 1979. Damage to Marram grass *Ammophila arenaria* by larvae of *Meromyza pratorum* (Diptera). Holarct. Ecol. 2, 182-185.

Larvae of *Meromyza pratorum* Meigen (Diptera, Chloropidae) were found to affect tillers of *Ammophila arenaria* (L.) Link in the dunes of Newborough Warren, Anglesey, U.K. The highest percentages of affected tillers were found in September. There were clear differences between the proportions of affected tillers in the various successional stages of the dunes. It was found that the proportion of affected tillers was reduced by a fertilizer treatment but slightly increased by removing associated species.

Huiskes, A.H.L. - 1979. The population dynamics of *Ammophila arenaria*. In: R.L. Jefferies and A.J. Davy (eds.). Ecological Processes in Coastal Environments; Oxford, Blackwell Scientific Publ., p. 637 only.

Populations of tillers of *Ammophila arenaria* (L.) Link were very stable in density during two years of observation. Higher densities occurred in the mobile areas of the dunes, as compared with more stabilized areas. The birth rates and death rates of cohorts of leaves showed a strong seasonal fluctuation. High birth rates and death rates were found in the higher areas of the dunes, as compared with lower lying dune grasslands and slacks. Fertilizer application increased both the population density and the population flux, but only if the nutrients could reach the root system of

A. arenaria, i.e. in areas where there was little root competition from other species.

Huiskes, A.H.L. and J.L. Harper, 1979. The demography of leaves and tillers of *Ammophila arenaria* in a dune sere. *Oecol. Plant.* 14 (4), 435-446.

Ammophila arenaria varies in shoot density and flowering frequency in different positions of sand dune successions. The flux of marked tillers and of leaves of *A. arenaria* was measured in populations in various stages of sand dune seres at Newborough, North Wales. The density and vigour of vegetative and flowering shoots of *A. arenaria* decreased with advancing stages of the sand dune succession.

Tillers carried twice as many leaves in summer as in winter. The birth rates and death rates of tillers and leaves were closely synchronous and showed marked seasonal periodicity. The highest birth and death rates occurred from May to August. Leaves produced in summer had a much lower life expectancy than those produced in autumn, winter and spring. The flux of tillers and leaves did not vary significantly in different phases of the dune seres. The synchrony of birth and death rates of leaves and tillers suggests that the two are causally interrelated and may represent density dependent responses that operate within different carrying capacities in the different parts of the seres.

Nieuwenhuize, J. and J.M. van Liere, 1979. Vergelijking van twee oplosbaar fosfaatanalyses in grond. *DIHO Rapp. en Versl.* 1979-7, 12 pp.

Two methods for the determination of total phosphorus in soil are discussed. We compared the ignition method with the wet digestion method and found 5.2% higher results with the wet digestion method. Recovery experiments proved that 99% from added PO_4 was found to be analysed. Without perchloric acid the wet digestion method seems to give incomplete destruction (97%).

Noordwijk-Puijk, K. van, W.G. Beeftink and P. Hogeweg, 1979. Vegetation development on salt-marsh flats after disappearance of the tidal factor. *Vegetatio* 39, 1-13, (Δ -172).

Vegetation succession on the tidal flats of the Middelplaten, isolated from tidal action after the construction of a barrage in 1961, has been studied with cluster analysis. Data of yearly cover-abundance estimates of species over 1963-73 produced from the Big Island have been partitioned at two levels yielding 7 and 17 clusters respectively. Mapping the 17 clusters for the successive years showed pathways in cluster distribution related with the desalinization rate and water relations in the soil. From the cluster transitions three successional lines could be recognized. They represent developments under different environmental conditions in which differences in desalinization rate,

water relations and availability of nutrients are deciding.

In the whole area of investigation two invasions could be recognized in the period of colonization, one of halophytes, and the other of glycophytes. In the early years the vegetational pattern is mainly temporal, probably related with unidirectional environmental processes and strategies of species. In later years the pattern is mainly spatial, partly due to 'fossilization' of earlier stages of succession for which reduction in availability of mineral nutrients may be responsible. Vegetational diversity expressed in numbers of clusters reaches a maximum about 10 years after the barrage had been constructed. It is suggested that a second period of succession will be initiated by long-term ecological processes, such as humification, decalcification and acidification, in which the vegetation will participate.

Watkinson, A.R., A.H.L. Huiskes and J.C. Noble, 1979. The demography of sand dune species with contrasting life cycles. In: R.L. Jefferies and A.J. Davy (eds.). *Ecological Processes in Coastal Environments*; Oxford, Blackwell Scientific Publ., 95-112 pp.

Sand dunes provide relatively simple and structured habitats in which the biological significance of different life cycles and growth forms can be analysed. In particular sand dune species can often be found in *r* and *K* phases of population development. Examples will be presented to illustrate the demographic analysis of annual and clonal perennial plants growing on the Anglesey dune system.

Five short-lived annuals have specialized life cycles and at least one of them has been shown to be subject to density-dependent regulation. The performance of individuals can be largely accounted for (69% of reproductive variance) as a function of distance from nearest neighbours. The absence of seed longevity is a feature of these species.

The demographic study of shoot systems in *Ammophila arenaria* and *Carex arenaria* reveals the high flux in the turnover of shoot systems that is disguised by conventional census methods. Application of fertilizers dramatically increases both birth and death rates in shoots of *C. arenaria*. The dynamics of the shoot population of *Ammophila arenaria* and of *Carex arenaria* appear to depend on the dynamics of the accumulated banks of meristems, the dormancy of which is controlled by the interaction of internal (correlative) inhibition and external (e.g. nutrient) factors.

X.4. MISCELLANEOUS

Duursma, E.K. (ed.) - 1979. Progress Report 1978 *DIHO*, Verh. KNAW, Nat., 2e reeks 73, 128-232, (Δ -181).

Results, obtained by three working groups and from

miscellaneous subjects of the Delta Institute for Hydrobiological Research, Yerseke are presented.

E.K. Duursma, F. Vegter and P. Kelderman - 1978. Concepts of hydrochemical water quality of the Delta waters. *Hydrobiol. Bull.* 12, 215-225, (Δ -174).

Some concepts of hydrochemical water quality in estuaries are discussed from the point of view of time and space. The relations of metal and other substances with chloride as conservative property are questioned with respect to time, space and presence of particulate matter. Some examples are presented for phosphorus budgets of the Dutch Wadden Sea and Lake Grevelingen in order to demonstrate the various impacts determining estuarine conditions. The effects of non-attached particulate metals and slow solid-binding processes of metals and radionuclides onto particles have to be considered for estuarine studies. At the same time a start should be made to determine the residence times of natural and anthropogenic substances in the estuaries which reflect also the human activities on the estuaries and thus the resulting changes in the natural equilibria.

Lambeck, R.H.D. - 1979. Rotganzen in het Deltagebied: de leeftijdsverhouding in het seizoen 1977/78 en meer gegevens over hun status binnen de totale rotganspopulatie. *Watervogels* 4: 36-39.

Brent geese in the Delta area: the age-ratio in the season 1977/78 and more information about their status within the total brent population. From 20 February till 6 March 1978 a survey has been made along parts of the Eastern Scheldt, Lake Veere and Lake Grevelingen (S.W. Netherlands) to determine the age-ratio in flocks of Brent geese (*Branta b. bernicla*). Among nearly 3100 birds checked only one was a juvenile. So there was a near complete breeding-failure in 1977.

At a total of ca. 2400 birds three wore colourrings. They were ringed in spring 1976 and 1977 on the island of Terschelling (western Wadden Sea). Sightings of those birds and as in 1976/77 the absence of English colourringed Brents confirm the idea that the Brent geese in the Delta area form an independent wintering population. In autumn at least a part seems to stay for some time in the Wadden Sea area. In spring the birds join the rest of the population on haunts in the Wadden Sea.

Lambeck, R.H.D. - 1979. Vogels en Oosterschelde (Birds and the Eastern Scheldt). *Zeeuws Nieuws. Natuur, Landschap en Milieu* 5, 54-58.

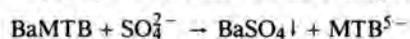
In this paper (in Dutch) a review is given of the ornithological knowledge about the Eastern Scheldt. Species, role of the area as habitat for breeders and migrants, numbers, ecological relationships and the international importance for birds (especially waders) of the Eastern Scheldt are discussed.

Merks, A.G.A. and J.J. Sinke, 1979. De analyse van opgelost sulfaat in zoute en brakke wateren met behulp van een automatisch analyse-systeem. *DIHO Rapp. en Versl.* 1979-8, 14 pp.

In many samples of brackish inland waters of the Delta area of the Netherlands sulphate was determined according to the classical gravimetric analysis. This method takes time and is not very reproducible. Another problem was the minimum volume of water needed for the analysis, at least 50 ml. Especially for the research of interstitial water where very small samples are common, this will be a hard to overcome case.

A method in which small volumes can be used, and based on a precipitation of BaSO_4 in glycerol-ethanol, can only be used in samples with very small differences in matrix. This now is not possible in inland or interstitial water samples.

We had the possibility of testing a method of the RID ('t Hart, pers. comm.). Also we did some test serials in saline and brackish waters. The method is based on the forming of methyl-thymol-blue (MTB) out of Barium methyl-thymol-blue BaMTB according to:



The resulting MTB can be detected in a colorimeter at 460 nm. Many cations disturb this reaction and therefore a sample should be washed in a cation-exchanger. All the chemical handlings of the sample and the measurement of the resulting color are done in an automatic analysis system. In our case we use an Auto Analyser AA II. The advantage of this analysis is in this way clear: completely automatic, about 30 samples per hour can be done and this is much quicker than with any of the older methods. Also the reproducibility is much better than especially the gravimetric method. The volume of sample which is needed for the complete analysis is very small, about 2-4 ml.

Parma, S. - 1978. Political aspects of the closure of the Eastern Scheldt estuary. *Hydrobiol. Bull.* 12, 163-175, (Δ -173).

A chronological survey is given of the discussion about the aspects of the closure of the Eastern Scheldt, an estuary in the SW Netherlands (Delta Area). Delta Plans were made already before World War II. After the Disaster of 1953 the main objective was to obtain a sufficient safeguard against superfloods. The ecological losses in the Delta Area were not yet recognized. Due to the activities of action groups this aspects was put into the interest of political parties. In 1973 a special commission investigated the possibilities of a synthesis between ecology and land protection. The commission proposed to maintain a reduced tidal range in the Eastern Scheldt by means of a storm-surge barrier. In Government and Parliament this pro-

posal was evaluated, adapted and finally accepted. Dike-raising, according to the pressure groups the most simple and cheapest way to get the necessary protection, was definitely rejected. The most important consequence of these decisions was the extra costs of 2000 million guilders.

Up till now discussion are focussed on the most acceptable tidal reduction. The Parliament accepted because of financial reasons 25% reduction. The action groups advocate at most 10%.

Vlasblom, A.G. - 1979. Aantal overlevende dieren, zuurstofverbruik en osmotische waarden van het bloed van *Marinogammarus marinus* (Leach) bij verschillende temperatuur en chloriniteit combinaties. DIHO Rapp. en Versl. 1979-4, 22 pp.

The aim of the investigations was to get insight into the survival capacity of *M. marinus* in water of less salinity than that in the habitat. Criteria in the in-

vestigations were the number of surviving animals, the osmotic value of the blood, and the oxygen consumption of the animal under various temperature and chlorinity combinations.

M. marinus can live in water with chlorinities down to 7‰ without any disturbance. When the chlorinity of the water drops from the normal value 16‰, the osmotic value of the blood decreases steadily, while the survival is reduced to 60% at 2‰ chlorinity.

The temperature has no detectable influence either on the osmotic value of the blood or on the survival of the animal. *M. marinus*'s confinement to habitats with chlorinities not less than 7‰ Cl⁻ is due to the fact that the reproductive capacity is negatively affected in lower chlorinities, rather than to the tolerance of the adult animals.

The oxygen consumption, generally, increases with decreasing chlorinity.

Institute for Ecological Research

Progress Report 1979

Edited by J.W. Woldendorp



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1. Task and function of the Institute

The Institute was founded in 1954 by the Division of Sciences of the Netherlands Academy of Arts and Sciences for the performance and promotion of ecological research in a broad sense and co-operation with other organizations engaged in such research.

The research projects are carried out by four departments, three of which are housed at the Institute's headquarters at Arnhem; the fourth, the Department for Dune Research, has its seat at Oostvoorne. Recently, a new seat for experimental research was established at Heteren, near Arnhem. Field work is carried out in various parts of The Netherlands (see Fig. 1.1). The main objects of study are the properties of plants and animals in relation to their specific occurrence. In this respect special attention is paid to plants of grasslands and to birds, particularly the Great Tit.

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.

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 is applicable to the management of nature, and this aspect is taken into account in the planning of research projects. A number of governmental services makes use of the results of these projects. In addition, the Institute administers the ringing of birds in The Netherlands. It is also the site of the Euring Data Bank, which all recoveries of birds ringed in Europe are to be assembled.

The Institute is supervised by a committee appointed by the Division of Sciences of the Academy, and is financed by the Government.



Fig. 1.1. Location of the Institute for Ecological Research and its field-work sites

1. Headquarters in Arnhem
2. Department for Dune Research 'Weevers' Duin'
3. Heteren (experimental research)
4. National Park 'De Hoge Veluwe', where most of the field-work on the Great Tit is done
5. Vlieland (additional field-work)
6. Oosterhout (additional field-work on the Great Tit)
7. Liesbosch (additional field-work on the Great Tit)
8. Westeinder Plassen, where most of the field-work on the Coot is done
9. Dunes of Goeree (additional field-work of the Department for Dune Research)

2. Scientific staff

Director

J.W. Woldendorp

Population Ecology

J.H. van Balen (Head, Ecology)
J.A.L. Mertens (Eco-physiology)
P.J. Drent (Behavioural ecology)
A.J. van Noordwijk (Guest worker, BION/ZWO)
Jolien den Boer-Hazewinkel (Guest worker)
Christina W. Eshuis-van der Voet (Guest worker)

Bird Migration 'Vogeltrekstation'

A.C. Perdeck (Head, Bird migration)
A.J. Cavé (Ecology)
B.J. Speek (Bird ringing)

Distributional Ecology

J.H. Mook (Head, Ecology)
J. Haeck (Ecology)
R. Hengeveld (Zoology)
J. van der Toorn (Botany)
Ph. Stoutjesdijk (Micrometeorology)
R. Soekarjo (Plant physiology)

P.J.M. van der Aart (Head, Ecology)
A.H.J. Freijsen (Experimental ecology)
C.W.P.M. Blom (Experimental ecology)
D. van der Laan (Synecology)

C. van Dijk (Plant-microorganism relationships)
P.A.I. Oremus (Plant-microorganism relationships)
S.R. Troelstra (Soil science)
J. van Groenendael (Guest worker, BION/ZWO)
M.J. Adriani (Guest worker)

3. General summary

In 1979, the Institute celebrated its twenty-fifth anniversary. To commemorate this event, a number of scientific activities were organized. The highlights of the past and present research were displayed on 55 posters which were exhibited at various meetings, most of them of a national character. In addition, an audiovisual series was composed to illustrate the multidisciplinary project on 'The characteristic properties of grassland plant species in relation to their environment'.

In September an international symposium was organized on 'The integrated study of bird populations'. In this symposium special attention was given to research on the Great Tit carried out in several countries. The *Proceedings* of the symposium will appear in the second half of 1980. The Department of Population Ecology contributed four papers to the symposium. Summaries of some of the papers are included in this Progress Report. In addition, the effects of inbreeding in the Great Tit are described.

The severe winter of 1978/1979 caused many Coots to die from starvation. In accordance with previous findings, the juvenile, non-autochthonous females proved to be the most susceptible to such starvation, due to their small size and low fat reserve.

In the preceding Progress Report results were given on survival rates obtained by the Bird Migration Department by analysis of recoveries of ringed birds. Continuation of this analysis showed that the survival rate of the Dutch Grey Heron is related to winter temperature, as has also been found for the British Grey Heron. The recovery data were also used to analyse the distribution pattern over the year of some species of ducks and geese. Comparison of recoveries of birds 'shot' and 'found dead' revealed that the picture of the distribution of the birds over the year is strongly biased by the distribution of hunters and finders. However, on the assumption that the bird species under investigation are influenced in the same way by these factors, comparison provided interesting results. For instance, the observation that the male ducks leave the breeding area before the females, was confirmed. However, in some duck species, e.g.

Wigeon, Pintail, and Teal, the winter quarters of the females lie farther south than those of the males. This allohiemy might be due to differences in the feeding behaviour of the sexes.

The departments of Distributional Ecology and Dune Research continued their work on the characteristic properties of grassland species in relation to the local environment. This project is being carried out in collaboration with the universities of Groningen and Utrecht. The research is concentrated mainly on the five *Plantago* species occurring in The Netherlands. Most *Plantago* species show little overlapping of habitat requirements. Only *P. lanceolata* has a wide ecological amplitude, and this species often grows together with one of the other species. Consequently, the properties of *P. lanceolata* could be compared with those of each of the other species. *P. major* and *P. coronopus* show larger fluctuations in population numbers than the other species do. *P. coronopus* seems to be better adapted to drought but less well to severe frost than *P. lanceolata*. The life span of adult plants of *P. major* ssp. *major* is more or less the same as that of *P. lanceolata*. The larger population-size fluctuations of *P. major* may be related to the large amounts of seed produced. For *P. media* the fluctuations in population size are relatively small, as in the case of *P. lanceolata*. However, the former species is more resistant to drought and frost, and reproduces mainly by seed. In *P. lanceolata* the relatively low resistance to drought and frost is compensated by a high capacity for regeneration. The species displays considerable differences in demographic behaviour in the various locations. Indications were obtained that these differences are correlated with the structure of the vegetation as measured by light extinction.

The characterization of the microclimate of the various *Plantago* species was continued. For this purpose, a small thermo-electric psychrometer was constructed; this instrument is described in the present report.

The work on methods to assess the capacities of the environment to provide the *Plantago* species with nitrogen and phosphorus showed a good correlation between the amounts of nitrogen found in a mineralization experiment in the field and the quantities taken up by the plants. The analysis of the cations and anions present in the plant material

demonstrated that considerable environment-related differences exist between the various species. From these cation-anion balances it could be concluded that in the field at least part of the nitrogen taken up by *P. lanceolata* is in the form of nitrate. The investigations on the growth of *P. lanceolata* and *P. major* at extremely low nitrate concentrations confirmed earlier results indicating that the former species is less vulnerable to the effects of changes and interruptions in the nitrate supply.

A detailed study was done on vegetative regeneration in the *Plantago* species. The formation of daughter rosettes was highest in *P. lanceolata*. However, the apical meristem of this species is located well above the ground level, unlike that of adult plants of *P. coronopus*, *P. media* and *P. major*. The latter species are therefore better protected from damage due to grazing and treading.

So far, no pronounced differences have been found in the amounts of mycorrhiza on the root systems of the various *Plantago* species growing on dune grassland. Consequently, there are no indications that these symbiotic structures specific adaptations to the environment.

The combined results of the studies performed by the participants in the *Plantago* project suggest that for *P. lanceolata* there are no important differences in the genetic composition of the various populations. Nevertheless, due to the high phenotypic adaptability of the individuals the species is adapted to a wide range of environmental conditions. The populations of *P. major* are more heterogeneous genetically but its individuals are less capable of adaptation to variations in environmental conditions. As a result, the species is found in places with relatively stable growth conditions. In conclusion, it can be stated that different life-strategies have evolved in the *Plantago* species.

4. Population Ecology

4.1. POPULATION DYNAMICS OF THE GREAT TIT, *Parus major*

4.1.1. Territory occupancy, feeding conditions, and local survival in the winter (J.H. van Balen, P.J. Drent)

The study on the importance of territory occupancy and winter feeding conditions for the local survival of the Great Tit population in the Hoge Veluwe area was continued. The behavioural part of the study was terminated at the end of the 1979 breeding season. In the winter of 1979/80 observations were made on the distribution of the foraging tits in relation to the weather and the small beechmast crop.

The results of the latter study, some of whose numerical data date back to 1959, were incorporated into two lectures given at the International Symposium 'The integrated study of bird populations', held in September at Wageningen. The summary of one of these papers, to be published in *Ardea* 68 (1980), is presented below.

Population fluctuations of the Great Tit and feeding conditions in winter

(J.H. van Balen)

Summary

The fluctuations in breeding numbers of the Great Tit population in the 1960-1972 period on a 320 ha study area in 'Hoge Veluwe' were analysed. This was done

by an analysis of the numerical relations between parameters of the population and some environmental variables. During part of this period extra seed food was provided in the winter.

A preliminary analysis showed that the size of the beech crop has a strong effect on juvenile survival, and hence on population size; the provision of extra seed food by the observers enhanced the survival of the older age classes in particular. Further analysis by multiple regression and canonical correlation techniques gave the following results:

– The number of autochthonous yearlings in the breeding population (*i.e.*, young born in the study area in the preceding year) is positively correlated with the number fledged the year before and with the size of the beech crop; moreover, it is negatively related to the number of breeding birds in the preceding year, which indicates that the yearlings' chances of settling depend on the amount of space left vacant by the older birds. The beech crop influences juvenile survival between fledging and first breeding.

– The number of older breeding birds is affected by the provision of extra seed food, which enhances the survival of this category. Furthermore, there are indications that density-dependent losses (due to mortality and/or emigration) occur, and that the severity of the winter plays a role.

– The different responses of yearlings and older birds to the two types of seed food can be understood from the distribution of the food sources, the degree of site-attachment, and the dominance relations of different categories of birds.

The fluctuations in numbers, foraging behaviour, and diet in winter are discussed next, on the basis of

observations carried out in 1973-1979. In these years the number of breeding pairs fluctuated considerably, which was in accordance with the prevailing feeding conditions and the severity of the winters.

In the winter of 1976/77 there was a very good crop of beechmast. Although beechmast was already available in September/October, large-scale feeding on these nuts only occurred in December-January, and especially on days with frost and snow cover. This suggests that beechmast functions as an emergency food source.

The impact of a short spell of unfavourable weather was very distinct in February of 1978, when the number of tits present suddenly decreased by about 80 per cent after a period of snow on the 14th. The absence of a seed food supply and unfavourable winter weather resulted in the largest decrease in breeding numbers (46%) found in this study.

A similar situation occurred in the winter of 1978/79, but now the unfavourable period started earlier and lasted much longer. During this period a large part of the study area was evacuated by day, and to a lesser extent by night. A large part of the population then foraged at feeding stations outside the area. The area was repopulated in March, and the over-all decrease in breeding numbers only amounted to 24 per cent. The tits that foraged in the area from September to December, and also in March, consumed mainly animal food. This too indicates that animal food is preferred, and that seeds serve as emergency food.

It is shown that part of the disappearance of the yearlings already occurs before November; on the other hand, processes that determine the size of the breeding population act throughout the winter.

There are regional differences in the extent to which beechmast is consumed in the autumn and winter. In southern Scandinavia the Great Tit seems to be much more dependent on beechmast than is the case in The Netherlands and (possibly) in England. The impact of severe winter weather on Great Tit populations differs considerably between countries in relation to the general climate and the feeding conditions.

4.1.2. *Inbreeding in the Great Tit* (A.J. van Noordwijk)

If a male and a female have a common ancestor, they will both carry genes that are copies of the genes of that common ancestor. Their offspring may receive two copies of the same gene from this ancestor. This higher level of homozygosity causes the well-known deleterious effects of inbreeding. Quantitative estimates of these effects can be made by comparing degrees of inbreeding, because the homozygosity endowed by descent is proportional to the degree of the parents' relatedness.

In all four populations analysed (Hoge Veluwe, Liesbos, Oosterhout, and Vlieland) several cases of inbreeding were detected, but a reliable estimate of the occurrence and effects of inbreeding can only be made for the Vlieland population. In the other populations this estimate would be strongly dependent on the assumptions made about immigrants, *i.e.*, birds whose parents are not known: if they come from far, they will not be related to individuals born in the study area, and if they do not, they can be involved in inbreeding that is not detectable.

The effect of inbreeding can be observed in the hatching rate of eggs. The Vlieland population showed a significant regression of the hatching rate on the degree of inbreeding, not only as a result of the egg genotype but also due to the female's being inbred. When the egg is inbred the hatching rate decreases by 7.5 per cent for every 10 per cent increase in the degree of inbreeding. Taken together, the failure of about 60 per cent of all non-hatching eggs during the study period can be ascribed to the detected part of inbreeding. In the total brood reduction (clutch size minus number fledged) the effect of inbreeding is slightly lower. This could be expected if deaths due to inbreeding do not continue to occur after hatching. If the deaths of nestlings are partially density dependent, they would be less likely to occur after an early brood reduction by inbreeding. Thus, there may be some compensation that masks the effects of inbreeding on the number of fledglings.

Thus, the contribution of related pairs to the next year's breeding population is not smaller than the contribution from other pairs, *i.e.*, the effect of non-hatching eggs is more than compensated for by a higher recruitment rate.

The recruits from nests of related pairs, being inbred individuals, might have a lower fertility. If the female is inbred, the proportion of clutches with non-hatching eggs increases with the degree of inbreeding. No effect of the male's being inbred on hatching failure was found. However, the recruitment from these clutches was also higher than expected on the basis of the recruitment rate of clutches with the same size in the same year. This means that the number of grandchildren of breeding age produced by a related pair is higher than the number produced by an unrelated pair. Therefore, there seems to be no basis for the view that inbreeding should be avoided or that avoidance of inbreeding will be selected for.

4.1.3. *Energy requirements for incubation* (J.A.L. Mertens)

In the preceding Progress Report a new technique for measuring the heat flow in nest boxes was presented (Plate 1). This method was used to measure the energy



Plate 1. Nestbox for measuring heat production of hole-nesting birds. In order to detect the heat flux density all over the surface, 35 sensors are mounted in the walls.

expenditure of a female Great Tit while roosting in an empty nestbox, at night during the nestbuilding period, in the egg-laying period, and during incubation. It was found that the heat loss increased considerably during the egg-laying period. The nocturnal heat loss during incubation was roughly three times greater than the heat loss incurred at the resting metabolic rate. These findings are in conflict with predictions based on recently published models of incubating birds. It is concluded that the energy requirements for incubation may impose restrictions on the onset of incubation.

4.2. ECOLOGY OF THE COOT, *Fulica atra* (J. Visser)

Selective mortality in the Coot during the severe winter of 1978/79

In a recent paper on fat and protein metabolism in the Coot (Visser 1978), it was shown that under adverse environmental conditions the loss of fat and protein is related to the size of the birds (measured by wing length). Large birds lose more fat than small ones do, but the percentage loss of large Coots is smaller, due to the large fat reserves of these birds under normal conditions. The fat reserves are exhausted at an earlier stage in small than in large Coots, which means that small individuals have to use their protein reserves sooner than large ones. Hence, mortality due to adverse environmental conditions could be expected to be related to body size.

Wing length is not an ideal measure of body size, because it is affected by a large number of factors (Visser 1976). In large samples, however, differences in wing length can indicate size differences between the sexes, age groups, populations, etc. In the Coot

males have significantly longer wings than females (Visser 1976). Moreover, the body weight, lean dry weight, and the weight of the components protein and water, are significantly higher in males than in females. So there appears to be a size difference between the sexes. However, the mean fat weight does not contribute to this size difference. In 18 females and 12 males killed in the autumn and winter the mean fat weight was 172 and 171 g, respectively (Visser 1978). Nevertheless there seems to be a size-dependent relation in the decrease of fat and protein during adverse environmental conditions. For this reason one would expect mortality to be greater in females than in males. Fredrickson (1969) investigated 293 American Coots *Fulica americana* that died during a cold spell in spring. The breast-contour measurements indicated that females were more emaciated than males. Although birds of both sexes lost about the same percentage of body weight during starvation, the females apparently utilized a higher proportion of breast muscle than the males.

The severe winter of 1978/79 offered a good opportunity to test the hypothesis of differential mortality in the Ringvaart Haarlemmermeer study area. It was possible to collect samples of both live and starved Coots during January and February, 1979. Body weight, wing length and age composition were determined for both, and the sex of the starved birds was determined by dissection. Table 4.2.1. gives data on wing length and body weight in the two samples. The starved birds show a significant difference in wing length between males and females, corresponding with the differences found earlier (Visser 1976). There is also a significant difference in body weight, males being 73 g heavier than females. The mean weights of

Table 4.2.1. Wing length and body weight of starved and live Coots during the winter of 1978/79 in the Ringvaart Haarlemmermeer area. The values between parentheses are the 95% confidence limits.

	sample size	mean wing length (mm)	sample size	mean body weight (g)
starved Coots				
♀♀	42	199.6 (197.8-208.4)	43	408 (388-428)
♂♂	24	213.0 (210.4-215.6)	24	481 (454-508)
♀♀ + ♂♂	66	204.4 (202.2-206.6)	67	440 (421-459)
live Coots				
♀♀ + ♂♂	142	212.1 (210.7-213.5)	143	661 (643-679)

Table 4.2.2. Sex ratios of Coots in different winters.

area	month	year	sampling method	sample size	number of females	number of males
Geestmerambacht	Jan.	1970	shot	88	44	44
Geestmerambacht	Jan.	1972	shot	54	27	27
Ringvaart Haarlemmermeer	Jan./Febr.	1979	observed	51	26	25
Ringvaart Haarlemmermeer	Jan./Febr.	1979	found dead	67	43	24

the starved birds are remarkably lower than those determined during a short cold spell in 1976 (females 474 g, $n = 7$; males 653 g, $n = 5$; Visser 1978).

However, the 1976 sample included some birds which had died from causes other than starvation. If wing length and body weight of the starved and live birds are compared a significant ($P < 0.01$) difference is found for both measures. Starved Coots have smaller wings and a lower body weight. This difference in mean wing length is the first indication that differential mortality occurred.

To investigate a change in sex ratio in starved Coots compared with that of live birds, the composition of normal winter populations should be known. However, this information is difficult to obtain, because the only reliable characteristic for sex determination in Coots is a difference in voice. Unfortunately, this feature can seldom be used in the winter. A second problem is that of the random collection of samples. The chances of catching males and females or juveniles and adults with the light-trapping method might differ. The best estimate of the sex ratio in wintering Coots is probably obtained by examining hunters' bags. This was done in the Geestmerambacht area in January of 1970 and 1972. Both samples of Coots had been shot by several hunters. The entire bag was taken to the laboratory, where the sex was determined by dissection. The results were striking: in both samples the numbers of females and males were equal (Table 4.2.2.).

Another attempt to determine the sex ratio in the winter population was made by observing Coots with coloured rings. These rings have been used since the 1976 breeding season to identify breeding birds individually. At the onset of the cold spell in 1978/79, 376 colour-ringed adult Coots of known sex were available for observation (204 females and 172 males). These frequencies do not differ from a 1:1 ratio (chi-square = 2.722, $P = 0.10$). During January and February of 1979, 51 of these Coots (26 females and 25 males) were observed in the Ringvaart Haarlemmermeer study area. The sex composition of this sam-

ple did not differ from the composition of the total population of colour-ringed birds (chi-square = 0.194, $P = 0.66$). For 34 birds the exact age was known. The mean age for both 18 females and 16 males was five years (= 6th calendar year).

We can conclude from this that the sex ratio of adult autochthonous Coots in January/February 1979 was about 1:1. Moreover, there are no indications that the sex ratio of this group was changed by selective mortality.

The last sample in Table 4.2.2. comprises the starved Coots found in January and February of 1979. The sex ratio of this group differs significantly (chi-square = 5.388, $P = 0.02$). from the expected 1:1 ratio. This means that starvation occurs more frequently in females than in males. The significant difference in wing length between starved and live Coots (Table 4.2.1.) can be partially ascribed to the sex composition of the two samples.

According to Table 4.2.2. there is no change in sex ratio in the observed autochthonous adults, which indicates that the differential mortality mainly occurs in juveniles. This can be checked from the age composition of the sample of starved birds. Age determination in winter is, however, rather unreliable. Since the age of both groups was determined with the same method, we assume that there is no difference in error between the two groups. It is clear from Table 4.2.3. that there is significant differential mortality in the juveniles but not in the adults. This results in an unexpectedly high number of starved juvenile females.

Table 4.2.3. Sex and age composition of the sample of starved birds.

	females	males	chi-square	P
adults	14	11	0.360	0.55
juveniles	29	13	6.096	0.01

The difference in age composition between live (80 ad. and 62 juv.) and starved Coots (15 ad. and 42 juv.) also indicates that mortality caused by low temperatures is not random (chi-square = 6.590, $P = 0.01$). For juvenile females the losses appear to be higher than in any other category.

There are several factors which can contribute to differential mortality in Coots. Possibly the larger males can withstand cold better than the smaller females, because of a lower surface-volume ratio. This reduces the rate of heat loss per unit area of body surface. Herreid and Kessel (1967) found that larger species have a more effective heat insulation (in terms of $\text{cal g}^{-1} \text{hr}^{-1} \text{°C}^{-1}$) than smaller species do. Removal of the feathers caused an increase in conductance which was greatest in large species, and this suggests that the greater insulation of the large species resides at least partially in the feathers. Moreover, Herreid and Kessel found that intraspecific variation in body weight in the Redpoll (*Acanthis flammea*) was accompanied by significant changes in conductance. Conductance was lower in heavy individuals than in light individuals. Kendeigh (1969) mentioned four advantages for tolerating cold associated with increasing body size: '1. reduction in relative amount of energy required for existence, 2. lower metabolic stress per degree drop in temperature, 3. extension of zone of thermal neutrality to a lower critical ambient temperature, and 4. lower extreme limits of tolerance'. This holds for interspecific differences in metabolism correlated with weight, but probably also for intraspecific differences.

Although there are several physiological advantages for large individuals within a species, it is clear that large individuals need a greater total food intake. This might counteract the apparent advantages.

Another advantage of large body size may be found in the dominance relations under conditions in which competition for food occurs. During periods of low

ambient temperatures, birds must increase their food intake. If the accessibility of food is very unfavourable, this can result in strong competition between the individuals of wintering flocks. In such a situation dominant individuals or dominant groups will claim most of the available food. This could easily apply to the feeding conditions of wintering Coot flocks.

The population of wintering Coots can be divided into eight groups, according to sex, status, and age. There are significant differences in mean wing length between these groups (Visser 1976), but also differences in mean body weight (Table 4.2.4). The weight : wing length ratio expressed as g.mm^{-1} can be considered as a crude condition index (Owen and Cook 1977). The data in Table 4.2.4 are presented in descending order of mean body weight. The differences in mean body weight are striking. Juvenile non-autochthonous females are 300 g lighter (30%) than adult autochthonous males. All differences in body weight between the successive groups are significant ($p < 0.05$). Within the autochthonous and non-autochthonous groups there is a close relationship between mean wing length and mean body weight of the sex and age groups ($r = 0.971$, $P < 0.05$ and $r = 0.995$, $P < 0.01$, respectively).

At the start of a cold spell the juvenile non-autochthonous females are in the worst position, having the lowest condition index. It seems likely that during adverse environmental conditions over a long period the proportional mortality in the successive groups of Table 4.2.4 will increase in correspondence with their decreasing condition index.

References

- Fredrickson, L.H. (1969) – Mortality of coots during severe spring weather. *Wilson Bull.* 81, 450-453.
 Herreid, C.F. II and B. Kessel (1967) – Thermal con-

Table 4.2.4. Mean body weight, mean wing length, and condition index of eight groups of Coots (weights collected over the period from September through March from 1967 to 1975; wing length after Visser 1976).

group	sex	status	age	sample size	mean body weight (g)	mean wing length (mm)	condition index weight:wing length (g.mm^{-1})
1	♂♂	autochthonous	adult	423	993	220.3	4.5
2	♂♂	autochthonous	juvenile	325	967	215.1	4.5
3	♂♂	non-autochthonous	adult	365	917	216.9	4.2
4	♂♂	non-autochthonous	juvenile	446	886	212.9	4.2
5	♀♀	autochthonous	adult	441	796	207.2	3.8
6	♀♀	autochthonous	juvenile	380	764	201.7	3.8
7	♀♀	non-autochthonous	adult	363	753	203.3	3.7
8	♀♀	non-autochthonous	juvenile	451	693	199.9	3.5

ductance in birds and mammals. *Comp. Biochem. Physiol.* 21, 405-414.

Kendeigh, S.C. (1969) – Tolerance of cold and Bernmann's rule. *Auk* 86, 13-25.

Owen, M. and W.A. Cook (1977) – Variations in body weight, wing length and condition of Mallard *Anas platyrhynchos platyrhynchos* and their relation-

ship to environmental changes. *J. Zool., Lond.* 183, 377-395.

Visser, J. (1976) – An evaluation of factors affecting wing length and its variability in the Coot *Fulica atra*. *Ardea* 64, 1-21.

Visser, J. (1978) – Fat and protein metabolism and mortality in the Coot *Fulica atra*. *Ardea* 66, 174-183.

5. Bird migration

FLYWAYS OF ANATIDAE MIGRATING THROUGH THE NETHERLANDS (A.C. Perdeck, C. Clason)

Introduction

Bean Goose, White-fronted Goose, Wigeon, Pintail, Shoveler, Teal, and Mallard have been ringed in great numbers in The Netherlands, and a large number of recoveries are available. A comparative analysis of the migration patterns will be published elsewhere. In this report attention is drawn to biases due to the manner of recovery and to differential migration according to sex.

5.1. COMPARISON BETWEEN RECOVERIES OF BIRDS SHOT AND FOUND DEAD

Recoveries of ringed birds may not reflect the true distribution (spatial and temporal) of the population in question. For geese and ducks, the majority of the recoveries concern shot birds, which represent a mixture of the distribution of birds and hunting. To obtain an impression of the biases involved an attempt was made to compare the recoveries of shot birds with other kinds of recoveries. 'Found dead' proved to be the only other category with sufficient data.

The recoveries of both categories were divided into four periods of recovery: April (spring migration), May plus June (nesting time), September plus October (autumn migration), and January plus February (winter). For each of the seven species an average (median) position in each period is given for both categories in Fig. 5.1.

The difference between the categories are striking. During nesting time, 'shot' extends farther to the north-east than 'found dead' whereas the reverse is true during the winter. In autumn there is no great difference, but in April the differences are again large. The same trend holds within the species. While this difference is clear without statistical analysis for the periods of April and May plus June, its possible occurrence in the other periods was studied as follows.

A common main axis (first principal component) of all positions concerned was calculated, and positions were projected on this axis. The results showed that during the winter, within each species, the value of 'shot' on the main axis is smaller than that of 'found dead' in 6 out of 7 cases, whereas during the autumn this was 3 out of 7 cases.

To compare the complete migration patterns, the positions of the seven species in six bi-monthly periods were averaged (centre of gravity from the median geographical co-ordinates) and plotted in Fig. 5.1 (bottom). The positions in April are also given separately. From this picture it is concluded that although migration is apparent for both 'found' and 'shot' birds, the patterns are quite different. 'Shot' shows a much longer migration route, which is due to an extension at both ends. Furthermore, 'shot' birds seem to migrate during the autumn along a path differing from that used in the spring, which suggests a kind of a roundabout route or *Schleifenzug*. This pattern is especially determined by the recoveries in April. However, this phenomenon is not observable in the recoveries of the 'found' birds.

The discrepancies between these categories can be explained by differences in the chances of being recovered as 'shot' or 'found dead'. 'Found dead' recoveries are concentrated in the area with the greatest human population density. In 1965 only Belgium, The Netherlands, the Federal Republic of Germany, and the United Kingdom exceeded a density of 400 inhabitants per square kilometre (Bogue 1969). It is obvious that the chance of a dead bird being picked up is greater there than in the less densely populated areas. This implies that the 'found' picture is biased toward a smaller range of movement. For 'shot', however, no more reliable picture can be expected. The hunting pressure is probably largest in the southwestern part of Europe during the winter which would explain the extension to the southwest. The extension to the northeast in May and June may not be real either. Although the hunting season is closed at this time of the year, it is likely that in the northeastern part of Europe control is more difficult and the hunting pressure is therefore heavier.

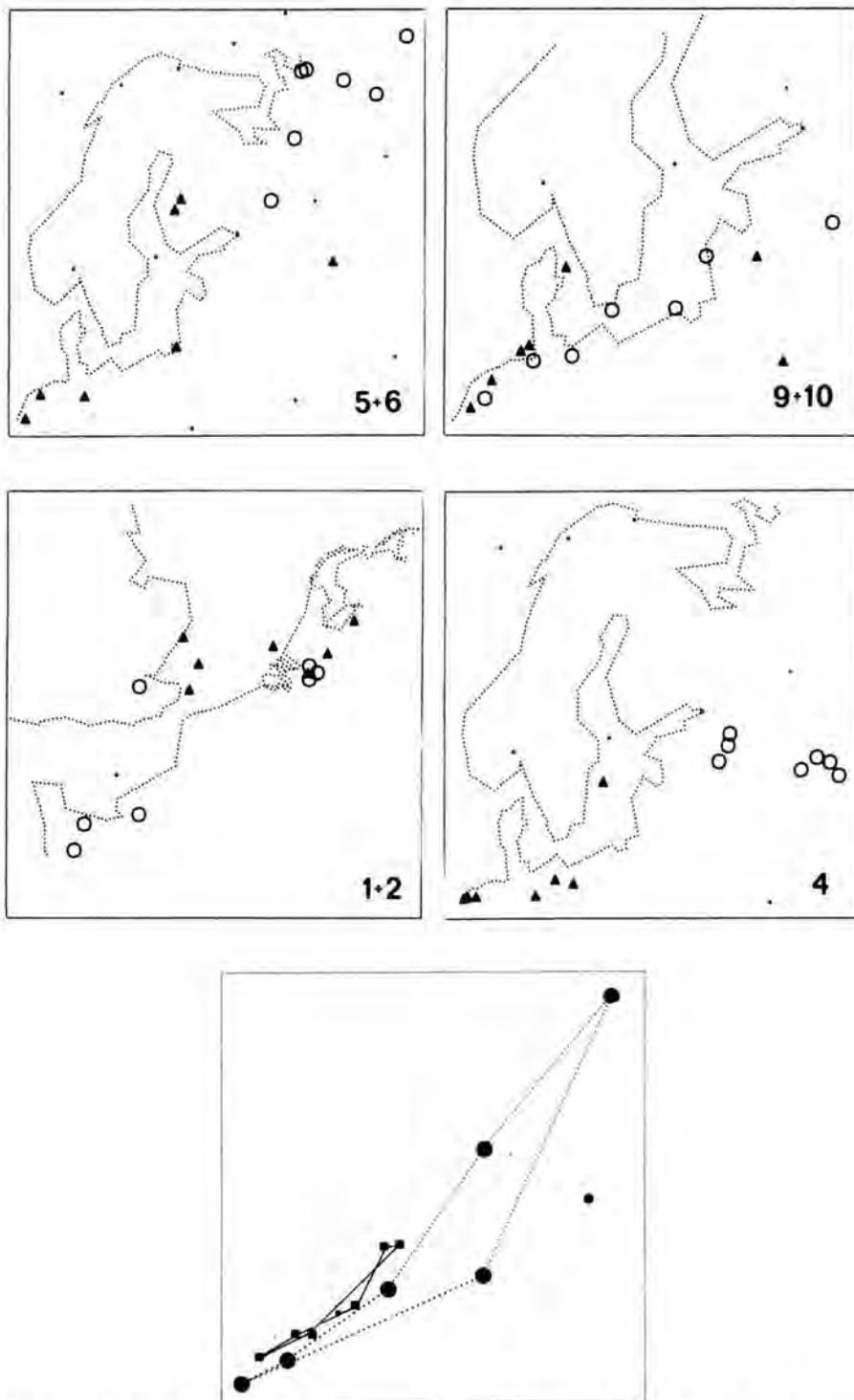


Fig. 5.1. Top four maps: average position of recoveries of ducks and geese 'shot' (circles) and 'found dead' (triangles) in two-monthly periods (numbers). Each symbol indicates the average position of one species. Bottom: average position (of the mean positions of 7 species of ducks and geese together) in two-monthly periods (numbers). Dots: shot. Squares: found dead.

5.2. DIFFERENTIAL MIGRATION ACCORDING TO SEX

A fairly large number of the ringed birds have been sexed, and therefore recoveries of these individuals can be used to analyse differences between males and females. For each species the median position of males and females in a certain period was calculated. The periods concerned are May and June combined, August, September, October, November, December, January, and February. Figs. 5.2 and 5.3 show these positions, with the male and female median positions connected by a line.

For each period the difference between the individual recovery-clouds of males and females were tested with the two-sample uniform scores test (Mardia 1972). Significant differences are found during the autumn (Mallard, Teal, Wigeon) and the winter (Pintail, Wigeon, White-fronted Goose). Apart from these significant differences, there seems to be, at least in the ducks, a trend that can be formulated as follows: from the breeding time onward the males are ahead of the females during the autumn migration, but the females catch up, say in November, and even overtake them in winter.

To test this pattern for each species, the main axis or first principal component (on the map) was calculated from the 16 median positions (8 for each sex). These positions were then projected on this axis and the value read for the female position was subtracted from that of the male position. Since the value on the axis increases from wintering area to breeding quarters, a negative difference means that the females are closer to the breeding area, and a positive difference has the reverse meaning. The result is given in Table 5.1. The concordance of the values was assessed with Friedman's test from the ranks given in parentheses.

The hypothesis that the seven species are not concordant (i.e., heterogeneous) could not be rejected ($W =$ coefficient of concordance $= 0.25, P = 0.09$).

When the geese are excluded, the ducks form a concordant group ($W = 0.53, P = 0.01$). The two geese species were not concordant ($W = 0.49, P = 0.45$). If the eight periods are numbered from 1 to 8, this sequence is correlated with the mean rankings of the ducks ($r_s = 0.76; p < 0.05$). This is not the case for the geese (Bean Goose: $r_s = -0.50; P > 0.10$; Whitefronted Goose: $r_s = -0.10; P > 0.10$).

It is therefore concluded that: 1. in the geese differences in position between the sexes are not related to the time of the year, and 2. in the ducks there is such a relationship that females tend to lag behind the males during the autumn, catch up with them in the winter, and then generally have more southwesterly positions than the males (sexual allohiemy).

It is well-known for the ducks that the males leave the breeding area before the females. This is in contrast with the geese, where male and female migrate together as pairs or families. Our data reflect this state of affairs, and thus suggest that on the whole, the ringers sexed the animals correctly.

Figs. 5.2 and 5.3 show that the early migration of the males is sometimes directed toward the winter quarters (Mallard) and sometimes more to the south (Wigeon, Pintail), indicating migration to certain moulting areas.

Differences in winter positions are most pronounced in the Wigeon, Pintail, and Teal. This seems to be in accordance with field observations and duck-wing studies.

During the winter, a preponderance of males was observed in The Netherlands for Teal and Pintail (Lebret 1950). The mid-winter sex ratio of the Mallard, however, approached equality (Eygenraam 1957).

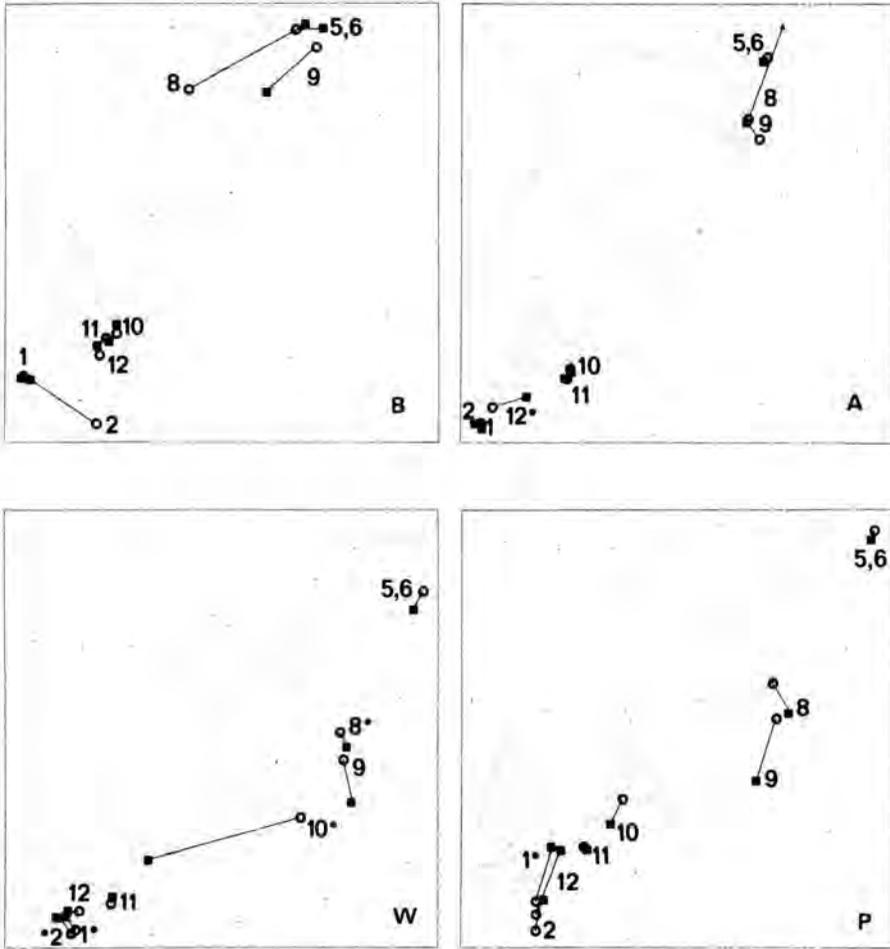
Boyd, Harrison and Allison (1975), Harrison and Allison (1977), and Harrison (1977) published results of analyses of duck wings collected from hunters in Great Britain. The adult male/adult female numbers

Table 5.1. Difference monthly median position of males and females on the main axis (kilometres). Values between parentheses are rank numbers.

	Bean Goose	White-fr. Goose	Wigeon	Pintail	Shoveler	Teal	Mallard	ducks
May & June	+110(7)	- 19(1)	-137(2)	- 54(3)	-258(2)	-350(8)	-296(3)	(4)
August	+760(8)	+576(8)	- 14(5)	- 53(4)	-543(1)	-210(2)	-353(2)	(2)
September	-404(1)	+ 41(6)	-135(3)	-378(1)	- 81(4)	-231(1)	-447(1)	(1)
October	+ 50(6)	- 4(3)	- 9(1)	-176(2)	+ 36(6)	- 58(3)	-247(4)	(3)
November	- 4(3)	+ 12(5)	+ 42(8)	+ 9(5)	+198(8)	+ 55(4)	- 9(5)	(6)
December	+ 47(5)	+174(7)	- 74(4)	+432(8)	+133(7)	+ 65(5)	+ 27(7)	(7)
January	- 13(2)	+ 4(4)	+ 17(7)	+351(7)	- 98(3)	+ 85(6)	+ 10(6)	(5)
February	- 3(4)	- 8(2)	0(6)	+192(6)	- 3(5)	+144(7)	+ 44(8)	(8)

Note: The periods of duck ringing are from July through March (Shoveler), October through March (Mallard), and August through March (Wigeon, Pintail, Teal).

Fig. 5.2. Average position of males (squares) and females (circles) in the various months (numbers). Asterisks indicate a significant difference between males and females. B = Bean Goose, A = White-fronted Goose, W = Wigeon, P = Pintail.



were as follows (number of seasons in parentheses): Wigeon: 2273/993(6), Teal: 1106/757(4), Pintail: 33/20(1), Shoveler: 18/4(1), and Mallard: 725/841(3).

The clear excess of males in the Wigeon led Harrison (1977) to suggest that 'the adult females have winter quarters elsewhere, quite possibly in France or further south'. This is confirmed by our analysis.

Duck-wing studies, field observations, and the present analysis all indicate that in the Mallard there is no difference in position between the sexes.

This lack of divergence might be explained by the early pair-formation of this species. According to Bezel (1959), 80 per cent of the individuals have already paired at the end of October. In the Teal this is not the case until the end of February.

Sexual allohiemy is also found in some species of diving ducks, e.g. the Goldeneye (Nilsson 1969),

Pochard, and Tufted Duck (Salomonsen 1968). Salomonsen suggests that the males, arriving first in the winter quarters, occupy all available space within the specific niches and pre-empt the food reserves. The females (and juveniles) are forced to continue their migration to areas beyond.

Another possibility is that the allohiemy is caused by different food requirements of the male and female. In Goldeneyes and Teal it has been observed that females feed in shallower water than males do, and that during cold periods the females left the area (Nilsson 1970; Willi 1970). In general, females seem to be more reactive to cold weather. This in itself could cause segregation of the sexes in the winter quarters. If this is true, a relationship between winter temperature and position would be expected, especially in the females. This question is under study at present.

References

Bezzel, E. (1959) – Beiträge zur Biologie der Geschlechter bei Entenvögeln. Anz. Ornithol. Ges. Bayern 5, 269-355.

Bogue, D.J. (1969) – Principles of Demography. Wiley, New York.

Boyd, H., J.G. Harrison and A. Allison (1975) – Duck Wings. A study of duck production. Wagbi Conservation Publication.

Eygenraam, J.A. (1957) – The sex-ratio and the production of the Mallard, *Anas platyrhynchos* L. Ardea 45, 117-143.

Harrison, J.G. (1977) – A summary of western European duck wing studies, 1974/75 to 1976/77. International Waterfowl Research Bureau, Bulletin No. 43/44, 78-86.

Harrison, J.G. and A. Allison (1977) – The duck production survey in western Europe 1976-77. Wagbi Magazine, Autumn 1977, 11-14.

Lebret, T. (1959) – The sex-ratios and the proportion of adult drakes of Teal, Pintail, Shoveler, and Wigeon in The Netherlands, based on field counts made during autumn, winter and spring. Ardea 38, 1-18.

Mardia, K.V. (1972) – Statistics of directional data. Academic Press, London.

Nilsson, L. (1969) – The migration of the Goldeneye in north-west Europe. Wildfowl 20, 112-118.

Nilsson, L. (1970) – Food-seeking activity of south Swedish diving ducks in the non-breeding season. Oikos 21, 145-154.

Salomonsen, F. (1968) – The moult migration. Wildfowl 19, 5-24.

Willi, P. (1970) – Zugverhalten, Aktivität, Nahrung und Nahrungserwerb auf dem Klingnauer Stausee häufig auftretender Anatiden, insbesondere von Krickente, Tafelente und Reiherente. Orn. Beob. 67, 141-217.

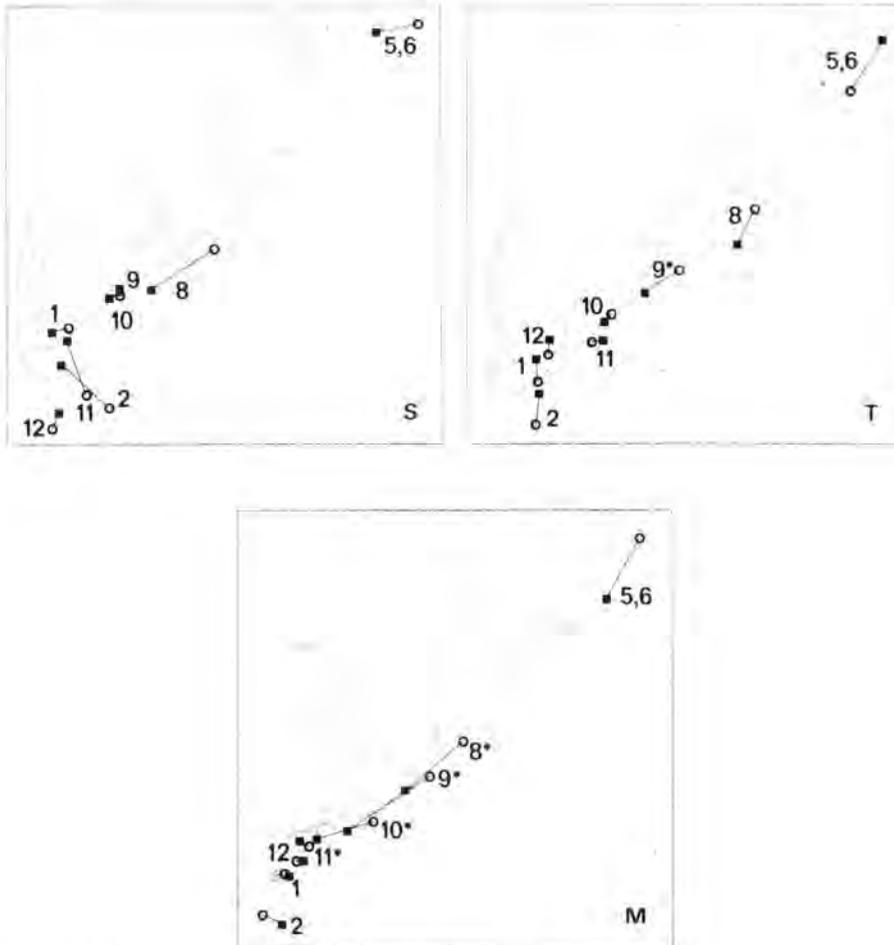


Fig. 5.3. As figure 5.2. S = Shoveler, T = Teal, M = Mallard.

6. Distributional ecology

6.1. SOME REMARKS ON THE DEMOGRAPHY OF *Plantago* SPECIES (J. van der Toorn, J. Haeck, J.H. Mook)

Demographic research has been concentrated on species of the genus *Plantago*, viz. *P. lanceolata*, *P. media*, *P. major* ssp *major*, and *P. coronopus*. The primary object is to investigate the differences in demographic behaviour between species growing in the same habitat and within species occurring in different habitats. Here, some preliminary results will be given on (1) the fluctuation in plants numbers, (2) germination and vegetative reproduction, and (3) the rate of mortality of small and large plants. Some of the data (concerning the location Westduinen) were collected by J. van Groenendael (Dune Research, 'Weevers' Duin').

The investigated populations occur in highly divergent grass vegetations (Table 6.1.1). Most *Plantago* species show little overlapping of habitat requirements. Only *P. lanceolata* has a wide ecological amplitude, and this species often grows together with one of the other species. Such locations were preferably chosen to permit comparison of the behaviour of a given species with that of *P. lanceolata*. One exception is the Uddel location, where the species grow only a few meters apart but in slightly different habitats (*P. coronopus* in an area which is somewhat more trodden than that of *P. lanceolata*). Another exception is the Heteren location, where both species (*P. lanceolata* and *P. major*) occur on the same soil but each in their 'own' environment (in a hay field and on a path, respectively).

6.1.1. Fluctuations in plant numbers

Fig. 6.1.1. shows the course of the total numbers of rosettes present on each observation date, as well as the numbers of new and disappearing rosettes (both expressed cumulatively). An indication of the magnitude of the fluctuation in numbers is provided by the 'relative fluctuation' (Table 6.1.2.), i.e., the ratio between the highest and lowest numbers of rosettes within a certain period (here October, 1978–October, 1979).

From Fig. 6.1.1 and Table 6.1.2 it is evident that *P. major* and *P. coronopus* show larger population fluctuations than the other species do. In the case of *P. major* the largest fluctuations occur in the growth season, because most of the seedlings (which form a large part of the population in the spring) die during the summer.

P. coronopus showed a large decrease in the winter 1978-1979, at both Westduinen and Uddel. This is in contrast to the increase of *P. lanceolata* numbers. This divergent behaviour of the two species had been seen earlier (in the Westduinen) in the (relatively dry) period of 1976-1977, when the total number of *P. coronopus* increased and that of *P. lanceolata* decreased (Vermuë 1979).

In contrast to the findings in the above-mentioned species, the graphs for *P. media* and *P. lanceolata* are strikingly similar for both locations. This is one of the points that deserves more detailed investigation.

6.1.2. Germination and vegetative reproduction

Fig. 6.1.2. shows the number of seedlings and new daughter rosettes present on each observation date. The percentages of daughter rosettes (relative to the total number of new rosettes) are given in Table 6.1.2. From Fig. 6.1.2. it is clear that most species have maximal germination in the spring. The Westduinen location is exceptional, because – in 1978 – the maximum occurred in the autumn (for both *P. lanceolata* and *P. coronopus*).

All species show vegetative reproduction by daughter rosettes (Table 6.1.2.), but percentages are low for *P. media* and *P. coronopus* and very low for *P. major*, whereas *P. lanceolata* shows wide variation. Daughter rosettes may be formed as a reaction to various forms of damage, e.g. caused by frost (winter 1978–1979; Westduinen, Uddel), predation by snails (Heteren), and trampling (Bruuk path). As a result, they are found in highly divergent habitats. The greater number of daughter rosettes formed by *P. lanceolata* might be due to the fact that its apex is not retracted below the ground level, as is usually the case in *P. media* and *P. major*, but remains exposed (Soekarjo 1979, and this report).

6.1.3. Mortality

In Table 6.1.2. the percentage of mortality is given for three categories of plants, distinguished according to their life stage in the spring of 1978. The mortality data concern three periods, viz. summer (May–October 1978), winter (October, 1978–May, 1979), and the whole year (May, 1978–May, 1979).

In general, the mortality of small plants (seedlings and 1-3 leaf stage) is higher than that of large (or 'adult') plants (≥ 4 leaf stage). In relation to this, the year mortality of seedlings is mostly high, but that of adult plants varies, both between and within species. *P. coronopus* (Uddel) has the highest 'adult year mortality', also compared with the co-habiting *P. lanceolata*. *P. coronopus* died mainly from frost

Table 6.1.1. Survey of investigated populations and description of habitats.

	Plantago species				Habitat	Structure	Soil
	<i>lanceol.</i>	<i>media</i>	<i>coron.</i>	<i>major</i> ssp. <i>major</i>			
Bruuk path	*			*	footpath; heavily trampled mown once a year	short and dense grass; a few open spots	peaty loam
Uddel	*		*		roadside <i>P. cor.</i> heavily trampled <i>P. lanc.</i> moderately trampled	<i>P. cor.</i> short and open <i>P. lanc.</i> short and moderately	sand
Westervoort	*	*			old railway embankment rough herbage; lightly grazed (rabbits)	dense grass moderately high, but open	gravel-rich sand and clay
Pannerden	*	*			small river dike; lightly grazed by ponies	moderately high and dense grass	sandy clay
Heteren hayfield	*				hay field; mown once a year (early July)	very high and dense grass	heavy clay
Heteren path				*	wheel tracks; rarely trodden	moderately high grass with open spots	heavy clay
Westduinen	*		*		old coastal dune grassland; in summer grazed by young cattle	short and open grass	dune sand

Table 6.1.2. Demographic data on *Plantago* populations

Population	Relative fluctuation in plant number ¹⁾	Percentage daughter rosettes ¹⁾ (1979)	Percentage mortality ²⁾								
			Seedling			1-3 leaf stage ³⁾			≥4 leaf stage ⁴⁾		
			S	W	Y	S	W	Y	S	W	Y
<i>P. lanceolata</i>											
Bruuk path		44	84	42	91	50	60	80	29	18	42
Uddel	1.9	38	27	71	79	16	6	21	11	41	48
Westervoort	2.1	2	74	49	87	35	28	53	14	16	28
Pannerden	1.6	35	79	75	95	22	32	47	3	19	21
Heteren hayfield	2.3	21	91	0	91	59	5	61	6	0	6
Westduinen	2.5	- ⁵⁾	-	-	-	-	-	-	-	-	-
<i>P. media</i>											
Westervoort	2.0	1	61	74	88	27	18	40	1	2	3
Pannerden	2.0	7	-	-	-	43	54	73	7	16	21
<i>P. major</i> ssp. <i>major</i>											
Bruuk path	6.6	0.4	95	25	96	31	8	36	12	12	22
Heteren path	7.1	0	60	89	96	32	33	55	17	8	24
<i>P. coronopus</i>											
Uddel	4.8	2	72	100	100	36	97	98	21	99	99
Westduinen	4.1	-	-	-	-	-	-	-	-	-	-

1) Ratio between highest and lowest numbers of rosettes in a given period

2) S = summer: April/May 1978-October/November 1978

W = winter: October/November 1978-April/May 1979

Y = year: April/May 1978-April/May 1979

3) for *P. coronopus* plants with a diameter of 0-10 mm

4) for *P. coronopus* plants with a diameter of ≥11 mm

5) data not yet available

damage in the severe winter of 1978-1979, as a result of which almost the whole population in Uddel was eradicated. *P. lanceolata* shows a wide variation in its adult year mortality. High values are found in open situations, where extensive damage is caused by frost (Uddel) and trampling (Bruuk path). The lowest mortality (especially in the winter) is found in places with dense and tall grass (Heteren). That this species has a low frost resistance in open situations, was also found in an experimental garden at Heteren.

Where *P. major* grows together with *P. lanceolata* on a foot path (Bruuk) its mortality (for both 1-3 and ≥4 leaf stages) was found to be considerably lower. Where both occurred in their 'own' habitat (Heteren), *P. major* showed a higher adult mortality.

In the relative dry and open Westervoort location, *P. media* has a considerably lower adult mortality than *P. lanceolata*, possibly due to a greater resistance to drought and frost. This difference was not found in the Pannerden location.

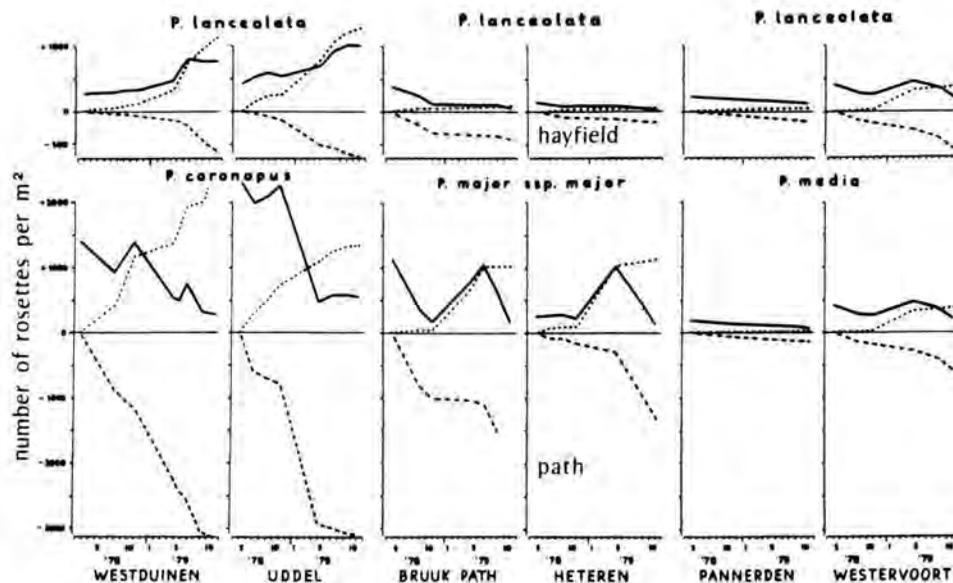


Fig. 6.1.1. Fluctuation in rosette number for several *Plantago* species. Solid lines: rosettes present. Dotted lines: new rosettes. Dashed lines: rosettes disappeared.

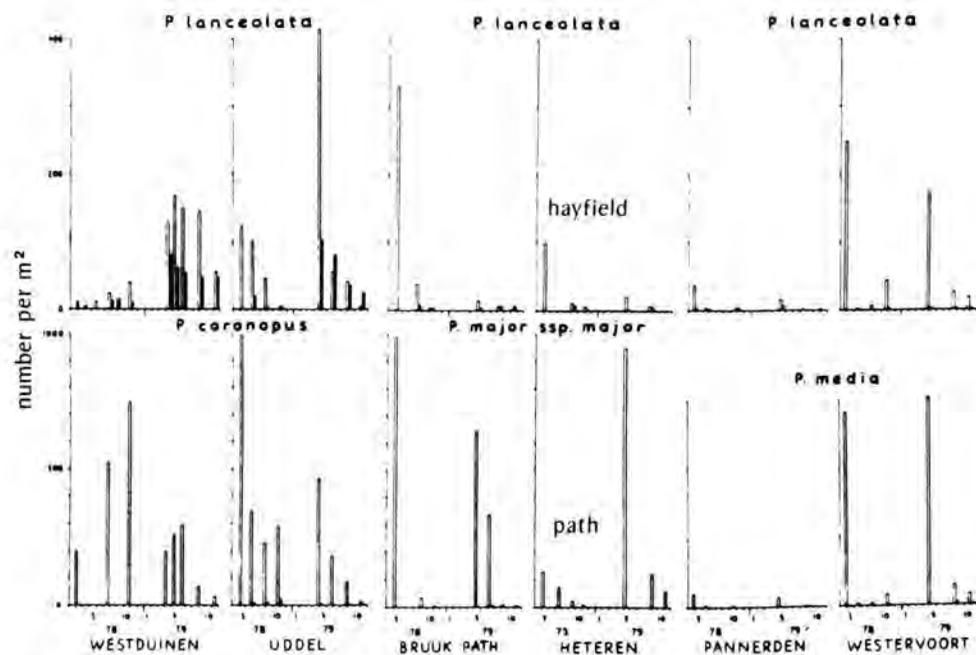


Fig. 6.1.2. Number of new seedlings and daughter rosettes present on each observation date for several *Plantago* species. Open columns: seedlings. Solid columns: daughter rosettes.

From the above, the following tentative conclusions may be drawn: *P. coronopus* (relative to *P. lanceolata*) seems to be better adapted to drought, but not to severe frost. The latter is perhaps due to its low capacity for regeneration. The species has a relatively short life span. Because the plants produce almost no seeds during their first growth season, there is a real chance of extinction of the population in a period with several successive severe winters. This might be one of the reasons why the species is so rare on inland sites.

P. major ssp *major*: Since the mortality of adult plants does not differ much from that of *P. lanceolata*, their life span will be about the same. Reproduction occurs by (many) seeds, which results in large fluctuations in population size.

P. media: Fluctuations in population size are relatively small, as in the case of *P. lanceolata*. Under dry and open conditions there is a relatively low adult mortality, which indicates a higher resistance to drought and frost compared with the last-mentioned species. In the experimental garden, too, a higher frost-resistance is evident. The species reproduces mainly by seed.

P. lanceolata: This species has a low resistance to frost in open situations. This is partially compensated for by its marked capacity for regeneration. As a result of this, the species can survive under other unfavourable conditions as well, such as trampling and predation. The demographic behaviour of this species differs between the various locations (see Figs. 6.1.1. and 6.1.2, and Table 6.1.2.). There are indications that these differences are correlated with the structure of the vegetation, as measured by light extinction.

References

Soekarjo, R. (1979) – Regeneration in *Plantago* species. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, 344-346.

Vermuë, J. (1979) – Demografisch onderzoek aan *Plantago coronopus* en *P. lanceolata* op de Westduinen. Internal report Instituut for Ecological Research.

6.2. VEGETATIVE REGENERATION IN *Plantago* SPECIES AND THE RELATION BETWEEN VEGETATIVE AND GENERATIVE REGENERATION (R. Soekarjo)

6.2.1. Introduction

Vegetatively formed daughter plants are generally in an advantageous position compared to seedlings. This is mainly due to the existing maternal support (Ginzo and Lovell 1973) in obtaining water, nutrients, and metabolites in the early stage of development. Therefore, vegetative regeneration may be of impor-

ance for the survival of a plant species in its habitat. This vegetative regeneration is of still greater importance, when we take into consideration that an increase of plant density by sowing seeds or planting young seedlings is not easily effected as is the case in *P. lanceolata* (Sagar and Harper 1960; Sagar and Mortimer 1976). Since this observation indicates that germination and further development of the seedling normally meets with critical phases, this phenomenon may be of general importance.

In earlier experiments (Soekarjo 1979) it has been shown, that *P. lanceolata*, *P. major*, and *P. coronopus* form daughter rosettes after removal of the apical meristem. Further work on the regeneration capacity of *Plantago* species is being done, to contribute to an understanding of the factors underlying their differences in occurrence in various habitats. Attention is also given to the development of daughter rosettes in intact plants and to the relation between vegetative and generative regeneration.

6.2.2. Formation of daughter rosettes in intact plants

The *Plantago* species belong to that group of rosette plants which maintain their rosette habitus. Inflorescences are borne laterally in the axils of leaves situated on the thickened stem base (caudex). The apical meristem remains in a vegetative condition, producing only leaves. Daughter rosettes are also formed on the caudex, as a result of the development of axillary buds. When the plants are grown in the greenhouse without supplementary nutrition, only in *P. lanceolata* the formation of daughter plants was observed. The plants were moved to the laboratory garden in autumn and sample groups were harvested in the next autumn. Winter mortality was only severe in *P. coronopus*, amounting to 50 per cent. Table 6.2.1. shows the number of rosettes in the four species under investigation, after the first and second growing season.

From these results it is clear, that *P. lanceolata* forms more daughter rosettes than the other species do, after both one and two years. The increase during the second year is even very strong. However, it should be noted, that in those *P. coronopus* plants that had withstood winter conditions, the number of rosettes formed was also distinctly higher, amounting to five per plant. In *P. major* and *P. media* no significant numbers of daughter rosettes were formed.

6.2.3. Effect of mechanical injury on the formation of daughter rosettes in *P. media*

Removal of the apical meristem caused the development of lateral buds into daughter rosettes (Soekarjo 1979). So in these true rosette plants the correlative in-

Table 6.2.1. Mean number of rosettes per plant in four *Plantago* species
(number of plants in brackets).

species	after one growing season		after the second growing season	
<i>P. major</i>	1.0	(20)	1.7	(17)
<i>P. media</i>	1.0	(20)	1.1	(20)
<i>P. lanceolata</i>	2.4	(61)	14.3	(9)
<i>P. coronopus</i>	1.0	(20)	6.0	(8)

Table 6.2.2. Formation of new leaves in *P. media* after mechanical injury.
Mean number of leaves of 5 plants per treatment.

treatment	after 1 week	after 2 weeks	after 3 weeks
debladed control	2.0	3.4	4.4
debladed and apical meristem removed	0	7.2	23.4
caudex removed	0.8	16.6	18.4

Table 6.2.3. Formation of new shoots in *P. media* after mechanical injury.
Mean number of shoots of 5 plants per treatment.

treatment	after 1 week	after 2 weeks	after 3 weeks
debladed control	0	0	0
debladed and apical meristem removed	1.2	6.4	7.2
caudex removed	3.2	6.4	8.0

Table 6.2.4. Long-term effects of the removal of the apical meristem in
P. major; situation after 7 months.
Mean number of 8 plants per treatment.

treatment	rosettes per plant	green leaves per plant	inflorescences per plant
debladed	1.0	9.3	7.8
debladed and apical meristem removed	3.5	16.8	9.5

hibition of lateral buds is similar to that in plants where the stem is normally elongated. But, as can be seen in Table 6.2.1., even on intact plants some daughter rosettes are formed in the first year by *P. lanceolata*. The degree of apical dominance is, therefore, different in these four species. The other three species show an absolute apical dominance in their first year, when left undamaged. In the second year there is a clear 'escape' from apical dominance in *P. coronopus*. As the response to the removal of the apical meristem was very clear, further experiments could be conducted using only five plants per treatment.

The plants were grown in the greenhouse, and the experiment proper was done under constant temperature (22°C) and a relative humidity of 80% in the light period and higher in the dark period. Low light intensity was used to obtain etiolated growth ($10 \pm 2 \text{ W.m}^{-2}$) as would occur in a closed grassland vegetation before the regeneration products reach the open light conditions. The plants were given a short day regime (8 hours light, 16 hours darkness), using TL 33/65 fluorescent light tubes. The regeneration process was followed for three weeks, to investigate the short-term responses.

Table 6.2.2. gives the numbers of new leaves formed after the removal of leaves only, of leaves and apical meristem, and of the caudex. A remarkable result was, that already after one week, those plants from which the caudex was removed, had formed some leaves. After two weeks, the number of leaves on these plants was significantly higher than in those from which only the apical meristem had been removed ($P = 0.05$). Because no part of the shoot is left in those plants whose caudex has been removed, the leaves must have originated from primordia formed on the main root. Moreover, it is likely that these primordia were present before the time of the treatment, as can be inferred from the rapid development. After three weeks, however, there was no significant difference any more between the numbers of leaves formed in these two sets of plants. If we now consider the number of shoots formed in the same experiment (Table 6.2.3), we see that more shoots were formed after one week on the plants without caudex ($0.025 < P < 0.05$). After two weeks, both treated sets had formed an equal number of shoots.

From these results it is clear that in the first week after the treatment, root-borne shoots develop in *P. media*.

6.2.4. Relation between vegetative and generative regeneration in *P. major*

Experiments similar to those reported for *P. media* were also done with *P. major*. No evidence was

found, however, for the formation of root-borne shoots in *P. major*. Here, the shoots newly formed after the removal of the apical meristem all originated from the axillary buds. When the plants were grown in the greenhouse for another seven months, the differences between debled plants and those from which the apical meristem had been removed, were still observable. The number of rosettes per plant as well as the number of green leaves on the plants, were higher in the plants from which the apical meristem had been removed seven months before (Table 6.2.4).

No daughter rosettes developed on the plants that were only debled, as could be expected. There was no significant difference in the numbers of inflorescences formed in each group. Both sets of plants were then debled again. The results after three more months, *i.e.*, ten months after the original treatment, are given in Table 6.2.5.

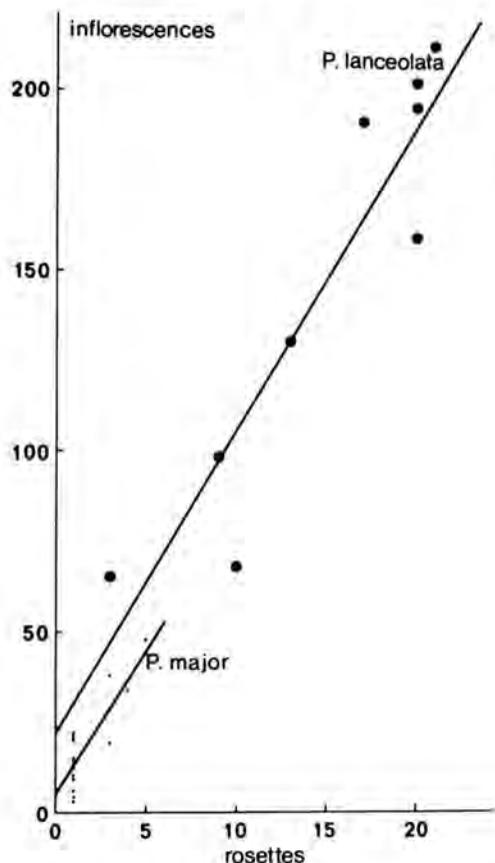
The differences between the two treatments were all highly significant ($P < 0.005$). From these results we may conclude that a plant deprived from the apical meristem, produces more leaves and inflorescences after the formation of new daughter rosettes than does a plant with only one rosette. The development after the second treatment suggests that this is a lasting effect. Since the ratio between inflorescences and green leaves is equal in both cases, the activity of the leaves may well be similar. The differences we observe, may well be due to the development of a larger root system in the plants with more rosettes. This larger root system in plants with more rosettes could be a favourable factor in the competition with other plants.

6.2.5. Relation between the numbers of inflorescences and the number of rosettes in uninjured plants

The tendency to produce more inflorescences with an increasing number of rosettes per plant, as described in the preceding section, was also observed in plants that were not injured. The basis of this phenomenon may again be the development of a larger root system in plants with more rosettes. In *P. coronopus*, where there was a great diversity in morphological characteristics between the plants, no evidence for this possibility could be obtained. In *P. lanceolata* it is beyond doubt that plants produce an increasing number of inflorescences, with increasing numbers of rosettes per plant, as can be seen in Fig. 6.2.1. Even in *P. major*, where not many plants with more than one rosette were found, this relation is evident.

Only for *P. lanceolata* it is certain that more rosettes may develop on one plant without being injured. In the other three species the occurrence of more than one rosette may be due to damage during the winter. The influence of various factors on the degree of apical dominance in these species will be studied fur-

Fig. 6.2.1. Relation between the number of rosettes per plant and the number of inflorescences per plant in *P. lanceolata* and *P. major*.



ther to obtain more evidence on the formation of daughter rosettes not related to injury and the successive formation of inflorescences.

6.2.6. The occurrence of root-borne shoots

As mentioned under 6.2.3, the occurrence of root-borne shoots was observed in *P. media*. This ability to form shoots on the roots is an important property enabling the plant to maintain its presence in its habitat. Another aspect of the formation of root-borne shoots is the possibility of obtaining cloned material. When the pots containing the plants were put on capillary mats providing a humid substrate, root-borne shoots were obtained in both *P. media* and *P. lanceolata*. These shoots developed on roots growing through the capillary mats. When the pots were moved, intentionally severing the roots, shoots also developed from the proximal end of the roots in the mats. These shoots together with a small part of the root, can be harvested and propagated individually. In this way the mother plant is preserved and can be used

repeatedly as a source of cloned material. The occurrence of root-borne shoots in *P. media* and *P. lanceolata* was already mentioned in the early literature (Irmisch 1857). They were observed to occur on the main root in *P. media*, and on small rootlets in *P. lanceolata* when root parts are uncovered.

6.2.7. Discussion

In an earlier report (Soekarjo 1979) it was suggested that the position of the apical meristem relative to the ground level might play an important role in the mechanism by which species maintain themselves in their specific habitat. The position of the apex in combination with the regenerating capacity provides alternative or even concurrent ways in which vegetative regeneration can occur. In those cases where the apical meristem is drawn below ground level by root contraction, damage to the leaves does not impede the formation of new leaves at the apical meristem. This situation occurs in well-settled plants of *P. major*, *P. media*, and *P. coronopus* when the ground is trodden upon or the leaves are eaten by animals or damaged otherwise. Under these conditions the apex of *P. lanceolata* is exposed to injury, because this is the only species under investigation in which the root contraction is not sufficient to compensate for the increase in stem length. The apical meristem remains well above ground level. If the apical meristem is damaged, axillary buds will develop into daughter rosettes.

Repeated damage, which is most likely to occur in this species, will eventually cause exhaustion and disappearance of the plant. These results explain the observation, that *P. major*, *P. media*, and *P. coronopus* (adult plants) are able to withstand treading and grazing, whereas *P. lanceolata* is not (Blom 1979). Under favourable conditions of soil aeration and an ample supply of water and nutrients, the formation of root-borne shoots in *P. lanceolata* may prolong the survival of a particular plant, for instance in well-kept lawns (von Arx-van der Brugge 1978). If one or more of these conditions is lacking, however, *P. lanceolata* will most probably disappear.

The formation of more inflorescences on plants having more than one rosette, both untreated and with the apical meristem removed, points to the possibility of an increased generative capacity after vegetative regeneration. The question whether this also holds for plants subjected to competition by other plants, requires further investigation. Also, damaged plants form their seeds later in the growing season than undamaged ones. This might be important under conditions of competition. If the competing species is not able to resume growth and development as fast as *P. lanceolata* does, the latter species will still be able to form seed before the end of the growing season, as occurs in hay fields (Berendse 1979).

Table 6.2.5. After-effects of the removal of the apical meristem in *P. major*, following a second treatment, i.e., deblading of both sets of plants. Mean number of 8 plants per original treatment.

original treatment	rosettes per plant	green leaves per plant	total leaves per plant	inflorescences per plant	inflorescences per green leaf
debladed	1.0	9.1	17.3	11.5	1.3
debladed and apical meristem removed	3.3	13.6	38.3	17.3	1.3

References

- Von Arx-van der Brugge, H.F.J. (1978) – Pers. comm.
- Berendse, F. (1979) – To be published.
- Blom, C.W.P.M. (1979) – Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. Thesis Nijmegen.
- Ginzo, H.D. and P.H. Lovell (1973) – Aspects of the comparative physiology of *Ranunculus bulbosus* L. and *Ranunculus repens* L. II. Carbon dioxide assimilation and distribution of photosynthates. Ann. Bot., N.S. 37, 767-776.
- Irmisch, Th. (1857) – Ueber die Keimung und die Erneuerungsweise von *Convolvulus sepium* und *C. arvensis*, so wie über hypokotylische Adventiefknospen bei krautartigen phanerogamen Pflanzen. Bot. Zeitg. 15, 465-474.
- Sagar, G.R. and J.L. Harper (1960) – Factors affecting the germination and early establishment of plantains (*Plantago lanceolata*, *P. media*, and *P. major*). In: The biology of weeds. J.L. Harper ed., Oxford, p. 236-245.
- Sagar, G.R. and A.M. Mortimer (1976) – An approach to the population dynamics of plants with special reference to weeds. Appl. Biol. 1, 1-47.
- Soekarjo, R. (1979) – Regeneration in *Plantago* species. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e reeks 73, 42-44.

6.3. A SMALL THERMO-ELECTRIC PSYCHROMETER AND ITS PERFORMANCE (Ph. Stoutjesdijk)

For the measurement of temperature and humidity it was necessary to use a small sensor performing acceptably over a wide range of humidity conditions, including those close to saturation point. From personal experience (Stoutjesdijk 1961) and data in the literature (Monteith 1954; Stigter and Welgraven 1976)

we expected that these requirements would be met by a thermo-electric device on the psychrometer principle. A further requirement was a reasonable resistance to mechanical damage, although this seemed hard to reconcile with good performance, because a thermocouple psychrometer performs better the more open the construction and the thinner the wires.

We finally arrived at the design shown in Fig. 6.3.1 (A, B). The thermojunctions constituting the dry and the wet 'bulbs' were made of chromel and constantan wire with a diameter of 0.03 mm. The wires were bent to form a V and interlocked as shown in the Figure. They were mounted somewhat recessed on

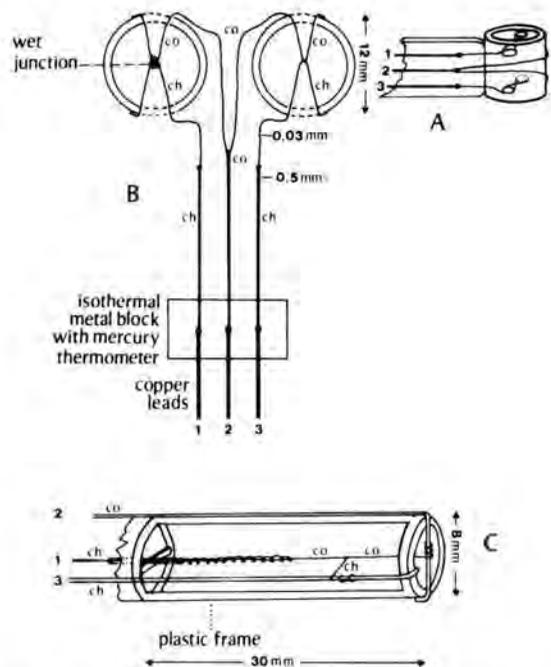


Fig. 6.3.1. Design and wiring scheme of droplet psychrometer (A, B) and small wick psychrometer. (C). co = constantan, ch = chromel.

both ends of a piece of perspex tube with an internal diameter of 10 mm and a length of 12 mm. The junctions were soldered with tin solder, and care was taken that only the spot where the wires made contact was tinned. The heavy extension wires (diameter 0.5 mm, length 1.5 m) made of chromel and constantan were connected to copper leads. These junctions were, together with a mercury thermometer, housed in a thermally isolated metal block.

One of the fine-wire junctions, the wet bulb, was provided with a tiny knot made of one strand of thin cotton sewing thread. This knot could hold a drop of water about 1.5 mm in diameter. Before use, the cotton was treated with a wetting agent.

The air temperature was measured in circuit 2-3, the mercury thermometer in the metal block being used as a reference. The difference between wet bulb and dry bulb temperature was measured in circuit 1-3. The performance of this instrument (droplet psychrometer) was compared with an aspirated Assmann psychrometer and also with a thermo-electric instrument (small wick psychrometer, Fig. 6.3.1, C). Here the wick consisted of cotton thread wrapped around the wires and extending 8 mm on each side of the junction. The wick was fed from a capillary reservoir. A larger version of this type of instrument (Stoutjesdijk 1961), with all sizes doubled and 0.05 mm thick wires, was also included in the test (large wick psychrometer).

Table 6.3.1. gives some representative results. The large wick psychrometer gave a stronger wet-bulb depression than the Assmann psychrometer did, its performance being virtually that of an ideal psychrometer (Stoutjesdijk 1961; Sonntag 1968). The small wick psychrometer was intermediary between

the large one and the Assmann instrument. The droplet psychrometer clearly showed a lower wet-bulb depression than the Assmann psychrometer or the other instruments tested. However, for the calculation of vapour pressure the normal psychrometer tables or formula can be used if a correction is made by adding 5% to the wet-bulb depression.

The droplet psychrometer was also tested in completely stagnant air over a saturated solution of NaCl in a 25-litre bottle. The vapour pressure measured in this way deviated less than 0.4 mbar from the expected value.

Although the droplet psychrometer did not approach the ideal instrument as closely as did the two types of wick psychrometer, especially the larger one, we still prefer it for use in dense vegetation because of its small size and remarkable ruggedness.

References

- Monteith, J.L. (1954) – Error and accuracy in thermocouple psychrometry. *Proc. Phys. Soc. B67*, 217-226.
- Sonntag, D. (1968) – *Hygrometrie*. Akademie Verlag, Berlin.
- Stigter, C.J. and A.D. Welgraven (1976) – An improved radiation protected differential thermocouple psychrometer for crop environment. *Arch. Met. Geoph. Biokl., Ser. B24*, 177-187.
- Stoutjesdijk, Ph. (1961) – Micrometeorological measurements in vegetations of various structure I. *Proc. Kon. Ned. Akad. Wetensch., Series C64*, 171-179.

Table 6.3.1. Wet-bulb depression ($^{\circ}\text{C}$) of different types of thermo-electric psychrometers (air temperature 22°C)

Assmann psychrometer	11.4
Droplet psychrometer in still air	10.8
Droplet psychrometer with ventilation	10.8
Small wick psychrometer in still air	11.3
Small wick psychrometer with ventilation	11.6
Large wick psychrometer in still air	11.8
Large wick psychrometer with ventilation	12.0

7. Ecological dune research 'Weevers' Duin'

7.1. SOME DEMOGRAPHIC CHARACTERISTICS OF *Plantago* SPECIES GROWN IN EXPERIMENTAL PLOTS (C.W.P.M. Blom, J. van Heeswijk)

Introduction and methods

The floristic composition and development of the semi-natural grasslands of coastal sand dunes can be strongly influenced by trampling and soil compaction. This study was performed to obtain more insight into the effects of these two environmental factors on the various stages in the life cycle of plants. For this purpose, *Plantago* species were chosen. *P. major* ssp. *major* and, to a lesser degree, *P. coronopus* are found on trampled and compacted soils. *P. lanceolata* occurs on moderately compacted and loose soils. For purposes of comparison *P. media*, which occurs on loose or moderately trampled clay and loam soils, was also included in the study.

The part of the study described in this report was performed in three groups of experimental plots where the effects of light, moderate, and heavy trampling on the growth and development of the four *Plantago* species were investigated. The trampling treatments were carried out with a trampling machine (see Blom 1979). In this experiment, the effects of compaction on these species, planted in April 1976 as young seedlings, were also studied.

The experimental design and many of the results have already been described in Blom, 1979. Because this experiment was continued in 1979, the results on survival and daughter-rosette formation are given in this report.

Results and discussion

A. Survival (Fig. 7.1.1.)

P. lanceolata

For *P. lanceolata*, the chance of survival is greatest on loose and moderately compacted soils. After three years, 80% of the planted individuals were still alive. On an untrampled, strongly compacted soil, the survival of this species was also found to be relatively high (70%).

The chance of survival decreased strongly with increasing trampling intensity. In October of 1979, only 37% of the heavily trampled *P. lanceolata* plants were still alive. During the entire research period the number of moderately and heavily trampled plants

decreased, whereas a decline of the number of lightly trampled plants was only observed in the beginning of the period.

P. coronopus

Of all the species under study, *P. coronopus* appeared to have the shortest life. In 1978 a strong decrease of *P. coronopus* was observed in all plots and in the severe winter of 1978-1979 all plants succumbed. This

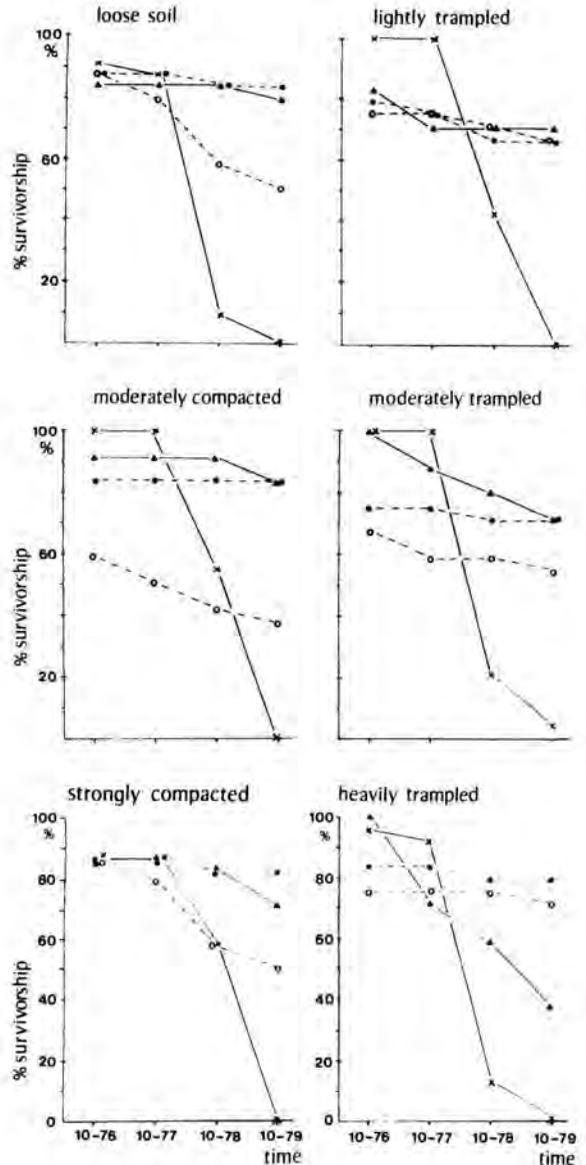


Fig. 7.1.1. The survival of four *Plantago* species in experimental plots.

phenomenon was also observed in the field outside the experimental plots.

Studies in the field indicate that plants of this species occurring on dune grasslands are short-lived (2-3 years). Thus, it is likely that the plants in the experimental plots (seed originating from a dune grassland population) would have died off even in the absence of extremely low temperatures.

The various treatments had a pronounced effect on the mortality rate. An increase in compaction of the soil led to an increasing number of surviving plants up to October, 1978. An increase of the trampling intensity led to a higher death risk. In 1978, the percentages of surviving plants were 42, 21, and 12, for the lightly, moderately, and heavily trampled plots, respectively. Compared with the untrampled plants on the loose soils, the chance of surviving was higher with light trampling.

P. major ssp. *major*

Comparison of the mortality rate of *P. major* plants in the untrampled and the trampled plots shows that under these conditions trampling strongly increases the chance of survival of this species. In 1979, the percentages of living plants lay between 37 and 54 in the untrampled plots, whereas for the trampled sites these values were 54 and 71. Remarkable were the relatively low numbers of living plants on the moderately compacted, trampled, and untrampled soils. The highest numbers of living plants were found on the heavily trampled plots (70.8% in 1979).

P. media

Between the treatments, no important differences in survival were observed for this species. Under these conditions, *P. media* had the longest life span of the *Plantago* species studied.

Comparison of the above results with the chances of survival of seedlings (Blom 1979) gives the following

picture. The effects of soil compaction and trampling on the seedlings of *P. lanceolata* are similar to those on mature plants. *P. coronopus* seedlings are very vulnerable to trampling and occur mainly on loose and relatively dry soils; the mature plants are more resistant to trampling. In the untrampled series the two-year-old *P. coronopus* plants survived in higher numbers on the compacted than on the loose soils. Seedlings of *P. major* and *P. media* succumbed in higher numbers in loose and relatively dry soils than the mature plants did. Both species have a high resistance to trampling in all stages of the life cycle.

Daughter rosette formation in *P. lanceolata* (Table 7.1.1.)

P. lanceolata showed a strong tendency to vegetative reproduction, which is in accordance with the findings of Soekarjo (1979). Only a few individuals of the other species formed daughter rosettes. No correlation was found between the vegetative reproduction of *P. major*, *P. coronopus*, or *P. media* and the treatment. It was remarkable that 7 plants (50%) of *P. media* formed rosettes in one of the untrampled, strongly compacted plots. However, daughter rosettes rarely occurred in the other plots of the same treatment.

As the results in Table 7.1.1 show, the number of rosettes per *P. lanceolata* plant increases with age. No important differences as to the number of daughter rosettes were observed between the trampled and the corresponding untrampled series. In 1979 there was a significantly higher number of rosettes per plant on the loose soils and on the lightly trampled plots compared with the untrampled strongly compacted soils and the heavily trampled plots. An increase of compaction and trampling led to a decrease in the rate of daughter rosette formation. The highest percentages of plants with more than one rosette were also observed on loose soils or on lightly and moderately trampled plots.

Table 7.1.1. Rosette formation by *P. lanceolata* plants in experimental plots.

A. Mean number of rosettes per plant ($n \pm S.E.$).

B. Percentage of plants with more than one rosette.

		loose soil	moderately compacted soil	strongly compacted soil	lightly trampled	moderately trampled	heavily trampled
A.	1976	1.4 \pm 0.2	1.0 \pm 0	1.3 \pm 0.1	1.2 \pm 0.1	1.2 \pm 0.1	1.1 \pm 0.1
	1977	5.6 \pm 0.7	2.5 \pm 0.5	1.8 \pm 0.4	4.6 \pm 0.5	3.0 \pm 0.4	3.4 \pm 0.5
	1978	7.2 \pm 0.8	4.1 \pm 0.6	4.6 \pm 0.6	6.8 \pm 0.7	5.9 \pm 0.8	4.5 \pm 0.9
	1979	12.4 \pm 1.9	8.4 \pm 1.0	9.5 \pm 1.5	14.5 \pm 1.7	8.7 \pm 1.5	6.8 \pm 1.8
B.	October 1979	95	82	76.5	100	100	89

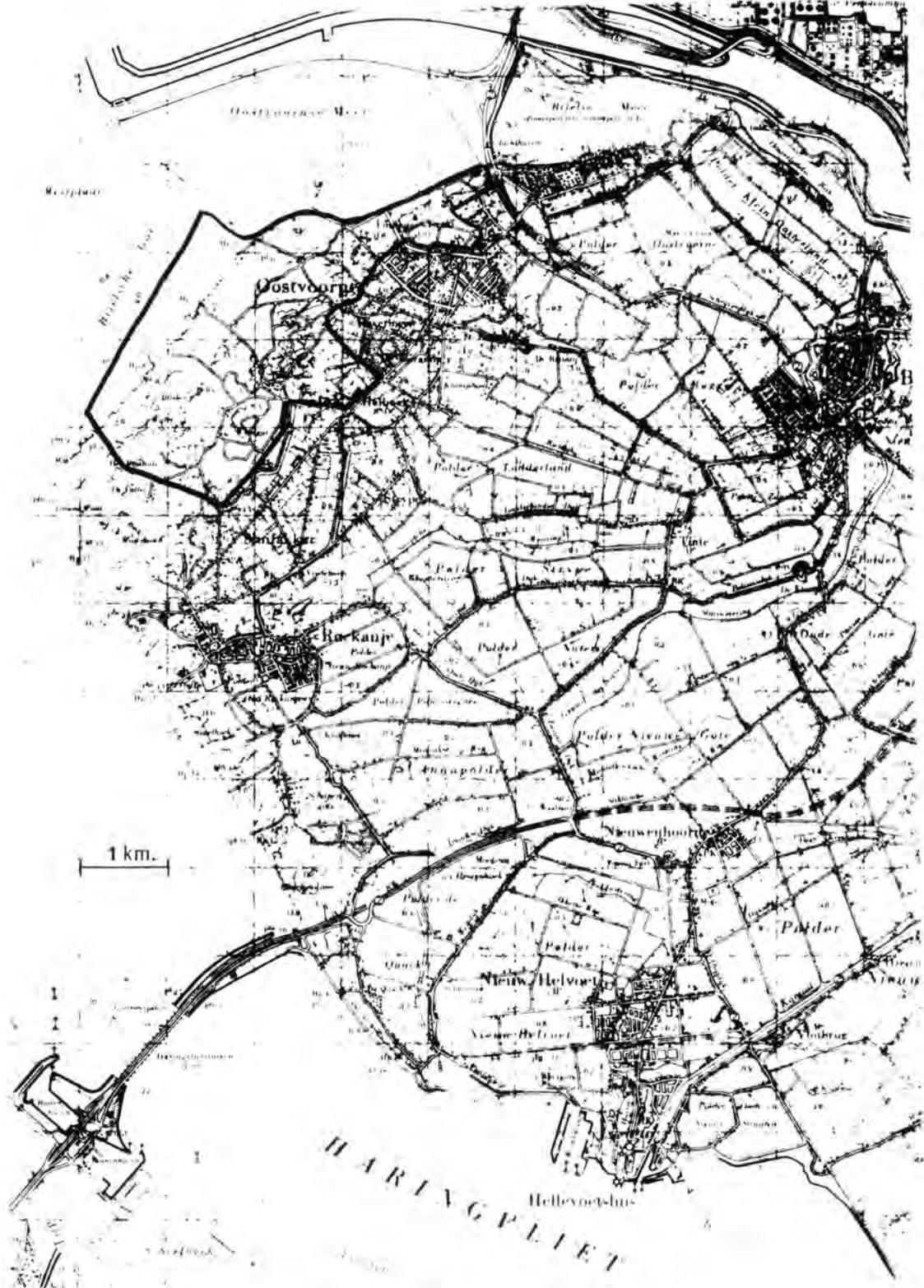


Fig. 7.2.1. Map on the coastal region of Vorne with indication of the inventoried area.

References

Blom, C.W.P.M. (1979) – Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. Thesis Nijmegen.

Soekarjo, R. (1979) – Regeneration in *Plantago* species. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 73, 344-346.

7.2. CHANGES IN THE FLORA OF THE VOORNE COASTAL AREA

(M. Boeken, D. van der Laan, P.A.I. Oremus)

Introduction

The coastal area of Voorne has always been of great biological value in many respects. Above all, this area could be considered outstandingly rich floristically. This richness was clearly demonstrated by an inventory of the vascular plants, which was started in 1962. The aim of this survey was to identify the species occurring and estimate their distribution in the area. For this purpose, van der Maarel suggested that the Voorne coastal area be subdivided into sections, each one homogeneous as to type of landscape and roughly equal in size (Adriani and van der Maarel, 1968). A hundred physiognomical sections were distinguished.

The main part of the inventory was executed between 1962 and 1964. Up to 1970, new localities of species were added. In 1979 part of the same area was inventoried again. This was done to make it possible to establish changes in the composition of the flora over these two periods, because a number of such changes might have taken place as a result of environmental changes in the area as well as in the surrounding area. This made it possible to detect the effects on the flora of such activities as the execution of the Delta Project; the construction of harbours, industrial plants, and roads; housing projects; increase of recreation pressure; and so on.

In this contribution the results of the second inventory of the flora (1979) are briefly reported and compared with the earlier results. More detailed information will be given elsewhere by the first author as part of his doctoral work at the University of Groningen.

During the period from April to August in 1979, the flora of part of the total area originally surveyed (31 of the 100 sections) in the dunes near the village Oostvoorne (Fig. 7.2.1) was inventoried. On the basis of the species list of the Dutch flora prepared by the Rijksherbarium in Leiden (Mennema 1976), all vascular plant species recorded in each section were checked off. To cover the seasonal variation, most of the sections were visited twice at different times of the year. The data of the first and the second inventories

were put on tape for computer analysis.

For comparison of the two inventories, use was made of a method introduced by van der Maarel (1971) and elaborated by Arnolds and van der Meijden (1976). According to this method, the Dutch species of vascular plants are classified in 9 categories, subdivided into 37 'ecological groups' and 10 frequency classes (0-9). The frequency values are based on the numbers of squares ($5 \times 5 \text{ km}^2$) in which a particular species is present in the Netherlands. By subtracting the frequency value of a given species from 10, a notation is obtained indicating the degree of rareness of that species in The Netherlands (Mennema 1973; Arnold 1975). The rareness values of all taxa occurring in a particular section taken together, determine the rareness value assigned to that section. Once rareness values are available for the various sections, the difference between them can be determined.

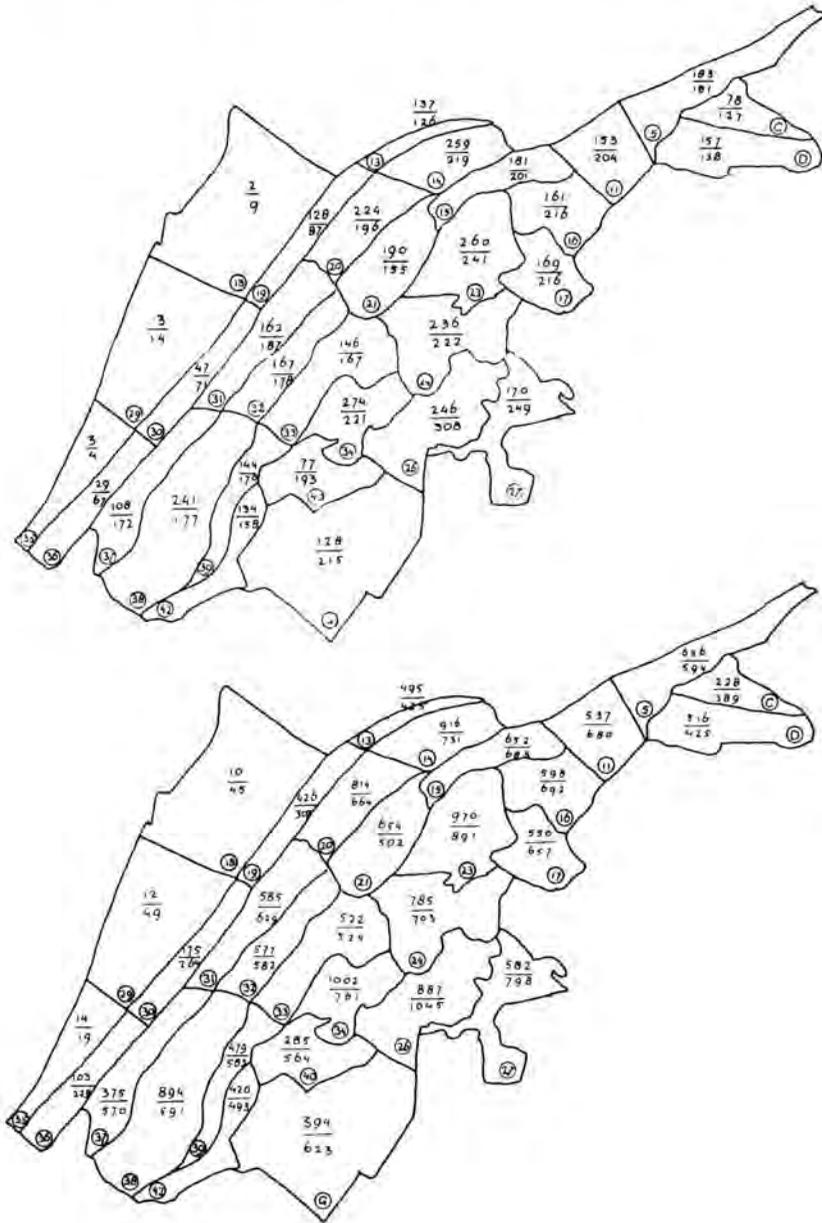
Another interesting aspect of the method devised by van der Maarel is that it makes possible to determine the ecological groups by which the floristical composition of a section is principally characterized. Calculation of the rareness values for each ecological group in each section indicates which particular group is the most important for that section (van der Meijden 1977). This method, called floristic analysis, has proved to be useful for the comparison of inventories of different areas made at the same time but is equally applicable to compare flora inventories of the same area made at different times.

To detect changes, if any, in the rareness value and the number of species in each ecological group, these characters of the respective inventories are compared. The data of both the 1962 and 1979 inventories were analysed to determine the extent to which the ecological groups were represented in each section. The amount of change could then be determined, and was expressed as:

1. the increase or decrease of the number of species of a particular ecological group in all 31 sections;
2. the increase or decrease of the rareness value of a particular group in all 31 sections; and
3. the increase or decrease of the number of sections in which species of a particular ecological group were found.

Unlike the 1962 inventory, where quantity was not recorded, in 1979 the occurrence of the species was quantified according to the Tansley scale (Tansley 1946). Of the three possibilities – i.e., increase, decrease, or equality in number of species and floristic composition – the first two are left because the equals were taken as half decrease and half increase.

Fig. 7.2.2. For each section (encircled figure) the number of species found (top map) and the rareness value (bottom map) are given, both for the first and the second inventory (top and bottom value, resp.).



Results

During the first inventory, 579 species were found in the 31 sections, whereas the 1979 inventory showed 540 species in the same area, a net decrease of 39 species. A hundred and four species occurring in the

1962-1970 period were not recorded in 1979, and 65 species were newly found. For both inventories, Fig. 7.2.2. shows the number of species and rareness value for each section. The number of species showed an increase 21 times and a decrease 10 times. For the

Table 7.2.1. Ecological groups or combinations of groups relevant to the coastal area of Vorne showing a significant change in:

		- number of species in 31 sections (A)	- rareness value in 31 sections (B)	- number of sections in which representative species of the ecological groups are found (C)
Ecol. group		A	B	C
1 (a/g)	plants of cultivated and dry waste places	+	+	+
1a	plants of cultivated and dry waste places on rich non calcareous cultivated soils	+	0	0
1d	plants of cultivated and dry waste, regularly trodden places on dry rich soils	+	+	+
1e	plants of cultivated and dry waste ruderal places, little trodden on rich dry non calcareous humusless soils	+	0	0
1f	plants of cultivated and dry waste ruderal places, little trodden on dry calcareous humusless soils	+	+	0
2a	plants of disturbed or open, damp to wet, rich soils poor in humus with fluctuating waterlevel or otherwise strongly fluctuating ecological conditions	0	0	+
3a	plants of beaches, sea dunes and sandy tide-marks	-	0	-
4d	plants of fresh water and neighbouring soils, wet ruderal places and riverine willow bush-swamps	0	+	0
6b	plants of grasslands on dry, moderately rich to rich, weakly acidic to weakly alkaline, moderately non or calcareous soils	-	-	-
6c	plants of grasslands on dry, moderately rich, neutral to alkaline calcareous or zinc bearing soils	-	-	-
7b	plants of moderately poor, alkaline, calcareous marshes	-	-	-
7c	plants of unfertilized grasslands on moist to wet, moderately poor and weakly acidic, peaty soils	-	-	-
2c+7a+b+c	plants of wet dune slacks	-	-	-
8a	plants of fell-fields on moderately moist to dry, moderately rich to rich soils	+	0	+
8b	plants of woodland margins on moderately moist, humus rich soils, poor in lime but rich in nutrients (especially nitrogen)	+	+	+
9 (a/e)	plants of woods	+	+	+
9b	plants of woods on nature moderately rich to rich, moderately moist to dry soils	+	+	0
9c	plants of woods on young, rich, moderately moist soils	+	+	+
9e	plants of woods and woodland margins, fairly to very poor, dry, non calcareous soils	+	+	0

+ = significant increase

- = significant decrease

0 = no significant change

rareness value, the increase is slightly less (20 increases, 11 decreases). Fig. 7.2.3 shows the decrease of the average rareness value. Table 7.2.1, which gives the results of the floristic analysis shows the changes in the various ecological groups as obtained with this method. The changes in a number of ecological groups are represented graphically in Fig. 7.2.4.

Discussion

Comparison of the two inventories showed that the total species number in the 31 sections decreased and that the species number per section increased. Furthermore, the average rareness decreased.

From these findings it may be concluded that the species still present remaining and/or newly appeared species must be more common in The Netherlands than those showing a decline. Analysis of the ecological groups that show increase or decrease indicates an increase for the groups representing species of habitats strongly influenced by man.

Except for the group representing woods, this increase might be related to the kinds of human interferences mentioned in the introduction. The increase found for ecological group 8a (woodland clearings) was due mainly to the recent spreading of *Senecio silvaticus* on Voorne. The reasons for this development are not known, but it is a striking fact that so far, this species has been much more common in some other dune areas in the Delta region than on Voorne. The increased contribution of species belonging to ecological

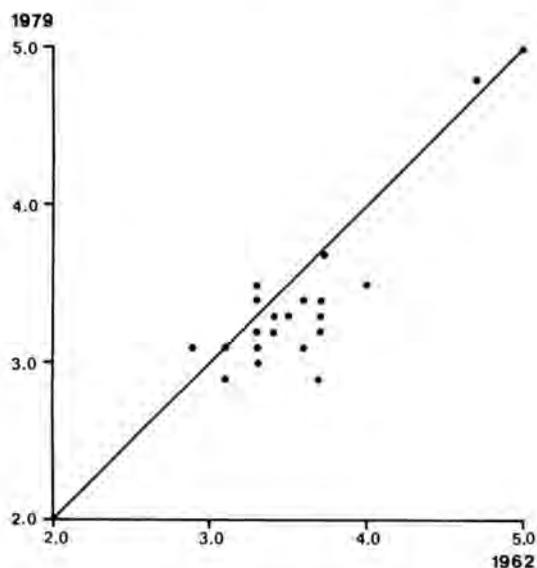


Fig. 7.2.3. Plot of the average rareness value showing the decreasing trend.

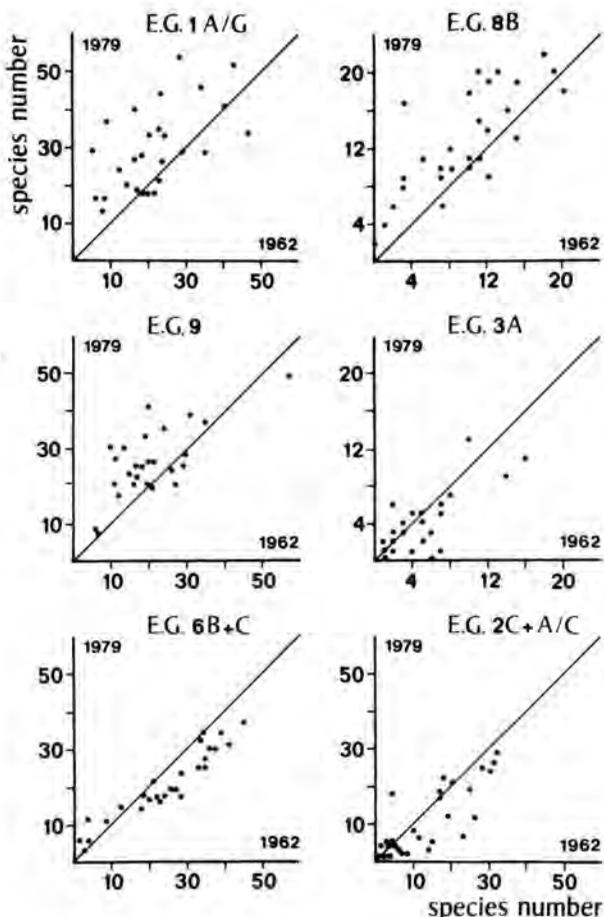


Fig. 7.2.4. Changes in a number of ecological groups between 1962 and 1979.

group 9 (woods) might be the result of vegetation succession. Groups representing species of the outer dune ridge (3a), dune grasslands (6b + c), and wet dune slacks (2a + 7a + b + c), decreased. The closure of the Brielse Gat, the construction of the Maasvlakte area after 1966, and technical improvement of the outer dune ridge as a sea defence, can be held responsible for the decrease of group 3a.

As a result of vegetation succession during the period between the first and second inventories, the soil surface of the dunes behind the outer dune ridge might have become more fixed. This may explain the reduction of such a typical representative of moving sand as *Ammophila arenaria* (Fig. 7.2.5). The decreased contribution of representatives of dune grassland (6b + c) is probably related to the increase of the recreational pressure and the development of scrub and woods.

Finally, the decrease of representatives of the

groups belonging to the wet dune slacks ($2a + 7a + b + c$) could have partially been caused by not only changes resulting from vegetation succession but also the very cold and wet winter and spring preceding the second inventory. In this respect a change in the ground-water table may also have exerted an influence, because series of records of the ground-water level in the part of the dune area under investigation showed that at eleven sampling points the average ground-water table over the years 1963 to 1970 was about 20 centimetres higher than the average level over the 1971-1978 period.

References

- Adriani, M.J. and E. van der Maarel (1968) – Voorne in de branding. Oostvoorne.
- Arnolds, E.J.M. (1975) – Een floristisch-oecologische waardebeoordeling nabij Utrecht ten behoeve van natuurbehoud en planologie. *Gorteria* 7 (11), 161-179.
- Arnolds, E.J.M. and E. van der Maarel (1979) – De oecologische groepen in de Standaardlijst van de Nederlandse flora 1975. *Gorteria* 9 (9), 303-312.
- Arnolds, E.J.M. and R. van der Meijden (1976) – Standaardlijst van de Nederlandse flora 1975. Leiden.
- Arnolds, E.J.M. (1971) – Florastatistieken als bijdrage tot de evaluatie van natuurgebieden. *Gorteria* 5 (7/10), 176-188.
- Mennema, J. (1973) – Een vegetatiewaardering van het stroomdallandschap van het Merkske (N.Br.) gebaseerd op een floristische inventarisatie. *Gorteria* 7 (11), 161-179.
- Mennema, J. (1976) – Een nieuwe streeplijst, een nieuw seizoen. *Gorteria* 8 (6), 113-117.
- Meijden, R. van der (1977) – De flora van de Dordtse Biesbos, Leiden.
- Tansley, A.G. (1946) – Introduction to plant ecology. 2nd ed. London.

7.3. THE ABUNDANCE OF VESICULAR ARBUSCULAR MYCORRHIZA IN *Plantago lanceolata* AND *Plantago coronopus* IN A NATURAL DUNE GRASSLAND (WESTDUINEN, GOEREE) (C. van Dijk),

Introduction

Although the majority of plant species on natural grasslands shows associations with vesicular arbuscular mycorrhizal (VAM) fungi, little is known about the extent to which these VAM associations contribute to the actual distribution pattern of a plant species.

Competition for low levels of nutrients by neighbouring plant species in situations with high densities of root systems might be influenced by the abundance of VAM associations of competing root systems.

As a first approach to the question of whether VAM associations play a part in the distribution of a plant species, the abundance of VAM associations was studied in populations of *Plantago lanceolata* in relation to the VAM density of competing root systems. In addition, the abundance of VAM associations was studied in *Plantago coronopus* without taking neighbouring plant species into account. The choice of these two *Plantago* species was based on the institutes participation in an integral study of life strategies in the genus *Plantago*.

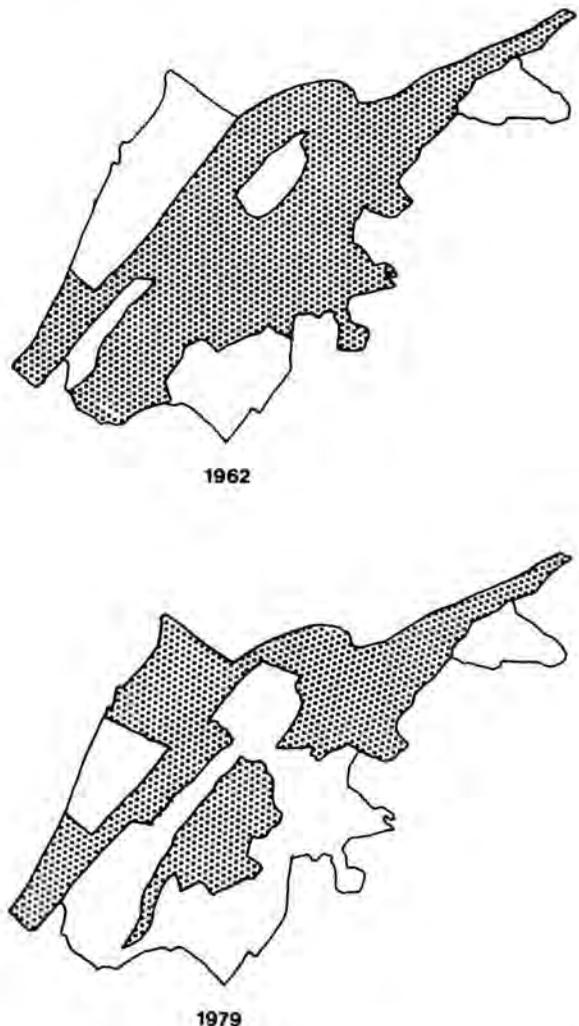
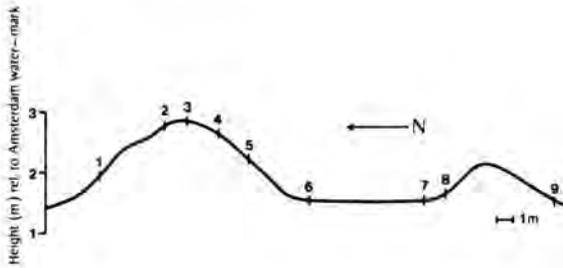


Fig. 7.2.5. Distribution of *Ammophila arenaria* determined in the two surveys.

Fig. 7.3.1. Position of sampling sites at Transect 2, Westduinen (Goeree).



Abundance of VAM in Plantago lanceolata and neighbouring plant species

A population of *P. lanceolata* in an old dune area (Westduinen) on the island of Goeree was studied. In this area, nine sites along 30 metres of Transect II (Troelstra 1977) were selected on the basis of the presence of *P. lanceolata* as well as their positions along the profile of the transect, which included a summit, slopes, and a valley (Fig. 7.3.1). At each site, one 15 cm² sod was dug out to a depth of 20 cm. Individual root systems were separated and rinsed. For each sod, 20% of the root system of each plant species was collected for the determination of VAM abundance. The samples, which consisted of root pieces of one cm, were obtained from the 2-3 cm root zone, the 7-8 cm root zone, etc. Root segments thus collected were cleared with KOH and stained with trypan blue in lactophenol (Phillips and Hayman 1970).

Infection density (I) was calculated as the percentage of all root segments with intracellular fungal hyphae in each sample. The mycorrhizal status of intracellular fungi was not always certain. Therefore, mycorrhizal infection was also expressed as VA, i.e., the percentage of root segments with either vesicles or arbuscules or both. The ratio VA/I serves as a standard for the abundance of vesicles and/or arbuscules in infected root segments.

I and VA were calculated for each of six of the most abundant plant species at the nine sites along transect II. I values as well as mean values for I and VA per species (\bar{I} and \bar{VA} , respectively), derived from combination of sites 1 to 9, are presented in Fig. 7.3.2.

P. lanceolata proves to share high density of fungal associations with *Ranunculus bulbosus* when both \bar{I} and \bar{VA} considered. Although \bar{I} is equally high for *Agrostis tenuis* and *Festuca rubra* and \bar{I} values for *Anthoxanthum odoratum* and *Cerastium arvense* are higher than 60%, the \bar{VA} values of these four species are considerably lower than those of *P. lanceolata* and *R. bulbosus*.

The results in Fig. 7.3.2 suggest that, from the viewpoint of mycorrhizal associations, the conditions of the habitat of transect 2 for competition and survival are favourable for *P. lanceolata*, at least if the abundance of mycorrhizal associations contributes to the competitive ability and stress tolerance of a species.

The same conclusion can be drawn when I and VA of the combined root systems of seven plant species, now including *Poa pratensis*, are compared with I and VA for *P. lanceolata* at each of the nine sites along Transect II (Table 7.3.1).

Especially the I values of the combined samples show little divergence within the transect, and no influence of local differences in elevation was observed. The relevance of differences in the VA value of these samples cannot be understood without more detailed study. The validity of the VA calculation as a reliable tool for the quantification of VAM associations is questionable, because formation of vesicles and arbuscules does not always accompany invasion of VAM hyphae along the root systems.

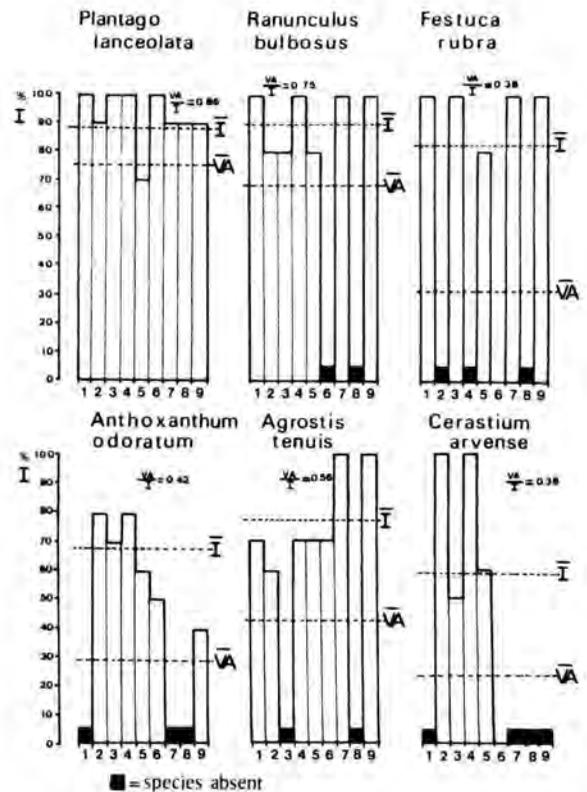


Fig. 7.3.2. Percentage of fungal root infection (I) for six plant species at sites 1 to 9, Transect 2, Westduinen (Goeree).

Differences in the soil conditions along Transect II as determined by Troelstra (1978) are probably too small to cause any significant difference in VAM density in *P. lanceolata*.

Abundance of VAM in *Plantago coronopus*

Determination of the I values of 77 individual plants of *P. coronopus* in the same habitat (Westduinen, Goeree) but distributed over a wider area, revealed that 80% of the plants have I values lying between 70 and 100%. Only 10% had an I value lower than 40%. Spatial variation of I in *P. coronopus* did not appear to be correlated with soil porosity or soil water content. The organic-matter content of the soil may have influenced I to some extent, because low to moderate values of I (I < 50%) were found at 3-9% soil organic matter, whereas for I > 50%, this content ranged from 3 to 18%. But too few scores indicating high contents of organic matter were obtained to conclude that high levels of organic matter promote VAM abundance.

Spore morphology studies of VAM abundance led us to conclude that *Glomus fasciculatus* (Gerdemann and Trappe 1974) is the most abundant VAM species in this area. It may also be concluded that soil conditions of the Westduinen are favourable for VAM development in *P. lanceolata* and *P. coronopus*. A more complete picture of the abundance of VAM associations in species of *Plantago* can only be obtain-

ed by extension of this study to more extreme habitats.

References

Gerdemann, J.W. and J.M. Trappe (1974) – The Endogonaceae in the Pacific Northwest. Mycologia Memoir no. 5.

Phillips, J.M. and D.S. Hayman (1970) – Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. mycol. Soc. 55, 158-160.

Troelstra, S.R. (1978) – Spatial variability of soil chemical properties in an old dune area (Westduinen) of Goeree. In: Progress Report 1977, Institute for Ecological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 71, 321-327.

7.4. THE GROWTH AND NITRATE UPTAKE OF *Plantago lanceolata* AND *P. major* IN CULTURE EXPERIMENTS WITH INTERMITTENTLY SUPPLIED NITRATE IN LOW CONCENTRATIONS (A.H.J. Freijisen, H. Otten)

The investigations are focussed on the question of whether the plant species *Plantago lanceolata* and *P. major* subsp. *major* have different nitrate requirements, and are based on the hypothesis that *P.*

Table 7.3.1. Density (I and VA) of VAM associations at location 1-9 along Transect II, Westduinen (Goeree). Calculated from the combined root systems of 7 dominant species. *

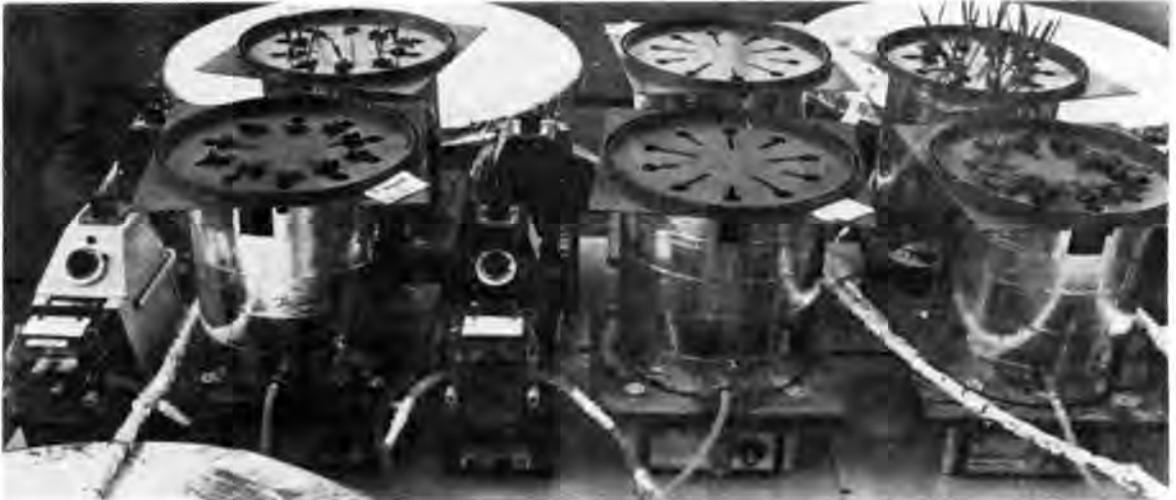
In brackets: values calculated for *P. lanceolata* only.

Location	Infection density		
	I (%)	VA (%)	VA/I
1	89 (100)	66 (100)	0.75 (1.00)
2	84 (88)	39 (56)	0.48 (0.64)
3	80 (100)	47 (100)	0.59 (1.00)
4	87 (100)	54 (94)	0.62 (0.94)
5	64 (68)	28 (53)	0.43 (0.77)
6	64 (97)	40 (72)	0.62 (0.74)
7	91 (88)	64 (71)	0.75 (0.81)
8	** (86)	** (27)	** (0.31)
9	78 (88)	62 (88)	0.75 (1.00)

* Plant species considered: *Plantago lanceolata*, *Ranunculus bulbosus*, *Festuca rubra*, *Anthoxanthum odoratum*, *Agróstis tenuis*, *Cerastium arvense* and *Poa pratensis*.

** Not determined.

Plate 2. The cultivation of *Plantago* species at low nitrate concentrations.



lanceolata is able to grow at a lower availability of nitrate than *P. major* can. In nature, the former species is occasionally found on poor soils, whereas the latter occurs mainly on rather rich soils. The investigations form part of a multidisciplinary project (van der Aart 1979).

In an earlier report on the study in question, Freijnsen and Otten (1979) gave a brief survey of some preliminary investigations, and described the flowing nutrient system used to grow juvenile plants of *P. lanceolata* and *P. major* (illustrated in Plate 2). The species were grown in separate experiments. To assess the requirements of the *Plantago* species, two forms of low nitrate availability were applied. In one of these, the plants were supplied with an external nitrate concentration ($25 \mu\text{M}$) at which the uptake system of the root may be regarded as not being saturated. In the second, an intermittent supply of nitrate was applied. The growth of both species was only slightly influenced by the former treatment. An intermittent supply of nitrate reduced growth more strongly, especially in combination with a low concentration.

In the experiments done in 1979, too, *P. lanceolata* and *P. major* were grown under intermittent availability of low nitrate concentrations. Plants of both species were grown simultaneously, to permit reliable comparison of their responses. The following results are worth mentioning. When the experimental plants were supplied with nitrate in a concentration of $50 \mu\text{M}$ on alternate days during the culture period, both species showed an equal reduction of the growth rate. When a similar intermittent regime combined with a nitrate concentration of $25 \mu\text{M}$ was applied, *P. major* reacted more strongly. Its internal levels of

nitrate and organic nitrogen fell more than those of *P. lanceolata* did, and, furthermore, its relative growth rate was reduced more. *P. major* was also more severely affected in an experiment in which one-day periods with nitrate were alternated with two-day periods without this nutrient. The results indicate that *P. major* is more vulnerable under suboptimal nitrate availability, which is in agreement with our hypothesis.

In all experiments plants treated with the various form of restricted availability of nitrate showed growth reduction to a greater or less extent and also a reduced shoot/root ratio. The latter phenomenon is generally seen as an adaptation to suboptimal nutrient conditions. In almost all cases, however, these plants still contained nitrate and rather large amounts of organic nitrogen. This means that under the shortage of nitrate in the environment, the plants adapted mainly their morphological structure. Only in one case, viz. when the plants underwent two-day breaks in the nitrate supply, was almost no nitrate present in the leaves and the organic-nitrogen level dropped to about $1500 \text{ mmol.kg}^{-1}$. Here, important chemical changes in the plants accompanied the morphological changes.

References

- Aart, P.J.M. van der (1979) – A comparative multidisciplinary approach to the demography, physiology, and genetics of plant species occurring in seminatural grasslands. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, 347-350.
- Freijnsen, A.H.J. and H. Otten (1979) – The growth and nitrate uptake of *Plantago lanceolata* and *P. ma-*

TABLE 7.5.1 Some selected soil properties (0-10 cm depth) of six sites in the Westduinen area.

site/relative height* within the area	pH**		% org. matter***	total N (ppm)	exchangeable cations (meq/100 g)					CEC (meq/100 g)	
	H ₂ O	KCl			Ca	Mg	K	Na	Σ	NH ₄ Cl	NH ₄ OAc (pH 7.0)
T1/low	5.3	4.5	4.6	1558	2.71	0.49	0.08	0.11	3.39	3.52	4.71
T2/intermediate	4.7	3.8	5.3	1957	1.70	0.54	0.12	0.07	2.43	2.47	4.70
T3/low	4.8	4.1	23.8	9097	9.41	1.54	0.45	0.40	11.80	10.91	17.87
T4/intermediate	4.4	3.4	5.0	1597	0.69	0.33	0.12	0.05	1.19	1.90	4.64
T5/high	4.1	3.2	3.6	1179	0.22	0.14	0.05	0.03	0.44	1.50	3.49
T6/high	4.2	3.2	4.0	1356	0.30	0.15	0.05	0.04	0.54	1.26	3.59

* maximal differences in height c. 5 m

** 1:2½ (w/v) suspension

*** loss-on-ignition, 430°C for 24 h

TABLE 7.5.2 Initial mineral N determination for six sites (0 to 10 cm soil depth) in the Westduinen area during the period from March, 1978, to November, 1979.

site	range of average moisture contents (% oven-dry soil)	total N* (ppm)	mineral N*,**		
			NH ₄ -N	NO ₃ -N	
			(ppm)		(mM) ⁺
T1	16- 52	1558± 227	2.45±1.534 (0.83-6.68) ⁺⁺	0.74±0.314 (0.30-1.39) ⁺⁺	0.16±0.080 (0.05-0.33) ⁺⁺
T2	3- 20	1957± 268	3.00±1.657 (1.45-8.05)	1.23±0.479 (0.54-2.34)	1.00±0.744 (0.19-3.04)
T3	95-156	9079±1683	6.40±2.445 (2.32-12.04)	2.08±0.996 (0.69-4.98)	0.12±0.049 (0.04-0.23)
T4	3- 22	1597± 144	2.02±0.553 (1.32-3.51)	1.09±0.297 (0.50-1.76)	0.89±0.628 (0.31-2.77)
T5	3- 15	1179± 127	5.81±5.630 (1.37-22.83)	2.06±1.598 (0.72-5.75)	2.60±3.459 (0.39-14.86)
T6	1- 14	1356±131	9.38±4.854 (3.12-18.17)	2.12±1.483 (0.51-5.66)	3.49±4.864 (0.54-20.54)

* mean ± standard deviation (n=17-19)

** complete soil cores

+ assuming all nitrate to be present in the soil solution

++ range

TABLE 7.5.3 Estimates of the amounts of nitrogen (expressed in g/m²) to a soil depth of 10 cm at six sites in the Westduinen area.

site	total N*	g/m ²			net production mineral N expressed as % total N
		net production mineral N during total period March 1978-December 1979		total	
		NH ₄ -N	NO ₃ -N		
T1	182	16.29	1.00	17.29	9.5 (7.2)**
T2	222	16.27	5.64	21.91	9.9 (7.9)
T3	471	9.19	0.54	9.73	2.1 (1.7)
T4	171	12.87	3.83	16.70	9.8 (7.6)
T5	134	11.35	3.00	14.35	10.7 (8.3)
T6	165	17.83	2.68	20.51	12.4 (10.1)

* mean value of 17-19 sampling dates

** after estimated correction for the difference intact soil cores - pre-treated soil material

for in experiments with continuously and intermittently supplied nitrate concentrations of 750 and 25 μM . Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, 364-367.

7.5. SEASONAL PATTERNS IN THE AVAILABILITY OF MINERAL NITROGEN IN A RELATIVELY OLD DUNE AREA (WESTDUINEN) ON THE ISLAND OF GOEREE (S.R. Troelstra, R. Wagenaar)

Introduction

A previous paper (Troelstra and Wagenaar 1979) reported preliminary results on mineral nitrogen and nitrogen net mineralization in six rather dissimilar locations in a relatively undisturbed inner dune area (Westduinen) with a natural grassland vegetation on the island of Goeree, during the period from March to December in 1978. This research was continued until the end of 1979, and the data presented here refer to the complete period of the study: March, 1978, to December, 1979.

The primary aims of the research were the following:

1. To obtain an impression of the relative importance of ammonium-nitrogen and nitrate-nitrogen; ammonium concentrations are expected to be higher than nitrate concentrations in the 0-10 cm soil layer of the area under investigation (pH-H₂O: 4 to 5).
2. To assess nitrate concentrations in the soil solution. For agricultural conditions, nitrate levels can be as high as 20 to 30 mM, and the normal range in fertile soils is 2 to 20 mM depending on the rate of mineralization (nitrification) and the uptake by plants (Mengel and Kirkby 1978); lower nitrate concentrations are expected to occur in our study area, which is a natural dune grassland influenced by extensive stock grazing and very local fertilizer applications (restricted to the flatter parts of the 176-ha area).
3. To estimate the 'plant-available' mineral N flushes during the season.

Since the parameters in question are strongly dependent on environmental factors and microbial activity and are unquestionably influenced by the experimental conditions, only a semi-quantitative meaning can be attributed to these values and they must be seen as expected trends and orders of magnitude.

Materials and methods

For details of the experimental and analytical procedures, the reader is referred to the Progress Report for 1978 (Troelstra and Wagenaar 1979). Site characteristics are shown in Table 7.5.1. With the exception of CEC(NH₄Cl), all values are means of a total of 17-19 sampling dates spread over the period from March, 1978, to November, 1979. The pH varia-

tion in the 0-10 cm soil layer is relatively small, i.e., pH-H₂O: 4.1-5.3. Total N and organic matter are closely related; for all sampling dates the mean N percentage in the organic matter varied from 3.3 to 3.7 for the six locations. 'Effective' cation-exchange-capacity values, CEC(NH₄Cl), are distinctly lower than 'potential' CEC values (NH₄OAc, pH 7.0); at pH-H₂O > ca. 4.5 the percentage base saturation is essentially 90-100. CEC(NH₄Cl) values of the six sites lie in the range of 1-11 meq/100 g. On a soil-volume basis the range is smaller: 1.5-6 meq/100 cc. Compared to the other cations, the maximal amount of NH₄⁺ adsorbed is virtually negligible, i.e., 0.01-0.10 meq/100 g.

Net production of mineral N was calculated on an oven-dry basis by subtracting initial amounts at sampling time from the quantities found after aerobic incubation in the field or in the laboratory. Calculated values are the result of at least duplicate determinations. Allowance was made for the average moisture content on each sampling date and at each location; mean coefficients of variation of the moisture-content determinations (n = 4-6) were ca. 10 per cent for T1 and T3, and 20 to 30 per cent for the other sites.

Sites T1 and T3 were flooded in the early spring of 1979 and were therefore not sampled in April (T3) or May (T1, T3).

Results and discussion

Mineral nitrogen concentrations at sampling time

Generally, the results of the whole 1978-1979 period were comparable with those for 1978 (Troelstra and Wagenaar 1979); for sites T1 and T2, the values are shown in Fig. 7.5.1a. Except for one case at T5, ammonium concentrations were distinctly higher than those of nitrate at all locations. Unlike nitrate, ammonium will occur mainly in the exchangeable form. At sites T1, T2, and T4, ammonium and nitrate values were on the whole never higher than 4 ppm N and 2 ppm N, respectively. Location T3 showed values about twice as high, but here bulk density was lower and, judging from the variation in organic matter content, spatial variation was considerable. At sites T5 and T6, values up to 17-23 ppm NH₄-N occurred, particularly for location T6 during 1979.

Combination of the data for nitrate (a ppm NO₃-N; T1 and T2: Fig. 7.5.1a) and soil moisture (b % soil water; T1 and T2: Fig. 7.5.1b), yields the nitrate-concentration values (100a/14b mM) shown in Table 7.5.2 and Fig. 7.5.1c (T1 and T2). Thus all nitrate is assumed to be dissolved in the soil water. Three groups can be distinguished on the basis of nitrate concentration. At the wettest sites, T1 and T3, nitrate concentrations were generally not higher than

TABLE 7.5.4 Net mineralization rates (ppm N week⁻¹) in the 0-10 cm soil layer for six locations in the Westduinen area during the period from March, 1978, to November (December), 1979.

site	net production of mineral N (ppm week ⁻¹)					
	field incubation*		laboratory incubation I**		laboratory incubation II**	
	NH ₄ -N	NO ₃ -N	NH ₄ -N	NO ₃ -N	NH ₄ -N	NO ₃ -N
T1	-0.73-8.50(1.68) ⁺	-0.14-0.98(0.11) ⁺	0.92-24.3(8.01) ⁺ (8.54) ⁺⁺	-0.35-12.4(1.28) ⁺ (1.51) ⁺⁺	0.69-17.4(7.71) ⁺	-0.30-4.14(0.72) ⁺
T2	0.17-5.65(1.67)	-0.16-1.82(0.60)	5.29-16.8(11.3) (11.1)	0.15-17.8(7.01) (6.56)	1.23-20.8(8.83)	-0.11-7.50(2.66)
T3	-0.55-7.71(1.85)	-0.35-1.62(0.16)	8.33-42.2(22.1) (22.6)	0.55-38.3(9.99) (4.19)	7.14-44.0(17.9)	-0.48-2.58(0.75)
T4	-0.05-4.77(1.44)	-0.11-1.84(0.45)	2.78-21.2(8.52) (8.61)	1.66-8.49(4.03) (3.95)	2.69-15.3(7.29)	-0.12-4.24(1.53)
T5	-3.04-4.37(1.06)	-1.05-1.23(0.28)	3.04-15.7(8.49) (9.49)	0.30-4.24(1.72) (1.50)	2.95-21.0(7.96)	-0.32-1.23(0.36)
T6	-0.24-5.91(1.74)	-0.31-0.84(0.28)	2.06-19.9(11.6) (10.8)	0.39-3.90(1.93) (2.04)	-0.16-14.6(7.85)	-0.74-1.64(0.48)

* generally periods of 4 to 5 weeks

** generally periods of 3 weeks at 30°C; I quartz sand-mixed soil cores at minimum moisture content of 15 per cent (dry weight)

II intact soil cores at field moisture content

+ mean

++ these values refer to the same period as laboratory incubation II

TABLE 7.5.5 Total uptake of N, P, K, Na, Ca, and Mg by the above-ground biomass (a) and soil availability indices for these minerals in the 0-10 cm soil layer (b) at four locations in the Westduinen area.

(a) site	total uptake by the aboveground biomass (g/m ²) during the March-November period in 1979					
	N	P	K	Na	Ca	Mg
T1	8.76	0.54	4.08	0.84	2.05	0.86
T2	6.10	0.65	3.35	0.42	1.10	0.48
T3	8.44	0.67	5.53	0.69	2.52	0.72
T4	3.42	0.37	2.25	0.18	0.34	0.24

(b) site	g/m ²								
	net production of mineral N after field incubation (8/3/79-19/11/79)			NaHCO ₃ -P*		exchangeable cations*			
	NH ₄ -N	NO ₃ -N	total	Olsen-P	labile organic P	K	Na	Ca	Mg
T1	10.08	0.54	10.62	0.34	1.04	4.09	3.76	66.3	7.23
T2	5.74	2.90	8.64	0.77	4.03	5.31	1.83	39.9	7.72
T3	4.39	0.20	4.59	0.62	1.78	9.50	4.77	98.1	9.83
T4	6.85	2.39	9.24	0.66	3.61	5.00	1.23	14.5	4.16

* mean value of 17-19 sampling dates (March 1978-November 1979)

0.3 mM. At locations T2 and T4, maximum concentrations were 3 mM, and T5 and T6 can be compared with T2 and T4, but here some extreme values of 16 and 20 mM occurred.

These calculations were made with average data for the 0-10 cm soil layer. Since soil-moisture and nitrate gradients often show opposite directions, higher concentration values may be expected on a small scale within the 0-10 cm soil layer. On the other hand, high

nitrate concentrations will frequently coincide with unfavourable nitrate-transport conditions. However, it might well be that some plants experience relatively high nitrate concentrations in their immediate root environment as a result of active nitrification processes in the rhizosphere.

Table 7.5.2 summarizes the initial mineral nitrogen analyses.

Net production of mineral nitrogen during aerobic incubation in the field

In this respect, too, the results for the total 1978-1979 period are comparable with those of 1978 (Troelstra and Wagenaar 1979). Seasonal effects were present, and net production values for locations T1 and T2 are shown in Fig. 7.5.2 (height of blocks refers to base-width = length of incubation period). At the six locations net production amounted to 10-22 g N/m² (× 10 = kg N/ha) in the 0-10 cm soil layer for the total period (March, 1978-December, 1979) and was predominantly in the form of ammonium (particularly at the low sites T1 and T3; Table 7.5.3).

These quantities represent up to about 10 per cent of total N. In theory the level is about 1-2 per cent a year (Schnitzer and Khan 1978) or 2-4 per cent for two seasons, but these theoretical values often refer to a soil-profile depth > 10 cm and a more stable humus pool. Furthermore, total N and net production values have different frames of reference, i.e., pretreated soil material and undisturbed soil cores, respectively. The amounts of material sieved during pretreatment of the bulk samples were recorded and after calculating a

rough correction on this basis, the values between parentheses in Table 7.5.3 were found: except for T3 and T6, 7-8 per cent of total N. If a certain root turnover is taken into consideration, these values can be regarded as being of the right order of magnitude, although they may be somewhat too high.

If one calculates the rate of mineralization (g N/m² × week) for the different periods at each location, a distinct seasonal pattern is found, with nitrate running parallel to ammonium in most cases. For T1 and T2, the results of this calculation are shown in Fig. 7.5.3.

Comparison of field incubation and laboratory incubation

Data on field incubation and laboratory incubation, expressed as ppm N week⁻¹, are summarized in Table 7.5.4. Generally, field-incubation values are lower than mineralization potential values (incubation in the laboratory). However, maximum field-incubation values – i.e., the more optimal field conditions with respect to soil moisture, temperature, microbial populations, etc. – approximate the average

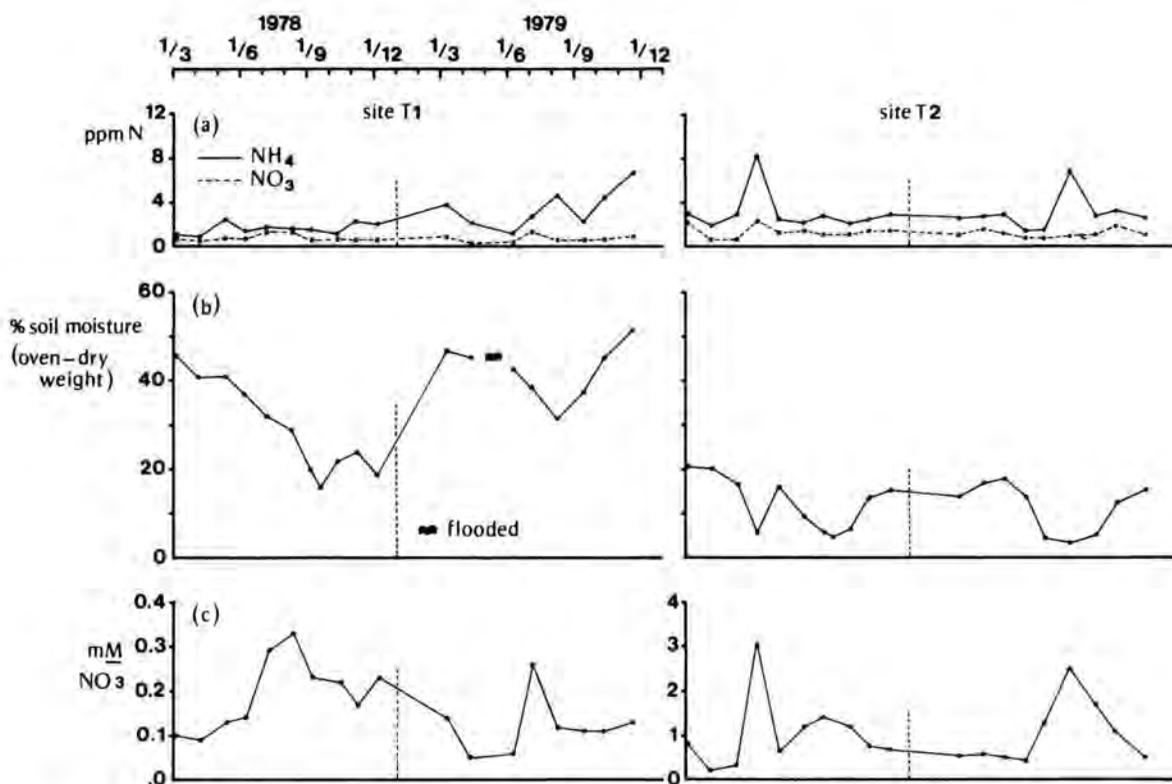
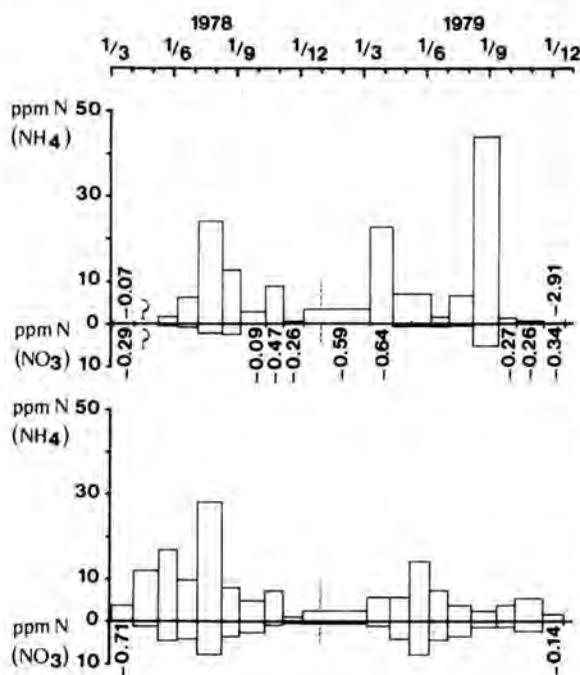


Fig. 7.5.1. Seasonal patterns of inorganic nitrogen levels at sampling time (a), soil moisture content (b), and soil solution nitrate concentration (c) in the 0-10 cm soil layer at locations T1 and T2 in the West-duinen area during 1978-1979.

Fig. 7.5.2. Seasonal pattern of net nitrogen mineralization at sites T1 and T2 in the Westduinen area during 1978-1979.



laboratory-incubation results, particularly in the case of nitrate (compare with laboratory incubation II in Table 7.5.4).

Comparison of net production of mineral nitrogen in the field with total nitrogen uptake in the above-ground vegetation

To estimate the above-ground biomass production and the total amount of nitrogen taken up by the above-ground vegetation, five 0.18-m² plots on each of the four locations T1-T4 were clipped at regular intervals during the period March-November in 1979. Dry matter production (g/m² × week) is shown in Fig. 7.5.4a. Successive clips were analyzed for N, P, K, Na, Ca, and Mg, and the total amounts (g/m²) taken up during the whole period are given in Table 7.5.5a.

Availability indices of the same elements are presented for the four sites T1-T4 in Table 7.5.5b. It becomes clear from Table 7.5.5 that for the macronutrients N, P, and K same orders of magnitude are found for the 'plant-available amounts' in the soil and the quantities taken up in the above-ground vegetation. This indicates that in the present area these nutrients are regularly circulated through the vegetation and that the labile organic P pool (see under 7.6) may contribute to the P nutrition of the plant.

However, it should be noted that the soil-availability indices in Table 7.5.5b refer to the 0-10 cm soil layer only, whereas the amounts of the elements taken up by the vegetation may relate to deeper soil layers as well. This holds especially for location T3, where the accumulation of organic matter goes beyond the 10 cm depth. Indeed, at site T3 the total net production of inorganic nitrogen in the 0-10 cm soil layer was lower than nitrogen uptake (Table 7.5.5).

Periodic values of both net production and uptake (g N/m² × week) are given in Fig. 7.5.4b. If it is taken into account that: (i) values do not refer to exactly the same plots within a site or to the same thickness of the soil layer, (ii) net production of mineral N may be somewhat over-estimated by the field-incubation technique, (iii) soil moisture boundary conditions (the process of leaching included) were not the same, (iv) grazing by rabbits cannot be excluded completely, (v) bulk densities used in the calculation of net production values (g N/m²) were rough estimates only and will no doubt show considerable spatial variation within a site, (vi) only the above-ground biomass was considered in the estimation of N uptake, and (vii) biomass production and N uptake may be over-estimated due to stimulatory effects of clipping (re-allocation of root nutrient reserves; increase in mineralization), the degree of agreement is very satisfactory, particularly for location T2.

References

Mengel, K. and E.A. Kirkby (1978) – Principles of plant nutrition, 593 p. International Potash Institute, Bern.

Schnitzer, M. and S.U. Khan (eds) (1978) – Soil organic matter. Developments in soil science 8, 319 p. Elsevier.

Troelstra, S.R. and R. Wagenaar (1979) – Mineral nitrogen and nitrogen net mineralization in an older dune area (Westduinen) on the island of Goeree. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, 367-376.

7.6. SEASONAL VARIATION OF LABILE ORGANIC PHOSPHORUS LEVELS IN A RELATIVELY OLD DUNE AREA (WESTDUINEN) ON THE ISLAND OF GOEREE (S.R. Troelstra, M.A. van der Meulen)

Introduction

Research on the organic phosphorus pool at six locations in the Westduinen area (Troelstra and van der Meulen 1979) was continued until the end of 1979,

Fig. 7.5.3. Rates of net mineralization ($\text{g N/m}^2 \times \text{week}$) at locations T1 and T2 during 1978-1979. Solid squares: ammonium-N; open squares: nitrate-N.

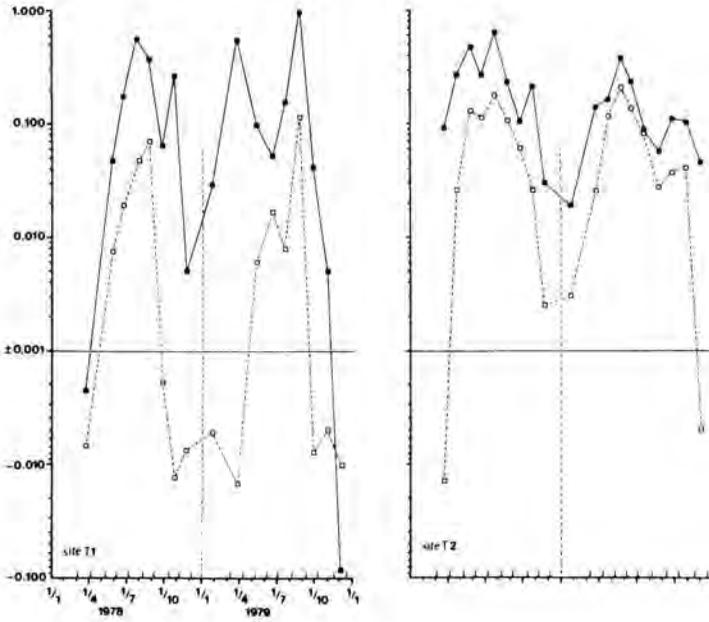


Fig. 7.5.4. Estimates of above-ground biomass d.m. production (a) and comparison of net production of mineral N (dashed line) with N uptake in the above-ground biomass (solid line) (b) at four sites in the Westduinen area during 1979.

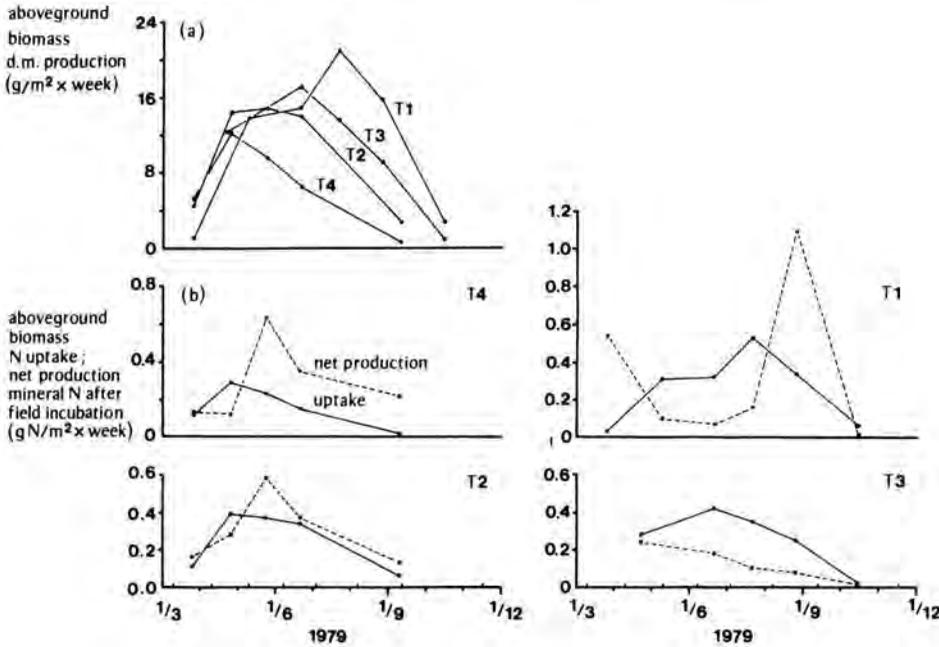


TABLE 7.6.1 Some selected soil properties (0-10 cm depth) of six locations in the Westduinen area. Values are means of 17-19 sampling dates unless otherwise indicated.

site/relative height* within the area	pH**		% org. matter ***	ppm		
	H ₂ O	KCl		total P ⁺	total organic P ⁺⁺	total inorganic P ⁺⁺⁺
T1/low	5.3	4.5	4.6	124	92	27
T2/intermediate	4.7	3.8	5.3	190	153	36
T3/low	4.8	4.1	23.8	480	401	100
T4/intermediate	4.4	3.4	5.0	140	119	21
T5/high	4.1	3.2	3.6	115	86	26
T6/high	4.2	3.2	4.0	117	90	28

* maximal differences in height c. 5 m

** 1:2½ (w/v) suspension

*** loss-on-ignition, 430°C for 24 h

+ mean of 10 sampling dates (3/78-12/78)

++ ignition method

+++ total P minus total organic P

TABLE 7.6.2 Estimates of the amounts of phosphorus to a soil depth of 10 cm at six sites in the Westduinen area, and total P uptake by the above-ground biomass.

site	g P/m ² *					total P uptake (g P/m ²) by the aboveground bio- mass during the period March-November 1979
	total	total organic	total inorganic**	labile organic	Olisen	
T1	14.5±1.28	10.7±1.42	3.15±0.93	1.04±0.25	0.35±0.12	0.54
T2	2.15±1.79	17.4±1.70	4.11±0.61	4.03±0.65	0.77±0.26	0.65
T3	24.9±4.89	20.8±3.73	5.20±1.70	1.78±0.38	0.62±0.26	0.67
T4	15.0±1.36	12.8±1.03	2.26±0.73	3.61±0.57	0.66±0.19	0.37
T5	13.1±1.13	9.72±0.99	2.89±0.66	2.90±0.26	0.71±0.12	-
T6	14.2±1.04	10.9±0.81	3.38±0.50	3.24±0.52	0.88±0.16	-

* values are means of a total of 17-19 sampling dates (3/78-11/79) except for total P and total inorganic P, which are means of 10 sampling dates (3/78-12/78)

** total P minus total organic P

and the present results refer to the complete period of the investigation, i.e., March, 1978, to November, 1979.

One of the objectives of this research was to establish a possible seasonal variation in the labile organic phosphorus pool (Halm *et al.* 1972; Bowman and Cole 1978b), which would indicate the importance of this soil phosphorus fraction for the within-season utilization of P by plants. According to Abbott (1978) and Bowman and Cole (1978a), the total value of extractable P (inorganic and organic) might be a better estimate of plant-available phosphorus in the soil, at least under certain conditions, than the extractable inorganic fraction alone.

Materials and methods

Soil samples were collected at more or less regular monthly intervals in six rather contrasting locations (T1-T6), which were also used in the nitrogen research program (see under 7.5). The sampling depth was 0-10 cm, and samples were dried (ca. 35°C) and sieved (1 mm) before analysis. Ground soil samples were used for the determinations of organic matter, total P, total organic P, and inorganic and organic fractions (except for labile organic P). Further details of the analytical procedures are given elsewhere (Troelstra and van der Meulen 1979). Some analytical data on the sites are given in Table 7.6.1.

To obtain information about possible shifts within and between inorganic and organic P fractions under laboratory conditions *in the absence of growing plants*, both nitrogen and phosphorus mineralization were studied with a laboratory-incubation procedure. The process of nitrogen mineralization can be clearly followed in such an experiment. For this purpose, dried and sieved soil material from three locations (inner dune area Westduinen (2: T2 and T3) and calcareous coastal dune area (1), Goeree) was aerobically incubated in polypropylene centrifuge tubes at 30°C and 20 or 50 per cent (T3) moisture content (dry weight). Twelve tubes were used for each location. At intervals the contents of one of two tubes were extracted with 1 N KCl for the determination of mineral nitrogen, and the contents of the others were immediately dried at 35°C.

After drying, half of each sample was ground in a mortar mill, and P analyses were performed as described elsewhere (Troelstra and van der Meulen 1979). Since analyses of P fractions are very laborious and it was intended to establish a possible trend in P mineralization only (in the same way as for N mineralization), replicates were not used.

Results and discussion

Figure 7.6.1 shows the seasonal pattern of the bicarbonate-extractable P pool (Olsen-P, labile organic P) for sites T1 and T2, and for the mean values of the pooled total of the six locations. From the organic matter variation, it may be concluded that much of the observed fluctuation of the bicarbonate-extractable P pool at all six sites is probably spatial, and that seasonal variation, if any, is not spectacular. Total organic phosphorus variation and organic matter variation were very similar; P contents (mean \pm standard deviation; $n = 17-19$) of the organic matter were 0.16 ± 0.01 (T3), 0.20 ± 0.02 (T1), 0.23 ± 0.02 (T6), 0.25 ± 0.02 (T4), and 0.29 ± 0.02 (T2) per cent. The strong fluctuation in the level of labile organic P at location T3 was considerably reduced when the results were expressed as percentage of total organic P.

The question may be raised whether this situation concerns short-term seasonal fluctuations on a small scale, with somewhat lower values occurring during the summer months, or a small-scale spatial variability of mainly steady-state situations. Harrison (1979) found a seasonal variation for the phosphatase activity in woodland soils. However, in a subsequent paper Harrison and Pearce (1979) also indicated that much of the within-plot variation of the phosphatase activity is spatial rather than seasonal.

Figures 7.6.2. and 7.6.3 show the relationships between Olsen-P and labile organic P for the Westduinen

area (locations T1-T6) and for 115 natural habitats in various parts of The Netherlands, respectively. In general, there is a fairly good positive correlation within a site or even within an area or soil type (R values of 0.39, 0.76, 0.79, 0.85, 0.76, 0.91, and 0.67 for T1-T6 and the pooled results of T1-T6, respectively). For the 115 natural habitats, the correlation was very poor (R = 0.33), although it is clear that many points show a relationship similar to that in the Westduinen area. This might mean that in certain situations the inclusion of the extractable organic P fraction does not necessarily improve the evaluation of the available P status of a soil. Indeed, Abbott (1978) pointed out that the organic P fraction normally measured with the NaHCO_3 extraction (Olsen-P) may include some easily hydrolysable organic P that is mineralized during the 30-min shaking period.

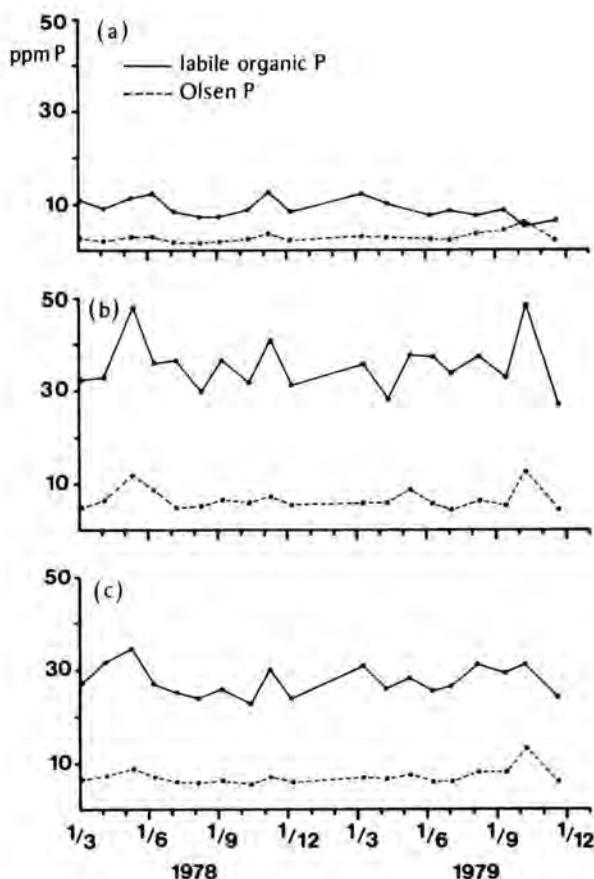
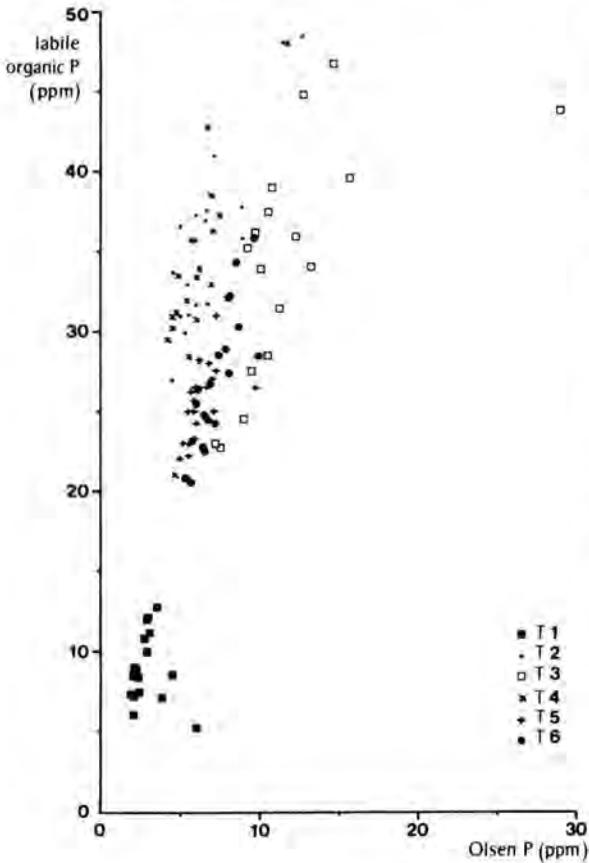


Fig. 7.6.1. Seasonal pattern of NaHCO_3 -extractable soil phosphorus for sites T1 (a), T2 (b), and for the mean values of the pooled results for locations T1-T6 (c) in the Westduinen area (0-10 cm soil depth).

Fig. 7.6.2. Relation between Olsen-P and labile organic P in the 0-10 cm soil layer at locations T1-T6 in the Westduinen area.



However, when different habitats comprising a wide range of soil types are compared, a better evaluation of soil-P availability might be obtained by using both the extractable inorganic and organic P fractions.

In Table 7.6.2, estimates of the amounts of P per square metre in the 0-10 cm soil layer are given ($\text{g P/m}^2; \times 10 = \text{kg P/ha}$). In the last column of Table 7.6.2 the total P uptake by the above-ground biomass during the period from March to November in 1979 is shown (see also under 7.5). Comparison of this column with P availability indices (Olsen-P) suggests a more or less active involvement of the labile organic P pool in the P nutrition of the plant. The question remains, however, whether this must necessarily be expressed in a distinct seasonal fluctuation of this pool.

Under the conditions of natural vegetations in temperate regions (low turnover rates), one could also imagine a kind of steady-state situation of this pool with seasonal fluctuations in the more resistant organic P pools, or even an over-all steady-state situa-

tion for all organic P fractions. If, on the other hand, a small seasonal fluctuation does occur, it will be hard to establish such a fluctuation definitely in a statistically reliable way, because of the substantial coefficients of variation of the organic P determinations themselves together with the spatial variation already present. Moreover, the area of a topographically uniform and more or less 'homogeneous' site available within the terrain, will also limit the intensity of sampling that can be applied.

The initial results of the incubation experiment are shown for the Westduinen location T2 in Fig. 7.6.4 (the sixth period of the incubation series has not yet been terminated).

In contrast to that of soil nitrogen, the chemistry of soil phosphorus is fairly complicated with different inorganic – soil minerals, adsorbed P, and soil solution P – and organic phases showing many interactions, including some with microbiological aspects. Phosphorus mineralization was much less pronounced than the accumulation of mineral nitrogen, and the effects could be observed best in material from location T2: (i) a small shift from organic P to inorganic P, (ii) within the organic P fractions mainly a decrease of the moderately and highly resistant fraction, and (iii) within the inorganic P fractions predominantly an increase of Al-bound P and (to a lower degree) Fe-bound P.

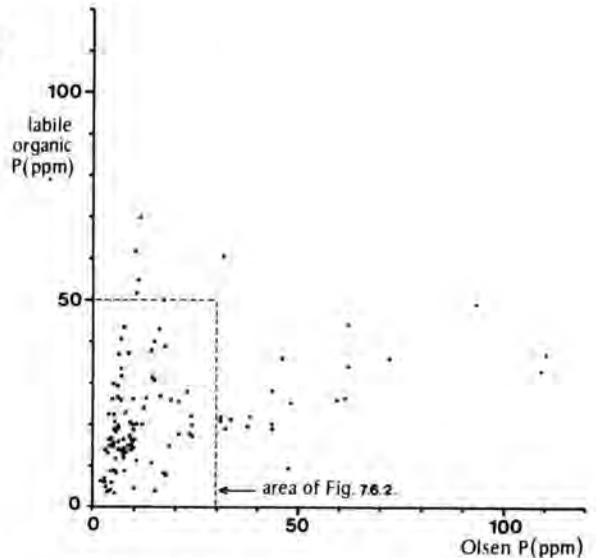
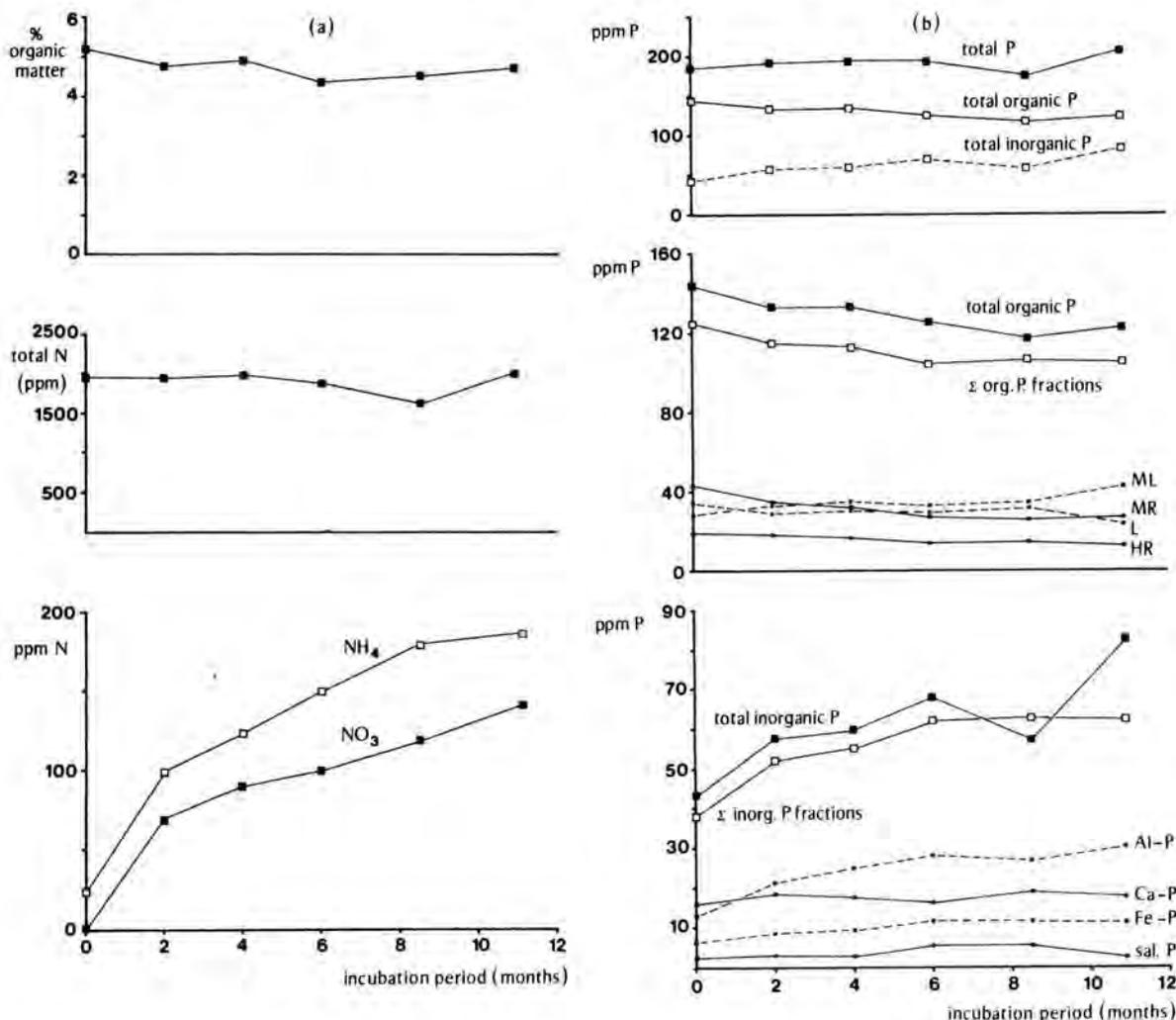


Fig. 7.6.3. Relation between Olsen-P and labile organic P in the 0-10 cm soil layer at 115 natural habitats, primarily of *Plantago* species, in various parts of The Netherlands.

Fig. 7.6.4. Time course of % organic matter, total N, mineral N (a), total P, total organic P, total inorganic P (=total P minus total organic P), and organic and inorganic P fractions (b) in soil from location T2 (Westduinen area, Goeree; 0-10 cm soil layer) during incubation at 30°C and 20% moisture (dry weight).



A remarkable finding is the rather constant level of the labile organic P pool during the experimental period of almost a year and under the experimental conditions applied (30°C, ca. 20% soil moisture, absence of growing plants).

However, the question remains as to a possible pretreatment effect of drying on the levels of the organic P fractions. Therefore, we plan 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 0-10 cm soil cores or pretreated material without previous drying.

References

- Abbott, J.L. (1978) – Importance of the organic phosphorus fraction in extracts of calcareous soil. *Soil Sci. Soc. Am. J.* 42, 81-85.
- Bowman, R.A. and C.V. Cole (1978a) – Transformations of organic phosphorus substrates in soils as evaluated by NaHCO₃ extraction. *Soil Sci.* 125, 49-54.
- Bowman, R.A. and C.V. Cole (1978b) – An exploratory method for fractionation of organic phosphorus from grassland soils. *Soil Sci.* 125, 95-101.
- 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.

Harrison, A.F. (1979) – Variation of four phosphorus properties in woodland soils. *Soil Biol. Biochem.* 11, 393-403.

Harrison, A.F. and T. Pearce (1979) – Seasonal variation of phosphatase activity in woodland soils. *Soil Biol. Biochem.* 11, 405-410.

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, 376-381.

7.7. THE IONIC BALANCE OF SOME PLANT SPECIES FROM NATURAL VEGETATIONS: COMPARISON OF PLANTS GROWN IN THE GREENHOUSE AND IN THE FIELD (S.R. Troelstra, W. Smant)

Introduction

The chemical analysis of plant species from natural vegetations was continued in 1979. Some general theoretical considerations with reference to the C-A concept, as originally proposed by De Wit *et al.* (1963), were given in a previous report (Troelstra and Smant 1979).

It has been pointed out that the *form* of the available nitrogen will have a considerable impact on the resulting (C-A)/org. N ratio of the whole plant (e.g., Jungk 1977). Since ammonium uptake has opposite effects on C-A and organic N accumulation, the (C-A)/org. N ratio will be smaller the more ammonium relative to nitrate is taken up by the plant.

The picture is made more complicated, however, by the effect of the (*nitrate*) nitrogen level (or rather the level of nitrate supply to the plant root, which is a combination of concentration and transport conditions) on the (C-A)/org. N ratio, particularly in the case of gramineous plants. Data of Banwart and Pierre (1975a) indicate a marked decrease in the (C-A)/org. N ratio of field-grown corn, oats, and orchard grass as a result of nitrogen fertilization, whereas P and K fertilizers produced only very slight or no changes in the (C-A)/org. N ratio of corn, oats, and alfalfa plants (Banwart and Pierre 1975b).

In fact, Pierre and Banwart (1973) and Banwart and Pierre (1975a,b) defined what they called an excess-base/nitrogen ratio, EB/N, but this ratio is actually comparable to the (C-A)/org. N ratio. In our calculation of C-A, H_2PO_4 is based on both inorganic and organic phosphorus. Therefore, combination of the

definitions for EB and C-A leads to the equation:

$$EB = (C-A) + NO_3 - org. S$$

For low levels of nitrate (NO_3 approximates zero), N and organic N are roughly equivalent, and we can write:

$$EB/N = (C-A)/org. N - org. S/org. N$$

According to Dijkshoorn and van Wijk (1967), organic S accounts for about 6 per cent of organic N, and the equation becomes:

$$EB/N = (C-A)/org. N - c. 0.06$$

Thus, EB/N and (C-A)/org. N are very similar. Where nitrate levels are low, these ratios will show a constant difference of about 0.06; with increasing accumulation of nitrate, EB/N progressively increases relative to (C-A)/org. N, and may eventually become the larger ratio.

Pierre *et al.*, (1970) mentioned the usefulness of EB/N ratios (or (C-A)/org. N ratios) for determination of the effect of the plant on the soil reaction in the case of nitrate uptake (see Progress Report 1978). For the (total) above-ground plant parts of 26 field-grown crop species, Pierre and Banwart (1973) found distinct differences in average EB/N ratios, ranging from 0.24 for cabbage to 1.09 for buckwheat; only 8 species had EB/N ratios ≥ 0.75 , i.e., red clover, alsike clover, carrot, table beet, tobacco, tomato, spinach, and buckwheat (in order of increasing ratio). All plant samples were collected from plots or fields that had received less than 225 kg N/ha.

It is a well-known fact that crop species such as buckwheat, spinach, sugar beet, and tomato, show little or no decarboxylation when adequately supplied with nitrate (van Egmond 1975; Argyriadis *et al.* 1976; Breteler 1977; Kirkby and Knight 1977). From water-culture experiments done by Kirkby and Knight (1977), a rather constant (C-A)/org. N ratio of about 1 can be calculated for whole tomato plants at 10 nitrate levels ranging from 0.25 to 12.00 meq/l: 0.95 ± 0.08 (nutrient solutions were renewed every 2 days!). Shortage of nitrate can even lead to HCO_3^- uptake – resulting in an excess carboxylation unrelated to nitrate and sulphate reduction processes – and (C-A)/org. N ratios > 1 (e.g. Argyriadis *et al.* 1976).

Below, a summary will be given of plant analyses carried out in 1979 at our institute, with special attention to the above-mentioned C-A aspects.

Materials and methods

The plant material was collected from natural habitats or made available from field trials and greenhouse experiments done by research workers in our institute and the universities of Groningen and Utrecht. In the May-June period in 1979 a soil-sampling program was carried out in various parts of The Netherlands, com-

TABLE 7.7.1 Ionic contents, sum of cations (C), sum of inorganic anions (A), carboxylates (C-A), organic nitrogen, total nitrogen, and (C-A)/org. N ratios in various field-grown *Plantago* species collected on different locations in The Netherlands (mean \pm standard deviation; n = number of samples)

	shoot/leaf					spike	
	<i>P. media</i> (n=7)	<i>P. major</i> (n=7-12)	<i>P. lanceolata</i> (n=36-37)	<i>P. coronopus</i> (n=1-4)	<i>P. maritima</i> (n=4-5)	<i>P. media</i> (n=5)	<i>P. lanceolata</i> (n=27)
K (meq/kg d.m.)	827 \pm 80	788 \pm 240	356 \pm 168	234 \pm 66	530 \pm 90	688 \pm 75	396 \pm 106
Na (meq/kg d.m.)	11 \pm 7	35 \pm 81	344 \pm 236	295	2006 \pm 251	4 \pm 2	155 \pm 101
Ca (meq/kg d.m.)	1660 \pm 139	1108 \pm 327	863 \pm 219	916 \pm 305	620 \pm 79	459 \pm 54	277 \pm 43
Mg (meq/kg d.m.)	242 \pm 34	257 \pm 35	206 \pm 40	525 \pm 218	590 \pm 53	144 \pm 23	124 \pm 21
H ₂ PO ₄ (meq/kg d.m.)	84 \pm 20	105 \pm 29	73 \pm 22	87 \pm 52	121 \pm 14	115 \pm 23	86 \pm 30
NO ₃ (meq/kg d.m.)	19 \pm 3	17 \pm 34	15 \pm 43	16 \pm 17	19 \pm 18	6 \pm 1	3 \pm 3
Cl (meq/kg d.m.)	459 \pm 309 (343 \pm 48)*	279 \pm 213	326 \pm 148	1145 \pm 542	2268 \pm 504	160 \pm 157	90 \pm 71
SO ₄ (meq/kg d.m.)	139 \pm 21	379 \pm 97	240 \pm 47	320 \pm 136	519 \pm 63	83 \pm 17	83 \pm 11

C (meq/kg d.m.)	2739 \pm 188	2214 \pm 329	1763 \pm 197	2002	3801 \pm 353	1296 \pm 89	952 \pm 92
A (meq/kg d.m.)	700 \pm 318 (583 \pm 71)*	803 \pm 248	653 \pm 149	1535 \pm 1192	2927 \pm 481	363 \pm 154	262 \pm 65
C-A (meq/kg d.m.)	2039 \pm 314 (2135 \pm 202)*	1378 \pm 162	1123 \pm 198	1310	731 \pm 114	932 \pm 125	690 \pm 72
org. N (mmole/kg d.m.)	1341 \pm 111	1372 \pm 373	1326 \pm 278	1169 \pm 269	1744 \pm 305	1235 \pm 335	1094 \pm 240
tot. N (mmole/kg d.m.)	1360 \pm 113	1380 \pm 380	1340 \pm 285	1187 \pm 288	1763 \pm 312	1242 \pm 335	1097 \pm 241
(C-A)/org. N	1.55 \pm 0.194	1.12 \pm 0.359	0.87 \pm 0.144	1.34	0.40 \pm 0.106	0.78 \pm 0.141	0.65 \pm 0.112

* if one extreme value is omitted

prising a total of 115 natural habitats, primarily of *Plantago* species. About 220 plant samples were taken at the same time: 133/90 (shoots and leaves/spikes). Plant species included (numbers of shoot and spike samples in parentheses, respectively) *Plantago media* (16/12), *P. major* (25/4), *P. lanceolata* (75/63), *P. coronopus* (4/3), *P. maritima* (5/0), and *Hypochaeris radicata* (8/8). We are still engaged in

analysing these samples; the results reported here refer to only part of this field material: (55-65)/32 (shoots and leaves/spikes).

Plant species other than the above-mentioned (i.e., field material, greenhouse experiments, etc.) were *Anthoxanthum odoratum*, *Hippophae rhamnoides*, *Cynoglossum officinale*, *Melandrium rubrum*, *Hypericum perforatum*, *Anthriscus sylvestris*, and *Triglochin maritima*. Chemical analyses were performed as described elsewhere (Troelstra and Smant 1979).

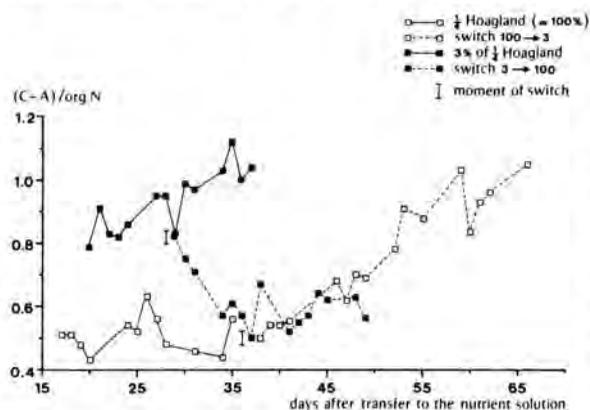


Fig. 7.7.1. Time course of the ratio of (C-A) to organic N in shoot material of *Plantago lanceolata* under various nutrient-level regimes.

Results and discussion

The preliminary results on 'natural-habitat plants' of the five *Plantago* species are given in Table 7.7.1. The potassium content of both shoot and spike material is significantly higher in *P. media* than in *P. lanceolata*. The sodium content increases from *P. media* to *P. maritima*, whereas calcium shows a distinct decrease in the same order. The magnesium levels in *P. coronopus* and *P. maritima* are relatively high. Average nitrate levels are of the same low order of magnitude for all species; a small number of relatively extreme values (100-300 meq/kg d.m.) occurred in *P. major* and *P. lanceolata*. Chloride contents of *P. maritima* and *P. coronopus* are very high, and sulphate is relatively low in *P. media*.

TABLE 7.7.2 Ionic levels in shoot material of plant species from natural vegetations grown under different conditions

Plant species	Experimental code*	Plant age (days)	Average dry matter per plant (g)	meq/kg d.m.								
				K	Na	Ca	Mg	H ₂ PO ₄	NO ₃	Cl	SO ₄	
<i>Plantago media</i>	f	-	0.590-0.000	726-938	3-22	1515-1931	181-277	57-114	15-25	287-1152	101-163	
	f1	-	0.070-0.920	594-759	1-5	373-520	120-174	83-140	5-7	40-394	62-108	
<i>Plantago major</i>	w(NF2)	c.30	-	1179-1288	12-245	417-1440	296-444	205-252	1027	23	184	
	w(NF3)	c.30	-	1235	23	1321	305	231	1103	39	212	
	f	-	0.117-1.000	378-1044	1-276	630-1747	221-348	60-142	2-120	17-67B	221-54B	
<i>Plantago lanceolata</i>	**w(I52)	c.35	0.060	1012	14	1269	313	227	964	42	278	
	w(I52)	c.35	0.064	964	26	1305	326	250	961	62	213	
	w(NF3)-t	c.30	-	981-1449	10-76	1196-1728	497-604	224-254	1488-1562	20-112	290-303	
	w(NF4)	c.30	-	1270	11	1592	528	248	1721	103	354	
	w1-1(HL/FH)-t	c.30-50	-	1120-1518	19-23	1365-1599	456-630	272-332	1349-1768	87-167	208-363	
	w1-2(HL/FH)-t	c.30-50	-	1332-1618	29-83	1307-1727	412-584	230-314	1287-1922	51-214	200-348	
	w1-1(SD)-t	c.15-35	-	938-1283	13-32	1392-1720	437-631	254-342	1107-1480	28-111	266-331	
	w1-2(SD)-t	c.25-55	-	1383-1573	20-36	1252-1496	377-539	239-279	1542-1847	37-102	233-304	
	w2-1(HL/FH)-t	c.40-60	-	511-913	30-100	1044-1381	254-418	56-93	8-123	44-401	148-245	
	w2-2(HL/FH)-t	c.30-50	-	460-968	28-49	847-1553	258-558	79-170	5-563	24-41	169-378	
	w2-1(SD)-t	c.15-35	-	728-955	29-50	1166-1508	302-524	77-135	251-308	35-162	104-178	
	w3-1(HL/FH)-t	c.50-70	-	845-1216	31-41	720-1452	218-406	95-298	242-1179	28-158	111-289	
	w3-2(HL/FH)-t	c.40-60	-	1048-1530	26-72	1396-1657	366-501	172-311	656-1615	27-140	206-319	
	w3-1(SD)-t	c.15-30	-	924-1279	24-49	1316-1656	430-603	163-337	891-1218	152-256	196-329	
	w4-1(HL/FH)-t	c.40-60	-	523-1008	23-27	670-1482	195-459	84-231	8-812	65-237	69-221	
	w4-2(HL/FH)-t	c.50-75	-	719-1460	72-373	1195-1620	243-479	103-211	28-1372	433-1122	142-252	
	w4-1(SD)-t	c.15-30	-	657-1044	17-42	1316-1754	343-594	162-291	538-1557	23-135	216-315	
	p(FB-A)	-	-	185-355	442-910	643-1258	254-489	58-124	5-14	259-971	272-522	
	p1(FB-A)	-	-	273-287	191-638	222-473	143-248	53-127	2-7	46-525	63-234	
	p(FB/3B/b)-t	-	0.424-7.314	658-874	211-260	754-1377	167-411	57-109	8-450	658-1000	85-177	
	p(FB/3B/c)-t	-	0.332-1.088	410-767	128-311	683-1202	132-305	50-99	4-28	363-1030	98-171	
	f	-	0.036-1.340	117-808	41-1026	483-1574	118-276	31-106	2-257	46-764	133-322	
	f1	-	0.017-0.867	163-587	40-364	168-369	70-163	35-130	1-16	7-258	61-107	
	<i>Plantago coronopus</i>	*w(Gr.)	c.40	0.008-0.014	407-918	878-4055	440-1624	292-448	265-303	996-1932	486-3537	102-115
		w(I52)	c.35	0.037	1131	349	1591	232	264	1639	80	94
		**w(I52)	c.35	0.041	970	327	1463	272	303	1613	90	153
		f	-	0.040	140	295	1365	202	50	5	450	187
<i>Plantago maritima</i>	*w(Gr.)	c.40	0.011	554	2531	658	356	185	1405	1833	286	
	f	-	(0.155-0.181)	447-683	1659-2233	542-707	529-668	106-139	7-50	1683-2805	426-573	
<i>Anthoxanthum odoratum</i>	p(FB-A)	-	-	323-543	52-258	120-290	139-235	58-124	3-6	179-496	80-229	
	p1(FB-A)	-	-	125-304	44-192	37-195	85-187	16-67	2-5	143-240	72-157	
	p(FB/3B/b)-t	-	0.157-1.293	696-1005	15-70	228-392	143-204	112-231	5-15	416-659	55-97	
	p(FB/3B/e)-t	-	0.581-2.451	359-970	16-107	108-350	114-153	66-194	3-7	200-672	27-69	
<i>Hypochaeris radicata</i>	f	-	0.363	477	121	1002	383	75	3	177	231	
	f1	-	0.174	525	60	487	238	104	3	137	107	
<i>Triglochin maritima</i>	f	-	-	893	2266	381	463	123	12	3219	101	
<i>Hippochaë rhamnoides</i>	p(P0/G)	-	1.40-2.48	272-507	7-13	212-496	78-304	36-76	2-12	7-35	34-93	
	p(P0/V)	-	1.72-2.13	218-387	6-13	254-525	83-176	29-95	1-31	7-32	26-94	
<i>Anthriscus sylvestris</i>	f(ST)-t	-c.100	0.2-21	740-1372	0-45	476-1023	124-242	69-187	10-351	103-459	21-58	
	f(ST)	-	-	680-1190	0-36	490-884	114-291	81-159	8-48	108-425	35-76	
<i>Cynoglossum officinale</i>	w(NF/Ca1)***	-	-	1027-1099	25-44	258-342	710-790	253-310	268	40	54	
	w(NF/Ca2)***	-	-	1117-1235	18-55	379-571	646-884	294-426	296	31	31	
	w(NF/Ca3)***	-	-	1076-1411	24-90	575-714	494-573	272-424	90-221	39-47	31-61	
	w(NF/Ca4)***	-	-	1209-1354	20-211	745-930	325-368	229-362	117-130	52-72	22-25	
	p(NF1)	-	-	797-1060	4-127	972-1375	363-500	37-55	8-11	93-116	36-48	
	p(NF1Ca)	-	-	711-1043	0-21	1209-1517	293-455	49-100	22-88	43-53	16-24	
	p(NF2)	-	-	659	29	956	538	43	18	0	184	
	p(NF2Ca)	-	-	619-736	27-29	955-1127	385-465	94-128	11	113	28	
<i>Melandrium rubrum</i>	w(NF/Ca1)***	-	-	945-1828	270-370	20-50	576-910	98-222	77-275	11-27	18-72	
	w(NF/Ca2)***	-	-	1332-1995	213-316	95-161	464-773	110-206	182-409	12-40	16-63	
	w(NF/Ca3)***	-	-	1325-1860	161-259	216-472	408-682	108-241	128-311	13-39	21-60	
	w(NF/Ca4)***	-	-	1237-2046	137-188	418-699	313-580	102-200	121-372	17-72	25-87	
<i>Hypericum perforatum</i>	w(NF/Ca1)***	-	-	645-717	3-16	3-20	487-560	229-264	59-110	23-42	40-69	
	w(NF/Ca2)***	-	-	629-804	10-24	29-47	299-474	132-221	23-118	20-51	22-60	
	w(NF/Ca3)***	-	-	588-877	10-47	46-220	208-315	118-189	10-119	35-82	32-52	
	w(NF/Ca4)***	-	-	589-790	7-33	225-353	157-219	125-172	14-79	23-140	35-72	

* w = water culture; w1 = nutrient level (nitrate) relatively high
w2 = " " low
w3 = nutrient level switch (low + high)
w4 = " " (high + low)
plants were harvested after the switch during a period of 2-3 weeks
p = pot experiment/experimental field plot
f = field material
p1, f1, p1f = spikes
-t = time-course measurement
** ammonium nutrition during last 24 hours only
*** Ca1-Ca4 = nutrient solutions with varying Ca levels (low + high)
* nutrient solution (i H) with varying (0-100 mM) NaCl levels (*P. coronopus*) or with 50 mM NaCl (*P. maritima*)

TABLE 7.7.5 Dry matter yields and (C-A)/org. N ratios of three successive harvests of *Plantago lanceolata* and *Anthoxanthum odoratum* plants (pot experiments)

Experiment	Harvest (date)	<i>P. lanceolata</i>		<i>A. odoratum</i>	
		Dry matter (g/plant)	(C-A)/org. N	Dry matter (g/plant)	(C-A)/org. N
1	1 (7/3/78)	0.76	0.56	0.33	0.47
	2 (4/4/78)	3.81	0.86	1.57	0.52
	3 (24/4/78)	5.64	1.22	2.22	0.58
2	1 (7/3/78)	0.69	0.91	1.15	0.30
	2 (4/4/78)	1.54	1.61	2.58	0.71
	3 (24/4/78)	2.07	2.03	3.58	0.92

Except for *P. coronopus* (only one value available so far), the results of the C-A calculation given in Table 7.7.1 show the same order within the group of *Plantago* species as indicated previously (Troelstra and Smant 1979). Generally, (C-A)/org. N ratios decrease in the sequence *P. media*, *P. major*, *P. lanceolata*, *P. coronopus*, *P. maritima*.

Most of the plant-analysis data are summarized in Tables 7.7.2, 7.7.3, and 7.7.4, which can be seen as supplementary to the Tables given elsewhere (Troelstra and Smant 1979). The differences between plant material from the field and from greenhouse experiments are again apparent, especially in the case of NO₃. Apart from competition effects with neighbouring plants, it is evident that nitrate-supply mechanisms (ammonification – transport of ammonium – nitrification in the rhizosphere; or ammonification – nitrification – transport (diffusion and mass flow) of nitrate to the root) can be severely restricted in natural vegetations. Soil physical conditions, particularly soil moisture, will be sub-optimal for most of these (non-agricultural) situations and will undoubtedly have an effect on nitrification and nitrate-transport processes.

Table 7.7.4 shows that (C-A)/org. N ratios of *P. lanceolata* plants from water-culture experiments are often in the range 0.40-0.60. This ratio increases strongly for material from pot experiments, experimental plots, and the field, as well as for plants from water-culture experiments performed at a low nitrate level. A nutrient-level-switch experiment with *P. lanceolata* (code 2(HL/FH)-t in the Tables) is very illustrative in this respect (Fig. 7.7.1). The (C-A)/org. N ratios of the shoot were about 1.0 and about 0.5 at a relatively low nutrient level (3% of ¼ Hoagland) and a relatively high nutrient level (¼ Hoagland), respectively. After a nutrient-level switch (low – high or high – low), the value belonging to the new nutrient

level was found. Obviously, decarboxylation (and a rhizosphere-pH increase) will be the usual process for *P. lanceolata* at sufficiently high nutrient levels, just as for the gramineous plants. At a relatively low nitrate level, the reverse will occur and the (C-A)/org. N ratio of the whole plant will increase to values > 1.

Analyses of successive harvests of roots and shoots of *P. lanceolata* and *A. odoratum* from two pot experiments (codes FB/ JB/b and FB/ JB/e in the Tables) revealed a similar effect. For three successive yields the values in Table 7.7.5 were calculated for whole plants. If nitrogen uptake is assumed to be 100 per cent in the form of nitrate during the experiments, *P. lanceolata* changes over from decarboxylation to extra carboxylate formation (in excess of nitrate and sulphate reduction), whereas decarboxylation in *A. odoratum* decreases. In terms of rhizosphere-pH effects this means that alkaline effects become less pronounced and may even shift to an acidifying effect.

From Table 7.7.3 it can be concluded that *Anthoxanthum odoratum*, *Triglochin maritima*, and *Hippochaë rhamnoides* are plants with a relatively low C-A content, whereas the chemical composition of *Cynoglossum officinale* and *Melandrium rubrum* indicates very high C-A levels for these plants.

In conclusion we can make some speculative statements which certainly require verification in the future. All *Plantago* species will probably behave in a similar way. However, the transitional point decarboxylation – extra carboxylation (or the formation of (CA)/org. N ratios > 1) presumably occurs at different (nitrate) nutrient levels and will be highest for *P. media* (because of the relatively high C-A content). Further, we still believe that *P. media* is more vulnerable to the harmful effects of ammonium-nitrogen than, for instance, *P. coronopus* or *P. maritima*. Therefore, the form of nitrogen must positive-

ly be taken into account, when one investigates the effect of (nitrogen) nutrient levels on the growth and development of *Plantago* species.

Concerning the distribution of *Plantago* species in the field, the following theory may be advanced. *P. lanceolata* will occur at relatively low pH values, whereas *P. media* and *P. major* generally prefer higher pH values because of the higher C-A level they require for optimal growth (under these conditions, more nitrogen in the form of nitrate will become available). On the other hand, *P. coronopus* and *P. maritima* also often occur at relatively high pH values, but here one is dealing with salinity effects, which cause only species with relatively low C-A contents to be better adapted.

Future research will include more detailed studies on the chemical composition of *Plantago* species in relation to the form of the nitrogen taken up. Moreover, attention will be paid to the specific composition of the (CA)/org. N ratios (> 1) presumably occur at different pectate) of the different *Plantago* species as well as to changes in this composition after, for instance, NaCl additions.

References

Argyriadis, G.A., W. Dijkshoorn and J.E.M. Lampe (1976) – Level and origin of carboxylate in buckwheat. *Pl. Soil* 44, 669-681.

Banwart, W.L. and W.H. Pierre (1975a) – Cation-anion balance of field-grown crops. 1. Effect of nitrogen fertilization. *Agron. J.* 67, 14-19.

Banwart, W.L. and W.H. Pierre (1975b) – Cation-anion balance of field-grown crops. 2. Effect of P and K fertilization and soil pH. *Agron. J.* 67, 20-25.

Breteler, H. (1977) – Aufnahme und Assimilation von

anorganischem Stickstoff bei Kulturpflanzen. *Landwirtsch. Forsch., Sonderheft* 34, 1-17.

Dijkshoorn, W. and A.L. van Wijk (1967) – The sulphur requirements of plants as evidenced by the sulphur-nitrogen ratio in the organic matter: a review of published data. *Pl. Soil* 26, 129-157.

Egmond, F. van (1975) – The ionic balance of the sugar-beet plant. Thesis Agricultural University, Wageningen.

Jungk, A. (1977) – Wirkung von Ammonium- und Nitrat-Stickstoff auf das Wachstum und die Zusammensetzung von Pflanzen. *Landwirtsch. Forsch., Sonderheft* 34, 18-26.

Kirkby, E.A. und A.H. Knight (1977) – Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation, and cation-anion balance in whole tomato plants. *Pl. Physiol.* 60, 349-353.

Pierre, W.H. and W.L. Banwart (1973) – The excess-base and excess-base/nitrogen ratio of various crop species and of parts of plants. *Agron. J.* 65, 91-96.

Pierre, W.H., J. Meisinger and J.R. Birchett (1970) – Cation-anion balance in crops as a factor in determining the effect of nitrogen fertilizers on soil acidity. *Agron. J.* 62, 106-112.

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, 381-389.

Wit, C.T. de, W. Dijkshoorn and J.C. Noggle (1963) – Ionic balance and growth of plants. *Versl. Landbouwk. Onderz.,* 69-15. Pudoc Wageningen.

8. Publications in 1979

Aart, P.J.M. van der – Dieren en beweging. *Zuidhollands Landschap* 4, 20-21.

Adriani, M.J. – Het mondingsgebied van de grote rivieren en de Biesbosch. In: *Spectrumatlas van de Nederlandse landschappen*. Het Spectrum, Utrecht, Antwerpen, p. 174-185.

Adriani, M.J. – Slufter en Oostvoornse Meer; hoe nu verder. *Zuidhollands Landschap* 1, 3-6.

Adriani, M.J. – Leven in beweging. *Dynamiek*. *Zuidhollands Landschap* 4, 3-4.

Adriani, M.J. – Het achterduinbos. *Zuidhollands Landschap* 4, 22-23.

Blom, C.W.P.M. – Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. Thesis, Nijmegen. 135 p.

Blom, C.W.P.M., L.M.F. Husson and V. Westhoff – Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. IV. The vegetation of two dune grasslands in relation to physical soil factors. *Proc. Kon. Ned. Akad. Wetensch., Series C*, 82, 245-273.

Dijk, C. van – Endophyte distribution in the soil. In: J.C. Gordon, C.T. Wheeler and D.A. Perry (eds.) – *Symbiotic nitrogen fixation in the manage-*

ment of temperate forests. Forest Research Laboratory, Oregon State University, p. 84-94.

Dijk, C. van – Dynamiek van het organisme. Zuidhollands Landschap 4, 5-11.

Haeck, J. and R. Hengeveld – Biogeografie en Oecologie: over verschillen in de mate van voorkomen binnen het areaal. Vakblad voor Biologen 59, 26-31.

Laan, D. van der – De gevolgen van enkele jaren met extreme grondwaterstanden voor de vegetatie van vochtige duinvalleien op Voorne. Duin 2, 22-44.

Laan, D. van der – Dynamiek van landschap en plantengroei. Zuidhollands Landschap 4, 11-19.

Laan, D. van der – Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. Vegetatio 39, 43-51.

Luijendijk, R. – De verspreiding van waterwantsen in relatie tot enkele milieufactoren op Voorne-Putten. In: J. Vangenechten and O. Vanderborgh (eds.) – Biology and distribution of waterbugs. Centre d'étude de l'énergie nucléaire, Brussel, BLG 529, 24-31.

Nip-van der Voort, J., R. Hengeveld and J. Haeck – Immigration rates of plant species in three Dutch polders. J. Biogeography 6, 301-308.

Oremus, P.A.I. – A quantitative study of nodulation in *Hippophaë rhamnoides* L. ssp. *rhamnoides* in a coastal dune area. Plant and Soil 52, 59-68.

Reinink, K. – Observations on the distribution of land snails in the woods of the IJsselmeer polders. Basteria 43, 33-45.

Visser, J. – Fat and protein metabolism and mortality in the Coot *Fulica atra*. Ardea 66, 173-183.

Woldendorp, J.W. – Het Instituut voor Oecologisch Onderzoek bestaat 25 jaar. Vakblad voor Biologen 59, 251-253.

Institute for Ecological Research – Progress Report 1978. Verh. Kon. Akad. Wetensch., Afd. Natuurk., 2e Reeks 73, 304-391 (1979).

Contents:

Woldendorp, J.W. – General summary. p. 306-309.

Balen, J.H. van – Feeding conditions, foraging behaviour, and diet in winter. p. 309-310.

Drent, P.J. – Territory occupancy in the autumn, seed food supply in winter, and local survival during the annual cycle. p. 310-316.

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Soekarjo, R. – Regeneration in *Plantago* species. p. 344-346.

Aart, P.J.M. van der – A comparative multidisciplinary approach to the demography, physiology, and genetics of plant species occurring in semi-natural grasslands. p. 347-350.

Blom, C.W.P.M. – Separate and combined effects of trampling and soil compaction on root patterns, root biomass and seed production of four *Plantago* species in experimental plots. 351-364.

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Troelstra, S.R. and M.A. van der Meulen – Organic soil phosphorus fractions in an older dune area (Westduinen) on the island of Goeree. p. 376-381.

Troelstra, S.R. and W. Smant – The ionic balance of some plant species from natural vegetations and its relation to nitrogen uptake and salt tolerance. p. 381-389.

Progress Report 1979

Edited by S. Parma



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1. Introduction (S. Parma)

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 fresh-water systems in the Netherlands. Due to the separate development of the two institutes 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, viz, 1. The 'Vijverhof' Laboratory at Nieuwersluis (Utrecht), at the original site of the Institute (See Fig. 1). 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.

1.2. ORGANIZATION OF THE INSTITUTE

The Institute is financed by the Ministry of Education and Science by means of the funds allotted to the Royal Netherlands Academy of Arts and Sciences.

The Institute had in 1979 on its pay roll 41 full time places covering a staffing of 45 persons. Additionally, one employee is paid by the Service Centrum Friesland. Twenty eight persons were working at the 'Vijverhof' Laboratory at Nieuwersluis and 16 at the Tjeukemeer Laboratory at Oosterzee, both including staff members. In addition, 9 guestworkers, 17 students and 12 trainees took part in the scientific programme of the Institute (Table 1). The general service departments, covering the administration, library, photography, ships and workshop, consisted of 13 people.

1.3. RESEARCH PROGRAMME

The hydrobiological research in fresh-water systems of the Netherlands was in its earliest phase of development when the Institute started its work. The research

in the first years had, therefore, mainly an inventory character. This was followed in the late sixties by a phase in which several processes in the fresh-water ecosystem under study were described more thoroughly. At Nieuwersluis, these two phases have paved the way for a major research effort in Lake Vechten, a mesotrophic, thermally stratified sandpit with a surface area of 4.7 ha and a maximum depth of 11.9 metres. The study is aimed at an analysis of the ecosystem of this lake.

At Oosterzee the investigations concentrate on algae and on the subsystem of fish populations and their food organisms. Both the studies are focussed on the Tjeukemeer, a shallow, peaty lake of c. 21 km² in area and with an average depth of c. 1.5 m.

The 'Ecosystem study of Lake Vechten', especially focussed on the carbon budget, involves three working groups: 'Primary and Secondary Production', 'Ecophysiology of Water Plants' and 'Mineralization of

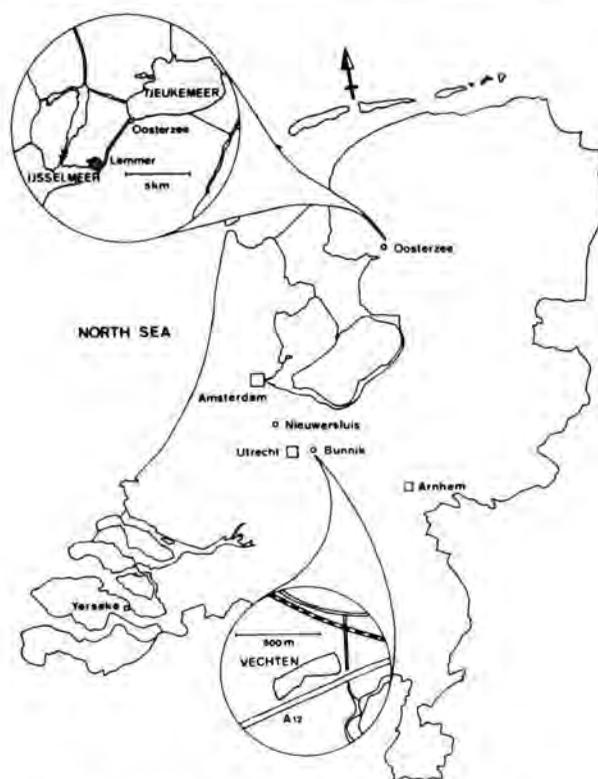


Fig. 1. Map of the Netherlands showing the study areas of the Limnological Institute - Tjeukemeer research at Tjeukemeer Laboratory, Oosterzee, Friesland, and Lake Vechten research at 'Vijverhof' Laboratory, Nieuwersluis, Utrecht.

Organic Matter'. The 'Tjeukemeer Research' is carried out by the working groups 'Algology' and 'Foodchain and Production Studies' (See Table 1).

Besides the main projects being carried out by the working groups three separate subjects are also being studied (See Table 1). The 'Vechtplassen' research will

be extended gradually in the next years and would concern the effects of a decrease of P-loading in a system of shallow peaty lakes. The Polder research is financed by the Ministry of Public Health and the Environment. It should be a basis for the management of the polders — typical Dutch fresh-water systems.

Table 1: Research scheme in 1979: s=student, t=trainee, g=guest worker

Tjeukemeer research	<u>Working group:Algology</u>	
	Dr. J. R. Moed (wg. leader)	R. J. A. Mevissen (g)
	Dr. H. de Haan	J. Geraerds (s)
	H. L. Hoogveld	B. van der Werf (s)
	H. A. Kramer	A. Baks (s)
	Th. de Boer	S. Kromkamp (t)
	J. Voerman	A. Moudstra (t)
	Ing. G. J. Schrottenboer	W. Bakker (t)
	P. J. Timmer (g)	
	<u>Working group Foodchain and production studies</u>	
	Drs. J. Vijverberg (wg. leader)	D. Fokkema (s)
	Drs. W. L. T. van Densen	J. W. Garding (s)
	Drs. H. W. de Nie	G. Nampink (t)
	Drs. E. H. H. R. Lammens	H. Hermans (t)
	P. J. Mac Gillavry	F. Mennes (s)
	Th. N. Frank	J. Meischke (s)
	A. G. Frank-Landman	B. Nieuwenhuis (t)
	A. Ypma (g)	C. Rentes (s)
	E. van Biemen (g)	A. Schoon (s)
	A. Baks (s)	R. Schouten (s)
	A. Bijlard (s)	
	Lake Vechten research	<u>Working group Primary and Secondary Production</u>
Dr. R. D. Gulati (wg. leader)		M. A. M. Kramer (t)
Drs. W. A. de Kloet		H. Vermeulen (t)
Drs. J. T. A. Vulto		W. Opstelten (s)
G. Postema		P. van der Linden (t)
K. Siewertsen		A. C. Jooren (t)
<u>Working group Ecophysiology of water plants</u>		
Drs. P. H. Best (wg. leader)		J. H. A. Dassen
Dr. C. L. M. Steenberg		M. D. M. Trommel
Dr. H. J. Gons		G. L. Wieggers (g)
R. van Keulen		R. Bergsma (t)
H. J. Korthals		J. T. Meulemans (s)
M. J. B. Bär-Gilissen		
<u>Working group:Mineralization of organic matter</u>		
Dr. Th. E. Capenberg (wg. leader)		M. D. M. Trommel
Drs. H. Verdouw	Ir. E. F. van der Heide (g)	
Drs. J. J. Olie	A. M. M. Jansen (g)	
A. G. Misselo	J. J. Stoffels (s)	
C. A. Hordijk	G. J. Jonkheer (t)	
E. M. J. Dekkers	A. C. Jooren (t)	
Bergumermeer research	<u>Research group:Biological consequences of cooling water discharge</u>	
	Dr. J. B. Wanders (g)	J. S. Swart (g)
Vechtplassen research	<u>Research group:Regional Limnology</u>	
	Dr. R. D. Gulati	A. Reins (s)
Polder research	<u>Project:Oxygen budget in a ditch</u>	
	Dr. S. Farma (project leader)	Ir. R. Veeningen (g)



The Limnological Institute at Nieuwersluis: (above) the main-building 'Vijverhof' and (below) the new building

The work of the Institute is based on five year plans of studies which are carried out in consultation with an Advisory Commission installed by the Royal Netherlands Academy of Arts and Sciences.

The Institute organizes training programmes for students from Dutch universities. Training and research facilities are also offered to students and scientists from abroad.

2. Tjeukemeer research in progress

Tjeukemeer is a shallow freshwater lake (area, c. 21 km²; mean depth, c. 1.5 m). It forms a part of the Frisian reservoir system. The surface of the bottom consists of a mosaic of peat (60%), sand (35%) and mud (5%). In winter the lake receives nutrient- and humus-rich water from the surrounding polders. The concentration of 'dissolved' humic acids (0.2 µm) varies from 3.4 to 13.7 mg.l⁻¹. Due to the high Ca⁺⁺ (range, 36–56 mg.l⁻¹) and HCO₃⁻ (range, 79–122 mg.l⁻¹) contents, 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 content of IJsselmeer water is much lower than that of the polder water but the chloride concentration much higher. The increase in the chloride concentrations up to 200 mg.l⁻¹ gives the lake an oligohaline character.

A study of the food chain in Tjeukemeer was started in 1966 within the framework of the International Biological Programme and finished in 1971. Subsequently, two working groups were set up.

2.1. WORKING GROUP 'ALGOLOGY'

(J.R. Moed, H. de Haan, Th. de Boer, H.L. Hoogveld, H.A. Kramer, G.J. Schrottenboer and J. Voerman.)

2.1.1. Introduction

Large populations of diatoms, blue-green and sometimes green algae are observed annually in Tjeukemeer. The monitor-programme is aimed both to establish the magnitude and periodicity of these algal populations and to obtain information about the factors co-regulating their wax and wane. The regulating factors are studied in the field and in the laboratory. The varying physical-chemical regime of the lake may influence the availability of, namely, P, N and Fe for algae. In other words, the hydrology may be co-regulating in the algal periodicity.

In order to study the different physical-chemical forms of Fe and P, gel filtration and ultrafiltration are being applied, and the availability for algae will be tested in chemostats. Bio-assays are being performed to detect the factors co-regulating algal periodicity.

The field and laboratory data thus obtained will be applied to an experimental model to simulate the algal periodicity in the Tjeukemeer.

The research programme for the period 1979–1983 is aimed at explaining the periodicity of abundant algal species in Tjeukemeer as well as at a quantitative description of the waterflows into the lake. In this regard, the working group has sought cooperation with Ir. J.D. Leenen and Ir. H.G. Verhagen, both affiliated with the Laboratory for Hydraulics and Catchment Hydrology, Agricultural University, Wageningen.

2.1.2. Hydrological field studies

General

Hydrology of the Tjeukemeer is determined by the deficit or surplus of water in the surrounding polders. The inflow and the concentration of the nutrients of the inflow have to be determined in order to calculate the nutrient load and its role in the algal growth.

Therefore, a study was started at the Laboratory of Hydraulics and Catchment Hydrology at Wageningen to determine the water inflow in the Tjeukemeer. Using a simple mathematical model, based on instantaneous mixing of chloride across the whole lake, the inflow in the Tjeukemeer during the 'dry' season in the years 1969, 1970 and 1971 was calculated, and compared with the intake of IJsselmeer water at Lemmer and Stavoren in order to find a relationship. The results appear promising. Sampling at a few more points is needed.

Due to 'saline' water flowing into the lake on the west side during the 'dry' season, the chloride concentration on the east side is always lower. To describe this phenomenon the model of instantaneous mixing has to be given up and dispersion effects have to be considered. A one-dimensional model is now being developed to calculate a longitudinal dispersion coefficient for the Tjeukemeer.

The hydrology in 1979

As in previous years, the temperature, turbidity, chlorinity, nutrients, chlorophyll-a and the algae in the Tjeukemeer were monitored.

Up to 7 March the lake was covered with a 25 cm thick ice-layer. On 11 March all ice had disappeared. Subsequently, up to the end of June, no IJsselmeer

water entered the Tjeukemeer since the polder had a surplus of water. On the other hand, the polderwater was pumped into the lake, as well as water from remote polders, and high grounds also entered the lake. The chloride concentration in (polders) samples taken halfway between Echten and Bantega was relatively high ($>100 \text{ mg} \cdot \text{l}^{-1}$). This is possibly related to an underground waterflow from the Tjeukemeer.

The inflow of IJsselmeer water to the Tjeukemeer, as indicated by the gradual increase of chloride concentration, started this year two months later than usual.

2.1.3. Algological field studies

Algal periodicity

The algal surveys in 1977, 1978 and 1979 show that algal species belonging to *Cryptophyceae*, *Bacillariophyceae*, *Cyanophyceae* and *Chlorophyceae* were mainly abundant and their composition did not vary from year to year. Moreover, the overall pattern of the algal periodicity in 1979 agrees with the general trend observed since 1972. In 1979 *Cryptophyceae* consisted mainly of *Cryptomonas* spp. and *Chroomonas* sp. Maxima in the period March–May (Fig. 2) were, respectively, 700 and 800 cells ml^{-1} . In a sample taken from below the ice on 9 February, the algal numbers were respectively 1,200 and 600 cells ml^{-1} .

The diatoms increased sharply in numbers during the second half of March and the first half of April (Fig. 3). Maxima for *Asterionella formosa* (400 cells ml^{-1}) and for *Melosira* spp. (4,300 cells ml^{-1}) were observed on 10 April. The maximum of *Asterionella* was rather low compared with that in 1977 and 1978 (respectively, 2,300 and 3,900 cells ml^{-1}). The maxima for *Diatoma elongatum* (6,700 cells ml^{-1}) and for the unicellular centric diatoms (20,000 ml^{-1}) were observed, respectively, on 24 April and 8 May. The latter species had a maximum two weeks after that of *D. elongatum* which is unusual. In summer an increase of unicellular centric diatoms with cell size larger than that in spring was observed (Fig. 3).

The blue-green algae started to increase in numbers during the last half of April, i.e. distinctly after the diatoms (Fig. 2). Maxima for *Aphanizomenon flos-aquae* (4,300 filaments ml^{-1}), *Lyngbya* spp. (3,500 filaments ml^{-1}), *Oscillatoria limnetica* (3,000 filaments ml^{-1}) and *Dactylococcopsis* spp. (1,500 cells ml^{-1}) were observed on 22 May. These values are, except for *Dactylococcopsis* spp., compared to those of previous years, relatively lower. *Dactylococcopsis* spp. is usually not abundant in the Tjeukemeer although in 1972 also a maximum of the same magnitude as in 1979 was counted. *O. redekei* (72,000 filaments ml^{-1}) had a maximum on 5 June. This is similar to that observed in 1978. As usual, *O. agardhii* increased in numbers

during summertime, but a density of 43,000 filaments ml^{-1} this year is double the levels reached in previous years.

The numbers of *O. limnetica*, *Lyngbya* spp. and *Aphanizomenon flos-aquae* increased again in July, leading, respectively, to maxima of 1,600, 4,300 and 9,100 threads ml^{-1} , on 11 September.

The green algae increased in numbers after the ice had melted (Fig. 3). The maxima for *Scenedesmus* spp. (17,700 colonies ml^{-1}) *Ankistrodesmus* sp. (3,600 cells ml^{-1}), unidentified green flagellates (6,800 cells ml^{-1}), and unidentified green single cell algae (1,600 cells ml^{-1}) were observed on 22 May. The value for *Scenedesmus* appears to be about 50 per cent higher than that observed in 1975. A sharp drop in numbers of the green algae in June is striking. For some species of the green algae a second increase in numbers was observed shortly afterwards. This resulted in the maxima of *Scenedesmus* spp. and the green flagellates in July and of the green single cell algae in September. The absence in 1979 of *Planctonema lauterbornii*, a filamentous alga abundant in 1977 and 1978, is noteworthy.

Variability in the length of cells and threads

The average cell lengths of *D. elongatum* in the periods of increase and decrease were, respectively, 79 μm on 10 April and 58 μm on 22 May. This decrease on the latter date is less marked than that observed in 1978. The role of environmental conditions in this regard is being examined. The average thread length of *O. agardhii* varied considerably. At the time of onset of growth it reached 380 μm (on 24 April); at the time of its maximum numbers it was 160 μm (in early June) and later about 200 μm (July–September). The average thread lengths of *A. flos-aquae* and *O. redekei* were, respectively, 210 and 285 μm . These values decreased, respectively, to 130 and 120 μm after 8 May.

Bioassay experiments

In 1979 enrichment bioassays were performed fortnightly to detect the nutrients likely to co-regulate the algal periodicity in the Tjeukemeer.

In each experiment 200 ml of a medium M26 (Table 2) containing all the nutrients (P, N, Si, Fe, Fe-EDTA) was used except that one of the nutrients was excluded each time. These were added to bottles containing 400 ml of filtered (120 μm) lake water. These batch cultures, together with the one to which 200 ml of complete medium i.e. containing all the nutrients, and the one to which 200 ml of distilled water was added, were incubated for at least six days at 18°C and exposed to natural light conditions (north window). The cultures were stirred constantly and aerated with wet air.

Fig. 3. Seasonal changes in the numbers of dominant Bacillariophyceae and Chlorophyceae in the Tjeukemeer during 1979.

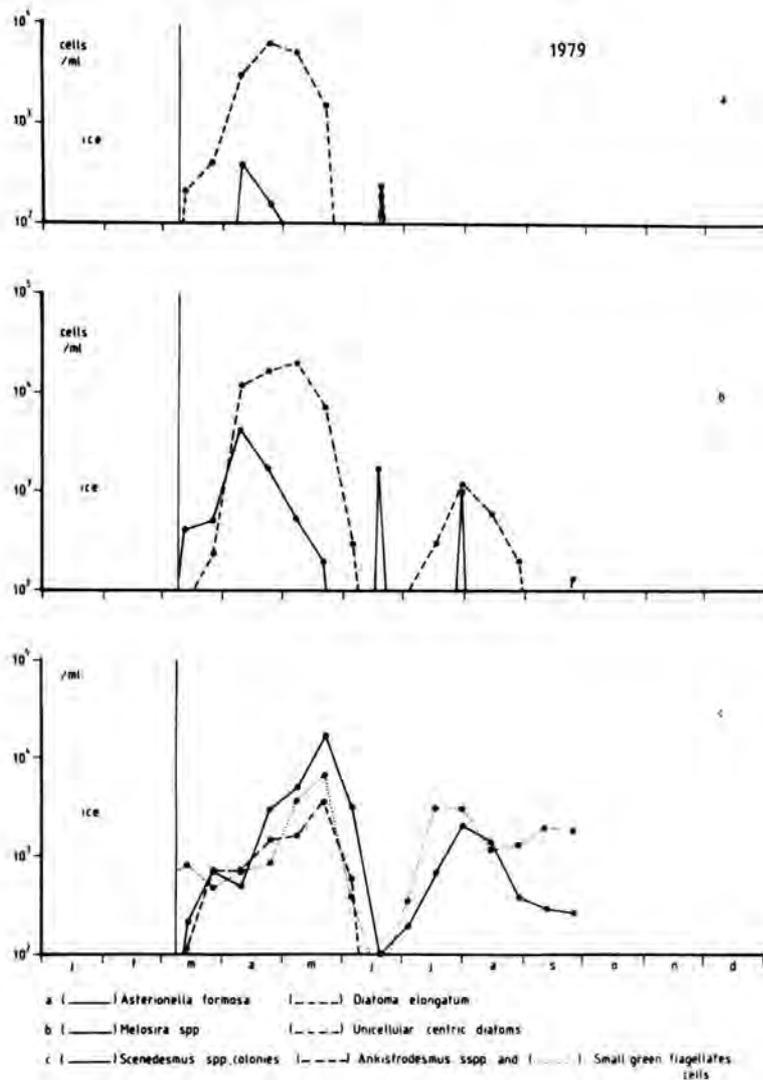
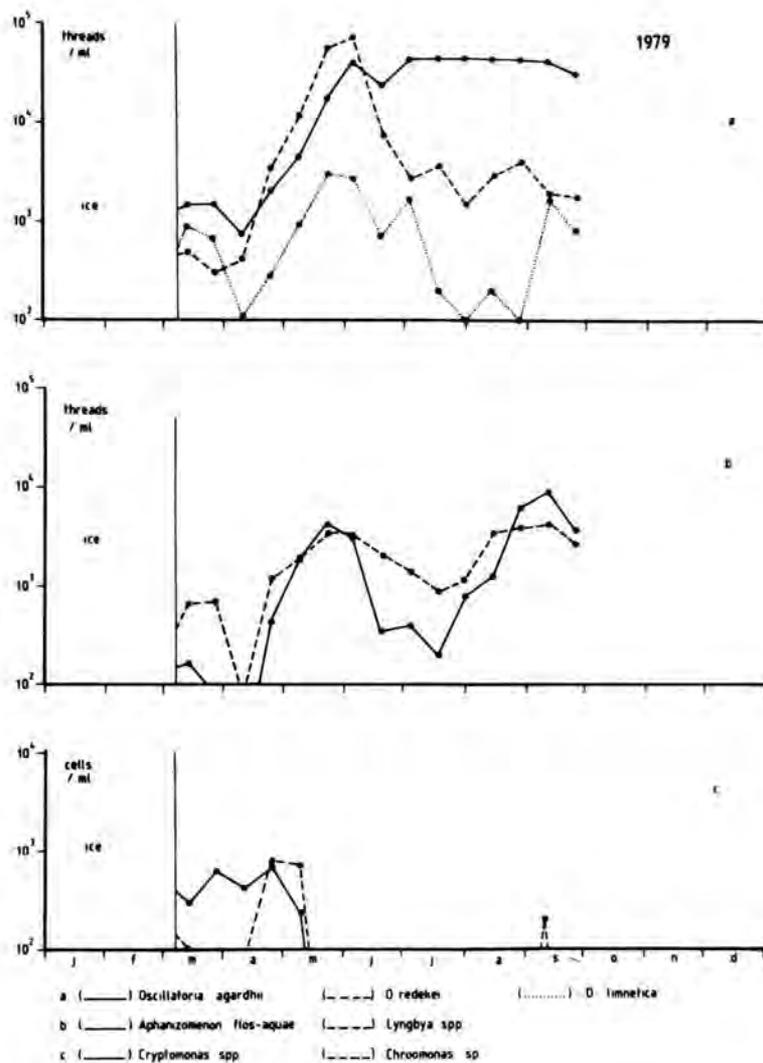


Fig. 2. Seasonal changes in the numbers of dominant Cyanophyceae and Cryptophyceae in the Tjeukemeer during 1979.



Both at the start and after six days of incubation samples were taken from each bottle for estimation of algal biomass (chlorophyll-a, dry weight, optical density at 720 nm) and composition. Since the results are not yet fully analysed only some preliminary remarks will be made. Up to April (ice period excluded) none of the nutrients taken into account appeared to limit algal growth in the bioassay. From this time until November in most of the experiments N was a limiting factor, sometimes together with Fe (+EDTA) and P. From the end of July, at which time IJsselmeer water entered the lake, the omission both of trace-elements and Fe seemed to stimulate algal growth. It is speculated that in such cases the EDTA left is available to chelate toxic substances, presumably Cu (See 2.1.4.), present in the Tjeukemeer water. However, extrapolation of the results to situations prevailing in the lake is not valid because culture conditions were quite different from those in nature (EDTA as chelating factor in the medium, no sediment present, etc.).

0.24 mg.l⁻¹, being 50 per cent higher than the average for the previous 3 years.

The other nutrients decreased to minimal values as follows: SiO₂-Si, (0.04 mg.l⁻¹) in April, NH₃-N (0 mg.l⁻¹) in May, and Tot-Fe_{diss}, Tot-P_{diss} and NO₃-N (respectively, 0.01, 0.003 and 0.02 mg.l⁻¹) in June. Shortly after their minima clear increases for Si, Fe and P were measured, due mainly to the polder water entering the lake. This is in agreement with the fluctuations in the intensity of yellow colour of the Tjeukemeer water.

From the bioassay experiments it is interesting to observe that following the minima of ammonia and nitrate an increase, as in the case of P, was not observed. Org-N_{diss} (data of one sampling point) showed a maximum of 3.1 mg.l⁻¹ on 22 May and a minimum of 0.3 mg.l⁻¹ on 3 July. This decrease might indicate the utilization by algae of Org-N_{diss} in addition to (the then less available) inorganic N.

The dissolved (0.2 μm) Cu content of the Tjeukemeer water was estimated by flame atomic ab-

Table 2. Composition of medium M 26 used for the bio-assay experiments (mg.l⁻¹)

CaCl ₂ ·2H ₂ O	44	HBO ₃	0.3
MgSO ₄ ·7H ₂ O	24.6	Mn SO ₄ H ₂ O	0.3
KCl	4	(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	0.06
Na HCO ₃	42*	Cu SO ₄ ·5H ₂ O	0.08
Na NO ₃	51	Co (NO ₃) ₂ ·6 H ₂ O	0.08
K ₂ HPO ₄	2.5*	Zn SO ₄ ·7H ₂ O	0.2
Na ₂ EDTA H ₂ O	5		
Fe SO ₄ ·7H ₂ O	2.78		
(C ₂ H ₅) ₄ SiO ₄	110		

*Sterilized separately

2.1.4. Hydrochemical field studies

General chemistry

During the ice period samples were taken from below the ice at one point on 30 January and 20 February and shortly after the ice period on 13 March. The values for Tot-P_{diss} (<0.2 μm) increased respectively from 0.03 to 0.20 and finally to 0.27 mg.l⁻¹. It is interesting that in the previous years a value of 0.3 mg.l⁻¹ was not exceeded. In contrast to the increase of Tot-P_{diss} that of Part-P was linear, values being, respectively, from 0.12 to 0.28 and from 0.28 to 0.46 mg.l⁻¹. This suggests an accumulation of P in Part-P.

During April Tot-P decreased to 0.26 mg.l⁻¹. The average value for 1979 remained (high) at

sorption, a self-constructed Cu electrode and using a colorimetric method. The latter method appears to be the most suitable. Preliminary results show the dissolved Cu content of Tjeukemeer to be about 20 μg.l⁻¹. This concentration appears to be high from an ecological point of view since values of this order of magnitude are reported to be toxic for algae. About half of the dissolved Cu could be bound by the cation-exchanger Chelex-100 and appeared to be labile Cu. More work is needed to prove the reliability of the Cu measurements. From the bioassay studies, particularly concerning toxic substances, the possibility of a rather high Cu content in Tjeukemeer water is quite evident.

Cu-Complexing Ability (CA)

Contrary to the previous two years a distinct max-

imum of CA of Tjeukemeer water in the period July–August was lacking. In June 1979 the algal biovolume dropped from 55 to 35 $\text{mm}^3 \cdot \text{l}^{-1}$. During comparable periods in 1977 and 1978 these maxima amounted to 75 and 100 $\text{mm}^3 \cdot \text{l}^{-1}$, respectively. In these years, the biovolume dropped significantly, namely, from 75 to 25 and from 100 to 25 $\text{mm}^3 \cdot \text{l}^{-1}$. It may, therefore, be deduced that even the absence of a distinct maximum for CA in the period July–August 1979 points at the relationship between the periodicity of the blue-green algae and the CA in the Tjeukemeer.

During the three years the CA was measured, the values for Tjeukemeer were consistently higher than those of the water from the polders. This is true even after a correction for the fulvic acids, present in both types of water, is applied. The algal populations in polder water never reach levels as high as in Tjeukemeer. This also suggested a connection between algae and metal-binding ability in our fresh water systems.

2.1.5. Laboratory studies

Determination of ammonia

The specificity of the salicylate method to determine ammonia in peaty waters was compared to the methods using, respectively, glutamate dehydrogenase and KBr/NaOCl.

Samples were neutralized, filtered (0.2 μm), passed through an Al_2O_3 column, and, in some cases, distilled at alkaline pH. In the latter case the three methods gave similar results. The highest content of ammonia was measured after alkaline distillation without passing through Al_2O_3 . Fortnightly data of one year confirm that the organic material, that yields ammonia using the distillation procedure, adsorbs partly onto Al_2O_3 and is thus present in the effluent as well. This latter (organic) fraction, at least for the greater part, is not coloured with salicylate.

The values measured with the KBr/NaOCl method may be somewhat higher than those with the salicylate method since, in addition to ammonia, hydroxylamine (literature data) seems to be determined with the KBr/NaOCl method.

Because of the relatively lower sensitivity of enzymatic determination of ammonia, comparison of this technique with that of the salicylate method is restricted to data in which the ammonia concentrations were high. The salicylate method does seem to have high specificity for ammonia in case of peaty waters. Studies will be continued.

Determination of nitrate

It was found that pre-heating (3 min. at 50°C) of zinc powder at alkaline pH in the presence of NaCl and

EDTA increases the sensitivity three-fold by lowering the blank. However, this also influenced the reduction capacity of the zinc powder, resulting in values about 85% of the controls without pre-treatment. Reports concerning the determination of nitrate both as nitrite (zinc batch method) and as ammonia (zinc column method) are in preparation.

Determination of organic nitrogen

The application of the persulphate method has been somewhat retarded due to troubles with the buffer added to avoid ammonia evaporation which could occur during filtration.

Determination of manganese

To determine manganese concentrations the method of Strickland and Parsons was chosen. According to this method Mn^{2+} ions are oxidized to MnO_4^- by KIO_4 in a sodium acetate buffer. The KMnO_4 is then used to oxidize leucomalachite green and the absorbance measured at about 620 nm. The NaCl content influences the final absorbance and since Tjeukemeer water has varying NaCl concentrations sufficient NaCl is added to eliminate the effect of NaCl in the samples. Thus, in addition, a lower blank can be obtained. It was found that lowering the concentration of leucomalachite green prevents the occurrence of a final turbidity.

To determine $\text{Tot-Mn}_{\text{diss}}$ an oxidative pre-treatment is necessary. Although persulphate or periodate degrade humic substances completely, apparently side-reactions occur which interfere with the determination of manganese.

Determination of amino acids

From previous work it is known that the ninhydrin method to determine amino acids in Tjeukemeer water gives 10 times higher values than the fluorometric method with fluram. Attempts to elucidate, namely Mn and Fe as factors responsible for the different results of both methods to date failed since the cation exchanger Chelex-100 used to remove metals, appears to release ninhydrin positive material.

Automation of chemical analysis

For many years the nutrients were analysed by manual techniques. It is expected that for the current and future research projects there will be a gradual increase both in frequency and numbers of the nutrient analyses. The manual analyses are time consuming and thus limit the pace of progress in research. It is, therefore, desirable to automate the analytical methods; for this purpose we are now applying a

Technicon Auto Analyzer II 'continuous flow system'. Further benefits of this system are: less use of chemicals, and higher sensitivity and better reproducibility. At present we can analyse P, Fe and Si partly automatically and two of these simultaneously. The classical and the automatic analyses of P, Fe and Si are being compared. For the Si analyses there is very good agreement between both the methods, so we have decided to determine Si on the AA II system (50 analyses h⁻¹). For the other two nutrients the agreement is less good. Research will be continued to solve this problem.

To optimize automation, it is necessary to simplify and standardize the pre-treatment of the samples. Destruction and hydrolysis problems can possibly be solved by a Block Digestor (uniform heating of samples, shorter digestion time and less sample transfer). The filtration step may be replaced by a dialysis step.

Curie-Point Pyrolysis-Mass Spectrometry (CPy-MS)

The content and the molecular weight distribution of fulvic acids in Tjeukemeer change seasonally. It has now been established by CPy-MS that the composition of fulvic acids changes seasonally too. These changes, as shown particularly by the pyrograms of the high molecular weight fulvic acids, can be explained in terms of fragment molecules attributable to polysaccharides, proteins and/or phenolic polymers. Whether the seasonal changes of the content and the molecular weight of fulvic acids are causally related or not needs further study.

Ultrafiltration (UF) experiments

The molecular weight distribution of the dissolved (<0.2 µm) organic matter of the Tjeukemeer, being mainly fulvic acids, has been studied by ultrafiltration and the results compared with those of Sephadex-G25 Gel filtration (SF). The average results of both methods are listed in Table 3. While SF only gives two

factors with a different molecular weight range, UF offers the possibility of obtaining more distinct fractions due to the extended choice of membranes with different pore sizes. Even though both the methods involve the same separation mechanism, i.e. molecular sieving, the results differ. According to UF the majority of fulvic acids has a molecular weight 2-3 times higher than that according to SF. Since the pore sizes both of the membranes and of Sephadex-G25 have been calibrated with globular solutes, it is difficult to explain the differences in the results in case of fulvic acids. It has been demonstrated by Sephadex Gel filtration and dialysis that the molecular weight of the fulvic acids is pH dependent. So far the preliminary results of UF experiments at pH 3.0 and 7.0 did not seem to be very pH dependent. Compared with SF, UF experiments showed very small shifts towards lower molecular weight ranges. This may be due to the possession of a fractionation range of Sephadex which the membranes do not have.

Effect of Cu on the primary production of natural algal populations in the Tjeukemeer

The effect of Cu on the primary production of phytoplankton in Tjeukemeer (June-July), measured by the Winkler method, appears to be light dependent (See Fig. 4). Cu has hardly any effect at low light intensities (<10 µE m⁻².s⁻¹), also at increasing light intensities Cu does not seem to inhibit production even at Cu concentrations exceeding 0.5 mg.l⁻¹. The high level of concentration (200 µg.l⁻¹) at which Cu appears to be maximally toxic may be connected with the relatively high Cu binding capacity of the Tjeukemeer, the average being 490 µg Cu l⁻¹.

Also the form of the Cu binding curves at high light intensity may be due to Cu binding (i.e. detoxifying) substances either already present in the water (See also 2.1.3. Bioassay experiments and 2.1.4. General chemistry) or due to excretion by photosynthesizing algae.

Continuous Cultures

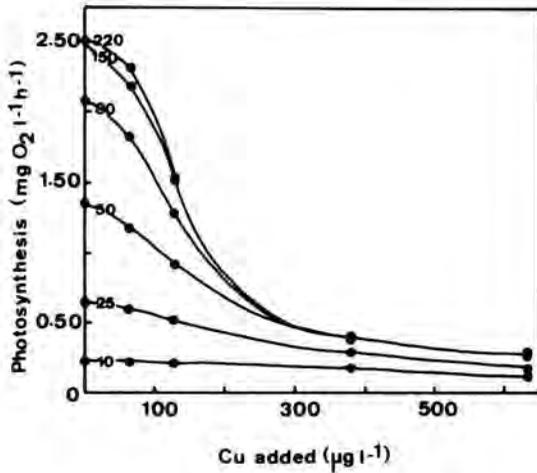
The growth limiting effect of Fe on *Scenedesmus quadricauda* and *Oscillatoria limnetica* in medium MD26 was studied by gradually lowering the Fe content in the chemostats (D=0.3 d⁻¹). The biomass and chlorophyll of the two organisms did not respond in the same way to the amount of Fe in the medium. *O. limnetica* appeared to become Fe limited at a four times higher Fe concentration in MD26 than *S. quadricauda*. In other words, the eucaryotic *Scenedesmus* cell has a much more efficient Fe uptake system than the procaryotic *Oscillatoria* cell.

The atomic Fe:N ratio in MD26 at which Fe became growth limiting was estimated to be 0.006:1 for *S.*

Table 3. Fractionations of organic matter of Tjeukemeer by ultra(UF) and sephadex 9-25 gel (SF) filtrations at pH 7.0.

Molecular Weight Range	Percentage of Total Organic Matter	
	UF	SF
> 30,000		10
30,000 - 300,000		0
20,000 - 30,000		35
10,000 - 20,000		35
2,000 - 10,000		10
<2,000		10
	5,000 - 10,000	75
	500 - 5,000	25

Fig. 4. Effect of copper on the photosynthesis of phytoplankton in Tjeukemeer water at different light intensities ranging from 10 to 220 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.



quadricauda and 0.023:1 for *O. limnetica*. These ratios may offer a tool to examine whether both the organisms grow under Fe limiting conditions in the lake. The lowest Fe:N ratio recorded in the lake in 1978 was 0.034:1. This means no Fe limitation. However, since for the calculation of this ratio total Fe and N data of the lake were used, undoubtedly, the Fe data overestimated the biologically available Fe. This emphasizes the urgent need for a separation method for algae, bacteria and other, non-living, suspended particles in the wind-exposed Tjeukemeer.

To reach light saturation during Fe limited growth in MD26, *S. quadricauda* and *O. limnetica* need light intensities of, respectively, c. 600 and 30 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. However, at growth rates below 0.2 d^{-1} in the case of *O. limnetica*, light appears to be limited due to the high turbidity of the culture. When the light intensity was increased to 60 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the culture became again Fe limited (Fig. 5). An X:D curve of *O. limnetica* at two different light saturated levels (30 and 60 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) indicates that the Fe-limited growth of *O. limnetica* depends on the level of light saturation. If this holds in general for nutrient limited algae, the data from nutrient-limited algal cultures grown at one light saturated intensity would not be conclusive.

The amount of EDTA, i.e. the complexing ability in MD26, drastically affects the pigment synthesis of *Scenedesmus* cells grown at Fe limitation. At 0.5 $\text{mg} \cdot \text{l}^{-1}$ of EDTA the chlorophyll content is c. 2 $\mu\text{g} \cdot \text{mg}^{-1}$, but at 2 $\text{mg} \cdot \text{l}^{-1}$ of EDTA, c. 8 $\mu\text{g} \cdot \text{mg}^{-1}$ of chlorophyll is synthesized. Further increase in the concentration of EDTA causes a rapid

drop in the chlorophyll content. Apparently, there is a maximal amount of complexing ability needed in MD26 to reach an optimal pigment synthesis in Fe-limited growing *Scenedesmus* cells. These results strongly suggest that not only the quantity of Fe but also its physical-chemical form affects the metabolism and growth of Fe limited *Scenedesmus* cells.

Multi-species competition experiment

Since 1972 *Oscillatoria agardhii* has become dominant over the other blue-green algae during summers in the Tjeukemeer (See Fig. 2). In a preliminary simulation experiment Tjeukemeer phytoplankton was grown in a continuous culture ($D = 0.125 \text{ d}^{-1}$). Tjeukemeer water was used as inoculum. In the medium Si and inorganic N were omitted and glutamine added as N source. Other culture conditions were; $t = 18^\circ\text{C}$; a light-dark

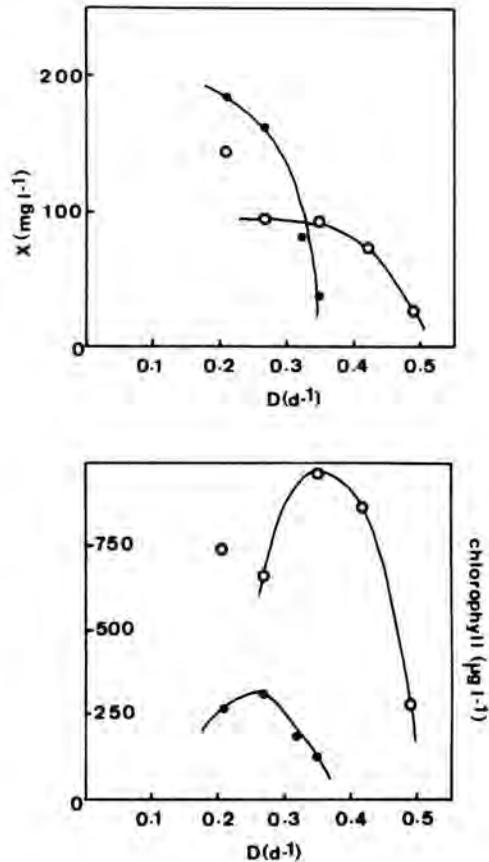


Fig. 5. Biomass and content of chlorophyll of *Oscillatoria limnetica* at $20 \pm 1^\circ\text{C}$ in an iron-limited chemostat at different dilution rates at two different light saturations: unshaded circles, 30 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and shaded circles, 60 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Table 4. Changes in algal composition (species as %) and optical density (OD) in a multi-species competition experiment after the indicated number of days (d)

Algae	start	7d	13d	20d	41d	50d	85d
<i>O. agardhii</i>	82	47.5	16	3	1.5	0.5	-
<i>O. redekei</i>	6	20	10	5	45	26	5
<i>O. redekei</i> <i>O. limnetica</i> ?	-	-	-	2	30.5	61	16
Lyngbya-like	4	12	6	4	18	7	1.5
<i>A. flos-aquae</i> (Ø 3-5 µm)	7	20	67.5	85	4	3	0.5
<i>A. flos-aquae</i> (Ø 2-3 µm)	-	-	-	-	-	-	0.5
Green algae + diatoms	1	0.5	0.5	0.5	1	1	1
<i>Chlamydomonas</i> sp.	-	-	-	-	-	1.5	75
rest	-	-	-	0.5	-	-	0.5
O.D. $\frac{5\text{cm}}{720}$	0.34	0.19	0.29	1.10	0.29	0.70	0.60

rhythm of 12 hours; and light intensity of c. $80 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ at the front of the vessel.

Some results are summarized in Table 4. Under the conditions applied the growth of diatoms and green algae was greatly limited, nor were these conditions favourable for the growth of *O. agardhii*. During the early phase of growth (0–20 days) numbers of *O. agardhii* had already decreased drastically, viz, up to 3%, but in case of *Aphanizomenon flos-aquae* the numbers were 85%, showing heterocysts within 7 days and finally akinetes. Thereafter, *A. flos-aquae* lost its dominant position (21–40 days) and threads looking like those of *O. limnetica* took over.

On the basis of the observed morphological features a separate investigation has been started to study possible similarities of *O. redekei* and *O. limnetica*. Both algae are now considered as distinct species and belong to the abundant blue-green algae in the Tjeukemeer. In the period from 50 up to 75 days the $E_{720 \text{ nm}}^{5 \text{ cm}}$ decreased slightly, i.e. from 0.7 to 0.6, and a population probably of *Chlamydomonas* sp. developed very well. Although the experiment did not fulfil the primary objective, useful information was obtained.

Cultivation of diatoms

For optimal growth of *Asterionella formosa* and *Diatoma elongatum*, and to prevent the growth of the

latter species against the walls of the culture vessels, the influence of the pH and EDTA is being investigated.

2.1.6. Published articles

Seasonal variations of fulvic acids, amino acids and sugars in Tjeukemeer, The Netherlands.

Haan, H. de and T. de Boer, Arch. Hydrobiol. 25: 30–40.

The summary was reported in the Progress Report 1977.

Curie point pyrolysis mass spectrometry of fulvic acids from Teukemeer, The Netherlands.

Haan, H. de, T. de Boer and G. Halma, Freshwat. Biol. 9: 315–317.

The summary was reported in the Progress Report 1978.

Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectrophotometric measurements.

Moed, J.R. and G.M. Hallegraef, Int. Revue ges. Hydrobiol. 63: 787–800.

The summary was reported in the Progress Report 1978.

2.1.7. Reports

Verkennde studie van het lichtklimaat in het

Tjeukemeer (Preliminary study of the light conditions of Tjeukemeer). Vroom, M., Trainee Report (in Dutch).

Limnologisch Instituut, Tjeukemeer laboratorium, Oosterzee, The Netherlands.

A laboratory model was designed to study the light climate in the Tjeukemeer. Apparatus used consisted of an ISCO spectroradiometer, model SR, a Kipp recorder, model micrograph BDS, and a black painted perspex tube with transparent lids. The perspex tube was part of a system through which Tjeukemeer water was circulated. A Philips MLR lamp (160 W) and the solar irradiation were used as light sources. Transmission spectra were obtained from the ratio of the transmission observed after passage through Tjeukemeer water and air.

The transmission observed at the blue side of the spectrum was the lowest. Scanning from 475 nm towards 550 nm the transmission increased gradually. In the region 550–670 nm increase observed was small. Around 675 nm most often a slight inflection was observed. Beyond this point the transmission increased again until 700–710 nm at which region a maximum was reached. From about 710 to 750 nm the transmission decreased.

Evidence is shown that the particles more than the humic acids contribute to the great reduction of transmission in the blue region. The rather slight increase of transmission in the region 550–675 nm probably is due to particles, whereas the inflection around 675 nm is likely to be caused by the light absorption resulting from chlorophyll-a in algae. It seems that the water component itself reduces the transmission beyond 710 nm.

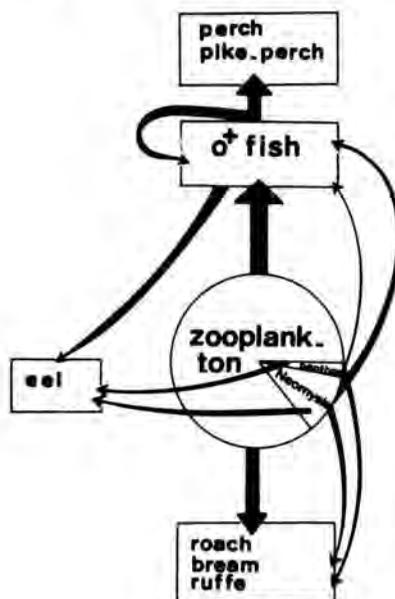
2.2. WORKING GROUP 'FOODCHAIN AND PRODUCTION STUDIES'

(J. Vijverberg, W.L.T. van Densen, E.H.R.R. Lammen, H.W. de Nie, E van Biemen, P.J. Mac Gillavry, Th. H. Frank, A.G. Frank-Landman and A. Ypma.)

2.2.1. Introduction

The working group is engaged in a study of the interrelations between the fish populations and their food organisms (See Fig. 6). Population structure, population density, fecundity, mortality, growth, standing crop biomass and production are estimated for both the fish species and the most important fish-food species. The latter include zooplankton (copepods and cladocerans) and macrofauna elements as chironomids, gammarids and the opossum shrimp, *Neomysis integer*. Much work is directed to the ecology of 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

Fig. 6. Field of research of the working group 'Food-chain and production studies' (1978–1982).



describe this subsystem and to get more insight in its dynamics.

The work is being carried out in phases. This year most work was done on the I^+ and older fish. The eel was included in the study for the first time. A detailed study was started on the predation of pike-perch on O^+ fish and on the competition between eel and bream for chironomids, and bream and O^+ fish for zooplankton.

2.2.2. Studies on zooplankton and macrofauna

The aim of this study is to calculate the weekly production of species of copepods, cladocerans, chironomids and *Neomysis integer*. The method used is based on the species population dynamics. Development rates of micro- and macro-crustaceans are assessed in the laboratory. Population densities, population structure and fecundity are determined by analyzing the lake samples. During 1979 special attention was given to *Daphnia hyalina*, *Leptodora kindtii* and *Neomysis integer*.

The inshore zone was sampled for chironomids. Seven taxa were observed regularly: *Chironomus*, *Cryptochironomus*, *Stictochironomus*, *Glyptotendipes*, *Pentapedilum*, *Polypedilum* and *Tanytarsus*. The *Chironomus* species is large (individual weight of 4th instar c. 40 mg fresh wt.), *Tanytarsus* and *Pentapedilum* are small taxa with an individual weight of

c. 0.5 mg. The other named taxa are of medium size, their individual weights vary between 2 and 6 mg fresh weight. Although in the sand the small taxa were dominant by numbers, the biomass was dominated by medium-sized taxa, namely, *Glyptotendipes* and *Stictochironomus*. *Chironomus* dominated the biomass in the mud. The annual mean biomass production in the inshore zone was c. 150 kg fresh wt. ha⁻¹; this is similar to that estimated earlier by Beattie. The annual mean production for the whole lake may be in the order of magnitude of 30 kg fresh wt. ha⁻¹.

The reed beds were sampled for *Asellus* and *Gammarus* during spring and autumn. Compared with the data of 1969 and 1970 by Chambers, population densities of these species now are very low.

The population densities of the opossum shrimp, *Neomysis integer*, were extremely low. The highest density recorded was 0.1 ind. m⁻² in October. The net annual production was c. 0.01 kg fresh wt. ha⁻¹, this is two to three orders of magnitude lower than the estimates of one of the preceding years.

Copepods and cladocerans were sampled regularly at five permanent stations in the lake. Thirteen species together represent 98% of the individuals encountered. The population densities of these species in 1979 generally differed from most other years since this work was started in 1968. Densities of *Acanthocyclops robustus* and *Cyclops v. vicinus* were relatively high, but those of *Mesocyclops leuckarti* and *Eurytemora affinis* were low. The densities of smaller cladoceran species such as *Bosmina coregoni*, *B. longirostris*, *Chydorus sphaericus* and *Ceriodaphnia pulchella* were exceptionally low, in contrast with the relatively high densities of the large *Daphnia hyalina*. Densities of the large predator *Leptodora kindtii* were only relatively high during early summer, but those in the second part of the growing season were relatively low. The annual net copepod production was c. 200 kg fresh wt. ha⁻¹, and that of the cladocerans c. 2000 kg fresh wt. ha⁻¹.

The vertical distribution, population structure and fecundity of *Leptodora* were studied. The vertical distribution was studied during the day at two depths and during two diurnal observations. The vertical migration behaviour differs with size. Most small individuals (2–5 mm) stay during day-time near the surface. At sunset they start to migrate downwards and during the night most of them concentrate at a medium depth. The large individuals (≥7.5 mm) are at day-time near the bottom. At sunset they ascend to the surface and at night most of them concentrate in the surface layer. The medium sized individuals (5.5–7 mm) do not migrate and are present in both surface and bottom layers; however, their densities near the surface are c. 50% higher than those near the

bottom. Thus, when the population consists of relatively many small and medium-sized individuals, at day-time most *Leptodora* are found near the surface. But when the large size class dominates the population, at day-time most individuals are found near the bottom. The number of eggs per female and the percentage of adult females with broods were recorded. The percentage of diapause eggs increased progressively from 3% during early August, to 50% in September and to 70–100% in October. Males appeared first during August and their frequencies increased during September and October. In October c. 50% of the population consisted of males.

Instar durations of two *Daphnia* spp. and four copepod spp. were assessed in laboratory cultures using lake water as food medium. *D. cucullata* developed through three juvenile moults and *D. hyalina* through four. The duration time of the juvenile instars is c. 50% of that of the adult instars at the same temperature. Growth in length was relatively slow in *D. cucullata*. The copepods developed through five juvenile copepodite instars. In *Eurytemora affinis* the duration times of juvenile instars were similar, while in *Acanthocyclops robustus*, *Mesocyclops leuckarti* and *Cyclops v. vicinus* the duration of the 5th instar lasted significantly longer.

The work on modelling the population dynamics and production of copepods and cladocerans, in cooperation with the Department of Bio-Informatica of the State University of Utrecht, was continued. A population dynamics simulation model based on the life cycle of *Daphnia hyalina* was developed. The egg development, birth of young and the discontinuous growth of adults and juveniles are important life cycle phenomena which the model accounts for.

2.2.3. Studies on fish

Young-of-the-year fish (O⁺)

The sampling programme for O⁺ fish started at the beginning of April and included, besides the Tjeukemeer, a transect from the lake to the water-inlet from the IJsselmeer near Lemmer. Because of the absence of older smelt in the Tjeukemeer the O⁺ smelt-production appeared in 1979 to be dependent upon the inlet of larvae from the IJsselmeer. Because of intensive precipitation in spring it was not before 13 May that the sluices were opened and smelt-larvae spread over the lakes. The Tjeukemeer received the lowest numbers due to a constant water-inlet from the hinterland. The numbers of pike-perch and perch larvae which originated from the older pike-perch and perch in the lake were extremely low; this holds also for the recruitment of later spawning roach and bream. This, together with the heavy predation

pressure by older pike-perch, resulted for the third consecutive year in a poor recruitment for all species at the end of the season.

The production of O⁺ fish in the Tjeukemeer was not more than 5 kg fresh wt. ha⁻¹. In the eight other Frisian lakes, sampled during the summer of 1979, it was about 100 kg fresh wt. ha⁻¹. This is because of a better inlet of smelt larvae in some lakes and better recruitment of pike-perch in the other lakes. Except for the smelt, the lakes differed remarkably with regard to growth of species, the Bergumermeer being the most favourable for all species. The studies on the daily ration of O⁺ fish including the laboratory experiments on the turnover-time of stomach contents were finished. Oxygen-consumption for pike-perch was also measured; minimum daily rations based on Winberg's balanced equation appeared to be higher than those based on the mean stomach contents and turnover-times.

Older fish

Older perch and pike-perch were sampled by means of a trawl and using different mesh-sized multi- and mono-filament gill-nets. Net efficiency for pike-perch appeared to be highest for multi-filament gill-nets (210/3). Comparison of catches of pike-perch in 48 and 51 mm (half mesh) gill-nets, according to the Holt-method, revealed a proportionality-factor (k) between mesh-size (m in mm) and optimal catchable pike-perch (l₀ in cm fork-length) according to l₀ = k · m of 0.93. The pike-perch population is dominated still by the strong year-classes of 1975 and 1976, and characterized by poor recruitment since then (See Fig. 7). The growth of the pike-perch during 1979 in the Tjeukemeer was very poor because of, as men-

tioned earlier, poor food supply and low water temperatures. A remarkable switch in feeding during the season from O⁺ smelt to O⁺ roach, instead of the more available O⁺ bream in the open-water zone, was observed.

Standing crop of O⁺ fish was high in Grote Brekken and low in Tjeukemeer indicating a better feeding condition for the older pike-perch in the Grote Brekken, which was also clear from their higher somatic weight. In the absence of commercial fishing on the older pike-perch the possibility of reduced predation-pressure on the O⁺ fish and thus for good recruitment in the near future does not exist.

During 1979 research was started on the populations of one year and older bream, silver bream and roach. The fish were sampled monthly with a trawl at five places. The total catch per sampling was 300–500 fishes. Depending on the season, weight, gonad weight, age and sex-ratio were determined, whereas length-frequency of the catch was determined on all occasions. Only the data on bream have been analyzed. The yearclass 1975 was the most abundant comprising 50 per cent of total numbers and had grown from 21.5 to 24.5 cm (See Fig. 8). Of this yearclass 50 per cent of the males and 5 per cent of the females became sexually mature, while the sex-ratio was 1:1. The other 50 per cent was dominated by the yearclasses 1970 and 1973; these had grown, respectively, from 32.5 to 34.0 cm and from 32.5 to 34.0 cm. They were all sexually mature and had a sex-ratio of 1:1. Bream spawned from the second half of May till half June, the larger fish (>30 cm) in May and the smaller ones in June. The silver bream spawned in the second half of June and the roach in the second half of May. The somatic growth of bream was in July and August, the gonadic growth in September and October. The condition parallels this process: it is at a minimum in June, constant in July and August and at a maximum in September and October. From June till September the stomach contents of bream, silver bream and roach were sampled and those of the bream analysed. In July and August, in particular, when there was little zooplankton available, the most important food item was chironomids; in June and September zooplankton, especially *Daphnia*, dominated the fish food. Based on the energy requirements of the bream population, starting from Winberg's balanced equation, this means a consumption of approximately 1000 kg per ha zooplankton and 500 kg per ha chironomids.

In 1979 also a study on the eel in the Tjeukemeer was started. The lake was sampled fortnightly between May and October. In total 2039 eels were caught during the night by means of a narrow meshed trawl, and fishing during the day yielded 600 fish. An intensive study of the diet of more than 900 eels revealed the

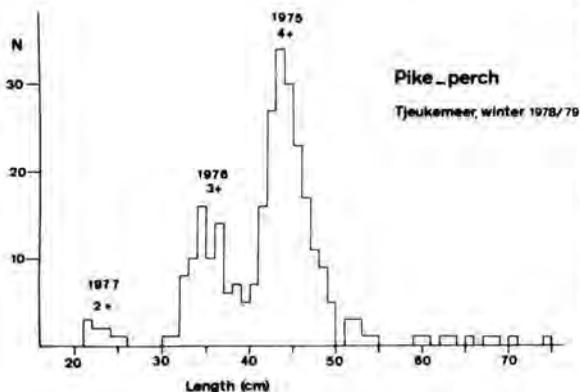
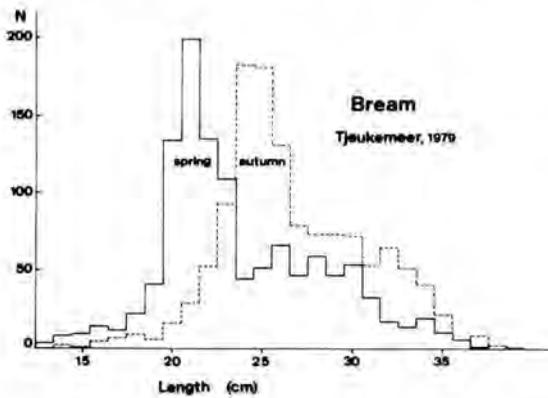


Fig. 7. Population structure of pike-perch (*Stizostedion lucioperca*) in the Tjeukemeer (1979).

Fig. 8. Population structure of bream (*Abramis brama*) in the Tjeukemeer (1979).



quantitative importance of the pupae and larvae of chironomids and the molluscs *Anodonta* and *Unio* as food organisms. The Fulton's condition index for the whole sample varied between 0.140 in May and 0.190 in August. The length classes between 24 and 28 cm dominated the population. The females comprised 12 per cent of the population of individuals longer than 28 cm. The work on growth and age using otoliths will be continued. Eel and bream shared the middle-sized chironomid larvae as an important part of their diets.

2.2.4. Articles in press

Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, The Netherlands.

Vijverberg, J., *Freshwat. Biol.* (1980) 10: 317–340. The summary was reported in Progress Report 1978.

Distribution patterns of zooplankton in Tjeukemeer, The Netherlands.

Nie, H.W. de, H.J. Bromley and J. Vijverberg, *J. Plankton Res.*

The vertical and horizontal distribution of Copepoda and Cladocera in Tjeukemeer were studied. Indices of dispersion are discussed, the sample variance of logtransformed data proved to be an appropriate way to quantify patchiness. Indices of dispersion were calculated to express species specific differences. Only during short periods of the year the population densities appeared to be influenced by water brought to the lake by canals and pumping stations. Dispersion values were influenced by wind-speed, inhomogeneities in the chlorophyll-a concentrations and by the population densities. Sample variances are relatively low, compared with those of other lakes.

2.2.5. Published articles

A modification of the Ekman–Birge bottom sampler for heavy duty.

Beattie, D.M., *Freshwat. Biol.* 9: 181–182.

The summary was reported in Progress Report 1978.

The fecundity of the smelt, *Osmerus eperlanus* L., in the Tjeukemeer (abstract).

Densen, W.L.T. van, *Neth. J. Zool.* 29: 623.

The population density, growth rate and production of roach *Rutilus rutilus* (L.) in Tjeukemeer, The Netherlands.

Goldspink, C.R., *J. Fish. Biol.* 15: 473–498.

A method of estimating the population density of roach in Tjeukemeer (21.3 km²) using 20,529 introduced fin-clipped fish is described. Fyke nets proved to be an effective method of sampling the population for marked fish during the spawning season. A total of 20,277 roach were processed during the recapturing period. The population density of roach (>14 cm was estimated to be 1,246,458. The growth rate of roach in the lake, although relatively poor (von Bertalanffy's L_{∞} for males and females, 22 and 26 cm respectively) for the species was similar to that recorded in three other Frisian lakes. The mortality rate of males was higher than that of females. Logarithmic length-weight regression analyses showed that the value of the coefficient varied both within and between the sexes, that of females being higher (range 3.038–3.375) than that of males (range 2.760–3.254). Seasonal changes in the size of the coefficient were due to a disproportionate loss of gonad weight in larger fish. The fecundity of the population was comparatively high for the species.

The total production of the population was estimated to be 95 kg. ha⁻¹ of which 39 kg. ha⁻¹ was contributed by fry. In older fish (>IV) the production of females (12 kg. ha⁻¹) exceeded that (2 kg. ha⁻¹) of males, due to differences in their growth and mortality rates. The relatively poor performance of roach in Tjeukemeer, in terms of biomass and production, was due to a scarcity of zoobenthos and competition from other species of fish. There is no evidence either from this study or the literature that the productive potential of roach in lakes is high, even though macrophytes and detritus can be consumed in significant amounts.

2.2.6. Reports

Productie en oecologie van zoetwatervis (Production and ecology of freshwater fish). (in Dutch) Densen, W.L.T. van, Internal Report, Section General Fish Culture and Fisheries, Agricultural University Wageningen, Wageningen, The Netherlands.

These lectures concern both the holistic and the analytic approach to freshwater fish production. The holistic approach is clarified by the sigmoid biomass

growth according to Graham (1935) and the morphoedaphic index. The analytic approach comprises besides stock-characteristics as abundance, population structure, individual size of members and fecundity, production and mortality and the speeds with which the stock changes as a function of time. The impact of fish on the stock of its food organisms, especially zooplankton, is given special attention. The theoretical frame-work is supplemented with the discussion of critically selected articles from the literature.

De oecologie van de O⁺ blankvoorn, *Rutilus rutilus* L. in het Tjeukemeer (Ecology of O⁺ roach, *Rutilus rutilus* L. in Tjeukemeer).

Schaick Zillesen, P.G. van, Student Report no. 1979-6 (in Dutch), Limnological Institute, Tjeukemeer Laboratory, Oosterzee, The Netherlands.

The ecology of roach of the age group O⁺ in the littoral zone of lake Tjeukemeer was studied. Growth, biomass, production and mortality of O⁺ group roach during the years 1976–1978 and food intake and selection during 1978 were estimated. Year class strength of O⁺ roach was correlated with environmental factors during the first year of growth. The growth in the years 1978 and 1977 was markedly less than that in 1976, while in 1976 a very low biomass, production and mortality were estimated. This may be due to unsuccessful spawning or high larval mortality in the beginning of May 1976 because of the very low water temperature. The production of O⁺ group roach was high in 1977 (376 kg.ha⁻¹) and low in 1976 (20 kg.ha⁻¹).

Smaller species of cladocerans were relatively eaten most and among the food-organisms, the largest individuals were prevalent. Predation on *Leptodora kindtii* during the night was remarkable. The food-intake of the population of O⁺ roach was estimated to be 432 Kcal.ha⁻¹, 96% of this had occurred by 6 August 1978 since the population declined sharply thereafter.

There is a positive correlation between temperatures of the water during the first year of growth and year class strength.

De bouw van de kop van O⁺ baars en O⁺ snoekbaars in relatie tot hun voedselopname, een morfologisch en ethologisch onderzoek (The construction of the head of O⁺ perch and pike-perch in relation to their feeding, a morphological and ethological study). Graaf, G.J. de, Student Report 1979-8 (in Dutch) Limnological Institute, Tjeukemeer Laboratory, Oosterzee, The Netherlands.

Previous field studies showed negative size selective feeding on copepods and cladocerans by very young O⁺ perch and pike-perch and positive size selective feeding by older and larger O⁺ fish. The purpose of this study was to explain this phenomenon. The func-

tional morphology of the fish head was studied in relation to fish size. Two factors probably limit the size of food particles which small O⁺ fish can ingest, i.e. the volume of the buccal cavity and power of suction.

De selectiviteit van staande netten voor snoekbaars, *Stizostedion lucioperca* (Selectivity of gill-nets for pike-perch, *Stizostedion lucioperca*). Schouten, R., Trainee Report no. LH stage 79-1 (in Dutch), Report of a training period carried out at the Limnological Institute, Oosterzee, for the Section General Fish Culture and Fisheries of the Agricultural University, Wageningen, The Netherlands.

Catches of pike-perch in 48 and 51 mm gill-nets were compared according to the method of Holt (1963) to reveal the optimum catchable fish-length for a given mesh-size and the standard deviation in the normal distribution describing the selectivity-curve.

Waarnemingen aan de voedselsamenstelling van O⁺ baars, *Perca fluviatilis*, snoekbaars, *Lucioperca lucioperca* en spiering, *Osmerus eperlanus*, gedurende 1978 in het Tjeukemeer (Observations on the food composition of O⁺ perch, *Perca fluviatilis*, pike-perch, *Lucioperca lucioperca* and smelt, *Osmerus eperlanus*, during 1978 in Tjeukemeer). Mesa-Menor, J., Trainee Report (in Dutch), Limnological Institute, Tjeukemeer Laboratory, Oosterzee, The Netherlands.

During the growing season of 1978 *Daphnia hyalina*, *Bosmina coregoni*, *Leptodora kindtii* and cyclopoid copepods appeared to be the most important food-items for O⁺ smelt, perch and pike-perch. Only pike-perch predated on *Neomysis integer* and occasionally on fish. Intensive fish-predation by pike-perch was hampered by its slow growth as a consequence of low water temperatures.

Groei- en ontwikkelingsnelheid van de aasgarnaal *Neomysis integer* (Leach) in relatie tot temperatuur (Growth and development rate of the opossum shrimp *Neomysis integer* (Leach) in relation to temperature). Schrottenboer, G.J., International Report no. 1979-1 (in Dutch), Limnological Institute, Tjeukemeer Laboratory, Oosterzee, The Netherlands.

A method to culture *Neomysis integer* in the laboratory is described. Mortality-rates and growth were determined at several temperatures. Some preliminary results on instar duration times are given.

3. Vechten research in progress

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; max. depth, 11.9 m; mean depth, 6 m) has no surface in- and outflowing streams and is thermally stratified from c. May to early November. It has a well developed littoral region. During the stagnation period an anaerobic zone extends maximally to the 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 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 co-ordinated and integrated approach to limnological problems in Lake Vechten. This new approach forms the basis of the present studies in progress since 1976. In this regard, a three-pronged attack constitutes a simultaneous study of the processes controlling the production of organic matter by the autotrophs, its consumption by the herbivores and its mineralization by bacteria.

The cooperation of the three working groups is especially focussed on a study of the carbon budget of the lake. The cooperation is realized by pooling the information on the budget collected by the three groups. A newly started research is aimed at a study of the interaction between the littoral and limnetic regions of the lake. Dr. J. Dvorák studied the littoral and benthic macro-invertebrates of the lake.

3.1. PROJECT 'CARBONCYCLE'

3.1.1. 'Raai' studies

(C.L.M. Steenbergen, R.D. Gulati, W.A. de Kloet, M.J. Bär-Gilissen, G. Postema, K. Siewertsen and R. Bergsma.)

Introduction

The main object of the 'Raai (=transect) studies' is to examine the pattern of horizontal distribution of phyto- and zooplankton in Lake Vechten. Such a study is essential before generalizations about the flow of energy in the lake's ecosystem are possible. This is, particularly, so since the studies until recently were aimed at understanding the limnetic and the littoral

regions separately rather than at the interactions between the two areas. Moreover, the research on phytoplankton primary production and zooplankton grazing was confined to the pelagic water. Thus, the conclusions based only on the limnetic data may be premature. This may be especially so in a stratifying lake like Vechten, with a well developed littoral region which extends roughly up to the 5 m depth contour, and comprises about 38 per cent of the lake's total area. Further, on calm days, one may expect local differences, e.g. in the temperature and light regimes, and the rates of primary production and zooplankton grazing, within the littoral and limnetic regions. Besides, the macrophytic and periphytic vegetation, and the fauna inhabiting it, and benthos, would tend to accentuate the differences between the two areas.

Sampling stations and parameters

The lake was sampled at 8 stations, along a transect stretching from the littoral on the north-east (station 1, 2), through the limnetic (station 3-7), to the littoral on the south-west (station 8) in the eastern basin (Fig. 9). The lake was sampled between 10 and 12 hours, at 1.2-1.8 m depth stratum at all stations using a 5 l. Friedinger sampler. At stations 2 and 5, the strata between 2.6 and 3.2 m were also sampled.

The parameters measured are: water temperature, light, dissolved oxygen, and chlorophyll-a and phaeophytin at all stations, and *in situ* rates of primary production and zooplankton grazing, sestonic (phytoplankton and detritus) and zooplankton concentrations as carbon, and dissolved organic carbon (D.O.C.) at stations 2 and 5. Also measured were zooplankton densities at stations 2 and 5, including station 8 (since recently). Dominant genera of algae in the littoral (station 2) and limnetic region (station 5) were also recorded. Wind speed data were obtained from the Royal Nederlands Meteorological Institute (K.N.M.I.) at De Bilt which is about 5 km from the lake.



Fig. 9. Map of Lake Vechten showing depth contours (m) and position of sampling transect. The numbers along the transect show the location of sampling stations.

Biomass parameters

The mean ($N = 18$) chlorophyll estimates of station 5 were similar ($P < 0.05$) to the average concentration for the transect, and thus this station is a representative sampling site for phytoplankton. Moreover, depth integrals of chlorophyll from four limnetic stations also support this conclusion. One exception to the above generalization is station 8. The chlorophyll concentration at this station departed significantly from the random distribution ($P < 0.02$). This was due to higher concentrations observed at this station, particularly, during July and August when the weather was calm. Probably some local factors in the littoral region promoted an increased plankton growth.

The chlorophyll and sestonic carbon ratios were slightly higher for open water (station 2) than for the littoral region (station 5), because of higher carbon and lower chlorophyll concentration in the open water (Table 5). The linear regression correlation between chlorophyll-a and sestonic carbon, both at station 2 and 5, was significant ($N = 10$, $P < 0.01$). Taking the intercept (a) as a measure of detrital carbon, it appears that detrital carbon constitutes about 49% of the total sestonic carbon in littoral samples, in contrast with 37% in the limnetic water. It is likely that the relatively

higher detritus concentration thus calculated for the littoral region is due to resuspension of particulates from the bottom, especially in view of the shallowness and the methods of sampling (rowing boat).

Primary production and zooplankton grazing

The mean primary production rates of phytoplankton at stations 2 and 5 were similar (Table 6). The activity coefficients (production rate per unit chlorophyll) was, however, higher in the open water than in the littoral.

Both the densities (nos. l^{-1}) and biomass of zooplankton were relatively higher in the pelagic region (Table 6). This was reflected in the significant differences ($P < 0.05$) in the distribution of *Daphnia*, in particular, and total filterfeeders, in general. Only nauplii densities in the littoral somewhat exceeded those in the open water.

The zooplankton grazing and feeding rates in the littoral region were, respectively, 58 and 66% of those in the limnetic water. The specific consumption rates of zooplankton (daily ration) would thus be somewhat lower in the case of littoral grazers.

The preliminary conclusions are: primary production rates appear to be similar but differences cannot be ruled out since the comparisons are based on

Table 5. Mean chlorophyll-a and sestonic carbon concentrations ($mg.m^{-3}$) with 95% confidence limits of the standard error in parentheses and their relationship

Parameter	station			
	2		5	
a) Chlorophyll-a	6.5	(\pm 2.4)	6.2	(\pm 2.5)
b) Seston carbon	745	(\pm 188)	786	(\pm 264)
c) Ratio b:a	130	(\pm 34)	153	(\pm 62)

Table 6. Means with standard errors (95% confidence limits) of chlorophyll, primary production and zooplankton and their grazing at the littoral and pelagic stations.

stations	region		N
	littoral	pelagic	
Chlorophyll $mg.m^{-3}$	7.7 \pm 2.9	8.2 \pm 4.3	6
Primary production $mgCm^{-3}.4hr$	26 \pm 15	27 \pm 14	6
Zooplankton $mg Cm^{-3}$	87 \pm 48	114 \pm 44	10
Grazing $\% hr^{-1}$	0.21 \pm 0.18	0.36 \pm 0.27	10
Consumption $mg Cm^{-3}.hr^{-1}$	0.95 \pm 0.84	1.44 \pm 1.11	10
Zooplankton ind. m^{-3} (10^{-3})	118 \pm 64	154 \pm 71	10

measurements in one stratum (1.2–1.6 m) rather than over the entire water column. This is also true for the zooplankton grazing data. However, based on the data so far collected, some tentative corrections of the zooplankton grazing as measured in the limnetic water are attempted. This correction factor (CF) may be calculated as follows:

$$CF = \frac{A1}{At} \times \frac{F1}{F2} + \frac{A2}{At}$$

where A1 and A2 are, respectively, littoral and limnetic areas of the lake, and At is total area; F1 and F2 are the means of the measured feeding rates ($\text{mgC} \cdot \text{m}^{-3} \cdot \text{hr}^{-1}$), respectively, in the littoral and limnetic regions. Considering that the littoral area is about 38% of the total area (4.7 ha) and F1 and F2 are 0.95 and $1.44 \text{ mgC} \cdot \text{m}^{-3} \cdot \text{hr}^{-1}$, the correction factor of 0.87 when applied to the limnetic data would give approximate rates for the whole lake.

3.1.2. Macro-invertebrate studies

The study was done by Dr. J. Dvorák, Czechoslovakian Academy of Sciences, Prague. His stay was financed by the Beyerinck–Popping Foundation.

The present study was carried out to get an impression of the rôle of the macro-invertebrate communities in the lake.

In May–June, an important period for the development of the macrophytic communities, different habitats were investigated with respect to the macrofaunal biomass and its species composition. The following substrates were sampled by means of SCUBA-diving: a) three groups of aquatic macrophytes, notably, the emergent species *Phragmites australis*, *Sparganium erectum*, *Typha angustifolia*, the floating species *Polygonum amphibium*, the submerged species *Ceratophyllum demersum*, *Elodea* sp., *Myriophyllum verticillatum* and the macro-alga *Nitella micronata*; and b) sediment (24 cm cores collected at several depths, either originally occupied by plants or lying-bare. The animals were identified by their group or species name. As to the phytophilous invertebrates, main attention was paid to the most frequently occurring groups, namely, Hydrozoa, Turbellaria, Hirudinea, Oligochaeta, Mollusca, Crustacea and the water stages of insects (mostly Ephemeroptera, Odonata, Trichoptera and Diptera). Determination of some chironomids was made on the basis of images reared from the sampled larvae.

Quantitative estimates of the biomass represented by the different plant-associated communities were made using standing crop data of the related macrophytes. Values are expressed in ash-free dry

weight per m^2 of the bottom. Quantification of the bottom fauna was also done on m^2 basis.

In May, still overwintering generations of some animal groups were found, and the fauna consisted largely of chironomids (60–80%), followed by molluscs and other insects. The June-samples showed the development of summer generations of the animals, and an increase in the biomass of carnivores compared to herbivores, due to the appearance of damselfly nymphs and larvae of Polycentropidae. The macro-invertebrate communities on emergent, floating and submerged macrophytes exhibited different species structure. In May the communities of different species of the submerged vegetation showed more similarity than those of the same macrophytic species at different depths. In June the species structure of the fauna was probably more affected by the plant species than in the spring and in June the type of vegetation showed to be the important factor in forming the macrofaunal communities. The benthic fauna of the non-overgrown areas was poor compared to the fauna of the plants, or the plant-occupied bottom (only some chironomids, Chaoborus and oligochaetes were found).

After identification of animals the species and trophic structure of the macrofauna will be analyzed with respect to the ecological types of the vegetation. The biomass relation of the animals and host plants will be examined (in ash free dry matter, organic matter or caloric content).

3.2. WORKING GROUP 'PRIMARY AND SECONDARY PRODUCTION'

(R.D. Gulati, W.A. de Kloet, T.J.A. Vulto, G. Postema, K. Siewertsen.)

3.2.1. Introduction

The routine studies on phytoplankton primary production and zooplankton grazing and assimilation in Lake Vechten were wound up in April 1979. The data collected during the last seven years are being further processed. However, several aspects relating to the above studies did not receive adequate attention even though their importance in the energy flow phenomena of the lake may not be underestimated. Foremost among these are:

- reliability of primary production data as calculated from the production values during the 4-hour incubation and irradiance during this period and the day;
- zooplankton excretory products, and their availability to primary producers, as well as their role in the mineralization of organic matter;
- role of bacteria as zooplankton food;
- role of the larvae of *Chaoborus flavicans* in

regulating the population dynamics of zooplankton; and

e. exchange or interaction between the littoral and limnetic regions and its significance in the functioning of the ecosystem of the lake.

Preliminary studies on these aspects were commenced in the course of 1979. For the last named study see under 3.1. Besides these areas of research activity, Gulati participated in the 'Parakrama Limnology Project' in Sri Lanka during August–September 1979 (See under 4.1.).

3.2.2. Studies on primary production

The routine work was continued till April 1979 to complete a two year study on the chlorophyll and production of the two size fractions (3–33 and $>33 \mu\text{m } \varnothing$) of phytoplankton. The production rates in March 1979 i.e. soon after the 2-month ice period were unusually low for this period. The measured rates during the period, i.e. 52 and $56 \text{ mgC} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, are less than a third of those in the preceding years. The absence of an early spring peak may be attributed to the low temperature following freezing and thaw, and not to the light climate. The production rates steadily increased and the peak of $126 \text{ mgC} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ was rather late in spring (April end) than is usually the case. This trend, though less marked, was also reflected in the chlorophyll concentrations and thus in the production per unit chlorophyll. The production data of lake collected so far, particularly of 1977–1979, is being analyzed.

Diurnal changes and calculation of daily rates

The measurement of daily primary production is limited to a 4-hour *in situ* incubation period and the day values are obtained by multiplying the four hour values with a light factor which is a ratio between total irradiance on the day and during the exposure period. This method of estimating the daily production is widely accepted, unless, of course, hourly or exposure period rates are expressed. The major draw-backs with such estimates of the daily rates are: first, it is taken for granted that there are no changes in spectral composition of light during the course of the day or that such variations, if they exist, do not affect the rates of photosynthetic processes. In short, changes in production rates are assumed to be proportionate to those in light intensity. Secondly, in such conversions to daily rates, the possible effects of diurnal changes in factors other than those of light are completely ignored. Important among these factors are changes in the physiological state of algae, zooplankton grazing rates, nutrient availability or exhaustion, water temperature, sinking of algae, extracellular excretion

and shifts in light saturated depth (Z_{1K}) and P_{opt} .

Some preliminary studies in 1978 had revealed that calculation of daily production rate based on a single 4-hourly incubation during mid-day is far from satisfactory. The result thus obtained significantly differed, i.e. when, instead of conventional mid-day measurement, early morning or late afternoon data were used to arrive at the day figure. Moreover, when compared with the day figure obtained by integrating the data of all the incubation periods between sunrise and sunset, anomalies became more apparent. Mr. W. Opstelten (State University of Utrecht, Utrecht) carried out a study on the problem outlined above during June–November 1979. In this period he made nine series of diurnal measurements. Each series comprised five to seven 2-hourly production measurements during the day commencing shortly before sunrise and concluding after sunset. Chlorophyll concentration was measured simultaneously. Besides, water temperature, light penetration and Secchi disc disappearance depths were also recorded at least three times during the day. Though the data needs to be processed further it is clear that daily production estimates based on early morning and afternoon measurements were up to 100 per cent higher than those obtained by summing up the production during the two-hour exposure periods (Fig. 10). This difference decreased steadily in the course of the day and noon-time exposures when converted using the light factor, on the contrary, underestimated the daily integral production by about 10 to 20 per cent. The daily estimates based on late afternoon measurements were a replica of the early morning situation, namely, about hundred per cent higher than the 'real' production.

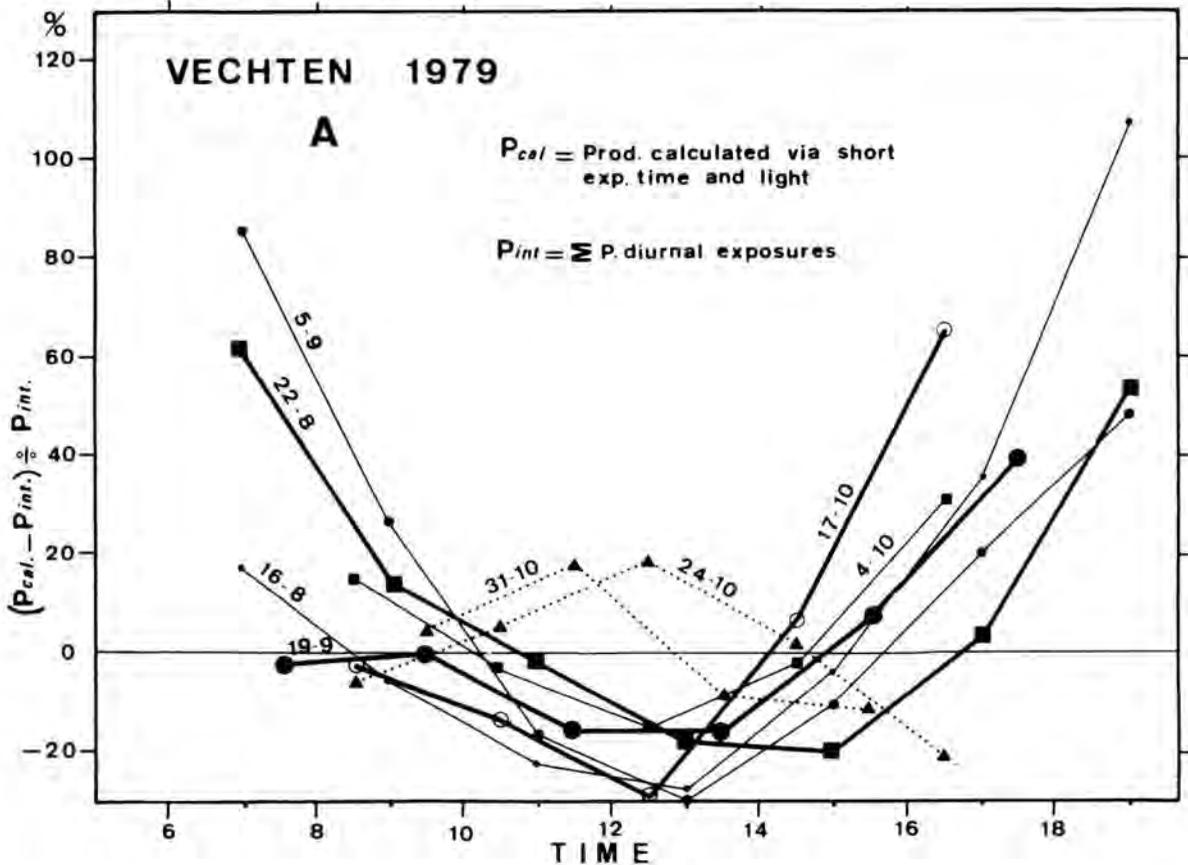
3.2.3. Studies on zooplankton excretion products and primary production

The grazing studies of zooplankton in the preceding years have repeatedly demonstrated that the annual maxima of zooplankton and their grazing in the period mid-May, early June coincide with a sharp fall in the phytoplankton concentration. However, despite this decrease, the primary production rates are either unaffected or even increase to exhibit a peak. In other words, the activity coefficient of phytoplankton, i.e. production rate per unit of chlorophyll, increases significantly. Both in 1977 and 1978 the activity coefficients were three to five times higher in May and June than those in the preceding or the following periods.

The increase in the zooplankton feeding rates results also in a concomitant increase in their egestion rates and increased transparency of the lake, namely, a 'clear phase' as evident from the maximum of Secchi disc disappearance depth (4–5 m) in this period. It is, therefore, likely that deeper light penetration, together

Fig. 10. Daily production rate calculated (P_{cal}) from the 2-hourly production measurements and the daily solar radiation as per cent of the integral production

(P_{int}) obtained by summing up the 2-hourly production measurements during the course of the day.



with increased nutrient availability — related to egestion, excretion and mineralization — stimulates photosynthetic rates, and thus the higher activity coefficients.

The main object of the study was to test if, indeed, there is an increase in nutrient supply related to zooplankton grazing, and to what extent these products play a role in enhancing production rates of phytoplankton. In this regard five preliminary experiments were conducted in the laboratory. Replicate lake water samples containing two to four times the *in situ* concentration of zooplankton were placed in conditioned cabinets. The light and water temperature conditions used were those prevailing in the lake. Parallel controls from which zooplankton was removed by filtration using a $105 \mu\text{m}$ (\varnothing) filter grid were also run. After a grazing period of 24 hours, zooplankton was filtered off and samples divided into sub-samples. In one series the primary production (C^{14} -technique) was measured soon after (one day), in the other series the filtrates were allowed to stand in

the conditioned cabinet for two days before measuring production (on the third day). The production in the parallel controls, i.e. in samples devoid of zooplankton, was measured both at the start of the experiment, and after one and three days. All measurements of production were carried out at *in situ* water temperature and at a light intensity of $125 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{second}^{-1}$ (light source TL 20 W/33). Chlorophyll concentration was also determined, except during the first experiment in June. The concentrations of nutrients, phosphorus, nitrogen, and silica, were examined both in the controls, and in the samples in which zooplankton had grazed.

The production rates in the experiments I and II were, respectively, 32 and 36% lower in the samples in which zooplankton had grazed than in controls, but the activity coefficients in the two cases were comparable. In the subsequent two experiments, the decrease in ^{14}C -fixation rates, respectively, 63 and 68%, were even more significant. The differences in the chlorophyll concentration between the controls

and experimental samples did not reveal a definite trend, but there was a decrease during the experiment II, increase in experiment III and no change with respect to controls in the last case. The chlorophyll content in the controls, on the other hand, tended to increase in comparison to the concentrations at the start of the experiment.

The data on the excretion products is erratic though there is evidence of ammonia excretion in all the cases. Since all the experiments were done in open plastic beakers, the possible effects of diffusion of nitrogen (NH_3) and carbon (CO_2) from the air, as well as leaching of these two elements and of phosphorus from the plastic were examined. Both diffusion from air and leaching from the vessels were found to have caused serious anomalies despite the correction via the controls. The work done so far has given an insight into the methodological deficiencies, and is therefore a useful basis for future research.

Role of bacteria as zooplankton food

The main components in the food menu of zooplankton are, of course, phytoplankton, detritus and bacteria. However, not all the components are equally important throughout the year. This is sequel to the fact that their relative concentrations, availability and nutritive value may vary seasonally. For a better understanding of energy flow in the food chain it is essential to understand their importance in the food of zooplankton. The studies of zooplankton grazing have so far concerned the phytoplankton and detritus constituents, including bacteria colonizing the latter; this is because the calculations of feeding rates are based on the product of grazing ($\% \text{ d}^{-1}$) and the prevailing seston concentration ($3\text{--}33 \mu\text{m}$). It follows that whereas bacteria colonizing detritus are considered in the calculations of zooplankton ingestion rates, the 'free bacteria' are not. In view of the rather high bacterial densities (up to $10^7 \cdot \text{ml}^{-1}$) encountered in the lake during the recent years their significance as a potential source of food or as an alternative food source, particularly when phytoplankton concentrations decline, cannot be ruled out.

Mrs. A.C. Jooren (Teachers Training School, Utrecht) studied in collaboration with the working group 'Mineralization of Organic Matter' the role of bacteria as zooplankton food. The magnitude of glucose- ^{14}C uptake was tested and found to be satisfactory for grazing studies. In the initial experiments laboratory reared *Daphnia magna* was used.

The results obtained are summarized:

length (mm) of animals:	1.2–1.8
F ($\text{ml} \cdot \text{hr}^{-1} \cdot \text{animal}^{-1}$):	0.006–0.011
uptake ($\text{ind}^{-1} \cdot \text{hr}^{-1}$):	
bacteria:	1.7–3.2 ($\times 10^6$)
$\mu\text{g C}$:	0.33–0.62

The extremely low filtering (F) and uptake rates are due to the abnormally high bacterial densities used as a trial food. Subsequently, the food concentrations used ranged from 1.1 to 9.4 $\text{mg C} \cdot \text{l}^{-1}$, representing the concentrations prevailing in most Dutch lakes. The filtering rates dropped by an order of magnitude from 0.045 to 0.005 $\text{ml} \cdot \text{ind}^{-1} \cdot \text{hr}^{-1}$, when the food concentration increased, respectively, from 1.1 to 4.0 $\text{mg} \cdot \text{C} \cdot \text{l}^{-1}$, but the rates were constant when the concentrations were further increased. In another experiment, *Daphnia* of different size classes (0.9, 1.2 and 1.5 mm) were used; the bacterial densities varied between 7×10^6 bacteria per litre and five times higher (food conc. 1.4–7.1 $\text{mg} \cdot \text{C} \cdot \text{l}^{-1}$). The changes in filtering rates were as follows: small *Daphnia*, from 0.09 to 0.02 $\text{ml} \cdot \text{ind}^{-1} \cdot \text{hr}^{-1}$; medium *Daphnia*, from 0.04 to 0.05 $\text{ml} \cdot \text{ind}^{-1} \cdot \text{hr}^{-1}$; and big *Daphnia*, from 0.006 to 0.09 $\text{ml} \cdot \text{ind}^{-1} \cdot \text{hr}^{-1}$. In short, the last two size classes of *Daphnia* did not exhibit an inverse correlation between the filtering rates and food concentration. It is quite likely that because of the small size of the food particles (bacteria), increased food concentration results in the expected 'depression' in filtering mechanism of the smaller animals. But in the case of relatively bigger animals (medium and big categories), size of food particles is a limiting factor, and, therefore, despite the increased food concentration, the animals are stimulated to filter maximally.

The subsequent work concerned the feeding of *Eudiaptomus gracilis*, especially in view of this animal's predominance in Lake Vechten during the study period (November to January). Moreover, during winter the phytoplankton concentrations are low but both the bacterial densities and *Eudiaptomus* numbers are still high. In the first experiment labelled lake algae ($\text{NaH}^{14}\text{CO}_3$) and bacteria (glucose- ^{14}C) were used separately, in filtered ($0.2 \mu\text{m}$) lake water in concentrations normally prevalent in the lake. The filtering rates of *Eudiaptomus* (length 0.25–0.90 mm) were: bacterial food, from 0.01 to 0.05; and algal food, 0.003 to 0.008 $\text{ml} \cdot \text{ind}^{-1} \cdot \text{hr}^{-1}$. It is apparent that both *Daphnia* and *Eudiaptomus*, when offered exclusively bacteria as food source are able to utilize bacteria in suspension.

The experiment was repeated using a range of animals, varying in length from 0.45 to 1.50 mm. Again the filtering rates on bacteria were higher. In both cases the size related increase was 8 to 9 times. In the last experiment labelled algae and bacteria were used as tracer, rather than both tracer and source of food. The main food source was the *in situ* lake water, containing 1.92 $\text{mg C} \cdot \text{l}^{-1}$ of seston material ($<33 \mu\text{m} \varnothing$). It is interesting that filtering rates employing algae as tracers were 10–25 times higher than when bacteria were used as tracers. In summary the preliminary indications are: first, animals do utilize

bacteria as food, and the extent of this utilization is possibly determined by the relative abundance of bacteria in water. Having a choice between bacteria on one hand, and algae and detrital material on the other, selection of particles would depend on the size of particles rather than the type of particles in the food.

3.2.4. Studies on *Chaoborus* larvae and zooplankton regulation

Our knowledge of the importance of predatory zooplankton and other secondary and tertiary consumers in Lake Vechten is very poor. Major predatory organisms in the lake are: Rotatoria, *Asplanchna priodonta*; Crustacea, *Leptodora kindtii* and *Cyclops* spp.; Diptera, larvae of *Chaoborus flavicans*; fish, several species feeding at different trophic levels. *Chaoborus* sp., considering its densities and year round presence in the lake, has apparently quite an important place among the predators: first, in regulating zooplankton and, secondly, as food for fish.

Miss J.T.A. Vulto started a study of the role of *Chaoborus* larvae in the food chain of Lake Vechten and sampled the benthic and limnetic populations mostly along a transect in the eastern part of the lake. The larval densities (IV instar) in the benthic region ranged from 210 to 970 ind. m⁻² between 8 and 10 m depth contours and from 1200 to 1400 ind. m⁻² in the deeper area. In the limnetic region, II, III and IV instar larvae were encountered in late September and early October; in shallow areas relatively few animals were caught.

A study of the pharyngeal contents revealed more guts full than empty. *Bosmina* sp., copepods, *Ceriodaphnia* sp. and *Keratella* sp. were recorded among the contents of pharynxes which in many cases comprised unidentifiable material, i.e. detrital debris. In the limnetic collections *Bosmina* carapaces were observed more frequently in the contents of the pharynxes than other zooplankton species. Also, copepod spp. and *Eudiaptomus* were encountered. Furthermore, in *Chaoborus* from the limnetic region the gut contents were relatively more easily recognizable in the animals collected in the early hours of night than in those caught during late night, or early morning. Lastly, in the benthic animals the gut was usually full soon after sunrise, and copepods and cladocera were equally important in the contents of the pharynx.

3.2.5. Articles in press

Remarks on the present status of limnology in India based mainly on the Indian publications in *Hydrobiologia*, and suggestions for future approach. Gulati, R.D. and G.H. Würtz-Schulz. *Hydrobiologia*. 72, 211–222 (1980).

A review of the Indian limnological studies published in *Hydrobiologia* up to 1979 reveals that the number of papers documented in this Journal far exceeds those in all other relevant journals on freshwater ecology/limnology. Of the more than 325 papers pertaining to different aspects of the freshwater bodies, so far published, nearly two-third appeared during the last one decade. Earlier studies in *Hydrobiologia* are characterized by emphasis on morphology, taxonomy and bionomical aspects. In the latter part of the sixties ponds, including fish farms, received relatively more attention than other water types. The majority of papers on ponds deal with general ecology, water chemistry, plankton and algae. Studies on lakes and reservoirs lack in ecosystem approach; moreover, energy budgets, and availability and regeneration of nutrients are attempted by few. Many man-made lakes, as well as rivers and streams, have not received due attention by the Indian limnologists. The studies on springs deal mainly with systematics of diatom flora and blue-green algae.

The number of papers pertaining to flora, i.e. algae, fungi and macrophytes, is large. However, but for those on the macrophytes, emphasis is usually on taxonomy, morphology, discovery and description of new species. This is also true for fauna, particularly, for publications on Protozoa, Annelida and Rotatoria. The publications on Crustacea deal with a variety of subjects, namely, taxonomy, life cycle, reproduction and development. There is a lack of studies on feeding of herbivores. Only four studies on aquatic insects are reported in the Journal. About a dozen papers deal with systematics, reproductive cycle of bivalve molluscs, and with spawning behavior, growth, effect of environmental factors, and hormones of snails. The fish papers do not reflect the trends of progress in India. They generally lack in ecological emphasis. Several fish papers deal with food intake, conversion and utilization, and metabolism. Among them, a large number relate to air-breathing fish.

Only about five per cent of the Indian papers in *Hydrobiologia* directly concern food chain aspects. Producer–consumer interactions, carnivores, and processes governing flow of energy culminating in fish are inadequately known. Approximately thirty papers concern the estuarine and marine environments.

Based on the achievements so far, lacunae and needs, a change in the approach is desirable. Limnologists should concentrate more on the problems related to utilization of water resources for food production, pollution control, public health, water supplies, and on interdisciplinary education programmes.

3.2.6. Reports

Een biochemisch planktononderzoek in het plasje

Vechten (A biochemical plankton research in Lake Vechten).

Siewertsen, K., Internal Report No. 1979-3 (in Dutch) Limnological Institute, Nieuwersluis, The Netherlands.

A biochemical study of plankton and dissolved organic matter in Lake Vechten was carried out from March 1972 to March 1979, more intensively between March 1975 and March 1978. The data have been statistically analyzed. Annual, seasonal and, in some cases, monthly means with standard deviation were calculated. The effect of water temperature and plankton biomass on the biochemistry of plankton was examined by regression and correlation analyses.

The techniques employed are those given in literature, but in some cases they were modified. The Chemical Oxygen Demand (C.O.D.) for measurement of carbon was improved by increasing the concentration of reagents, i.e. by adding 30 ml (distilled) water instead of 70 ml. It can be broadly generalized that the water temperature plays an important role in determining the biochemical composition of plankton. The effect of seasonal changes both in quantity and in biochemistry of seston (zooplankton food) on that of zooplankton, except for a direct relation with the fat content, appeared to be not significant. There are indications, however, that biochemical composition of seston, particularly, the nitrogen (N) — carbon (C) ratio varied directly with biomass.

The mean N:C ratios of zooplankton and seston were, respectively, 0.23:1 and 0.17:1. The means with standard deviation (%) of biochemical composition were as follows: zooplankton, proteins 65 ± 7.2 ; fats 18 ± 5.6 ; and carbohydrates 17 ± 5.7 ; and for seston, proteins 49 ± 11.5 ; fats 20 ± 6.4 ; and carbohydrates 31 ± 10.5 .

Rise in water temperature was correlated inversely with decrease in per cent fats in zooplankton but with an increase in that of proteins; in case of seston, the rise in water temperature was accompanied by a decrease in % of proteins but by an increase in that of carbohydrates. The protein:N ratios were: zooplankton, 4.9 ± 0.8 :1 and seston, 5.0 ± 0.7 :1. The heat of combustion per mg organic dry weight was: zooplankton, 24.15 ± 1.46 joules; and seston, 19.17 ± 1.63 joules. The ash contents (%) were, respectively, 6.4 and 43.0 for zooplankton and seston.

The ash per cent in seston fluctuated markedly with season, i.e. from 24% in late summer to 58% in early winter. In both zooplankton and seston the variations in energy content were related negatively with the water temperature.

Despite some discrepancies between the ratio dry weight/chemical oxygen demand (C.O.D.), the latter appears to be a reasonably good biomass parameter.

De planktonbiomassa in het plasje Vechten (The plankton biomass in Lake Vechten).

Siewertsen, K., Internal Report No. 1979-4 (in Dutch) Limnological Institute, Nieuwersluis, The Netherlands.

The routine measurements of plankton mass in Lake Vechten were carried out from March 1972 to March 1979 as a part of research studies of the working group 'Primary and Secondary Production'. From March 1976 the spatial variations in the biomass were also studied. The grand annual mean of seston mass for the different years was 3.7 ± 0.42 g C/m²; on the average 28% of the biomass consisted of seston fraction $>33 \mu\text{m}$ (\emptyset). The grand annual mean for zooplankton was 0.91 ± 0.21 g C/m². The mass of both seston and zooplankton differed significantly with depth. The highest zooplankton concentration during the summer stratification period was recorded adjacent to the anoxic layer. The zooplankton concentrations at night were higher than those during the day. This may be attributed to migration, errors of sampling techniques or both.

The seston fractions, $<33 \mu\text{m}$ and $>33 \mu\text{m}$, had maxima, respectively, in spring and late summer. There was a direct linear (regression) correlation between the seston mass (C. mg/l) and water temperature (t, °C) during 1976 and 1977; the regression equation was as follows: $C = 0.551 + 0.018t$; $R = 0.331$; $N = 56$ and $P < 0.02$, where R is correlation coefficient, N = no. of seston and temperature measurements, and P is the probability level. This is due to a strong positive correlation between temperature and the seston fraction $>33 \mu\text{m}$ ($C = -0.103 + 0.0309t$; $R = 0.59$, $P < 0.001$) rather than to the fraction $<33 \mu\text{m}$ which, on the contrary, exhibited a negative correlation with the water temperature ($C = 0.652 - 0.013t$, $R = -0.36$, $P < 0.01$). A correlation between the water temperature and zooplankton mass was lacking. This is due to the absence of marked seasonal fluctuations in the concentration of zooplankton; except in May and June, when the mean concentrations of zooplankton are relatively the highest, i.e. respectively, 1.56 and 1.22 g C/m², the annual variations in zooplankton are low and the biomass is around 0.8 g C/m². Nevertheless, the variations are correlated with the food (seston $<33 \mu\text{m}$) conditions prevailing five to six weeks earlier. Occasionally, the zooplankton concentration, particularly, in May exceeds that of seston $<33 \mu\text{m}$.

Een onderzoek naar de voedselselectiviteit van *Eudiaptomus gracilis* (An investigation of the food selectivity of *Eudiaptomus gracilis*).

Vermeulen, H., Student Report No. 1979-3 (in Dutch), Limnological Institute, Nieuwersluis, The Netherlands.

The filtering rates and food selection of *Eudiaptomus gracilis*, an important filter feeder in Lake Vechten, were investigated in the laboratory employing the Coulter Counter technique. Both *in situ* lake food and laboratory cultured algae were used as food. The calanoid filtered the food particles in the diameter range of 2.7–21 μm , maximally filtering the particles measuring 13–14 μm .

In the lake water, the filtering rate of *E. gracilis* doubled with the increase of particle size (\varnothing) from 5 to 10 μm . The mean filtering rate of 0.20 ml ind⁻¹.d⁻¹ calculated from the decrease in the volume of particles of all size classes was comparable to that estimated from the decrease in the particular organic carbon. In another experiment *Chlorella vulgaris* was used as food source in the filtered lake water. The selection of particles measuring 10 μm in diameter was more pronounced than that observed for particles of this size in the *in situ* lake food.

Lastly, in a series of four parallel experiments, respectively, single celled *Microcystis aeruginosa* culture (obtained from the Laboratory of Microbiology, University of Amsterdam, Amsterdam), *Chlorella* sp., *Diatoma elongatum* (kindly provided by working group Algology, Tjeukemeer Laboratory) and *Scenedesmus quadricauda* were used as food species. The ranges and means of diameter of particles of the algal species used were:

species	range (μm)	mean (μm)
<i>Microcystis</i> sp.	2–7	4
<i>Chlorella</i> sp.	2–11	7.9
<i>Diatoma</i> sp.	2–16	11.6
<i>Scenedesmus</i> sp.	8–20	12.6

The mean filtering rates of these algae differed markedly, with 0.44 ml.ind⁻¹.d⁻¹ on *Microcystis* and *Scenedesmus* species on one hand, and about one and two ml.ind⁻¹.d⁻¹, respectively, on *Chlorella* and *Diatoma* on the other. These differences were related to an increase in filtering rate related with increase in the particle diameter up to about 14 μm and a sharp decrease thereafter.

Voedselopname van *Daphnia magna* (Food uptake of *Daphnia magna*).

Kramer, M.A.M., Trainee Report No. 1979-1 (in Dutch), Limnological Institute, Nieuwersluis, The Netherlands.

The effect of food concentration, particle size and temperature on the filtering rate of *Eudiaptomus gracilis* and *Daphnia magna* were studied. Particles smaller than 3 μm were not filtrated by both the species. Above 3 μm the filtering rate of *E. gracilis* increased with the increasing particle size. *Daphnia magna*, on the contrary, has a mean rate between 3 and 6 μm .

Moreover the filtering rate appeared to depend on the feeding condition of the animal and on the age of the phytoplankton culture: in older *Chlorella* cultures the filtering rates were relatively lower.

3.3. WORKING GROUP 'ECOPHYSIOLOGY OF WATER PLANTS'

(P.H. Best, C.L.M. Steenbergen, H.J. Gons, H.J. Korthals, M.D.M. Trommel, G. Wiegers, M.J.B. Bär-Gilissen, J.H.A. Dassen.)

3.3.1. Introduction

Within this working group several ecophysiological aspects of three different groups of primary producers are being studied, i.e. aquatic macrophytes, periphytic algae associated with macrophytes and phytoplankton. Particular attention is given to the role of the macrophyte-periphyton community in the carbon and nitrogen cycles of Lake Vechten. This year also emergent macrophytes and macrophyte-associated invertebrates were studied. In the pelagic zone, the behaviour of the dominant phototropic microorganisms, occurring in the anaerobic hypolimnetic water layers, was investigated.

3.3.2. Studies on macrophytes

Species distribution and production

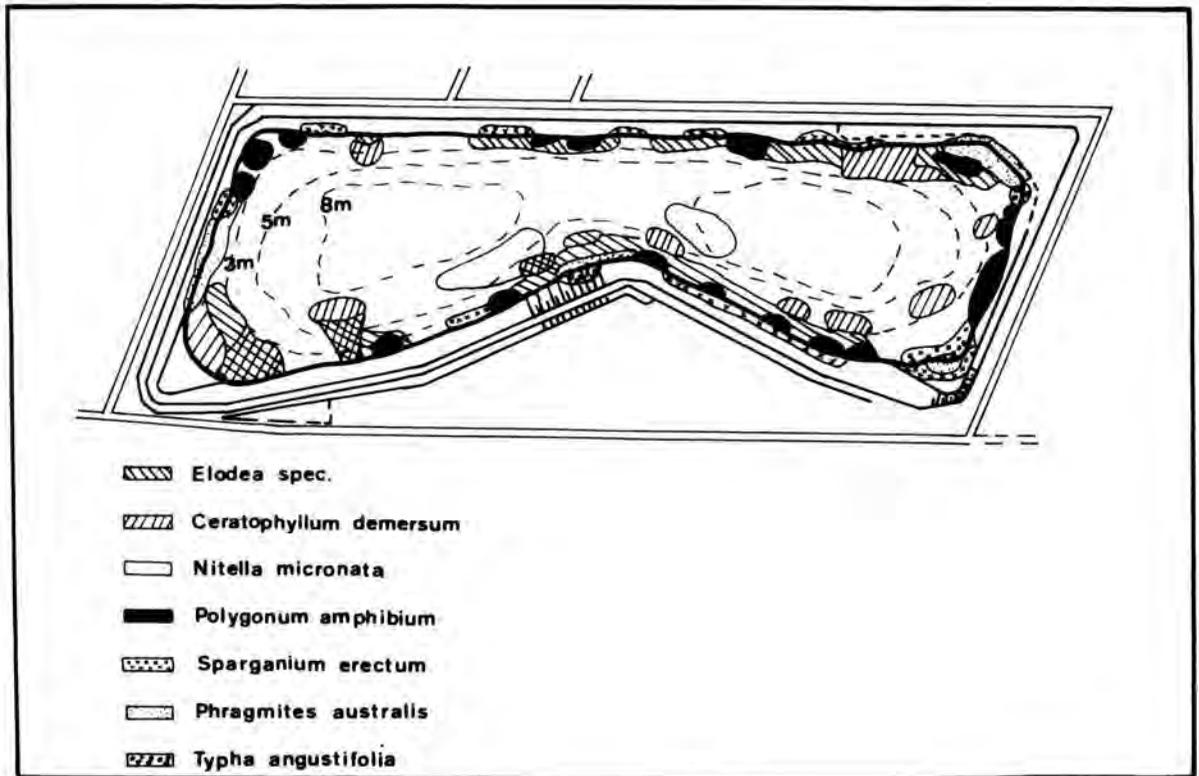
The distribution of the main emergent, floating and submerged macrophytes was recorded (Fig. 11). In the zone of the submerged macrophytes, *Elodea* sp. was the main dominant taxon, followed by *Ceratophyllum demersum*; the two species occupied, respectively, 8.9 and 4.1 per cent of the total lake surface.

Myriophyllum verticillatum had almost disappeared from the lake, although some remains were present in spring. The macro-alga *Nitella mucronata* occurred only in two small meadows in the southern part of the lake. As regards the floating macrophytes, several patches of *Polygonum amphibium* were present along the shoreline of the lake, covering 1.5 per cent of the lake surface area. In the emergent vegetation three species occurred in well-developed monotypic stands, namely, *Phragmites australis*, *Sparganium erectum* and *Typha angustifolia*, occupying, respectively, 1.6, 0.4 and 0.2 per cent of the lake surface.

In order to quantify the role of the primary producers of the littoral zone in lake metabolism, their production rates (standing crop measurements, see also the section on *Phragmites*) and respective nutrient contents (C, N and P) were estimated.

The relative contributions of the submerged, floating and emergent macrophytic communities to their total organic matter input into the lake were, respectively, 17.5, 8.4 and 74.1 per cent (representing

Fig. 11. The distribution pattern of the main submerged, floating and emergent macrophytes in Lake Vechten during 1979.



values of 151.6, 72.6 and 640.8 kg ashfree dry weight .lake⁻¹). When the organic matter values are converted into C, N and P for the three different plant groups it is observed that the relative importance of the submerged macrophyte communities is greater with respect to the nitrogen and phosphorus cycles than to that of carbon. This is caused by their higher N:C and P:C ratios than those of the emergent and floating species.

Compared with the observations in 1978 the importance of the submerged macrophytes decreased in 1979, i.e. decrease of the organic matter by about 55%. Although this tendency was noticed already earlier, the sharp decrease in production in 1979 might partly be due to the severe winter in 1978–1979.

Production and mineralization studies on Phragmites australis

This study was done by Mrs. M. Zippin (State Foundation for Environmental Engineering, Rio de Janeiro, Brazil) on a post-graduate grant from the Ministry of Education and Science in The Netherlands.

The production rate of *Phragmites australis* in the littoral zone of Lake Vechten was determined during the growing season of 1979. The standing crop was measured every two months in a quadrat of 0.1 m². The plant material was divided into three main portions, namely, shoots, roots and dead material. The growth curve thus obtained was compared to that derived from the plants tagged at the onset of the growing period and harvested in the course of the year. The plant material was analyzed for C, N and P.

The biomass of *Phragmites* increased to a maximum at the end of July when the plants have a maximum shootweight of 4.5 g ashfree dry weight .m⁻² (respective percentages for dead plant material, roots and shoots were: 15.2, 15.9 and 68.9 (See Fig. 12). The C, N and P contents were higher from April to July than during rest of the year, respectively, 47, 2.8 and 0.36 per cent of the ashfree dry weight.

Most plant material ends up in the lake at the end of the vegetative period; consequently, a nutrient input of 536 kg ashfree dry weight for the whole lake was estimated.

Decomposition of *Phragmites* leaves was measured

under experimental conditions (light-dark cycle of 12:12 hours, temperature 15°C) in closed systems for 147 days. Containers filled with a) lake water, b) lake water and sediment, and c) lake water plus sediment and plant material (in nylon litterbags, 1 mm mesh size). The plant material was collected in March from the lake bottom; initial leakage of the nutrients had thus occurred in the lake. It was freeze-dried and 'fragmentized' before the start of the experiment. In the course of the experiment the ashfree dry weight, and N and C contents of the plant material decreased gradually to 64, 54 and 66 per cent of the initial values, respectively. The P content fluctuated due to accumulation of bacteria and their excretion products. Almost all the C and N that disappeared from the plant material during the first 100 days was recovered in the water phase. Thereafter, however, the nutrients accumulated in the sediments. Only about 10 per cent of the total C and N content of the water phase was soluble (i.e. <math><45 \mu\text{m}</math>). The ortho-phosphate content in the water phase increased substantially from 60 to 100 days incubation in the low-oxygen vessels compared to that in the control; this was probably released by the sediments or originated from the decaying algae and bacteria. Only a small part of the plant-P was recovered as ortho-P in the water phase.

The changes in bacterial numbers and photosynthetic organisms were recorded by epifluorescence counting and pigment chromatography. In the water

phase only obligate heterotrophic bacteria were encountered whereas in the sediment also photoheterotrophs were found. Initially (0–60 days) the activity of both groups was greatly stimulated by the decaying plant material as demonstrated, respectively, by the production of considerable amounts of methane and by the increase in pigments specific for photoheterotrophic bacteria (Fig. 13). Later on, the growth of algae, particularly diatoms, increased but the number of photosynthetic bacteria decreased.

Growth and photosynthesis of Ceratophyllum demersum

A simulation model for the growth and production of *Ceratophyllum* is being developed. In addition to data collected in previous years, special attention was paid to the effect of temperature on the carbon fixation rate in relation to the plant's physiological stage. The optimum temperature for photosynthesis was found to depend on age, being about 18°C for full-grown plants (under laboratory conditions). The carbon fixation rate was measured and the relationship between this process, the chlorophyll content and the total non-structural carbohydrates (TNC) of the plants was determined. Photosynthetic rate and chlorophyll content were strongly correlated in the first half of the year, but less so later on (Fig. 14). The TNC content fluctuated and tended to increase from spring to summer. In mid-summer low photosynthetic rate coincided with a high TNC content, indicating a decrease in net photosynthesis concomitant with a diminution of the sink for assimilates.

Experimental work on the initial photosynthetic products and excretion is in progress.

Nitrogen effects

The role of the submerged macrophytes in the nitrogen cycle of Lake Vechten is being studied in cooperation with the working group 'Mineralization of Organic Matter'. As found already earlier in short term experiments, the $\text{NH}_4\text{-N}$ uptake rate of *Ceratophyllum* plants in early growth stages is substantial and much higher than in older plants.

In 1979, ^{15}N labelled ammonium chloride and potassium nitrate were used as nutrients, in experiments with a longer duration, to measure nitrogen uptake. *Ceratophyllum* plants in two different growth stages were compared in this respect, notably a) plants in which dormancy was broken by low temperature treatment, and b) plants grown for two weeks under experimental summer conditions. Initial uptake rates (V_i) were measured in the plants incubated for one hour. Nutrient concentrations ranged from 20 to $500 \mu\text{g.l}^{-1}$. Nitrate uptake was not detected with the present method (optical measurement of ^{15}N enrich-

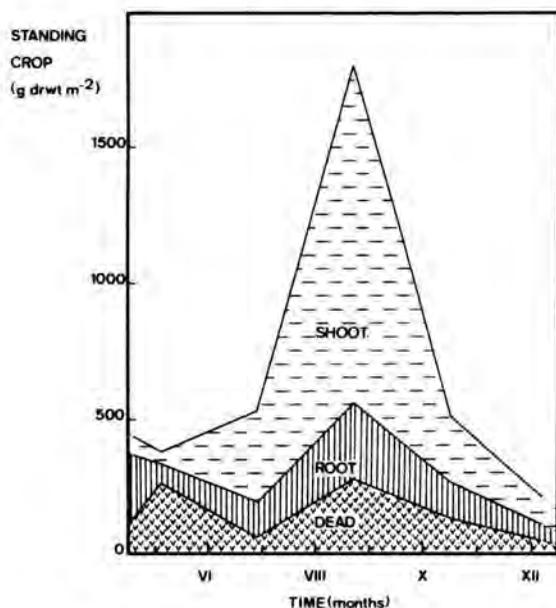
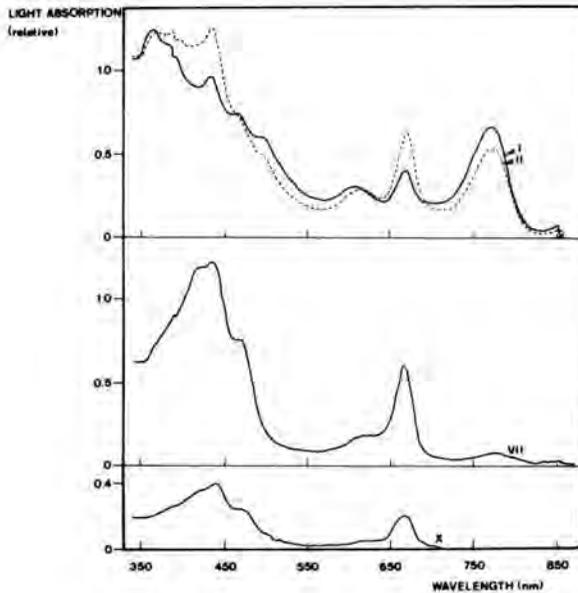


Fig. 12. Biomass (ashfree dry weight) of *Phragmites australis* in the course of the year.

Fig. 13. Absorption spectra of the water phase collected from the vessels in which decomposition of *Phragmites*-leaves was studied. Comparison of vessels originally filled with: X, water; VII, water + sediment; and I-II, water + sediment + plant material. Species-specific absorption of photoheterotrophic bacteria occurs at about 770 nm.



ment in the plant) both in the dormant and full-grown plants. In the case of ammonia considerable differences were found between the two physiological stages. For dormant plants the relationship between uptake rate and ammonia concentration was linear over the entire concentration range; at $500 \mu\text{g} \cdot \text{l}^{-1} \text{NH}_3\text{-N}$, V_i was $90 \mu\text{g N g}^{-1}$ ashfree dry weight hr^{-1} . Full-grown plants reached a maximum V_i of $270 \mu\text{g N g}^{-1}$ ashfree dry weight hr^{-1} at $200 \mu\text{g NH}_3\text{-N l}^{-1}$. At higher concentrations V_i remained constant. Half saturation level was reached at about $80 \mu\text{g NH}_3\text{-N l}^{-1}$.

In addition to the short term uptake measurements, uptake rates for longer periods (i.e. 18 hours) were determined. Since the labelled nutrient disappeared from the incubation medium and was not fully recovered as plant ^{15}N (especially in the case of nitrate), control experiments are in progress to determine whether ^{15}N , after uptake by the plants, is excreted as organic nitrogenous compounds.

3.3.3. Studies on periphyton

Observations on the relationship between submerged macrophytes and periphyton, with respect to their

masses, were similar to those in the previous years. Also, elemental analyses, microscopic enumerations and paper chromatographic analyses showed the known characteristics, i.e. high ash content and C:N ratio due to carbonate, and predominance of filamentous green algae and pennate diatoms.

Efforts were directed to gain insight mainly into the functional properties of the periphyton of *Ceratophyllum* at a fixed location in the SE part of Lake Vechten.

Water samples were collected from fixed depths above and within the plant stand. Temperature, pH, and concentrations of oxygen, dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were measured in the same sample. Irradiance was measured using a sensor assembly, which could be lowered without changing the position of the plants. In Fig. 15 generalized results are given for the period July till October in daylight conditions. Light attenuation due to self shading resulted in virtual darkness in the lower half of the plant stand. Only oxygen consumption could be expected at this depth; this was confirmed by the oxygen profile. Light attenuation, resulting in conversion into heat, may also explain the observed small temperature differences. The oxygen and pH profiles showed peaks at the same depth at considerable distance from the plant apices. Evidently, this indicated the location of major photosynthetic activity within the community. DIC remained constant with depth, but DOC (incidental measurements) showed an increase towards the sediment. Thus the low pH values near the sediment may have been due to the presence of organic acids.

Secondly, the depth distribution of macrophytes and periphyton biomass was determined. Three plants of similar length were collected and divided into three portions; the top 15 cm and equal lengths of middle and lower part, 50 cm in the given example (Fig. 16). The major plant mass was found in the upper and middle portions, periphyton largely on the middle one. There was a striking difference between the periphyton upon the lower plant parts and that upon the upper parts; below, the pigments were almost exclusively derivatives, and live algae were very low in number. This was due, of course, to the absence of light at this depth. Also, the carbonate content in the lower portion was reduced markedly. In view of the observations on the light and pH (Fig. 15), this is expected, i.e. the rise in pH accompanying photosynthesis precipitated carbonate, while on the lower plant parts, with zero irradiance and lower pH, there was resolubilization of carbonate. This occurred with the release of organic compounds adsorbed onto the carbonate, as indicated by the remaining appreciable amounts of hydrogen and nitrogen after periphyton was washed at 450°C .

Fig. 14. Photosynthetic rate (●—●), measured under 'standard' summer conditions, and chlorophyll content (▲—▲) of *Ceratophyllum* tips, both expressed per ashfree dry weight basis.

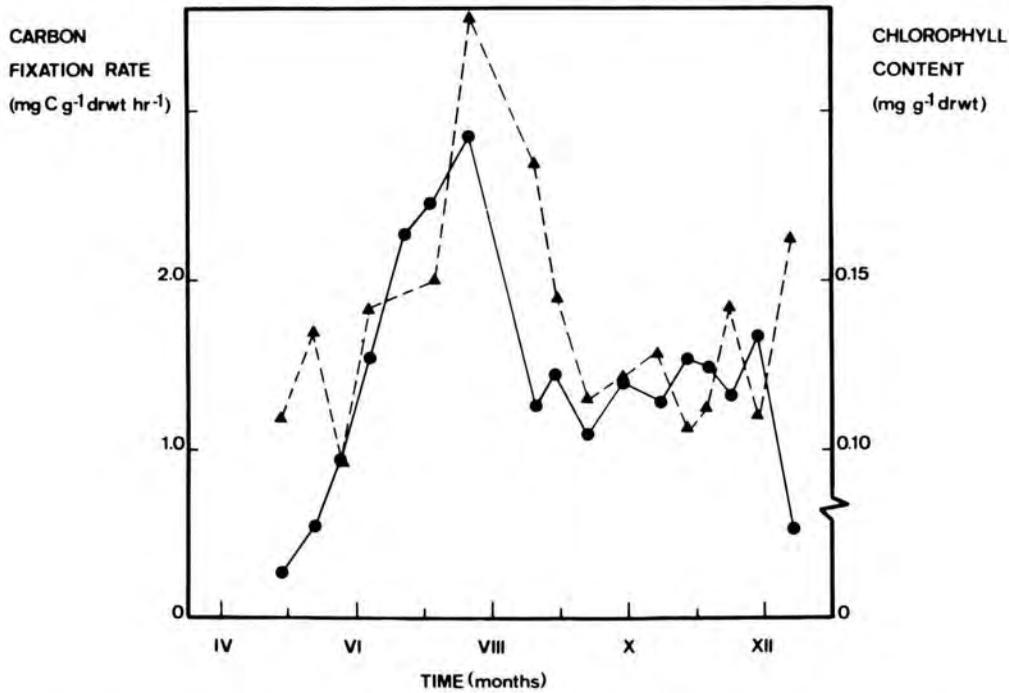
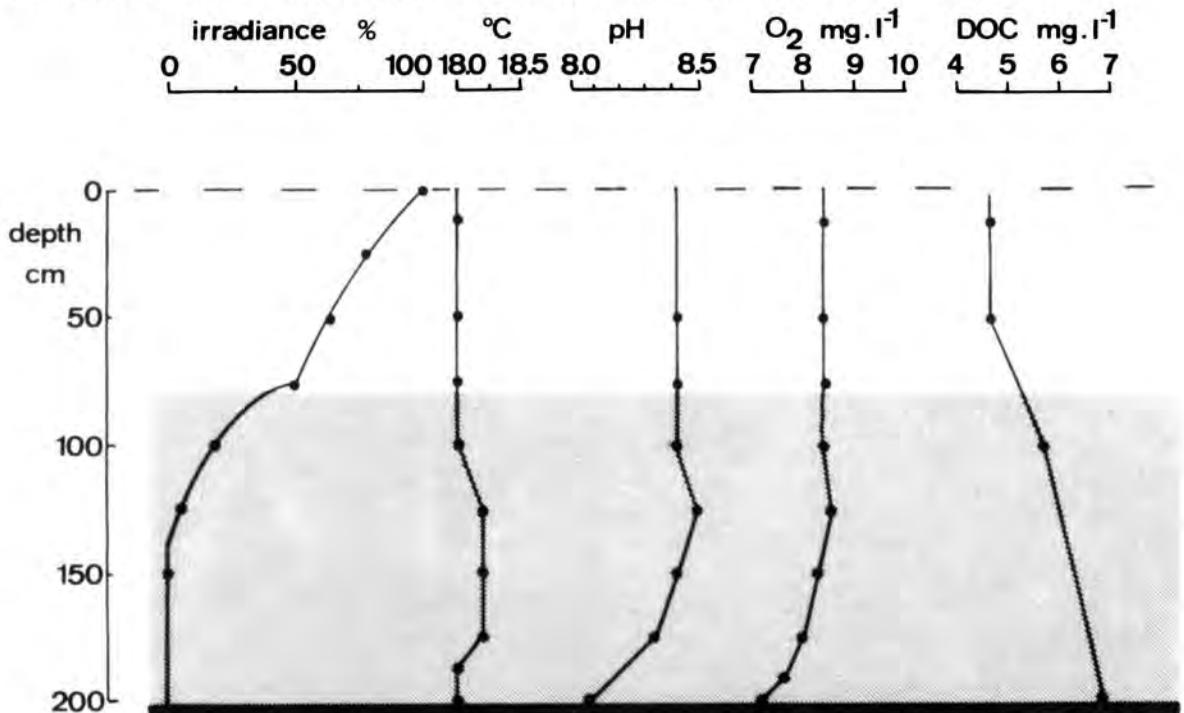


Fig. 15. Depth curves of irradiance, temperature, pH, dissolved oxygen and dissolved organic carbon (DOC) in the littoral zone during daylight. The shaded area represents the *Ceratophyllum* plant stand.



Thirdly, the relationship between specific oxygen exchange rate and irradiance was determined for both periphyton and the plant parts. This was done using a laboratory incubator, using the field temperature and light from fluorescent lamps. Except for the lower compartment, the periphyton, on ashfree dry weight basis, expressed higher photosynthetic capacity and markedly higher respiration rates than the macrophyte. Based on these data and those on light measurements in the field (Fig. 15 and 16), the net oxygen exchange rates of the plants and associated periphyton were calculated for nighttime and two daytime conditions of irradiance (Fig. 16). Obviously, during the night there was respiration only, mainly in the middle compartment, which contained most biomass. At moderate daylight (150 W . m⁻²), the net oxygen exchange of the community was positive, with most production at the top, but light was sufficient to allow for net oxygen production by the periphyton in the middle compartment. With full sunlight irradiance (300 W . m⁻²) even the middle compartment produced as much as the top compartment. From the integrated values it appears that, though macrophytes and

epiphytic algae represent considerable productivity potential, the total community, i.e. including heterotrophic organisms, may be oxygen consuming on a 24 hours basis. This may be explained by accumulation of organic matter from outside the community, the sources of which are still a matter of speculation.

During SCUBA dives, the littoral zone of Lake Vechten showed a marked patchiness in the distribution of submerged macrophytic and epipellic algal populations. At all depths wide stretches were found with sparse or even without macrophytic vegetation. Such places often had dense populations of epipellic algae, displaying macroscopic features of either diatoms, filamentous green algae or blue-green algae. Therefore, the significance of epipellic algae might be greater than previously assumed. Chlorophyll showed only weak correlation with depth. The amount of chlorophyll per unit area was quite substantial even down to the lower border line of the littoral zone; at 6 m depth the value was 52 mg chl. a m⁻²; this is within the mean range of column integrated values for the epilimnetic phytoplankton.

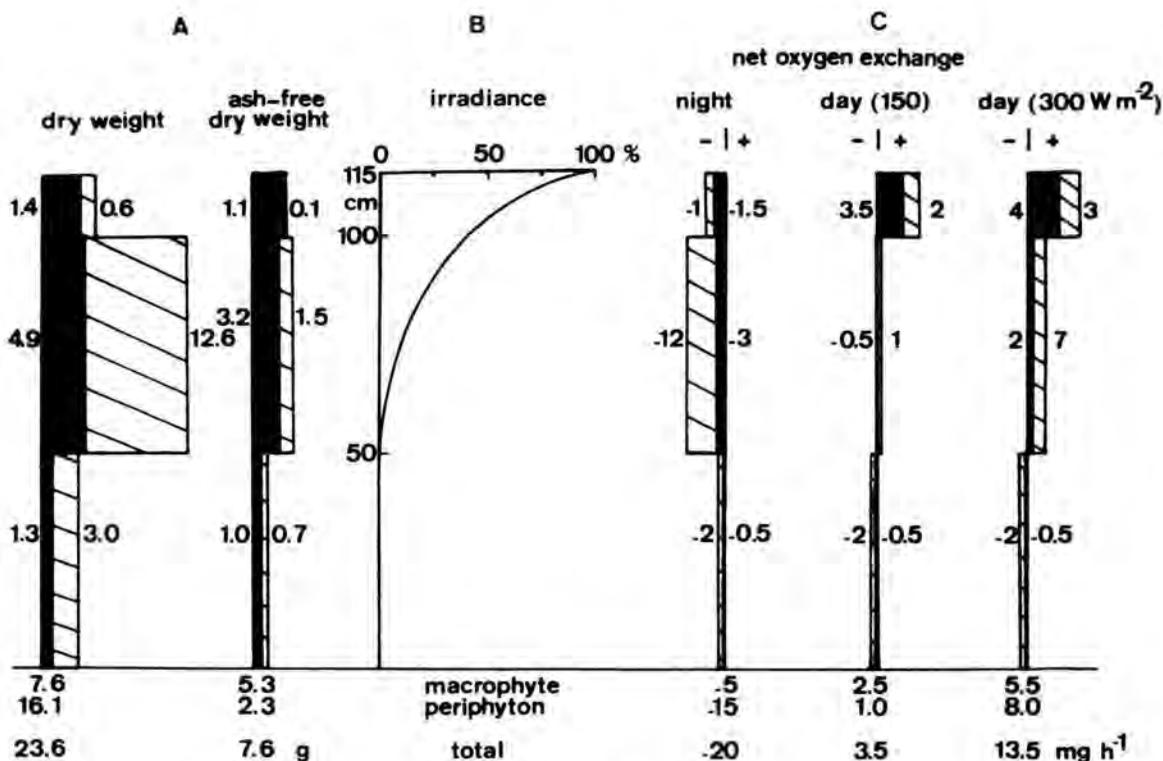
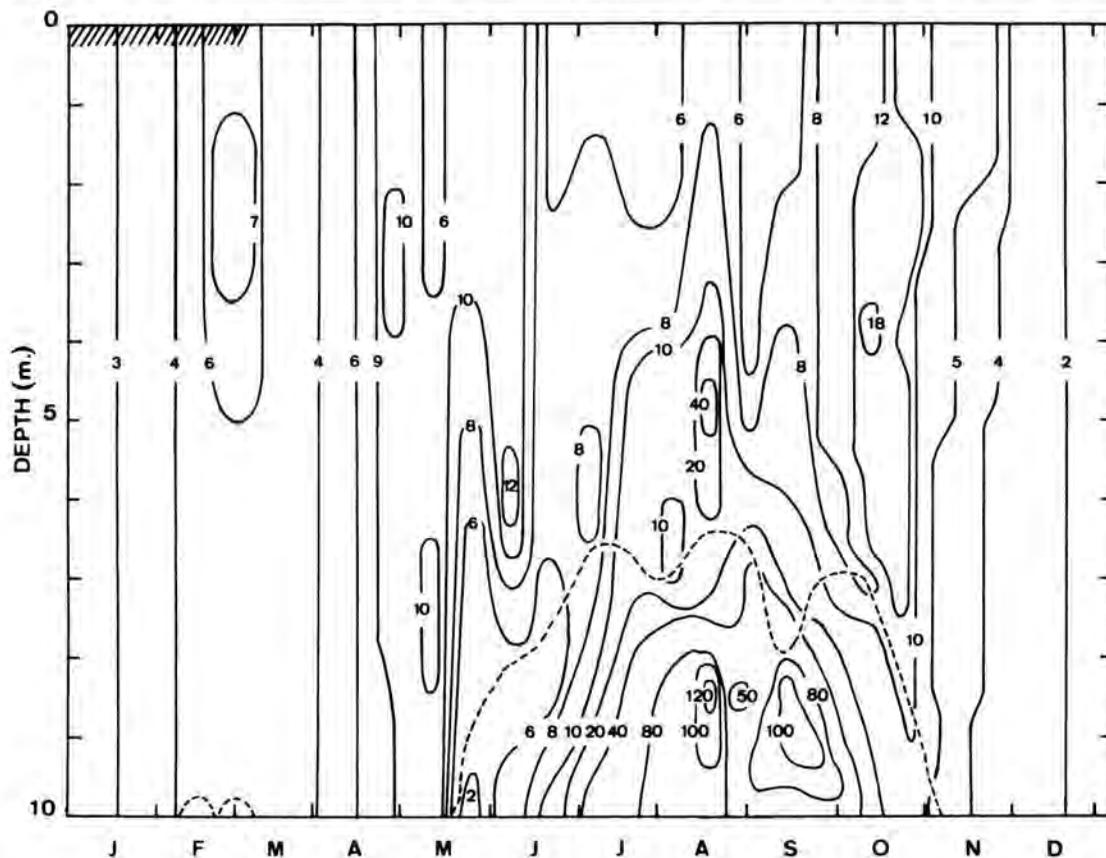


Fig. 16. Depth distributions of mass (A), irradiance (B), and net oxygen exchange in three light conditions (C). Shaded and hatched areas represent mass and activity, respectively, of macrophytes and periphyton. The figures below are the depth integrated values.

Fig. 17. Depth-time distribution of phytoplanktonic chlorophyll concentrations ($\text{mg}\cdot\text{m}^{-3}$), in Lake Vechten during 1979. Striped area indicates ice cover. Broken lines represent the oxygen zero isopleth.



The epiphytic algae appear to be potentially good producers. The carbon fixation rates reach values up to $100 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, depending largely on the population density of the macrophytes. Unfortunately, due to the heterogeneous species distribution, it is not possible to give a precise value for littoral algal productivity over the whole lake. Therefore, it is better to attempt and establish boundary limits for the parts of the littoral zone exhibiting well developed communities of macrophytes with epiphytic algae, and the parts occupied only by epipelagic algae.

On the basis of the oxygen exchange data, the epiphytic algae have much higher growth rates than the macrophytes. Yet, it was shown previously that there exists a rather fixed relationship between algal and macrophytic biomass, which indicates grazing might be a major factor in the population dynamics of these algae.

Finally the decomposition rate in the periphyton may be estimated. This yields a value of approximately $4 \text{ mg C}\cdot\text{g}^{-1}\text{C}\cdot\text{h}^{-1}$, or up to $45 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$,

again depending on the population density of the macrophyte. The question regarding the sources of the organic matter relates to the general problem how imports, and possibly exports, affect the processes studied in Lake Vechten.

3.3.4. Studies on phytoplankton

Vertical distribution

The vertical depth distribution of the phytoplanktonic biomass in Lake Vechten varies greatly from season to season with the shifts in the species composition. Using the chlorophyll concentration as an estimate of phytoplanktonic biomass conspicuous temporal changes are seen (Fig. 17). The biomass is low in winter, increases in spring (April–May), and then increases at different strata during the period of summer stratification. Phytoplankton is composed of diatoms, e.g. *Stephanodiscus* sp. and small green algae, e.g. *Chlorella* and *Scenedesmus* species, predominating in the winter. In spring, small flagellate algae, e.g.

Dinobryon sp. became important also, shifting mainly to dinoflagellates (*Ceratium* sp.) in summer. The metalimnetic maximum in summer was related largely to *Mallomonas caudata* (*Chrysophyta*). The development of two deep (8 to 10 m) maxima, one in August and a second one in September, was related to very dense populations of cyanobacteria and to green and purple sulphur bacteria.

Characterization of hypolimnetic phototrophs

Further insight was obtained in the species composition of the populations present in the anaerobic strata of the lake. The pigment composition of the hypolimnetic population was compared with that of the different phototrophic micro-organisms isolated from the hypolimnion. Main pigment components in samples taken from the hypolimnion were: chlorophyll-*a*, the bacteriochlorophylls *a*, *d* and *e*, the carotenoids β -carotene isorenieratene, okenon, zeaxanthin, caloxanthin and nostoxanthin. Two *Chromatium* strains were isolated. Both strains lacked the carotenoid okenon, but contained large quantities of aliphatic carotenoids not encountered in the lake. The attempts to obtain pure cultures of the dominant, okenon containing, Chromatiaceae (e.g. *C. okenii*, *Thiopedia*) did not succeed. Partially successful were the attempts to isolate representatives of Chlorobiaceae. A mixture of filamentous gas vacuole containing species possessing Bchlorophyll *d*, β -carotene and isorenieratene was lost in sub-cultures. These filamentous micro-organisms were notably numerous in the hypolimnion. Apparently, they belong to the group of green-coloured Chlorobiaceae and probably to the genus *Chloronema*. Finally, a representative of the brown-coloured Chlorobiaceae occurs in the lake; this could be deduced from the occurrence of bacterio-chlorophyll *e* and isorenieratene, but unfortunately this organism did not show up in the enrichment cultures.

A representative of the chroococcean cyanobacteria, isolated from the hypolimnion during the foregoing year, completes the picture. The cells of this strain contain the pigments chlorophyll *a*, β -carotene, zeaxanthin, caloxanthin and nostoxanthin.

Ecophysiology of hypolimnetic phototrophs

Besides characterization of the populations and study of their spatial distribution, the carbon fixation rates of the hypolimnetic phototrophs *in situ* under different incubation conditions using the ^{14}C technique were measured in preliminary experiments. The experiments were performed in collaboration with the working group Primary and Secondary Production. The C-fixation rates under aerobic and anaerobic conditions were compared. Light incubations were done

both with and without DCMU. DCMU inhibits oxygenic photosynthesis, carried out probably by the cyanobacteria, but does not affect the anoxygenic photosynthesis, carried out by the strictly anaerobic green and purple bacteria. Samples were collected at a depth of 8.5 m. Labelled bicarbonate ($\text{Na H}^{14}\text{CO}_3$) was injected and the bottles lowered to the sampling depth and incubated from 11.00 to 15.00 hours. During the September production maximum (See Fig. 17) the effect on the C-fixation rate of 'adding oxygen' to the bottles was clear. The mean C-fixation rates of aerobic and anaerobic samples were, respectively, 103 and 230 $\text{mgC m}^{-3} \cdot 4 \text{ hr}^{-1}$. In the presence of oxygen DCMU completely inhibits the photosynthetic C-fixation; however, in the anaerobic bottles with DCMU the C-fixation rate was only 20 per cent of that measured in anaerobic bottles without DCMU. This low rate of anoxygenic photosynthesis may be due partly to a shortage of electron donors such as sulphide and simple organic compounds needed by the green and purple bacteria. Only traces of sulphide could be detected in the hypolimnion during this experiment in September.

At the end of August, coinciding with a temporary low chlorophyll concentration (See Fig. 17), the sulphide concentration in hypolimnetic water collected at 11.00 hr varied between 2 and 6 μM . The mean assimilation ratio ($\text{mgC hr}^{-1} \cdot \text{mg}^{-1}$ chlorophyll) of the hypolimnetic phototrophs for the period 11.00 to 15.00 hours was 0.4.

3.3.5. Articles in press

Effects of nitrogen on the growth and nitrogenous compounds of *Ceratophyllum demersum*.

Best, P.H., Aquat. Bot. 8

The effects of high concentrations of nitrogen on *Ceratophyllum demersum* L. were studied. Nitrogen was added in the form of nitrate or ammonia.

Growth and morphology were not affected by nitrate up to a concentration of 105 mg l^{-1} . Ammonia, supplied in low concentration during a short period, stimulated growth. Higher concentrations, applied for a prolonged period, were toxic.

Increased levels of both nitrate and ammonia in the ambient water enhanced the nitrogen content of the plants. The amino acid concentration and composition, however, remained relatively constant.

Photosynthesis in *Ceratophyllum demersum*: Carbon fixation rates in relation to the plants' physiological stage, and the contents of chlorophyll and non-structural carbohydrates (summary).

Best, P.H., Hydrobiol. Bull. 13

Attention is paid to several aspects of the productivity and role of the submerged aquatic macrophytes in the carbon cycle of Lake Vechten. *Ceratophyllum*

demersum, a predominant macrophytic species in the littoral zone of this lake, occurs mainly from three to five metres depth.

In situ measurements of its photosynthetic rate demonstrated a maximum in spring (May), followed by a distinct decrease early in summer and a second maximum in late summer. The spring maximum of the photosynthetic rate coincided with the maximum increase of plant biomass in time. Maximum biomass occurred in early autumn, August–September.

Although *in situ* measurements pointed to the upper plant portions (70 cm) as the most important with respect to photosynthetic activity, it was demonstrated under standard experimental conditions that lower portions might be equally active depending on the plant's age. In the lake, light reduction with depth is one of the major causes in the commonly observed decrease in photosynthetic activity in lower plant parts.

Comparison of the photosynthetic rates in plant tips *in situ* and under standard experimental conditions indicated that in spring both water temperatures and the plant's chlorophyll content limit photosynthesis. This was concluded since under experimental conditions: a) photosynthetic rate and chlorophyll content were strongly correlated during the first half of the growing season, and b) higher rates were measured than *in situ*. Subsequently, however, light and the distribution of non-structural carbohydrates (TNC) in the plant also play an important role.

During the second half of the growing season, the lower parts of plants growing in the lake gradually lose their buoyancy; this is caused by a deteriorating light climate resulting in a decreasing photosynthetic activity.

Periphyton in Lake Vechten, with emphasis on biomass and production of epiphytic algae (summary). Gons, H.J., *Hydrobiol. Bull.* 13.

Characteristics of the periphyton associated with the submerged macrophytes *Ceratophyllum*, *Elodea* and *Myriophyllum* were similar. During the whole growing season the periphyton dry weight often exceeded that of the macrophytes. The periphyton contained much detritus and carbonates, and the epiphytic algae were accompanied by large numbers of bacteria, protozoa and rotifers. Attached algae were scarce, but motile diatoms were abundant throughout the year. Thus the periphyton showed the features of a benthic community. Consequently, the role of the macrophytes would be extension of the bottom area rather than actively promoting the growth of micro-organisms by providing minerals or excreting organic substances. During the peak of the littoral development the epiphytic algae had a high photosynthetic capacity, while at the same time the periphyton dark respiration was intense.

From data on the depth distribution of biomass and light in a *Ceratophyllum* stand, it may be concluded that the contribution of the epiphytic algae to the production in the lake's littoral zone was substantial. Furthermore, on a daily basis, the total association of macrophyte and periphyton would be oxygen consuming due to heterotrophic activity, notably, the mineralization of the detritus.

3.3.6. Published articles

Growth substances and dormancy in *Ceratophyllum demersum*.

Best, P.H., *Physiol. Plant.* 45: 21–28.

Ceratophyllum demersum L. occurs in winter in the dormant form, in summer in the vegetative form. Factors that affect growth and dormancy in *Ceratophyllum* were studied. After several weeks of severe winter conditions the plants changed from dormant to quiescent state. Under natural conditions *Ceratophyllum* plants remain quiescent for several months, due to unfavourable growth conditions. Experimentally the dormancy could be broken by high and low temperature treatments (shocks), and most effectively by addition of GA. An attempt to induce dormancy in full-grown plants by the addition of ABA under extreme summer or winter conditions proved unsuccessful.

The IAA and ABA contents in the plants were measured during the year. In winter the concentration of ABA was high and that of IAA low, whereas in summer the IAA concentration increased and that of ABA was variable. IAA only slightly antagonized the inhibition of growth by ABA. Both the growth regulators were readily taken up from the culture medium, as was confirmed by a study with radioactive labelled compounds. The uptake rate of IAA was significantly higher than that of ABA, being 762 μg and 3.26 μg per plant in 24 h, respectively. GA was found to have a strong antagonistic effect on the ABA induced growth inhibition.

The total GA activity in dormant and quiescent plants was similar, in full-grown plants it was much lower. In the dormant state a large part of GA was in a bound form, whereas during quiescence relatively more GA occurred in a free state in the plants.

Photosynthesis in relation to dormancy in *Ceratophyllum demersum*.

Best, P.H. and J.T. Meulemans, *Aquatic Bot.* 6: 53–65.

Growth and photosynthetic activity in the different developmental stages of *Ceratophyllum demersum* L. were measured with particular reference to the physiological maxima of these processes and the factors influencing the carbon fixation rates.

The elongation of the main axis and growth of the adventitious buds were used as parameters for growth. The rate of photosynthesis was determined by measuring the uptake of radioactivity from ^{14}C -bicarbonate labelled medium.

The growth of directly harvested and pre-conditioned plant material was measured under standard laboratory conditions. In the directly harvested plants, maximum elongation was observed in February, whereas sprout growth occurred particularly in April, subsequently, the growth slowed down until November and the plants went into innate dormancy. In the pre-conditioned plants, the sequence of developmental events and the increase in photosynthetic activity happened earlier. The photosynthetic carbon fixation rate of *Ceratophyllum* plants was measured in several stages of post-dormancy under standard conditions. From the dormant period up to the spring, the rate of photosynthesis increased steadily. A sharp increase occurred in spring after the maximum development in elongation and the sprout growth.

In the pre-conditioned plants, variations in length of the incubation period did not affect the carbon fixation rates, though such variations caused significant differences in full-grown plants. When the plants were adapted for 24 hours to the standard conditions, the carbon fixation rates were, however, affected.

In the pre-conditioned plants, adapted for a long period to standard conditions, respiration rate was much higher in the dark than in the light, indicating efficient refixation of the $^{14}\text{CO}_2$ evolved in the light. In the directly harvested, full-grown plant material, however, the fixation rate was much higher than that in pre-conditioned plants, which might explain the decrease in the carbon fixation rate commonly reported in mid-summer.

Only a small percentage of the released radioactivity from the plants consisted of the excreted non-gaseous organic products.

Ecophysiological studies on growth and development of the aquatic macrophyte *Ceratophyllum demersum* L.

Best, P.H. 1979. Ph.D. Thesis, State University of Utrecht, Utrecht. Summary. 104 pp.

Shallow lakes provide a suitable habitat for aquatic macrophytes. In the littoral zone the submerged plants and the submerged parts of the emergent ones may serve as: a) a substrate for colonization by periphyton; b) a habitat for macrofauna; and c) a nutrient source or sink.

In Lake Vechten, a small lake near the city of Utrecht (The Netherlands), the carbon and nitrogen cycles were investigated. A component of this was the study on the growth and development and the factors

affecting these processes of the predominant submerged aquatic macrophyte *Ceratophyllum demersum* L.

Ceratophyllum exhibits two distinct morphologies during the year. Perennation which occurs in the dormant form is characterized by short internodes with densely packed whorls of thick, brittle leaves. In spring, with the initiation of growth, the main axis elongates and the leaves expand. In early summer, vigorous growth occurs, but by autumn dormant buds are formed progressively.

It was demonstrated that growth regulators affect the growth of *Ceratophyllum* in different physiological stages. The effects on growth of indole acetic acid and gibberellic acid were tested. Subsequently, the endogenous contents of indole acetic-, gibberellic- and abscisic acid were measured in freshly harvested plants in order to establish a relationship between their concentrations and the growth stage of the plant. The gibberellins were actively involved in the interruption of dormancy. Since the endogenous concentration of IAA increases only after dormancy is broken, it is unlikely that IAA is directly involved in dormancy interruption. The role of ABA in the induction of dormancy in *Ceratophyllum* is not clear, although the high ABA concentration in dormant plants prevents growth in winter. Induction of dormancy may be regulated by another growth inhibitor, not yet identified. Dormancy was broken by both high as well as low temperature treatment and by GA.

Hormonal effects only become visible when growth occurs. Growth is largely dependent on photosynthetic activity and on the nutrient status of the plant. Therefore, the photosynthetic and morphogenetic capacities were determined simultaneously during the transition from dormancy to the full grown stage. It was demonstrated that the period in which morphogenetic processes such as elongation, bud initiation and sprouting occur precedes that of maximum photosynthetic activity.

Distinct seasonal changes occurred in the nutrient contents of *Ceratophyllum* plants harvested from Lake Vechten. In winter, high carbon, nitrogen, starch and sugar contents were found. Preceding vigorous growth in early summer, a large part of the nitrogen was bound as protein pointing to rapid synthesis of enzymes involved in morphogenesis. By late summer, carbon, nitrogen, protein, sugar and starch had declined to low levels.

Comparison of the lowest levels measured of nitrogen and phosphorus of the plants with values indicated as growth limiting, suggests that nitrogen might be the growth limiting factor in Lake Vechten. Therefore, the effect of increased nitrogen concentrations on the occurrence and growth of *Ceratophyllum* was tested. Growth and morphology were not affected

by nitrate, but stimulated by ammonia added in low concentrations and inhibited by ammonia added in high concentrations.

Although an increased nitrogen concentration in the ambient water caused an increase in the nitrogen content of the plants, almost no effect was detected on the contents of protein and amino acids, or on the amino acid composition. Thus, eutrophication, coupled with an increase in the nitrate concentration of the water and sediments, does not affect the occurrence and growth of *Ceratophyllum*, whereas an increase in the ammonia concentration might cause the disappearance of this plant.

3.3.7. Reports

Pigmentsamenstelling van fytoplankton en periphyton gedurende de jaarcyclus (The pigment composition of phytoplankton and periphyton in the course of the year).

Stekete, J.J., Student Report No. 1979-1 (in Dutch) Limnological Institute Nieuwersluis, The Netherlands.

Paper chromatographic and spectrophotometric analyses of photosynthetic pigments in the phytoplankton of epi- and hypolimnion and in the periphyton obtained from *Ceratophyllum* plants were made. The principal aim of this study was to describe the respective algal communities in terms of pigment composition. Most groups of photosynthetic micro-organisms possess specific chloroplast pigments, which can be used as marker pigments. The share of the different groups of organisms in the light absorption of the total population can be calculated based on their concentrations.

The epilimnion was studied from August 1977 to June 1978. Dinophyceae (*Ceratium hirundinella*) dominated (share of 70%) until November as calculated on the basis of their specific pigment peridinin. During winter and early spring the algal population was dominated by Bacillariophyceae (*Stephanodiscus* sp.) and Chrysophyceae (*Dinobryon* sp.) as was obvious from the occurrence of fucoxanthin. Also Chlorophyceae (*Chlorella* sp.) were present as seen by the chlorophyll *b* spot on the chromatogram.

In the hypolimnion, studied from May till the overturn in November 1977, the bacterio-chlorophylls *a*, *d* and *e* were discovered, indicating the occurrence of Chlorobiaceae and Chromatiaceae. The occurrence of zeaxanthin was indicative for a bloom of Cyanobacteria.

The periphyton was studied from August to November 1977. That Bacillariophyceae and Chlorophyceae were dominant was calculated from the concentrations, respectively, of fucoxanthin and chlorophyll *b*. The results of pigment analyses were

correlated with those of microscopic analyses of phytoplankton, and seston dry weight, ash free dry weight and elementary analyses (H, C and N).

Het effect van stikstof, in de vorm van ammoniak, op de groei en het N-metabolisme van *Ceratophyllum demersum* L. (The effect of nitrogen, in the form of ammonia, on the growth and the nitrogen metabolism of *Ceratophyllum demersum* L.).

Bosman-Rekers, M.M., Student Report No. 1979-2 (in Dutch)

Limnological Institute, Nieuwersluis, The Netherlands.

Ammonia was added to *Ceratophyllum* plants in concentrations ranging from 0 to 5 $\text{NH}_4\text{-N mg. l}^{-1}$ in the culture solution, and its effect on the growth, and nitrogen and carbohydrate metabolism investigated. Ammonia addition caused a decrease in dry weight. A concentration of 0.5 $\text{NH}_4\text{-N mg. l}^{-1}$ stimulated growth. A concentration of 5 mg. l^{-1} $\text{NH}_4\text{-N}$ initially had a similar effect, but it became toxic after several days of N-treatment. This toxicity might be due to: a) uncoupling of the photophosphorylation and thus a decrease in energy supply, and b) a rise of the cell pH and a consequent damage to cell components.

During the early stages of growth, more nitrogen was absorbed than later on; this was reflected in the nitrogen and the protein contents of the plants. At the same time the carbohydrates in the plants decreased by about 25%. By the ammonia addition protein synthesis probably was intensified, and the concomitant degradation of the carbohydrates provided skeletons for amino acid synthesis and energy for the protein synthesis.

3.4. WORKING GROUP 'MINERALIZATION OF ORGANIC MATTER'

(Th.E. Cappenberg, H. Verdouw, J.J. Olie, A.G. Wisselo, E.M.J. Dekkers, R. van Keulen, C.A. Hordijk, A.M.M. Jansen and E.F. van der Heide.)

3.4.1. General Introduction

As part of the coordinated and integrated ecosystem studies in Lake Vechten the aim of these studies is to investigate the dynamics of the cycling of carbon, nitrogen and phosphorus in the limnetic region and in the sediments. Emphasis is laid on aerobic and anaerobic mineralization of organic carbon (C), nitrogen (N) and phosphorus (P) compounds produced during primary production processes, to their inorganic states. Attention is given particularly to the kinetic aspects of these mineralization processes, as related to their relevant field observations. Also, ecological interrelations between micro-organisms observed in the field, are being tested in the

laboratory. In this regard, the organisms are cultured under defined conditions using chemostats and their kinetic relationships (using labelled compounds) are studied.

3.4.2. Aerobic mineralization

General

For the third year in succession the relationship between release rates of extracellular organic products of the phytoplankton, and numbers and activity of heterotrophic bacteria using these compounds as their energy and carbon sources were studied. This relationship is one of the important phenomena in the aerobic breakdown of organic matter in the upper layers of Lake Vechten. The photosynthetic carbon fixation, excretion of photosynthetic products, bacterial cell counts and chlorophyll *a* concentrations (used as an index of phytoplankton biomass), were simultaneously measured at various depths in the lake.

Field observations

Due to the severe winter conditions the total carbon fixation (sum of carbon fixation and carbon excretion), was measured (^{14}C -carbon method) only from March onwards. Therefore, the spring maximum was missed. The carbon fixation rate increased steadily from March till June and highest values were observed in August ($80 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$). Both the total carbon fixation and the per cent extracellular release (p.e.r.) of photosynthetic products ($40 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$) as well as the concentrations of chlorophyll *a* showed maxima in August.

During this period, the highest production rate ($50 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$) was observed in the water stratum between 4 and 6 metres. In this stratum, the biomass of phytoplankton consisted mainly of *Mallomonas caudata*, *Ceratium hirundinella*, *Phacotus lenticularis*, *Dinobryon divergens* and *Chlorella* sp. A bloom of the last named species was indicated by an increase of chlorophyll *a* concentration (maximal values of $50 \text{ mg} \cdot \text{m}^{-2}$). In August the productivity at this depth was extremely sensitive to solar radiation (fixation rate of $50 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$ on a sunny day in contrast to $6 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$ on a cloudy day). Furthermore, the excretion of assimilated carbon was notably high at this 4 to 6 m layer and amounted from 40 to 60 per cent of total fixation. During September the rates of C-fixation and of p.e.r. decreased gradually, and after the overturn in the first week of November these values declined to low levels.

The p.e.r. appeared to be independent of depth; there was no increase in p.e.r. in the surface layers where high light intensity inhibited C-fixation. Also, there was no significant correlation between photosyn-

thetic C-fixation and chlorophyll *a* concentration in these upper layers. A significant correlation was found between productivity, chlorophyll *a* concentrations and numbers of heterotrophic bacteria (estimated by microscopic epifluorescence techniques) at deeper layers of the lake only during stratification.

Methodological studies

Besides the field observations, several methodological investigations were made. This, in order to obtain further substantial evidence to test the hypothesis if extracellular carbon sources form an important substrate for bacterial growth. Analyses of the excreted carbon compounds for their chemical structure were attempted, notably by means of Sephadex-gel filtration with ^{14}C -labelled excretion products.

Separation and characterization of the excretion products was done by Sephadex G 25 column chromatography, after calibrating this column for molecular weights. Finding the appropriate buffer system in which the used calibration substances dissolve and separate into separate peaks was difficult. For instance, Bacitracin (molecular weight of 1410) can polymerize in contact with oxygen under certain conditions. By eluting this compound in 0.05 M NaHCO_3 -buffer at pH of 9.6 under N_2 -flushing the following elution pattern was found (See Fig. 18a). Diminishing the oxygen content of the eluting buffer by reducing agents, no effect to the pattern was seen. According to Merck Index, Bacitracin is not stable in basic solutions. By eluting this compound with distilled water at pH 6.7 and without N_2 -flushing, an elution pattern as seen in Fig. 18b was recorded. In conclusion the pH is more important in this respect than anaerobic flushing conditions as can be seen from the different elution patterns of peak No. 2. Further analysis of the photosynthetic excretion products is in progress.

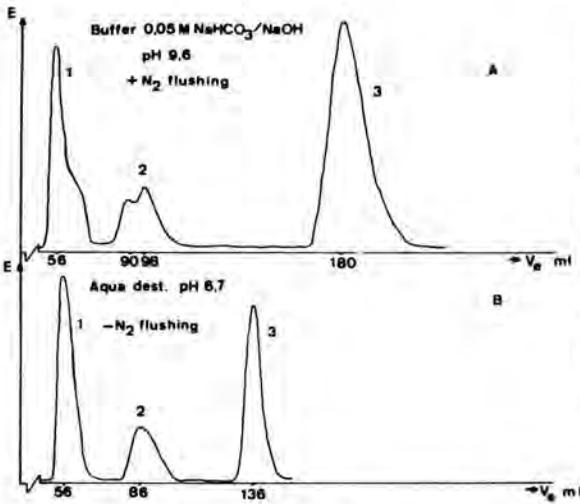
In the coming year studies will be extended to the turnover rates of mineralization and breakdown of the pool sizes of dissolved and particulate organic carbon in the pelagic zone of the lake, and to direct uptake studies of photosynthetic products by the heterotrophic bacteria attached to algae. Also, measurement of *in situ* activity of heterotrophic bacteria by the use of various autoradiographical and chromatographical techniques will be attempted.

3.4.3. Nitrogen and phosphorus cycle

General

In 1979 studies on the phosphorus cycle in Lake Vechten were started. Their main purpose is to follow the course of phosphorus mineralization in organic material together with that of carbon and nitrogen.

Fig. 18. Influence of pH and N₂-flushing on the elution patterns of Bacitracin and 2,4-dinitrophenylalanin from a Sephadex G 25 column with a flow rate of 10.1 ml·hr⁻¹. Peaks 1, 2 and 3 are, respectively, Dextran blue (MW >5000), Bacitracin (MW = 1410), and 2,4-dinitrophenylalanin (MW = 255).



These studies will include mass balance and turnover rate measurements of relevant compounds in the limnetic region and in the sediments.

Field observations of nitrogen compounds

For the third successive year nitrogen compounds were measured in the pelagic region of the lake in 1.2 m water strata from surface to bottom. The field work was extended with samples closer to the shore, to check if the transport of particulate material along the slopes of the lake bottom, for which indications were found last year, would have an appreciable effect on the figures of the total nitrogen content as calculated from samples in the deepest area only. At three sampling points on the 8 m contour around the normal sampling station (See Fig. 19), the concentrations and sedimentation rates (using sediment-traps) were measured monthly.

The results show, that close to the bottom sometimes significant differences in concentration occurred, which might locally affect the subsystems of the overall nitrogen cycle. For the basic mass-balance model of the cycle these edge-effects are apparently unimportant. Sedimentation measurements, however, showed that considerable differences in sedimentation rates existed between the point at 8 m depth in the middle and the point near the shores. An unquantified

amount of particulate matter seems to be transported to the hypolimnion along the edges of the lake, thus escaping measurement by the sediment-trap method. In conclusion, the sediment-trap data would underestimate the sedimentation rates.

Nitrogen transport and transformation studies

The data of three complete year cycles concerning concentration patterns of ammonia, nitrate, nitrite, P.O.N. (particulate organic nitrogen) and D.O.N. (dissolved organic nitrogen), together with the sedimentation data collected so far, would be the basis for more detailed studies of different processes involved in the nitrogen transport and transformation. A major problem regarding the cycle, especially in connection with sedimentation, is the transport of nitrogen compounds to the epilimnion during the stratification period. The nitrogen content of the epilimnion decreases during this time, but the measured sedimentation of nitrogen exceeds the amounts involved, particularly in the middle of the stratification period.

Transport of ammonia will be considered in the first place, as a strong concentration gradient of this compound develops in the hypolimnion: an increase of 4 mg.l⁻¹ is observed between 8 m depth and the bottom. Next to the transport calculations (e.g. eddy diffusion), the turnover rate of this nitrogen fraction will be measured, using the ¹⁵N labelling technique. Ammonia transport from the sediment is also important during the circulation period, since besides nitrification, this is the main nutrient-restoring process in the lake. Experiments will be conducted in the field and in laboratory systems, to determine actual rates of these processes within the nitrogen cycle. In addition to the transport studies, ¹⁵N-tracer experiments on nutrient uptake by macrophytes were continued and are mentioned in part 3.3.2.



Fig. 19. Location of sampling stations in Lake Vechten. V = sampling station in the deepest area of the eastern depression (10.5 m) and X, Y and Z are the sampling stations on the 8 m contour.

The changes in the amount of different phosphorus compounds were measured at the same sampling stations and time intervals as mentioned for nitrogen. The following determinations were carried out: $PO_4\text{-P}$, P_{diss} and P_{tot} , from which $P_{\text{org}} (=P_{\text{diss}} - PO_4\text{-P})$ and $P_{\text{part}} (=P_{\text{tot}} - P_{\text{diss}})$ were calculated. Taking into account the volumes of the different water layers, the total amounts of the P-fractions at different depths were calculated. Since Lake Vechten may be considered a closed system with respect to phosphorus, the sum of all the fractions must be constant in time. However, this was not found true, especially during the stratification period. It is likely that exchange processes at the water-mud interface would explain these observed discrepancies. Studies on these kinetic aspects and more field observations are needed.

Also the sedimentation of organic and inorganic P-compounds, as for the nitrogen compounds, was measured. The amount of phosphorus reaching the bottom during the year is estimated to be about 18 kg of P, giving a downward flux of about $50 \text{ g P} \cdot \text{day}^{-1}$ from the photogenic zone in the eastern part of the lake. The calculations based on the measurements along the profiles V, X, Y and Z (See Fig. 19) showed that the average annual total P-content of the photogenic zone was about 2.5 kg of P, and that of the hypolimnion about 1 kg of P. The differences are only due to those in the volume of the epi- and hypolimnion, the latter containing about 25% of the total water volume. Taking into account the annual downward flux of P and the actual pool size of P, there must be an upward flux towards the epilimnion. The transport of P-compounds from the bottom may be explained by a steep concentration gradient, which develops during the anaerobic conditions in the hypolimnion. Release rates of P-compounds will be investigated in laboratory systems e.g. by the use of ^{32}P -isotope techniques.

3.4.4. Anaerobic mineralization

General

Anaerobic mineralization is an important but still little understood route of carbon flow in aquatic ecosystems. Production of methane becomes the terminal step when alternative electron acceptors are absent, this also is the case in Lake Vechten. We have estimated that as much as one fourth of the primary production could be recovered as methane in the summer season. The kinetic aspects of the breakdown pathways and turnover rates of algal cell walls and their components as important metabolic intermediates in methanogenesis were investigated.

With an improved Hungate roll-tube technique actively growing bacteria were counted on an algal cell wall substrate (*Chlorella* sp., a dominant algal species in Lake Vechten with maximal numbers of about $3,000 \text{ cells} \cdot \text{ml}^{-1}$). The number of bacteria was about $2 \cdot 10^6$ per ml of wet mud, in the sediment stratum of -3 cm throughout the year. Products formed from the isolates (from the lowest dilution series growing on algal cell walls) were mainly acetate, propionate, butyrate, valerate, and to some extent lactate. Also the gases CO_2 and H_2 (correlated with the formation of butyrate) were formed; in these monocultures the products formed frequently inhibited growth. This quantitative formation of end-products gave some evidence of a coupled oxidation-reduction reaction system between two or more interacting anaerobic bacterial species. During fermentation of one initial substrate the so-called interspecies hydrogen transfer; occurred; it will be studied in more detail using chemostat culture-systems.

Microscopic observations (epi-fluorescence techniques) of the sedimented material, collected from sediment traps or from the topmost layer of sediment cores, showed only algal cell wall debris, skeletons of diatoms and zooplankton, and to some extent dead leaf material which had undergone substantial biodegradation. The organic content of these sedimented materials was about 18 per cent of dry weight or 30 mg per ml of wet mud, while CHN-analysis of algal cell walls gave a C:N ratio of 8.3:1 which is comparable to the ratio in sedimented organic matter. As the content of N and C in freshly harvested algal cells is about a three fold higher, a considerable amount is broken down during and after settling of the cells. Nevertheless, more information is needed about the turnover rates, with respect to carbon cycling in the anaerobic environments.

The output of carbon from the sediments was measured by incubating mud-samples, collected from undisturbed mud-cores obtained from the sampling points V, K, Y and Z (See Fig. 19) under *in situ* circumstances. There were no significant differences among the four points: the rate of production of the gases CH_4 and CO_2 was about $1.2 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{day}^{-1}$ which is equivalent to about 0.14 mg of $C \cdot \text{cm}^{-2} \cdot \text{day}^{-1}$. This figure is comparable to the rate of C input measured using sediment traps close to the bottom. But more detailed information is needed concerning the rate of production and the apparent diffusion coefficient of methane *in situ* using dialysis probes.

For these methane production studies spectrofluorimetric determinations of cofactor F_{420} in sediment samples were conducted in collaboration with

M.D. Delafontaine, University of Louvain. This factor, because of its specificity for methane-producing bacteria, can be used in monitoring the potential methanogenesis. The mean values for the four sampling stations did not significantly differ (19.3 nmol $F_{420} \cdot g^{-1}$ sediment). In contrast to this, in the mud cores higher values for the -5 cm layer than for the -2 cm layer (mean values of 12.4 versus 7.6 nmol $F_{420} \cdot g^{-1}$ sediment) indicated a higher production potential of methane in this layer. This is in agreement with earlier observations in that at this depth maximum numbers of methanogens were found. In conclusion, for estimating the output of carbon for the total anaerobic bottom area (the zone below the 8 m contour) one sampling point seems sufficient.

Methodological studies

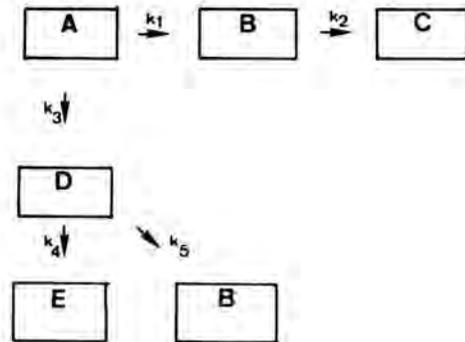
The methods to isolate pure cultures of sulphate-reducing bacteria and for bacteria growing on algal cell walls were successful by the use of strict anaerobic techniques in combination with 'Coy' anaerobic chamber. With these isolates symbiotic relationships between bacteria involved in the breakdown of organic matter in sediments will be studied.

Qualitative and quantitative analyses of non-volatile lower fatty acids in mud-cores were attempted using various catalyzed derivatization techniques for gas chromatography analysis. The technique was found unsatisfactory for the low concentration ranges occurring in the natural environment; this was especially so with respect to lactate, a key intermediate through which most of the decomposition processes are channelled. High speed liquid chromatography and radiochemical methods will be used to follow the actual mineralization rates and breakdown pathways (for schematic representation see Fig. 20). Thus, the use of a few tracer substrates should be sufficient to quantify, by measuring respective pool sizes and turnover rate constants of these substrates, the major part of the carbon flow in the detritus food chain in sediments.

3.4.5. Miscellaneous

Cooperation with Dr. J.W. de Leeuw and co-workers of the Organic Geochemistry Unit of Delft University of Technology, Delft, was continued. During the stratification period samples were taken from various water layers and from the sediment traps and sediment surface for pyrolysis mass spectrometric and other analysis. The chemical composition of the particulate material was compared to study the fate and breakdown of organic matter in the lake. In principle, upon pyrolysis the non-volatile organic material is thermally fragmented under well-defined conditions, yielding a mixture of largely volatile fragments, which

Fig. 20. Simplified schematic representation of lactate breakdown in anaerobic mineralization; A = lactate, B = acetate, C = $CH_4 + CO_2$, D = propionate, E = butyrate; k_1 , k_2 , k_3 , k_4 and k_5 are their respective turnover rate constants, and D/A_0 an example of first-order kinetic equation for calculating k_3 .



$$\frac{D}{A_0} = \frac{k_3}{(k_1 + k_3) - (k_4 + k_5)} \left[e^{-(k_4 + k_5)t} - e^{-(k_1 + k_3)t} \right]$$

is characteristic for the original material under investigation. Thereafter, the series of mass spectra (finger-prints) are compared accurately by computer-multivariate analysis. A preliminary comparison of the different size classes of the particulate organic matter with Py-MS analyses, their C:N ratios and their microscopic observations, combined with specific colour reactions, was done. This gave more substantial information about the mineralization in the stratifying lake. Further analysis of the data is in progress.

3.4.6. Articles in press

Use of radio gas chromatography in studying breakdown processes of organic matter in aquatic ecosystems.

Cappenberg, Th.E., In: Agrochemical Residue-Biota Interactions in Soil and Water with particular reference to isotope studies.

Panel Proceedings Series. International Atomic Energy Agency, Vienna, 1979.

The kinetics of anaerobic breakdown of organic matter in the sediments into methane and carbon dioxide was investigated. Rates of dissimilation of important intermediate metabolites, i.e. acetate and lactate, were measured by the disappearance from mud-samples of ^{14}C -labelled substrates using a gas chromatography-gas proportional counting system. The turnover rate constants (k -values) were calculated and rates of disappearance given by the product of the

turnover rate constant and its pool size. The breakdown of intermediates to methane and carbon dioxide is followed by experiments with uniformly and non-uniformly ^{14}C -labelled substrates in which the labelled compounds are not broken down fully; the conclusions drawn are based on the distribution of the radioactivity. Finally, attention is paid to the use of radio gas chromatography as a tool to study the effects of specific inhibitors in degradation studies.

Microbial breakdown processes of organic matter in anaerobic freshwater ecosystems.

Cappenberg, Th.E., *Advances in Microbial Ecology*, Vol. 4. New York, Plenum Press.

The chapter aims to evaluate present knowledge of anaerobic mineralization of organic matter by micro-organisms in relation to the carbon cycle in freshwater environments. Attention is paid to the availability of organic constituents for microbial utilization and the consequent mineralization of this organic matter to the inorganic state in sediments and in the overlying water layers. Emphasis is laid on the dynamic behaviour of typical electron acceptors, and the concentration of electron donors in a stratifying lake-system. The kinetic aspects of these processes in these ecosystems are strongly emphasized and are related to relevant field observations. Turnover rates of important intermediate metabolites are given and methods for calculating these rates evaluated. The use of ^{14}C -labelled substrates to follow breakdown of intermediate metabolites and, finally, the significance of the anaerobic mineralization in the carbon cycle in freshwater ecosystems is discussed.

Microbial interactions in anaerobic mineralization: A case of interspecies hydrogen transfer.

Cappenberg, Th.E., L. van Breemen and J. Kaper, *Microbiol. Ecol.* 7.

The coupled oxidation-reduction reaction between two interacting anaerobic bacteria during fermentation of one initial substrate is described. This metabolic coupling is manifested as interspecies hydrogen transfer of electrons in the form of molecular hydrogen from H_2 -producing heterotroph to H_2 -utilizing terminal electron acceptors such as methanogenic bacteria. The influence of the H_2 -producing, sulphate-limited *Desulfovibrio desulfuricans* on the fermentation of limiting amounts of H_2 by *Methanobacterium formicicum* is investigated using mixed continuous culture studies. In addition, results are given of relevant field observations obtained from Lake Vechten. The case of a symbiotic relationship described would supplement the existing information on the ecological interactions between bacteria in the breakdown of organic matter in sediments.

Cerimetry, a combined method for COD and dissolved O_2 .

Golterman, H.L. and A.G. Wisselo, *Freshw. Biol.*

A cerimetric method is described in which Ce^{3+} salts are used for the determination of dissolved oxygen and Ce^{4+} salts for the determination of COD. A solution is presented for the COD interference in the O_2 determination, a common feature of most Winkler determinations. The precision is $100 \pm 1\%$, the accuracy 100%. The method is simple, quick and reliable. The precision is compared with that of the Winkler determination.

Iron and manganese in Lake Vechten (The Netherlands); dynamics and role in the cycle of reducing power.

Verdouw, H. and E.M.J. Dekkers, *Arch. Hydrobiol.*

The concentration patterns of iron and manganese in the hypolimnion of Lake Vechten have been followed during three annual cycles. Supersaturated conditions with respect to FeCO_3 and MnCO_3 were observed in the hypolimnion during the stratification period, suggesting concentration control by carbonate formation. In the case of iron also FeS precipitation was observed.

Of the total iron present in the 0–12 cm sediment layer only about 1% appeared in the hypolimnion; for manganese this was about 25%. For the mechanism involved in the transport from the sediment to the hypolimnion bioturbation by *Chaoborus* larvae is suggested.

Only a small fraction (in the order of 1%) of the amount of reducing equivalents, generated in the primary production process, is represented in the hypolimnetic iron/manganese system.

3.4.7. Published articles

Kinetics of breakdown processes of organic matter in freshwater sediments.

Cappenberg, Th.E., *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 12: 91–94.

The anaerobic breakdown processes of organic matter can play an important role in the carbon cycle in aquatic environments. They are considered as a two-stage process, consisting of organic acid formation and gas formation. In the first stage a heterogeneous group of micro-organisms is active, in the second stage, the end-products of the metabolism of the first stage are converted by methane-producing bacteria to CH_4 and CO_2 . Emphasis is laid on the kinetic aspects of these processes in sediments of Lake Vechten. By gas chromatography-gas proportional counting system simultaneous analysis of the turnover and breakdown of lower fatty acids in bottom deposits was possible. Since the turnover rate constants, pool sizes and

breakdown pathways of acetate are depth-dependent, the metabolism of acetate in mud is rather complex. Lactate is broken down simultaneously into propionic, butyric, acetic acid and CO₂. This is a kinetically complex system, the kinetic parameters being depth-dependent in the mud.

3.4.8. Reports

Vergelijking van stikstofsedimentatie op verschillende punten in de plas 'Vechten' (maart t/m november 1979) (A comparison of nitrogen sedimentation at different stations in Lake Vechten (March–November 1979)).

Dekkers, E.M.J., Intern. Report No. 1979-2 (in Dutch)
Limnological Institute, Nieuwersluis, The Netherlands.

During the period March–November 1979, sedimentation rates were measured in the eastern depression of Lake Vechten at 8 m depth at 4 sampling locations, one in the middle in the deepest area about 2 m above the bottom and three along the shore at about 0.5 m above the bottom.

The amount of sedimentating material collected near the shore was larger than in the middle in terms of dry weight per cm² per day. The nitrogen content, however, was higher in the middle. The above mentioned differences were, therefore, smaller, when expressed as amount of nitrogen per cm² per day. When the nitrogen content of the organic fraction of the col-

lected material was considered, only small differences remained between the sampling points. The material collected near the shore had a relatively low percentage of organic matter.

Anaerobe afbraak van algenwanden in de modder van 'Vechten' (Anaerobic breakdown of algal cell walls in the mud of Lake Vechten).

Stoffels, J.J., Student Report No. 1979-4 (in Dutch)
Limnological Institute, Nieuwersluis, The Netherlands.

The study is a contribution to our knowledge of the first stage (acid formation) of anaerobic mineralization of carbon compounds in Lake Vechten mud. By means of a slightly modified Hungate roll tube technique algal cell walls (*Chlorella* sp.) fermenting, anaerobic bacteria were isolated from Vechten mud. An attempt was made to isolate cellulose converting bacteria, but growth on MN 300 cellulose could not be detected. The number of algal cell wall-splitting bacteria was about 10⁶ per ml of wet mud. A depth gradient in the mud (1 to 6 cm) was lacking. Gas liquid chromatography analysis showed acetic acid to be the most important end-product of the first stage of mineralization. Also found were: fatty acids, propionic, isobutyric, butyric, isovaleric and lactic acid, and the gases, H₂ and CO₂. At maximal acid concentrations the bacterial growth stopped and sporeforming took over. Attempts to analyze the breakdown pathways and study the kinetics of ¹⁴C-labelled algal cell walls after incubation of mud samples did not succeed.

4. Miscellaneous

4.1. VECHTPLASSEN RESEARCH

(P.H. Best and R.D. Gulati)

All the lakes in the 'Vechtplassen' area except the Wijde Blik and the Lake Maarsseveen, are shallow; they have been formed as a result of excavation for peat. Dredging of sand resulted in a few deep and thermally stratified lakes. The lakes are limnologically different, particularly due to their hydrological regimes, location, morphology and due to their recreative use. They range from nearly unpolluted and thus relatively less eutrophic waters, to moderately polluted and to grossly contaminated and highly eutrophic ones. The lakes in this area are the objects of limnological studies at the Limnological Institute since 1968.

Since 1975 some staff members participate in the research programme of the Limnological Laboratory

of the University of Amsterdam, focussed on the ecosystem of Lake Maarsseveen.

4.1.1. Studies in Lake Maarsseveen

The aim of this project started in 1977 is to investigate several aspects of the littoral zone in order to find possible relationships between different organisms, plants as well as animals. This year the changes in biomass of some macrophytic communities were studied. The communities were also examined by the National Institute for Nature Management (RIN) with respect to their macro-invertebrate inhabitants, notably 'meadow-forming' Charophytes and *Potamogeton lucens*.

4.1.2. Studies in the Northern Vechtplassen

Mr. G. Cevaal (State University of Utrecht), investigated the differences in the grazing activity — food assimilation rates — of laboratory-grown *Daphnia magna* in waters from the lakes in the River Vecht area. The waterbodies chosen were the

same as for an earlier study using zooplankton from Wijde Blik.

The main objective of the present study was, first, if the effective filtering rates by *Daphnia magna* were indicative of differences in the *in situ* food concentrations, quality, or both; secondly, by using a standard, laboratory-cultured *Chlorella* food in filtered lake waters, the differences in feeding rate, if any, could be correlated to differences in water quality.

The water quality of the lakes in the area differs significantly due to the prevailing hydrological regime, and pollution by water from the River Vecht via the Hilversum Canal. The seston (<33 μm \varnothing) concentrations in the water bodies examined are summarized in Table 7.

The data so far analyzed indicate that the effective filtering rates of *Daphnia magna* are inversely correlated to food concentration, i.e. lowest in Wijde Gat, and highest in Wijde Blik; the filtering rates in the canal and sluice waters lay between the extremes in the lakes (Table 8). Further, the results obtained do show that these differences between Wijde Blik and Wijde Gat increase with size of the animals (See Table 8), particularly, because of relatively much higher filtering rates of the large animals in Wijde Blik. It is likely that the lakes differ also in their size spectra of the food particles available for bigger animals. A relative scarcity of large food particles in Wijde Blik would tend to stimulate the bigger animals, and would also contribute to their increased effective filtering rates in the lake.

The filtering rates of *Daphnia* in 'standard' foods, i.e. filtered lake waters to which laboratory cultured *Chlorella* was added, were similar. This would point at the similarities in the water quality (if the *in situ* food concentration and its quality are excluded) of the water bodies including the canal and sluice.

In addition to the grazing studies, the growth of *Daphnia magna*, expressed as increase in length, and reproduction were also measured in different waters during a three-week period. Though the results are of a preliminary nature, both the growth and reproduction were relatively the best in Wijde Gat, followed by that in the sluice, and Hilversum Canal waters.

Table 7. Seston concentration (mgC.l^{-1}) in the water bodies

Waterbody	Min.	Max.	Mean	\pm	S.D.	N
Wijde Blik	0.4	0.9	0.6	\pm 0.2		6
Wijde Gat	6.7	11.2	8.3	\pm 1.6		6
Hilversum canal	1.4	5.7	3.0	\pm 1.6		6
Sluice	1.3	5.2	2.4	\pm 1.7		5

4.1.3. Articles in press

The submerged aquatic macrophytes in Lake Maarsseveen: species composition, spatial distribution and productivity.

Best, P.H., Hydrobiol. Bull. 14.

The submerged littoral zone of Lake Maarsseveen is well-developed occupying about 25% of the total surface area of the lake. Several macrophytic species which have become more or less rare in the Netherlands occur, notably the angiosperms *Alisma gramineum* and *Najas marina*, and 5 charophytic species. The plant cover can be divided into 5 main vegetation types.

Although charyophytes occupy the largest area of the littoral zone the *Potamogeton lucens* vegetation contributes the most to the total organic matter input of the lake. The productivities of *Potamogeton lucens*, *Elodea* sp. and the charophytes were high but that of *Zannichellia palustris* considerably lower.

4.1.4. Reports

Het effect van de voedselconcentratie, de grootte van de voedseldeeltjes en de watertemperatuur op de filtratiesnelheid van herbivore zoöplanktonsoorten in twee Vechtplassen (The effect of food concentration, size of the food particles and water temperature on the filtration of herbivorous zooplankton species in two 'Vecht' lakes).

Reins, A., Student Report No. 1979-5 (in Dutch) Limnological Institute, Nieuwersluis, The Netherlands.

In the period September 1978–February 1979 the effects of changes in food concentration, water temperature and particle size on the food uptake of some zooplankton species were studied in both 'Wijde Blik' and 'Wijde Gat'. The species were: *Eudiaptomus gracilis*, *Daphnia hyalina* and *Chydorus sphaericus*. A decrease in filtering rate at increasing seston concentrations proved to be evident for all species especially in the low seston concentration range, namely, 0.2–1.1 mg C.l^{-1} (Wijde Blik). The decrease in filtration rate as a result of an increased food concentration was the lowest in the case of *Chydorus sphaericus*. A stimulation of the filtering rate at decreasing food concentrations was compensated by the decrease in water temperature: in the period September–December the filtering rate even decreased despite decrease in the food concentration.

The temperature effect was strongest in the case of *Chydorus sphaericus*. This species was relatively insensitive to changes in food concentration.

Eudiaptomus gracilis and *Daphnia hyalina* reacted similarly to changes in temperature.

Eudiaptomus gracilis filtered seston particles bet-

Table 8. Means of filtering rates ($\text{ml}\cdot\text{hr}^{-1}\cdot\text{ind.}^{-1}$) of *Daphnia magna* of three size classes in different waters, as derived from regressing filtering rate (y) on dry weight (μg) of animals of six separate measurements between August and November, 1979.

Waterbody	Size class ($\mu\text{g}\cdot\text{animal}^{-1}$)			N
	10	50	100	
Wijde Blik	0.11	0.33	0.54	6
Wijde Gat	0.01	0.02	0.03	6
Hilversum canal	0.04	0.09	0.14	6
Sluice	0.03	0.11	0.18	5

ween 3 and 16 μm with a maximum rate for c. 7.5 μm particles.

4.2. BERGUMERMEER RESEARCH

(J.B.W. Wanders, A.F. Richter and J.S. Swart)

The research programme, financed by the Ministry of Public Health and the Environment, was finished at the end of June. A report will be published in 1980. A.F. Richter, working under the supervision of Dr. P. Hogeweg (Department of Bio-informatica, State University of Utrecht), finished his mathematical simulation model describing the effects of temperature and artificial heating on the population dynamics of the most abundant pelagic cladocerans (See also paragraph 2.2.2.).

4.3. PARAKRAMA (SRI LANKA) LIMNOLOGY PROJECT

(R.D. Gulati)

The Institute was invited to participate in the joint limnology project of the Chair of Limnology, University of Vienna, Vienna, and Universities and Research Institutions of Sri Lanka. R.D. Gulati, together with eight other workers from Austria (6), Germany (1) and England (1), was in Sri Lanka during the Phase I (August–September 1979). During this phase of studies the team investigated the general limnological characteristics of Parakrama samudra one of the largest man-made reservoirs in Sri Lanka. Gulati's travel to and stay in Sri Lanka was financed by a grant from the foundation 'Stichting Wetenschappelijke Organisatie voor de Tropen' (WOTRO).

Lake Parakrama, situated about 200 km NE of Colombo, near the ancient town of Polonnaruwa has existed since the 4th century A.D. However, it owes its present form to restoration for irrigation purposes about three decades ago. The lake has three main basins, north, middle and south, and forms one of the

biggest reservoir systems in Sri Lanka (area, 22 km^2 ; water shed, 75 km^2 ; maximum depth, 10 m and maximum water volume $264 \times 10^6 \text{ m}^3$). The lake is also known for rather high fish production (c.

$400 \text{ kg}\cdot\text{ha}^{-1}$), particularly, after the introduction of exotic species of fish, e.g. *Tilapia* spp. For a further rational exploitation of fishery potential and conservation of the lake a thorough background knowledge of limnology of the lakes is essential. In this regard the present study is the first major, coordinated effort.

During the summer of 1979 the water level was extremely low due to the abnormally high discharge at the outlet sluices in connection with repair work on the breach in the dike due to heavy storms of November 1977. The work by different workers was concentrated in the shallow northern basin, particularly, in view of the inaccessibility of the other two basins and limited time and facilities available. Major areas of work included studies of water movements, thermal stratification, light regime, phytoplankton and zooplankton sampling, primary production, zooplankton grazing and egg development time, benthic fauna and benthic respiration; in addition, the nutrient concentrations, feeding behaviour of fish, role of birds (cormorants) in the ecosystem of the lake through their predation on fish, and estimates of cattle grazing and faecal matter released in the lake's surroundings were also studied.

Gulati worked in cooperation with Dr. A. Duncan (England) and Dr. Kurt Bauer (W. Germany) in the production studies besides the assistance imparted by him in the training of graduate students from the Universities of Colombo and Kelaniya. In the first three weeks of August and again in later part of September, the weather was stormy and the lake rough. Thermal homogeneity prevailed despite high water temperature. On calm days in late August, and in early September, however, the lake was diurnally stratified. This resulted in a decreased oxygen concentration in the deeper water strata. In contrast, a supersaturation of the surface layers, resulting from an increase in the oxygen concentration of up to $4 \text{ mg}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, occurred.

The primary production, $420\text{--}740 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{hr}^{-1}$ in the lake was much higher than that often observed in eutrophic temperate lakes. The factors promoting this in Parakrama lake are: high water temperature ($28\text{--}34^\circ\text{C}$) solar radiation (up to $2250 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), besides the favourable nutrient availability due to fast regeneration (higher temperature) and continuous circulation. Moreover, continuous inflow from the river in the south-end is another source of constant nutrient input. Phytoplankton most abundant in the northern basin of the lake, consists of Chlorococcales, diatoms (*Melosira* spp. and *Synedra* sp.) and blue-greens (*Microcystis* sp.). *Anabaena* and *Oscillatoria* species were predominant in the middle and southern basins.

The chlorophyll *a* content in the north basin fluctuated between 50 and 80 mg. m⁻³. The primary production in the deeper water layers is apparently light limited (Secchi disc depth 30–50 cm; extinction coefficient, 2–5) rather than nutrients in view of the factors mentioned.

The crustacean zooplankton was surprisingly absent in the limnetic waters, except for a few individuals of *Phyllodiaptomus* encountered in the Clark-Bumpus hauls on two occasions. The rotifers, nevertheless, seem to fill the niche. A detailed sampling on 27 August revealed both their abundance and the existence of both vertical and horizontal gradients. This was inversely related to flow-in, and in the southern part of the north basin receiving the water from the middle basin the numbers were relatively much lower than in the northernmost part of the lake. The average densities at six sampling stations, which were under the influence of flow-out situation, were around 2000 ind. l⁻¹, in contrast with about twice this concentration found at a station more north-wards. The rotifer fauna was dominated by *Polyarthra* sp., 3 species of *Brachionus*, *Filinia* sp., *Trichocerca* sp. and *Asplanchna* sp. Among Protozoa, *Difflugia* sp., *Didinium* sp. and stalked ciliates were common in samples from deeper strata. The benthic zooplankton was comprised mainly of *Macrothrix* sp. in the northern basin and *Cyclops* spp. in the middle basin. There is circumstantial evidence for mass mortality of benthic molluscan fauna in the period preceding our study of the lake. Exposed dry bed (near shore line) was profusely littered with bivalve molluscan shells. Both fish predation and exposure to dryness, when the water level receded, could have caused this. In the deeper middle basin, molluscs were relatively more abundant, and formed a 'ring' like distribution between the deeper central part, and shallower outer area. Other important components in the bottom fauna, e.g. *Chaoborus* sp., though reported in the earlier works to be abundant, were found lacking.

The fish seems to be apparently abundant; besides *Tilapia mossambica* several other complementary species have been introduced into the lake since 1952. Fish production in the lake (400–450 kg. ha⁻¹) is reported to be one of the highest for water bodies in South-East Asia. The fish captured using gill nets were predominantly *Puntius dorsalis*. There are indications that besides bottom fauna, algae and detrital material constituted important supplementary fish food. It is likely that the drastic drop in water level that resulted in increased fish concentration — and thus increased zooplankton predation — has contributed, besides other factors, to a complete wiping out of the limnetic Crustacea in the lake.

It is proposed to continue the studies on Parakrama limnology, after the monsoon rainy period of

November–December, in February–March, provided, as scheduled, the repair work on the dike is completed and the lake refilled to the level normally prevalent. Further participation is dependent on the provision of the necessary funds.

4.4. POLDER RESEARCH

(R. Veeningen)

4.4.1. Dynamics of the concentration of dissolved oxygen in polder ditches

The project is financed by the Ministry of Public Health and the Environment and has been started in August. The main object is to find out if and how the dynamics of the concentration of dissolved oxygen can be used for water quality criteria for polder ditches. The programme of research is planned as follows:

- to get information about the temporary and spatial pattern of the dissolved oxygen concentration;
- to set up an oxygen-budget for a representative polder ditch;
- to determine the most important factors for the oxygen-budget, i.e. the exchange of oxygen between water and atmosphere and the production and consumption by the autotrophic and heterotrophic subsystems in the water- and sediment-phase;
- to estimate the influence of allochthonic (in)organic pollution on the dynamics of the concentration of dissolved oxygen.

Methods for sampling and measurement of oxygen in micro-gradients in the ditches were tested in laboratory systems. The field-work will be started in ditches in the vicinity of Nieuwersluis in spring 1980.

4.5. SPECIAL PUBLICATIONS

Outside the scope of the working groups and research projects some articles were published, some of them dealing with research from other Institutes.

Growth of *Scenedesmus protuberans* Fritsch in light-limited continuous cultures with a light-dark cycle. Gons, H.J. and L.R. Mur, Arch. Hydrobiol. 85: 41–56.

The chlorococcal green alga *Scenedesmus protuberans* Fritsch was grown in light-limited continuous cultures with a light-dark cycle of, respectively, 16 and 8 hours. The growth was studied by means of electronic particle counting and sizing and measurements of dry weight. Also the algal chlorophyll content and heat of combustion were measured. There are described a number of experiments on the growth during one light-dark cycle, in steady state cultures at a range of dilution rates. The observed fluctuations in the biomass of these cultures are discussed.

Waterbloei.

Liere, L. van, H.J. Gons and L.R. Mur, *Stikstof* 8: 252–257. (in Dutch)

'Waterbloom' is the name given to discoloration of the water of lakes by a superabundance of micro-organisms. Its origin is due to eutrophication, i.e. the 'enrichment' of the water with nutrients. Blooms, in particular of bluegreen algae may have serious detrimental effects on water quality. The actual mechanism by which these algae, instead of other groups, such as green algae, may predominate in a water, has not been revealed by long term intensive research on natural systems; recently, however, important progress has been made by comparing the growth of the green alga *Scenedesmus* and the blue-green alga *Oscillatoria* in continuous cultures. These species quite

differ with respect to the use of light energy; compared with *Scenedesmus*, the growth of the blue-green alga was favoured by light limitation. This was confirmed in the experiments when both organisms were inoculated in the same culture. *Scenedesmus* grew faster than *Oscillatoria* only at relatively high irradiances. With either low incident light or low average light (due to mutual shading at higher population densities) *Oscillatoria* always outgrew *Scenedesmus*. This suggests that the cause of predomination of *Oscillatoria* species after excessive loading of fresh water with nutrients, say phosphates, is the increase in algal biomass by which the distribution of light in the water is changed in such a way that growth of these blue-green algae is favoured over growth of species belonging to other groups.

5. List of articles

5.1. PUBLISHED IN 1979

Beattie, D.M. - A modification of the Ekman-Birge bottom sampler for heavy duty. *Freshw. Biol.* 9: 181–182.

Best, P.H. - Growth substances and dormancy in *Ceratophyllum demersum*. *Physiol. Plant.* 45: 21–28.

Best, P.H. - Ecophysiological studies on growth and development of the aquatic macrophyte *Ceratophyllum demersum* L. Ph.D. Thesis, Utrecht, 104 pp.

Best, P.H. and J.T. Meulemans - Photosynthesis in relation to dormancy in *Ceratophyllum demersum*. *Aquat. Bot.* 6: 53–65.

Cappenberg, Th.E. - Kinetics of breakdown processes of organic matter in freshwater sediments. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 12: 91–94.

Densen, W.L.T. van - The fecundity of the smelt, *Osmerus eperlanus* L., in the Tjeukemeer (Abstract). *Neth. J. Zool.* 29: 623.

Goldspink, C.R. - The population density, growth rate and production of roach *Rutilus rutilus* (L) in Tjeukemeer, The Netherlands. *J. Fish. Biol.* 15: 473–498.

Gons, H.J. and L.R. Mur - Growth of *Scenedesmus protuberans* Fritsch in light-limited continuous cultures with a light-dark cycle. *Arch. Hydrobiol.* 85: 41–56.

Haan, H. de and T. de Boer - Seasonal variations of fulvic acids, amino acids and sugars in Tjeukemeer,

The Netherlands. *Arch. Hydrobiol.* 25: 30–40.

Haan, H. de, T. de Boer and G. Halma - Curie point pyrolysis mass spectrometry of fulvic acids from Tjeukemeer, The Netherlands. *Freshw. Biol.* 9: 315–317.

Liere, L. van, H.J. Gons and L.R. Mur - Waterbloei. *Stikstof* 8: 252–257 (in Dutch).

Moed, J.R. and G.M. Hallegraef - Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectrophotometric measurements. *Int. Revue ges. Hydrobiol.* 63: 787–800.

5.2. IN PRESS

Best, P.H. - Effects of nitrogen on the growth and nitrogenous compounds of *Ceratophyllum demersum*. *Aquat. Bot.* 7.

Best, P.H. - Photosynthesis in *Ceratophyllum demersum*. Carbon fixation rates in relation to the plants' physiological stage, and the contents of chlorophyll and non-structural carbohydrates (Summary). *Hydrobiol. Bull.* 13.

Best, P.H. - The submerged aquatic macrophytes in Lake Maarsseveen: species composition, spatial distribution and productivity. *Hydrobiol. Bull.* 14.

Cappenberg, Th.E. - Use of radio gas chromatography in studying breakdown processes of organic matter in aquatic ecosystems. In: *Agrochemical Residue-Biota Interactions in Soil and Water with particular reference to isotope studies. Panel Proceedings Series. International Atomic Energy Agency, Vienna, 1979.*

- Cappenberg, Th.E. - Microbial breakdown process of organic matter in anaerobic freshwater ecosystems. *Advances in Microbial Ecology* Vol. 4. New York, Plenum Press.
- Cappenberg, Th.E., L. van Breemen and J. Kaper - Microbial interactions in anaerobic mineralization: A case of interspecies hydrogen transfer. *Microbiol. Ecol.* 7.
- Golterman, H.L. and A.G. Wisselo - Ceriometry, a combined method for COD and dissolved O₂. *Freshw. Biol.*
- Gons, H.J. - Periphyton in Lake Vechten with emphasis on biomass and production of epiphytic algae (Summary). *Hydrobiol. Bull.* 13.
- Gons, H.J. and L.R. Mur - Energy requirements for growth and maintenance of *Scenedesmus protuberans* Fritsch in light-limited continuous cultures. *Arch. Microbiol.*
- Gulati, R.D. and G.H. Würtz-Schulz - Remarks on the present status of limnology in India based mainly on the Indian publications in *Hydrobiologia* and suggestions for future approach. *Hydrobiologia*.
- Nie, H.W. de, H.J. Bromley and J. Vijverberg - Distribution patterns of zooplankton in Tjeukemeer, The Netherlands. *J. Plankton Res.*
- Verdouw, H. and E.M.J. Dekkers - Iron and manganese in Lake Vechten (The Netherlands); dynamics and role in the cycle of reducing power. *Arch. Hydrobiol.*
- Vijverberg, J. - Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, The Netherlands. *Freshw. Biol.* 10.
- 5.3. STUDENT AND INTERNAL REPORTS (IN DUTCH WITH ENGLISH SUMMARIES)
- Bosman-Rekers, M.M. - The effect of nitrogen, in the form of ammonia, on the growth and the nitrogen metabolism of *Ceratophyllum demersum* L. Student Report No. 1979-2.
- Densen, W.L.T. van - Production and ecology of freshwater fish. Internal Report, Sect. General Fish Culture and Fisheries, Agricultural University, Wageningen.
- Graaf, G.J. de - The construction of the head of O⁺ perch and O⁺ pike-perch in relation to their feeding, a morphological and ethological study. Student Report No. 1979-8.
- Dekkers, E.M.J. - A comparison of nitrogen sedimentation at different stations in Lake Vechten (March–November 1979). Internal Report No. 1979-2.
- Kramer, M.A.M. - Food uptake of *Daphnia magna*. Trainee Report No. 1979-1.
- Mesa-Menor, J. - Observations on the food-composition of O⁺ perch, *Perca fluviatilis*, pike perch, *Lucioperca lucioperca* and smelt *Osmerus eperlanus*, during 1978 in Tjeukemeer. Trainee Report.
- Reins, A. - The effect of food concentration, size of the food particles and water temperature on the filtration of herbivorous zooplankton species in two 'Vecht' lakes. Student Report No. 1979-5.
- Schaick Zillesen, P.G. van - Ecology of O⁺ roach, *Rutilus rutilus* L. in Tjeukemeer. Student Report No. 1979-6.
- Schouten, R. - Selectivity of gill-nets for pike-perch, *Stizostedion lucioperca*. Trainee Report no. LH stage 79-1, Sect. General Fish Culture and Fisheries, Agric. University, Wageningen.
- Schrotenboer, G.J. - Growth and development rate of the opossum shrimp *Neomysis integer* (Leach) in relation to temperature. Internal Report No. 1979-1.
- Siewertsen, K. - A biochemical plankton research in Lake 'Vechten'. Internal Report No. 1979-3.
- Siewertsen, K. - The plankton biomass in Lake 'Vechten'. Internal Report No. 1979-4.
- Stoffels, J.J. - Anaerobic breakdown of algal cell walls in the mud of Lake Vechten. Student Report No. 1979-4.
- Steketee, J.J. - The pigment composition of phytoplankton and periphyton in the course of the year. Student Report No. 1979-1.
- Vermeulen, H. - An investigation of the food selectivity of *Eudiaptomus gracilis*. Student Report No. 1979-3.
- Vroom, M. - Preliminary study of the light conditions of Tjeukemeer. Trainee Report.

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