Institutes of the Royal Netherlands Academy of Arts and Sciences

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Netherlands Institute for Brain Research

Progress Report 1980

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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. In 1904 this resulted in the formation 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 Netherlands 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 in the comparative anatomy of the nervous system. Much of the material used in his research, including a considerable number of human and animal brains, is still 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 first 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 a new building for the Institute next to the new Medical Centre of the University of Amsterdam.

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 electronmicroscopic 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 electronmicroscopic 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 rythms, 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, while as a new aspect their changes during aging are studied; 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 plasticity 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. Biomathematical and computational aspects of neurobiology

This workgroup has an essential task in providing experimental and mathematical support to all other research groups. In addition, scientific collaboration projects with group I and II were started, implying (1) methods for analytical description of neuronal growth and (2) modelling of bioelectrical activity in neuronal tissue cultures. From 1981 the group will be equipped with a new very powerful computer (DEC-VAX 11/780).

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 (group I), electrophysiological techniques for the study of the properties of the vasopressinergic synapse (groups II and III). Hormone assays, immunocytochemistry 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 in addition to the study of developmental aspects in the behaviours of these animals. 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. Following pharmacological suppression of REM-sleep throughout infancy, various behaviours, sleep physiology, brain chemistry and morphology have been examined in adulthood. A spectrum of abnormalities has been found, and the question now being pursued is the extent to which this 'syndrome' may in fact be attributed to deprivation of active-sleep in early life.

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, Prof.Dr. F.H. Lopes da Silva and Prof.Dr. J.L. Slangen convened frequently in 1980 together with Prof.Dr. D.F. Swaab, K.E. de Roos and Dr. D. van der Mei (K.N.A.W.), while Prof. R. Balàsz (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 electron microscopic 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.

Experiments were carried out to document the effects of neonatal undernutrition upon the rat brain and to determine the extent to which deleterious abnormalities could be reversed by postweaning refeeding and by postweaning environmental enrichment. Wistar rat pups were undernourished from birth until 30 days post partum (dpp). At 30 dpp, both underfed and normally suckled control animals were either sacrificed or weaned onto an *ad libitum* diet and permanently exposed to differential environments, being housed either in pairs in standard cages or in groups of 10 in large cages containing numerous objects (the large cages are on loan from the Department of Comparative and Physiological Psychology of the Catholic University of Nijmegen, Dr. W. Raaymakers). The latter groups were sacrificed at day 150. Their brains were weighed and Golgi Cox and cresyl violet stained sections were prepared. At the end of the acute period of undernutrition, whole brain weight was decreased by about 23% and forebrain by about 22%. Brain weight-deficits persisted, although alleviated to an extent of 11%, in both male and female animals maintained under standard conditions during refeeding period. Housing in enriched conditions, however, produced a small but statistically significant restoration of these deficits in the brain weight (in cerebellum and forebrain weight) of refed female animals, and not of refed male animals. Whole brain weight, e.g. was 5% greater in refed-enriched females than in refed-standard

females. In behavioural tests male and female underfed animals reacted to enrichment in the same way as controls (see for report on behavioural testing of these animals workgroup IV). This favours the argument that underfed animals are capable of responding positively to environmental stimulation.

Measurements of neocortical area and thickness taken at various standardized points along the length of the corpus callosum, showed no significant differences between refed-standard males and control-standard males at 150 dpp, whereas at 30 dpp they were significantly different especially in the occipital cortex (see Progress Report 1979). Detailed studies of pyramidal and multipolar non-pyramidal (MNPN) cell dendrites were therefore carried out in the occipital region. The somatic areas of both cell types were reduced in 30d-old undernourished male animals. However, only pyramidal cells showed significant dendritic alterations, with deficits in total dendritic length and mean terminal segment length. These parameters in the dendritic arborisations of MPNP cells were unaffected by undernutrition. Refeeding to 150 dpp restored the deficits in somatic size of the two cell types. In the MPNP cells the dendritic patterns were again completely comparable. However, in pyramidal neurons of the refed rats terminal segments were 20% shorter than their control. Thus, pyramidal cells (layers II-III), previously thought to be more resistant to neonatal modification, show greater susceptibility to the effects of early undernutrition than non-pyramidal neurons, since they develop a number of dendritic abnormalities which are not completely restored by refeeding. The (additional) effects of enriched environment are currently under investigation.

The effect of undernutrition on the ultrastructure of the rat cerebellum is investigated with quantitative electronmicroscopy. The initially applied perfusion method was modified according to personal instructions from Dr. V. Chan-Palay (Harvard, Boston) and Prof. C. Sotelo (INSERM, Paris). This resulted in an improved preservation of the ultrastructure such that most of the problems concerning the identification of the cellular processes in the cerebellar molecular layer were eliminated. For the measurements synapses between parallel fibres and Purkinje dendrites (pfP) were distinguished from synapses between parallel fibres and Golgi dendrites (pfG). Other types of synapses which constitute a small and heterogeneous minority were ignored. Comparison of the pfP-synapse profile lengths in 30 day old undernourished and control rats showed a small though significant decrease in mean profile length in the underfed animals. The total amount of pfP-synaptic contact area per unit volume of molecular layer was about the same. With respect to the pfG-synapses these parameters were not different. Very recently an appropriate procedure to estimate the number of non-convex structures in a reference matrix was published by Cruz-Orive, which will be applied to determine the synaptic number. Furthermore measurements are currently carried out to determine the total amount of dendritic area in relation with the synaptic parameters. Measurements of pfP profiles in the molecular layer of 150-day-old rats kept in enriched conditions from 30 days pp reveal that the mean profile length is lower compared to the value that was found at 30 days of age. This applies to both previously undernourished and control animals. A likewise lower pfP profile length was found in the enriched refed rats than in the enriched controls. However, the total amount of synaptic contact area per unit volume was slightly higher in the refed animals. The ultrastructural parameters were measured using a MOP-AMO2 (Kontron) digitizer device directly connected to the computer (Interdata M70). A general acquisition program for this connection comprising simple statistical analysis was developed (see report of group V).

THEME 2. THE DEVELOPMENT OF PREFRONTAL CORTEX IN THE RAT

In relation to the behavioural study of the role of the prefrontal cortex (pfc) (see workgroup IV) a start has been made with the study of the structural development of the pfc in October 1980.

The first approach was to define the cytoarchitectonic criteria on which these areas could be discerned in the Nissl-stained preparations. Because of differences in size of the different cells and their relative density, there is a certain lamination in the cortex. The differences in lamination between certain parts of the cortex can serve as a discriminatory criterion. On the 6th day pp it is possible to define the two projection areas of the nucleus mediodorsalis thalamis from the

bordering areas, as well as most of the sub-areas within the pfc with these criteria. However, because this criterion refers to more than one layer of the cortex, it is difficult, if not impossible, to discern the particular areas near the pole due to the curved structure of this part of the brain. This leaves a part of the pfc indistinguishable from other areas in coronal sections. In horizontal and sagittal sections it is even more difficult. In order to examine the discriminative power of other histochemical techniques, coronal sections will be stained with Timm's sulphide silver method, and for acetylcholinesterase and monoamines.

Until now the Timm's sulphide silver method for the localization of heavy metals and the AChE staining have been applied. The Timm's method stains two bands in most of the cortex, which correspond to the layers I, II and III, and layer V respectively. The silver precipitation, however, is more pronounced in the medial and orbital pfc in paraffin sections. In vibratome and cryostat sections this difference is less pronounced. The border between the cingular and retrosplenial cortex, however, is clear in these sections. With the possible exception of the paraffin sections the Timm's method does not add to the solution of the problem of distinction of the pfc in the polar area. Furthermore, there is only one sub-area within the pfc (area 32) which shows an AChE activity in layer V. Both the Timm's, the AChE staining and the Nissl staining show (each in its characteristic way) that the prefrontal cortex is heterogeneously structured. This will be of importance for behavioural studies of pfc lesioned animals (see group IV).

A start has been made with the staining of monoamines.

THEME 3. THE STRUCTURAL DEVELOPMENT OF THE VISUAL CORTEX IN THE RAT

After the study of the development of layer IV non-pyramidal neurons in rat visual cortex, this project is continued by the examination of the layer II-III pyramidal cell development, in collaboration with Dr. J.G. Parnavelas (University of Texas) and group V. Measurements of the basal dendrites of pyramidal neurons were made from camera lucida drawings using a Kontron MOP-AMO2 digitizer. These drawings included the z-coordinates of points such as the centre of cell body, origins of dendrites, bifurcation points and end points. Programs were developed by group V to facilitate the procedure of collecting and storing the data of the basal dendrites. Counts of primary basal dendrites showed that the mean number at day 6 post partum is 4.3 and increases to 5.2 by day 14. The number of dendritic segments per cell increases markedly in early life and reaches adult values at day 14. The total dendritic length shows a continuous increase from day 6 to adulthood with the largest increase occurring after day 10. The change in the radial distance of dendritic terminal tips from the cell soma shows a small significant decrease from day 6 to day 10, which is visualized by the orientation analyses according to the Ruiz-Marcos method. After day 10 there is a continuous increase to adulthood. A similar course of development is shown by the terminal dendritic segments, while the intermediate segments change only slightly throughout postnatal life. It appears, therefore, that the large increase in the length of the terminal segments between days 14 and 90 accounts for the continuous increases throughout life in total length and radial distance values. In conclusion, these data and the earlier data of the non-pyramidal neurons indicate that the principle that pyramidal neurons mature earlier than non-pyramidal neurons cannot hold in this general formulation. This is in contrast with Jacobson's hypothesis.

Data are currently collected on the group of small and medium and of large layer V pyramidal neurons.

Topologies

Dendritic trees can be characterized by their topological structure. The topological structures can be ranged according to the degree of 'number of terminals' and subdivided into classes of the topologically different dendritic trees. If we assume that the dendrites branch according to a special mode of growth it is possible to calculate the probability of occurrence of any dendritic tree under the condition that it belongs to a certain class of topological structures. For two extreme growth models (segmental and terminal branching) Berry and Bradley generated approximate values for the conditional probabilities of a number of

topological classes by computer simulations. We have derived a set of rules for each of the growth models by which the exact conditional probabilities are easily calculated. Thus for a dendritic tree for which degree and topological type are specified we can determine the probability that it occurs with respect to all other topological types (which we do not need to know!) of the same degree can be determined.

THEME 4. THE INFLUENCE OF VASOPRESSIN DEFICIENCY ON THE RAT BRAIN

This study is continued in collaboration with group III. For the results of the analysis of the cerebellum, medulla oblongata, bulbus olfactorius, colliculi, hypothalamus, hippocampus, cortex cerebri and the rest of the brain (mainly formed by the basal ganglia and the thalamus) in developmental series of HET-DI and HOM-DI Brattleboro rats (aged 8, 12, 16, 20, 24, 30 and 180 dpp) we refer to the report of group III. A quantitative study of Purkinje cell dendritic trees in 180 d HOM-DI and HET-DI is in progress. Nissl-stained preparations are prepared from cerebella of HOM-DI and HET-DI rats of 12, 24 and 180 days old. The areas of the different cerebellar layers in midsagittal sections are currently measured and analyzed.

THEME 5. BRAIN INDICES, A MEASURE FOR ENCEPHALIZATION

A start has been made (by M.A. Hofman) with the study of encephalization of mammals. By relating brain size to body size for a static interspecific series of mammals (249 species) an allometric relation has been developed with an exponent of 0.732, significantly deviating from the usual 0.667. This outcome has important implications because much theorizing on the significance of relative brain size is based on 0.667 exponent along lines of a brain volume-body surface relationship. This relationship is, however, only correct when geometric similarity is maintained with size increase, which rarely happens. On the other hand the new 0.732 exponent fits well in a theoretical framework about relative brain size in which the surface of the cerebral cortex is the main parameter instead of the volume of the whole brain. From an evolutionary point of view this relationship is significant since the cerebral cortex contributes most to encephalization. The new index is also a function of the thickness of the cerebral cortex. In the previous theories the cortical thickness was kept constant, independent of brain size. With the new index a dimensionless measure has become available for relative brain size, emphasizing the meaning of the cerebro-cortical volume in evolution, which enables us to classify mammalian species to their progressive development of the cerebral cortex and to relate the increase in structural complexity to a complexity in function.

Miscellaneous

a. A new semi-automatic measuring system for measuring 3-D neuronal tree structures is designed by the electronics and mechanics department, since the life-span of the present system is too short and the vulnerability is too large (see Progress Report 1979). See for report electronics and mechanics department. The construction of this system is currently executed.

b. A study has been started to determine the factors which induce fading of Golgi-Cox impregnated neurons. Different dehydration procedures and several developers and fixatives were used. Thin and very thick (± 250 µm) sections were prepared. The results show no significant differences between the different procedures used, but they point into the direction that the mounting medium Malinol (especially when it is diluted with toluene) may induce fading. Therefore, we are currently examining different mounting media for Golgi-Cox sections.

c. The effect of differential life-environments has been studied also upon the sleep patterns of rats (group II). At the end of the experimental periods the brains of the different groups were dissected into eight different parts. For the report of the results we refer to group II.

II. Interaction of the nervous system and behaviour during maturation

Our interest in: (1) the neuronal basis of early behavioural rhythms, specifically those related to sleep/waking cycles; (2) the influence of such spontaneous

neuronal activities, especially during active ('REM') sleep, on further nervous development; and (3) mechanisms underlying the formation of selective interconnections among nerve cells (-vide infra: themes 3 and 4) continued unabated in 1980. As was the case last year, *in vitro* tissue cultures were employed as a model system for these phenomena (theme 3), supplementing our studies on the intact organism (mostly using the rat, but some research on the frog was also carried out: theme 4).
All aspects of our program are represented, in the form of three non-subsidized projects, in the FUNGO workgroup 'Development and Aging of the Brain and Behaviour'.

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

Activity of neurons in the pontine reticular formation (PRF)

Two important improvements of recording techniques have been carried out which increased the stability of multiple unit activity (MUA) recording in free-moving animals. First of all, a miniaturized field-effect transistor circuit, small enough to be mounted on the head of very young rat pups, enabled us to record MUA from the pups even during active wakefulness. Secondly, implantation of microwires (ϕ 25 μm) that were glued to a guide-probe by means of soluble sucrose syrup increased the yield of preparations giving a stable signal. Results obtained with this technique corroborated the general pattern described in the literature for reticular neurons in adult rats, as well as our own findings from earlier experiments. At all ages studied (range: 9-39 days) state-related firing of PRF neurons has been found. Both MUA and the activity of selected single units were at their highest level during active sleep (AS) and wakefulness. Especially striking differences in firing levels between active and quiet sleep (QS) were found in animals older than 14 days, the increase during AS often being more than ten-fold. Since chlorimipramine (CLM), a monoamine reuptake blocker, was successfully used to suppress AS in developing rat pups (see below) we also studied the effect of this drug on the activity of PRF neurons. CLM not only suppressed AS for several hours in our animals, but also induced a concomitant reduction of PRF firing activity to QS levels or lower. This result is consistent with a role for PRF neurons in the generation of AS, and for the existence of a serotonergic inhibition acting upon them during QS.

Kaïnic acid lesions of brainstem neurons

In order to directly test the involvement of PRF neurons in sleep-wake regulation, we started a series of lesion experiments using locally applied kaïnic acid (a neurotoxic agent). With this technique we are now trying to destroy as selectively as possible the giant neurons of the PRF. In the course of working out the dose-effect relationships in developing rats, serious survival problems were encountered, due to the extreme brainstem excitation caused by the kaïnic acid. Epileptiform motor discharges could be controlled with the help of anti-convulsant drugs, but the closeness of vital respiratory centres to the target cell area still poses a serious problem. Until now only relatively small lesions have been obtained in the PRF, none of which gave rise to any obvious changes in sleep-wake organization. This is in contrast to electrolytic lesions which we made within the same area, and which resulted in striking changes during both REM sleep ('oneiric' behaviour) and wakefulness (hallucinatory behaviours).

Activity of midbrain raphe neurons

The experiments involving registration of MUA in the dorsal raphe area were rounded off this year. Regular slow discharge rates, characteristic of anesthetized animals, proved to be very rare in awake free-moving rats. All animals showed the highest firing levels during active wakefulness, with a progressive decline of the mean firing rate in quiet wakefulness, drowsiness and quiet sleep, respectively. In a majority of cases the slowest activity occurred during the 'pre-REM' (AS) periods and during motorically inactive portions of AS. The general pattern found during AS was an increase in activity over the QS level, chiefly due to clustering of neural discharges during epochs of phasic motor activity. An overall decrease in MUA during AS as compared to QS was observed

in a few preparations. These results are more variable than those reported for the adult rat, and provide little justification for an extension of this approach to earlier stages of development.

THEME 2. POSSIBLE INVOLVEMENT OF ACTIVE SLEEP (AS) IN BRAIN AND BEHAVIOUR MATURATION IN THE RAT

Neuronal activity in developing cerebral cortex

The results of these experiments were worked out this year and prepared for publication. It was found that, in addition to higher firing rates during AS than during QS, the single and the multiple unit registrations both revealed different *patterns* of discharge from one state to the next. Whereas in AS long barrages of action potentials (sometimes lasting more than 1 sec) occurred frequently, bursting was less frequent and much shorter lasting in QS, due to phasic inhibition of neuronal firing during the large amplitude EEG slow waves. A striking peculiarity in 12-day-old rats was the presence of continuous quasi-periodic fluctuations of bioelectric activity which caused a very high variance in all phases of the sleep cycle.

Longterm sequelae of chronic drug administration in infant rats

Last year's study using clomipramine (CLM) was repeated (in collaboration with group IV), using a larger number of animals, both male and female. In addition, sleep patterns were monitored weekly following the termination of drug treatment, in order to determine the onset of the adult sleep disturbance described last year. As yet, however, no abnormalities have been found up to 6 months of age. The CLM-treated rats in young adulthood showed comparable patterns of behaviour on open-field, dark-preference and masculine sex tests as in the earlier series. The control animals this time, in contrast, showed severely deficient sexual performance and an unusually high anxiety level in the test situations. Significant (drug) treatment effects could thus be confirmed, but, as compared to the controls, they were opposite in direction to those reported earlier! It becomes important now to identify the factor(s) accounting for such inconsistencies in the behavioural development of intended control groups of rats.

Environmental rearing effects on sleep patterns

Male Wistar rats were chronically implanted, at the time of weaning, with electrodes for recording the EEG and EMG. At 1 month of age they were randomly assigned to either *enriched* (EC), *social control* (SC) or *isolated* (EC) rearing conditions. The EC group showed the following changes with respect to both the SC and the IC groups: (a) increase in the amount of quiet sleep; (b) increase in the amount of active sleep; and (c) shortened active sleep latency. The changes were already significant after 3 weeks in the enriched environment, and became progressively more pronounced during the rest of the 2 months exposure. In a second series of experiments, in which the EC treatment was limited to 2 hr per diem, no statistically significant differences in sleep pattern could be found. Nevertheless, these animals showed the well-known increase in weight of the cerebral cortex, as well as a significantly enlarged hypothalamus. We may therefore conclude that environmental enrichment during infancy causes a significant increase in sleep time, but that this effect probably is unrelated to the observed changes in brain morphology. (Collaborative investigation with groups I and III.)

Alternate AS-deprivation procedures

A number of unsuccessful attempts were made to develop an alternative to the clomipramine approach (vide supra) for producing chronic AS-deprivation during infancy. The following procedures were evaluated:

a. local injection into the brain of antibodies made to (cat) brain proteins released during sleep (courtesy of Dr. R. Drucker-Colin, Autonomous University of Mexico);

b. injection into the brain of the acetylcholine inhibitor, scopolamine;

c. localized electrolytic and kaïnic acid lesions in the brain stem reticular formation;

d. environmental conditions such as extremes of temperature and continuous noise of flashing lights.

THEME 3. TISSUE CULTURE MODELS OF DEVELOPING CENTRAL NERVOUS SYSTEM ORGANIZATION AND FUNCTION

Selective sensory innervation of spinal cord explants

Organotypic spinal cord cross sections with attached dorsal root ganglion (DRG) were explanted from 13-14 day mouse embryos and cultured for 2-6 weeks either in serum-supplemented medium or in a chemically defined medium (CDM). CDM supported excellent retention of the *in vivo* cytoarchitecture of the explant through out the culturing period, whereas explants grown in serum-supplemented medium always flattened and often developed necrotic areas, with frequent blurring of the internal structures. Both media supported the development of spontaneous bioelectric activity in all cultures studied. Between cultures, and sometimes within the same culture or even at the recording site, the firing patterns varied from continuous (sometimes very regular) patterns of firing to strongly phasic. Evoked potentials upon stimulation of the DRG were recorded from 41 out of 45 cultures, demonstrating that functional connections were established between DRG and cord explants in either of the media used. Such connections were observed throughout the cord explants, sometimes diffusely spread over widely separated areas (dorsal, ventral, ipsilateral and contralateral), while in other preparations only restricted regions of the cord were functionally innervated.

Two age groups were investigated: 18-23 days and 25-42 days *in vitro* CDM cultures showed an increase with age in the number of points from which evoked activity could be found, whereas the serum-supplemented cultures showed no such increase. Age *in vitro* turned out to significantly influence the distribution of points showing evoked activity, such that in the older explants grown in the presence of horse serum most of the functional sensory connections were made with dorsal cord regions. In contrast, spontaneous activity remained just as abundant in the dorsal as in the ventral half. Iontophoresis of horse radish peroxidase (HRP) onto DRG neurons resulted, in some of the preparations, in the uptake and transport of the enzyme throughout the cell and its processes. Stained fibres were observed to enter the cord explants, and usually could be traced only to those areas of the cord from which evoked bioelectric activity had been obtained.

Growth of cerebral neurons in serum-free, chemically defined medium (CDM)

In order to circumvent the inherent variability of serum-supplemented media, a completely defined serum-free medium has been developed for culturing dissociated and organotypic fetal rat cerebral cortex tissues. Starting from literature data, many experimental trials had to be done before arriving at the proper composition for a serum replacing 'cocktail'. Preincubation in serum-supplemented medium for the first 24 hours after inoculation could be omitted by the addition of corticosterone and triiodo-L-thyronin. Each of these hormones had been found to exert a strong growth promoting influence on the neurons, and in combination a synergistic effect was suggested. The hormones vasopressin and testosterone failed to show any such neurotrophic effect. The general ultrastructure of the cultures as well as the numerical development of 6 different types of synapses on day 12 in this hormone-supplemented, chemically defined medium compared very favourably with the picture seen in serum-grown cultures. Forty cultures grown in CDM, aged 9-23 days *in vitro*, were recorded extracellularly by means of saline-filled micropipettes. Spontaneous firing of action potentials was observed in 33 of these preparations (11-22 days old). Striking was the frequent appearance of large, longlasting slow waves in clumps of tissue (up to 600 μV and 150 ms) (the causal relationships between structural development and functional activity in neuronal networks are being studied theoretically in collaboration with group V).

Chronic suppression of spontaneous bioelectric activity by xylocaine

The xylocaine experiments of last year were completed with a culture series (rat cerebral cortex) grown in serum obtained from Boehringer-Mannheim. This serum gave equally luxuriant outgrowth and synaptogenesis in controls as in

preparations bathed continually in the same medium plus 50 µg/ml xylocaine. The numerical density of synapses also appeared to be unaffected by the absence of neuronal excitation in the treated preparations. Bioelectric activities measured after transfer to the normal recording medium revealed a normal development as compared with the control cultures. These results indicate that neuronal functioning is quite unnecessary for many important aspects of cerebral development.

THEME 4. DEVELOPMENT OF MISDIRECTED WIPING REFLEXES IN FROGS

Topography of sensory ganglion cells

The topographic arrangement of cells within the DRG of the frog *Rana pipiens* appears to be only weakly polarized, with large individual variations in the size and number of cobalt-filled profiles occurring within control and experimental (early skin-rotated) groups. In the sham-grafted group the ratio of filled profiles in the dorsal as opposed to the ventral half of the ganglion was 1:9, whereas the ratio in the skin-rotated group was 1:6. No differences in DRG neuron sizes were observed between control and operated *Rana pipiens*. A significant difference in the size of DRG neurons of similarly operated Discoglossus pictus frog led us to conclude that one cell population probably had replaced another, and that this was responsible for the appearance of misdirected wiping responses in skin-rotated animals.

Functional innervation patterns of the skin

Behavioural responses to mechanical stimulation of the skin were observed in unoperated, in 180° skin-rotated and in sham-grafted *Rana pipiens* frogs. In the two control groups, wiping responses directed towards the animal's dorsum were solely mediated via dorsomedial (DM) and dorsolateral (DL) nerve trunks. In skin-rotated frogs, DM and DL nerve trunks were responsible for most of the misdirected responses elicited from dorsal body areas. Six frogs (2 sham- and 4 skin-grafted) possessed areas of plical skin from which wiping responses were mediated via more ventrally located nerve trunks. However, such dorsal extensions of ventral receptive fields never included midline skin areas, from which misdirected responses had been elicited. These experiments thus support previous studies using the frog in which it was concluded that selective (re)establishment of peripheral connections could not be the mechanism for the ontogeny of normal skin innervation patterns.

III. Interaction between the nervous system and hormones during maturation

In this project the production and secretion of peptides by nerve cells and their actions on the brain are studied. Emphasis is laid upon (1) vasopressinergic and oxytocinergic cells, i.e. on the sites of their production, transport, release and reception in the rat and human brain in relation to central processes, (2) upon the possible involvement of these neuropeptides in brain development and (3) their changes during aging. Furthermore (4) new methods are being developed to enable the study of specificity in immunocytochemistry, and (5) special attention is paid to the pineal by Dr. P. Pévet. The main disciplines include immunological techniques, electrophysiology, light- and electronmicroscopy and biochemistry while clinical material is obtained 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 astrocytes (glial cells) in the human brain and in brain tumors. Parts of this project are represented in the FUNGO project nrs. 13-35-07 and 13-35-20 and ZWO 91-106 (the latter two with financial support).

THEME 1. THE SITES OF PRODUCTION, TRANSPORT, RELEASE AND RECEPTION 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 fibres. This route has been revealed specifically by immunohistochemical techniques using the unlabeled antibody

enzyme method and purification of the first antiserum. Arginine-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 various other parts of the central nervous system. By means of 100 μm vibratome sections a Golgi-like image of the peptidergic neurons and their processes was obtained. This technique enabled a more exact and detailed description of the AVP and OXT containing projections from the PVN, SON and SCN into the brain. Thus additional areas of innervation were demonstrated, especially in the hind brain regions (see thesis R.M. Buijs). In collaboration with workgroup IV the possible functions of these peptidergic innervations are under investigation.

By means of immunoelectronmicroscopy, synaptic structures that stained positively for vasopressin, 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. The morphological appearance of these peptide synapses does not seem to differ in any respect from that of the 'classical' transmitter containing synapses, suggesting that these peptides might act as a neurotransmitter. The electrophysiological characteristics of vasopressinergic synapses are currently under investigation in collaboration with workgroup II.

The vasopressin containing synapses on oligodendrocyte-like cells in the amygdala are investigated using LM- and EM-immunocytochemistry with anti-carbonic anhydrase as a marker for oligodendrocytes. Light microscopical results indicate indeed that in the medial part of the amygdala oligodendrocytes are present. However, the first immunoelectronmicroscopical results suggest that the vasopressinergic fibres do not terminate on these oligodendrocytes.

In an attempt to determine under which circumstances AVP is released in the lateral septum, an *in vitro* incubation was set up using lateral septum slices from which the K^+-stimulated AVP release in the medium was measured. The first radioimmunoassay results indicate that AVP is indeed released in a Ca^{++}-dependent way.

Since in the literature evidence was reported for a regulatory effect of opioids on AVP and OXT release, enkephalin was localized immunocytochemically in the neurohypophysis using an antibody of Prof. R. Miller (University of Chicago, Ill., U.S.A.). Only after a short perfusion with paraformaldehyde as fixative, numerous positive fibres were found. Pre-embedding staining for E.M. revealed enkephalin positive granules of ± 100 nm, while staining was also found outside the granules. Most enkephalin-positive fibres were found in the perivascular space in the vicinity of pituicytes.

In collaboration with L. Gross (medical student, University of Rochester, N.Y., U.S.A.), the localizations of AVP and serotonin have been compared within the rat suprachiasmatic nucleus using serial semithin Epon sections. Anti-serotonin was supplied by Dr. H. Steinbusch (University of Nijmegen, Nijmegen). In addition, sections were double-stained (in collaboration with Dr. F. Vandesande, State University of Ghent, Belgium). AVP-positive cell bodies and fibres appeared to be localized mainly medio-dorsally. Serotonin-positive fibres were found only in the basal parts of the SCN. It appeared that AVP-positive cell bodies were only scarcely surrounded by serotonin fibers. However, axo-dendritic contacts cannot be excluded. Only immunoelectronmicroscopical studies using double-staining techniques may solve this problem.

A start was made to visualize the receptor sites for AVP. In the first instance (un)fixed cryostat sections of rat kidney and brain were incubated with AVP (in the nM range) and subsequently stained immunocytochemically. However, in concentrations near the dissociation constant (K_d) no staining was found, while at higher concentrations a diffuse background staining was found all over the section. This failure of staining might be due to either (a) the low amount of AVP bound, (b) the inability of antibodies to bind receptor bound AVP, or (c) rapid dissociation of hormone-receptor complex. However, after fixation of the bound hormone with glutaraldehyde-paraformaldehyde no specific reactivity in the kidney was found with various antibodies raised to AVP and LVP. A second attempt was made with biotinated AVP (performed by Dr. M. Wilcheck, The Weizmann Institute of Science, Rehovot, Israel). This conjugate is known to bind at receptors and might subsequently be detected with avidin-HRP, which has an extremely high

affinity for biotin ($K_d=10^{-15}M$). Unfortunately, it appeared that different preparations of avidin-HRP (synthetized by Dr. D. Boorsma, Free University, Amsterdam) showed also a high affinity for the tissue section resulting in a high background staining. In conclusion, at present no ICC technique seems to enable the visualization of receptor sites for AVP. Therefore a radiohistochemical technique will be applied using [^{3}H]-LVP (obtained from Prof. S. Jard, Collège de France, Paris).

THEME 2. NEUROPEPTIDES IN DEVELOPMENT

The development of the hypothalamo-neurohypophysial and extrahypothalamic system of the *rat* were followed both qualitatively by means of immunocytochemistry and quantitatively by means of radioimmunoassay (RIA). The use of vibratome sections enabled the localization of vasopressin containing cells in the region of the SON from fetal day 16 onwards and from day 18 in the region of the PVN. The fibre outgrowth of these cells towards the pituitary and extrahypothalamic brain sites seems to be well synchronized as on day 17 vasopressin containing fibres could be demonstrated in the olfactory bulb as well as in the median eminence. During the entire fetal period no positive staining for oxytocin could be obtained either in cell bodies or in fibres, while RIA of fetal brain samples indicated the presence of small amounts of oxytocin already at day 14 prenatally, which gradually decreased to fetal day 17. Radioimmunoassayable vasopressin in the fetal brain drops between day 14 and 15 and then remained unchanged between day 15 and 17. Radioimmunoassayable vasopressin and oxytocin were identified in the neurohypophysis by isoelectric focussing at day 17 at the isoelectric point of the synthetic peptides. For brain vasopressin the same was true, but sofar fetal brain 'oxytocin' appeared to have a different isoelectric point.

The first immunopositive vasopressinergic neurons of the SCN were found on the 2nd postnatal day, but the nucleus had an adult appearance only from the 14th postnatal day onwards. The exohypothalamic fibres derived from the SCN neurons were visible in the periventricular nucleus on the 7th postnatal day, while on postnatal day 10 fibres were detected in the lateral septum and the lateral habenular nucleus. From the 12th day onwards a marked and persistent sex difference developed with respect to the density of the fibre network in the lateral septum and the lateral habenular nucleus. In male rats the fibre density was much higher in both areas than in females. Experiments are in progress in collaboration with workgroup IV in which the organizing and activating influences of sex steroids on this sex difference are investigated.

In formalin-fixed adult and fetal *human material*, studied by means of vibratome sections, it appeared to be possible to stain the SON, PVN and SCN for neurohypophysial hormones, and to visualize extrahypothalamic sites of termination. The suprachiasmatic cells stained positively in a fetus of 34 weeks of gestation, while extrahypothalamic neurohypophysial hormone containing fibres were present in the medulla and spinal cord at 17 weeks of pregnancy. The optimal fixation procedures for human material are currently under investigation.

Earlier findings on *Brattleboro* diabetes insipidus rats have revealed a pre- and postnatally retarded brain development in this vasopressin deficient mutant, which persists into adulthood and suggests a role of vasopressin in brain development.

A developmental series of brain area weight measurements (cerebral cortex, cerebellum, olfactory bulb, hippocampus, hypothalamus, colliculum, medulla oblongata and the residue) has been completed for homozygous (HOM) and heterozygous (HET, i.e. control) diabetes insipidus Brattleboro rats, in collaboration with workgroup I, which also currently studies the morphological brain differences between HOM and HET animals. Between postnatal day 8 and 30 weight differences were found only in cerebral cortex, cerebellum and medulla, the latter two areas showing the most severe and most consistent differences for both sexes. From these areas only the cerebellum appeared to be permanently reduced in size at adulthood (180 days), and this structure has thus been chosen for further investigations. Retarded DNA synthesis in cerebral cortex, cerebellum and olfactory bulb tissue, as determined by DNA assays, and reduced [^{3}H]-thymidine incorporation into DNA, explains these differences (this study is performed in

collaboration with Drs. A.J. Patel and R. Balàzs, MRC Developmental Neurobiology Unit, London).

Since daily suppletion of vasopressin in adult (150 days) male and female HOM-DI Brattleboro's, given either peripherally or intracerebroventricularly, appeared not to restore the deficit in brain and cerebellar weight, the possible effect of vasopressin on brain size took place early postnatally. Sofar, a preliminary experiment with intraventricular injections of vasopressin (0.01 or 1 µg/2 days) between day 4 and 16 of postnatal life, did not prevent the brain growth impairment in HOM-DI pups. Also another approach, subcutaneous implantation (at day 4 postnatally) of a vasopressin loaded microporous polypropylene tubing (Accurel; kindly supplied by AKZO Chemie, The Netherlands, see below) from which a constant release of 4 ng vasopressin/day was obtained in the test tube, had no effect on brain and cerebellar weights as measured on day 55. However, a permanent further enhanced diuresis appeared to be induced by this last treatment. Since the kidney response for vasopressin of the postnatally vasopressin-treated HOM-DI animals was found to be normal, this seems to be a central effect. Experiments with lower dosages of vasopressin suppletion, either given intraventricularly or peripherally, and grafting of normal hypothalamic fetal tissue are in progress.

The possible potencies of *Accurel* (microporous polypropylene), as a device for continuous release of peptides, were was investigated using the AVP as a model (project subsidized by AKZO Chemie, The Netherlands).

Test tube experiments with several types of Accurel (pellets, tubing and powder) together with implantations of vasopressin-loaded Accurel into Brattleboro rats (which lack vasopressin because of a genetic defect) show that short- as well as long-lasting release can be obtained in a physiological way. Especially Accurel tubing, lumen-filled with a vasopressin solution and sealed at both ends, appears to give a rather constant and fortnight-lasting release. The use of Accurel in young (pre- and postnatal) and/or small laboratory animals has therefore potentialities that are currently not met by any other application technique. The 'spoilage' of peptide, probably due to surface adsorption, will get attention in a follow-up study.

Immunoreactive α-MSH has been found throughout the central nervous system. Cells and fibres of the dorsal root ganglion showed a very intense immunoreactivity. Using ultrathin frozen sections and the protein-A gold method (in collaboration with Dr. J. Slot, State University of Utrecht), this reactivity was found to be present over neurofilaments, which partly confirms previous work.

In order to see whether this α-MSH-like material was synthetized by the dorsal root cells or rather derived from other sources (*i.e.* the pituitary) explants of cervical and thoracal dorsal root ganglia (fetal day 14) were incubated (in collaboration with workgroup II) during 14 days in minimal essential medium containing 20% horse serum and subsequently stained by immunocytochemistry. Although cell cultures stained indeed positive with anti-α-MSH this can hardly be used as an additional argument for the production of α-MSH-like material since ganglia from the day-14 donor already showed some immunoreactive cells and fibres. It does show, however, the potential use of antibodies against peptides as cell markers in tissue culture.

THEME 3. VASOPRESSIN AND OXYTOCIN IN THE AGING HUMAN BRAIN

In formalin-fixed adult human material of 51-81-year-old individuals (obtained from the Free University, Amsterdam, Prof.Dr. F.C. Stam and Drs. W. Kamphorst, and the University of Amsterdam, Dr. A.C. Jöbsis), studied by means of vibratome sections, it was possible to stain the SON, PVN and SCN for neurohypophysial hormones and to reveal extrahypothalamic sites of termination in the amygdala, hippocampus, substantia nigra, medulla oblongata and habenula. The material revealed two types of neurosecretory cells; one with clearly distinguishable stained cell bodies and neurites which appeared mainly to contain oxytocin, and a second type which was larger, more weakly stained and from which fewer neurites were stained and that mainly contained vasopressin. This subdivision was confirmed in formalin-fixed paraffin embedded material. MOP measurements confirmed the impression that the vasopressin cells were larger than the oxytocin

cells, while in addition an unstained area in the periphery of the cytoplasm of the AVP cells was found. In order to investigate whether areas might contain lipofuscin, several lipofuscin staining procedures were compared from which the autofluorescence procedure gave the best results.
Lipofuscin accumulation seemed to be stronger in vasopressin cells than in oxytocin cells. A procedure is currently developed to compare quantitatively the amount of lipofuscin autofluorescence in these cells to their size and their oxytocinergic or vasopressinergic nature from childhood into senicum, while a comparative study is initiated in the aging rat.

THEME 4. METHODOLOGICAL DEVELOPMENTS CONCERNING SPECIFICITY IN IMMUNOCYTOCHEMISTRY

Immunocytochemical localization of a compound requires specificity of the first antiserum. The specificity can be investigated by separating all immunologically active compounds present in the tissue and measuring the affinity of the antiserum for each of these compounds. None of the available techniques allows, however, to test for antibody binding with small peptides (MW < 2000D) present in the tissue. SDS-gelelectrophoresis which is commonly used in these approaches as separation step of tissue proteins as well as the immobilizing techniques used for proteins could not be applied to small peptides. Therefore, isoelectric focussing on polyacrylamide gels has been chosen as separation technique.

After testing various fixation procedures, a new fixation technique has been developed which gives good results with respect to immobilization and preservation of immunological properties of the peptide. Isoelectric focussing was performed with Servalyt 2-11 on 350 μm thick polyacrylamide slab gels. After focussing sheets of Whatman paper both wetted with 25% glutaraldehyde were placed on the gel followed by several sheets of dry filter paper and finally a plexiglass plate with a weight. By this procedure the peptides are fixed within one hour, partly in the gel and partly in the 540 paper. After immobilization, the gel adheres to the paper and the gel-paper can be used in an immunocytochemical procedure. Synthetic AVP, OXT and α-MSH in amounts of 10 ng have been focussed, immobilized and immunocytochemically detected successfully using the unlabeled antibody enzyme method. The efficiency, universality of the fixation procedure and its application on tissue are currently under investigation.

Another aspect in a procedure to define specificity in immunocytochemistry, is the quantification of the ICC-reaction in both the tissue and a model system. This is necessary in order to establish the predictive value of a testsystem for the tissue section. As a model system the Defined Antigen Substrate Sphere (DASS) has been selected. The currently used PAP procedure allows a more sensitive detection of the first antibody than the indirect fluorescence method, which means that also the specificity characteristics have to be determined by means of this procedure instead of by the immunofluorescence procedures we have used until now. Currently vasopressin has been visualized using the PAP procedure on neurohypophysial paraffin sections while the same antibody was used to stain vasopressin in the bead model. Quantification on the tissue was obtained via a scanning spectrometer in order to avoid the influence of distributional errors on the absorbance values (in collaboration with Dr. P.C. Diegenbach, Zoological Laboratory, University of Amsterdam). Optimal incubation and measuring conditions for the quantification of the immunocytochemical reaction in tissue sections were determined accordingly, resulting in a direct relationship between the amount of diaminobenzidine (DAB)-precipitate and the amount of the first antibody bound to the tissue section. Similar optimalization was reached for the modelsystem by measuring spectrophotometrically the formation of soluble oxidized O-phenyldiamine complexes. This turned out to be an important improvement not only with respect to the sensitivity, but also because it was possible to avoid the laborious and expensive scanning procedure. The predictive value of this procedure for the serum specificity in a tissue section is under investigation.

THEME 5. PINEAL HORMONES

In most species there is a seasonal change in reproductive activity such that the young are born during the season in which the probability of survival for both adults and offspring is maximal. Environmental factors such as ambient temperature,

rainfall and food supply influence the reproductive cycle of mammals, while in temperate zones the length of the day is the primary environmental signal. The pineal responds to and transduces photic information. However, also an intense pineal synthetic activity exists in species living near the equator as was confirmed this year in the Malaysian rat (a study made in collaboration with the University of Kuala Lumpur, Malaysia). Our current concept is, therefore, that the pineal could integrate information not only about daylength, but also about other environmental factors such as temperature and food, and transduces this information to the neuroendocrine reproductive axis.

Chemical factors involved in this process are supposed to be (a) peptides and/or proteins and (b) indoleamines.

(a) By combination of different techniques we continue trying to identify the pineal *peptides* and to determine how their synthesis and release are regulated (in collaboration with Drs. I. Ebels (Utrecht), A. Reinharz (Geneva), C. Neacşu (Bucharest) and B. Vivien-Roels, F.C. Holder and J.M. Guerné (Strasbourg)). Last year, using ICC and RIA, we concluded that arginine vasotocin (AVT), which was regarded as the antigonadotropic hormone, is in fact not present in the mammalian pineal, a result which has been confirmed this year by ourselves and by other teams. Using a new bioassay, we have found vasotocin-like biological activity in a number of mammalian species which is very low in bovine pineal (3-5 pg/pineal) and high in that of other species (for example, 100-150 pg/pineal in the rabbit). In the E_5 fraction from bovine pineal (Dr. Neacşu, Rumania), which is free of AVT, AVP, OXT and α-MSH as determined by RIA, a biological activity similar to that of AVT has been found (40 pg equivalents of AVT/1000 pg of E_5 fraction). According to the results obtained in collaboration with Dr. Reinharz (Geneva) the compound in this fraction has a carboxyterminus similar to AVP and AVT (*i.e.* Pro-Arg-GlyNH_2). A chemical analysis of this E_5 fraction (made by Organon, Oss) has identified a total of 16 different amino acids in the fraction. By combination of chromatography and electrophoresis we have demonstrated that at least 3 'peptides' appeared to be present in the E_5 fraction, but after this separation procedure all AVT-biological activity had disappeared while immunoreactivity remained in two of these peptides. This year we have tried to develop new bioassays related to pineal physiology but, until now, without success.

Ultrastructural studies on cultured pineals and on the 'eyeless' mutant mouse (in which the pineal synthetizes practically no melatonin) indicate that indoleamines and particularly melatonin may control the synthesis and release of the proteic/peptidergic pineal 'hormones'.

(b) *Melatonin* has been and is still considered as the pineal hormone 'par excellence'. In collaboration with Dr. Balemans (Utrecht), the synthesis of melatonin and 5-methoxytryptophol (another 5-methoxyindole considered to be a pineal hormone) by the Harderian gland and the retina has been demonstrated. In the hamster the production of melatonin and 5-methoxytryptophol by the retina plus that by the Harderian gland is higher than that by the pineal alone. In the mole-rat the production of the Harderian gland is even 100 times higher than that of the pineal. Pineal rhythm of melatonin production was different from that of 5-methoxytryptophol and melatonin, while the 5-methoxytryptophol rhythm in the retina was different from that in the Harderian gland and in the pineal.

As it appears that (1) the 5-methoxyindoles (melatonin, 5-methoxytryptophol, etc.) synthesizing organs (retina, Harderian gland, intestine, pineal, etc.) are all organs receiving information, directly or indirectly, from the outside world (light, temperature, food); (2) that the rhythm of melatonin and 5-methoxytryptophol production by these organs is different and influenced by external parameters; and (3) melatonin probably controls the pineal peptide synthesis, methoxyindoles might integrate the environmental information in the pineal. This concept will be investigated in detail in the near future.

Miscellaneous

Mice that suffer from severe nephrogenic diabetes insipidus, obtained from Prof. H.W. Sokol (Dartmouth Medical School, Hanover, N.H., U.S.A.), are studied in order to see whether this strain is of interest for receptor, developmental, behavioural or immunocytochemical studies. Immunocytochemically, a rich extrahypothalamic vasopressinergic innervation appeared to exist, in which pre-

liminary observations reveal no obvious difference between diabetic and non-diabetic animals.

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.Dr. F.C. Stam and Drs. 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. The morphology and distribution of GFA containing glial cells was studied immunocytochemically using the PAP technique in 9 normal brains from patients of 15 to 91 years of age. Foetal and neonatal brains are currently under investigation. A morphological continuum from GFA negative glial cells, via slightly GFA positive gracile cells, to strongly positive large cells with the classical appearance of fibours astrocytes was found. In Bergman cells only the radial fibers were stained except in the brains where sometimes a GFA positive cellbody was found. In the subpial layers sclerotic GFA positive cells were present. In the taeniae of the chorioid plexus positive bipolar glial cells were seen, while in the ependyma some positive cells, sometimes with positive processus (tanycytes), were found. GFA positive neurons and their axons were found to stain sometimes in the visual cortex, thalamus and pallidum, while a few times the baskets around Purkinje cells were stained strongly positive.

In the 91-year-old brain a clearcut increase in the number of GFA positive glial cells was found in almost all areas, while in the younger age-group no age-dependent change was observed. The thickness of the GFA positive subpial and subependymal glial layers did not change with age. However, the number of positive glial cells in the molecular layer of the cerebrum was distinctly larger in the older age-group. In the cortices of the older brains a GFA positive glial corona was found around senile plaques. Only in these cortices positive glial cells with the classical aspect of protoplasmatic astrocytes were observed. In the final stage of neurofibrillar degeneration positive glial fibres seem to grow between the remnants of parallel neurofibrils. The antibodies of Eng and Delpêch revealed the same results. The types of staining in brain tumors are currently compared to those in human brain development.

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, while the data are analyzed by workgroup V. the projects with Dr. R.F. Drewett (Durham) on human lactation were continued, as was the project with Prof. B.T. Pickering (Bristol) and Dr. C.F. Goodfellow (Leeds) on human labour, with Drs. H.J.L.A. Ruis and R.S. Corbey (Den Bosch) on the effect of oxytocin administration per spray upon lactation in women, while a project was started with Drs. H.P. Oosterbaan (Amsterdam) on the question whether intrauterine growth disturbances can be determined by peptide hormone levels in the maternal or fetal compartment.

IV. Development and plasticity of behaviour

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

During 1980 the work continued along three lines: (1) analysis of motivational aspects of sexual and aggressive behaviour, (2) sex differences in avoidance learning and emotionality in rats and (3) the neural substrate underlying aggressive and sexual behaviour. Moreover, an increasing interest on the part of other groups at the institute in the study of behaviour, stimulated research in joined projects together with group I (effects of underfeeding and enriched environment on behavioural development), group II (behavioural effects of REM-sleep deprivation) and group III (vasopressinergic innervation of the brain and avoidance learning). Parts of this project are represented in FUNGO ('Development and aging of brain and behaviour' and 'Behavioural Mechanisms'), BION (Ethology),

and PSYCHONOMY (Comparative and Physiological Psychology). In 1981 behavioural research on the sexual motivation of rats will be subsidized by ZWO (Psychonomy project nr. 15-25-09) whereas a 'twinning' project was started together with the University of Swansea, granted by the European Training Programme for Brain and Behaviour.

THEME 1. ANALYSIS OF MOTIVATIONAL ASPECTS OF SEXUAL AND AGGRESSIVE BEHAVIOUR

Hormonal factors determining masculine sexual responses are almost exclusively studied in tests in which animals are tested against females artificially brought into behavioural oestrus with ovarian hormones. Females are tested with males of proven sexual vigour and activity. This common use of partners with a standard stimulus quality and the quantification of aspects of sexual behaviour by the use of behavioural parameters observed in the interaction of the two animals, obscures observation and study of aspects of behaviour related to sexual motivation. Detailed observations of receptive, proceptive and aggressive behaviour in an interaction between females treated with testosterone (TP), oestrogen (EB), oestrogen + progesterone (EBP) or OIL and males of proven sexual vigour were compared with data of these animals in tests determining attractivity and the reward of being sexually approached. Hormonal dosages were chosen below the values reliably influencing behaviour measures in the standard tests for sexual behaviour. The results indicated that although no consistent levels of receptive and proceptive behaviour were observed when tested with a male, attractivity of EBP but also of TP treated females was significantly higher than in EB or OIL treated females. In an Y-maze where each of 10 runs led to access to a receptive female or to a male, and this male, if chosen, was allowed one intromission, these same groups consistently chose male companionship. Further research was aimed at standardizing the testing procedures using the 'semi-open field' and the Y-maze. Johan Scholtens, already working on this project as a student, continued these experiments starting from September as alternative military servant.
At present the same behavioural measures are studied in groups of neonatally castrated males and androgenized females treated in adulthood with oestrogen or testosterone.
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 or OIL. Following a 24-hour isolation period pairs of animals were tested for aggressive behaviour. The hormonal status of both animals varied systematically, thus resulting for both males and females, in three groups: OIL vs OIL, OIL vs TP and TP vs TP. Aggression was observed in all four test conditions, in both males and females. In females (WEzob and S3) and in S3-males, TP treatment increased aggression. A further analysis of qualitative differences in aggressive behaviour using discriminant analysis, pointed to sex differences in aggression in the WEzob strain only. The results therefore cast serious doubt on the hypothesis of organizing effects of gonadal hormones with respect to intraspecific fighting in rats. Further experiments, however, comparing activating effects of lower dosages of TP with activating effects of EB in males and females, proved that significant sex differences in aggression are observed when males and females are treated with EB (10 µg for 14 days) and tested against a TP treated or EB treated opponent. High levels of proceptivity and receptivity are induced in females instead of the frequent periods of fighting observed in males. Interestingly, however, a second test on the subsequent day did not reveal any sex differences in these hormone treated animals.
The influence of the oestrous cycle of the female on her own and her partner's sexual and aggressive behaviour was followed in WEzob and S3 rats to compare these two strains of rats in encounters between intact males and females. Males as well as females of both strains showed aggression, the males being the more aggressive. Little agonistic behaviour was seen on the day of oestrus. On other days, male advances resulted in defensive kicking by the female, often followed

by lateral threatening. The escalated aggression usually ended with the larger male holding down the female. This sequence occurred more frequently in S3 rats thus confirming that this strain is more aggressive than the WEzob. Interestingly no detectable differences in aggression of the females were observed between the three days when the females were not receptive. Thus, although the endocrine profile varied between met-oestrous, di-oestrous and pro-oestrous days, these changes in hormonal balance were not reflected in behaviour. Familiarity with the male did not alter the female's aggression.
The relatively stable dominance relations within groups of rats and the easily observed behavioural changes accompanying defeat in an agonistic encounter, strongly suggest that the aggressive tendencies in an individual should at least partly be determined by previous experiences. In the present year methods of investigation and data were obtained on effects of winning and defeat on subsequent aggressive behaviour. WEzobs are always defeated very quickly when tested against S3's, whereas WEzobs become dominant when tested against WIstars. An experimental group of WEzob males was tested on three subsequent days against S3's or against WIstars thus creating winning and losing WEzobs. On the fourth day and again after a period of two weeks, winners were tested against losers. The results clearly indicate that significant and relatively stable behavioural changes are induced by this method. Losers showed less social initiative and aggression than winners whereas significant differences in autogrooming were observed, winners showing more grooming than losers. Although no differences in activity were noted, winners significantly localized most of their activity on the loser's side of the cage. The body weights of losers were reduced in contrast to those of the winners. Further work on this line used this testing procedure in testosterone or OIL treated males and females. The results seem to indicate that the behavioural inhibition induced by this procedure is a specific characteristic of the male's agonistic behaviour.

THEME 2. AVERSIVELY MOTIVATED LEARNING AND EMOTIONALITY IN RATS

Earlier results on passive avoidance learning indicated that in males avoidance tendencies are dominant whereas in females approach tendencies are dominant. Memory deficits per se do not account for these sex differences in learning. Last year experiments were started in the skinner box in which an approach-avoidance conflict can be studied in which spatial aspects are irrelevant for learning. A food pellet as a reinforcement 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 in addition to the food when the criterion was met. This stage of experimentation lasted for two daily sessions. Thereupon the original program was resumed, for 24 days. Preshock no sex differences were found, about seventy percent of the responses meeting the criterion for food reward. Punishment with a shock suppressed responding very effectively. Responding resumed only gradually, and many animals still had not reached preshock levels at the end of experimentation. Females, however, resumed responding significantly sooner than males did. These results indicate that the effect of response-contingent foot shocks extinguished more slowly in males than in females as revealed by their lower number of responses and reinforcements earned in the postshock stage. In addition, it could be demonstrated by use of special computer programs for analysis, that males showed more abortive responding, i.e. termination of the response before reinforcement criteria are met.
Male and female rats were studied in a discriminated lever press avoidance experiment. In contrast to what is commonly assumed, it could be demonstrated that rats are able to acquire this task fairly quickly if the parameters are chosen adequately. Females showed superior avoidance performance, less inter-trial responding and shorter durations of lever holding. Castration of the males resulted in a marginally significant improvement whereas ovariectomy of the females had no effect. When these same animals were tested for step through passive avoidance, more males than females showed avoidance responses. Furthermore it was demonstrated that performance on the retention trial of passive avoidance correlated significantly with lever holding in the leverpress avoidance task. The results of the former two lines of experiments demonstrate that spatial aspects are not particularly important for the manifestation of sex differences in

avoidance learning. The positive correlation of lever holding and response inhibition in passive avoidance, together with the finding that males showed more abortive responding after punishment, support the hypothesis that males and females differ in emotionality, males being the more fearful sex.
Previous work on the effects of gonadectomy and subsequent administration of gonadal hormones indicated that with a post-operative interval of one month, the sex differences in passive avoidance, although still apparent, were no longer significant without supply of hormones. Activating effects of estrogen and testosterone only could be demonstrated by the finding that sex differences were again present in these condition in contrast to the oil condition. Comparison of oil and hormone treated groups failed to reveal significant effects. In order to establish better base line levels of passive avoidance learning in non-treated gonadectomized rats and thus provide improved procedures for testing activating effects of these hormones, rats were gonadectomized prepuberly, and tested in adulthood (at the age of 125 days). The results replicated our earlier findings. Significant sex differences were found in sham operated and testosterone treated animals but not in oil injected groups. In contrast to the previous experiment no sex differences were found in the oestrogen treated animals. The effects of gonadectomy did not point to a further reduction of the differences between males and females. The results of both experiments demonstrate that circulating gonadal hormones, especially testosterone, play a role in the manifestation of sex differences in passive avoidance learning, but at the same time suggest that the influence of circulating gonadal hormones is not of principal importance. Apparently additional factors are involved as testosterone affected males and females differentially. It seems most likely that the activating effects of testosterone are related to the organizing effects of gonadal hormones.
To test for organizing effects of gonadal hormones during development on avoidance learning in rats neonatal males were castrated or sham operated and females were androgenized or treated with oil. All animals were gonadectomized prepuberly and treated with testosterone or oil in adulthood. Behavioural testing consisted of the open-field test, dark-preference and passive avoidance. Test experience as an experimental variable was part of the experiment as half of the animals were tested between day 25 and 30 of age. The results are being analysed.
The sexual dimorphism of pituitary-adrenal activity is well documented. Experiments were started aimed at investigating whether these differences contribute to the sex differences in aversively motivated learning and open-field behaviour in rats. In the first experiments dexamethasone was used to pharmacologically block the pituitary-adrenal system. Several retention intervals were studied: 0, 0.25, 1.0, or 24 hours. Dexamethasone had no effects on the percentage of animals which showed passive avoidance behaviour. However, in those animals which did reenter the shock compartment, significantly shorter latencies were noticed for dexamethasone treated males and females. In the open-field test no effects of dexamethasone nor any sex by treatment interaction were observed. Assuming that side effects affect both sexes to the same extent, the present data fail to support the hypothesis that sex differences in response to novel or aversive stimulation emerge from sexual dimorphism in the activity of the pituitary-adrenal system.

THEME 3. THE NEURAL SUBSTRATE UNDERLYING AGGRESSIVE AND SEXUAL BEHAVIOUR

The prefrontal cortex (pfc), defined as the cortical 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. Its structural development, possibly different between the sexes, is also part of this research.

Previous experiments demonstrated a functional dissociation between the two pfc-regions in male rats of the WEzob-strain (WE). Orbitofrontal pfc lesions resulted in an enhancement of aggressive behaviour, studied in male-male interactions. No differences could be witnessed between males which had received either medial pfc lesions or control operations. Neither lesioning of the orbitofrontal, nor the medial pfc resulted in noticeable changes in male sexual behaviour.

Further experiments on the regulatory role of the orbitofrontal pfc in social behaviour included additions to the testing situation for aggression and involved both males and females. This was partly based on the previous experiments in which the increase in aggression in males following lesioning of the orbitofrontal pfc was clear in encounters with more or less equivalent opponents, but hardly noticeable towards opponents which were more or less easily defeated. Moreover, studies in primates demonstrated the importance of social rank and gender as influential variables in determining the effects of brain damage on social aggression.

Both male and female rats of the WE-strain received bilateral orbitofrontal pfc lesions or were control-operated. Encounters were staged between WE-rats and rats of two other strains, S3 (Tryon maze dull) rats and WI (Wistar) rats. Rats of the S3- strain are *i.a.* characterized by their vehement aggression, and usually defeat WE-rats in symmetrical contests. Rats of the WI-strain show low levels of aggression and are usually defeated by WE-rats in symmetrical contests.

In male-male and female-female encounters between S3 and WE-rats vehement aggression was observed. Defeated WE-rats showed a considerable amount of submissive freezing; sexes differed in that aggression in S3-males was higher than in S3-females, and defeat in WE-males more severe than in WE-females. In both sexes, S3-rats showed less holding down towards the lesioned that the control-operated WE-rats, indicative of a slight effect of the lesion. In subsequent encounters with WI-rats, both male and female WE-rats showed aggressive acts more frequently than their WI-opponents. Surprisingly, aggression in WE-females surpassed that in WE-males. This might be due to the less severe defeat of females in the S3-WE encounters. The effect of the lesion was only obvious in the amount of non-social behaviour, which was higher in both lesioned males and females. This is in agreement with literature data on primates where damage to the pfc is reported to increase social withdrawal.

When encounters were staged between lesioned and control-operated WE-rats, aggression in male-male encounters was higher than in female-female encounters. Whereas behavioural differences between lesioned and control-operated females were only slight, a clear lesion effect in males was noticed in that lesioned males became dominant over their sham-operated opponents, confirming the outcome of a previous experiment.

As an alternative means of causing brain damage the neurotoxin kainic acid was administered to the frontal cortex (together with H. Pronker). The extent of the effect of this neurotoxin was evaluated in transversely cut Bodian-Ziesmer stained sections. Almost all neurons within a certain radius of the needle tip were destroyed and a neurotransmitter-specific effect could not be witnessed. Therefore, within the frontal cortex, application of kainic acid can be considered to be a useful and more restricted way of causing local brain damage.

BEHAVIOURAL EXPERIMENTS IN COLLABORATION WITH OTHER WORKGROUPS

Together with group II, the behavioural consequences of chronic deprivation of active sleep by means of chlorimipramine, during postnatal development were investigated. In earlier experiments, AS-deprived animals and controls were tested in various behavioural tests. This line of investigation was continued. For details see reports of workgroup II.

In collaboration with workgroup I experiments were carried out to determine whether any deleterious effects of pre-weaning undernourishment could be wholly or partially reversed by post-weaning environmental enrichment. Sex differences in response to malnutrition and/or environmental stimulation were also investigated.

Rats of both sexes were undernourished from birth to 30 days by restricting access to the lactating mother, and then fed *ad libitum*. After weaning, both previously underfed and normally suckled controls were permanently exposed to differential environments, being housed either in pairs in standard cages or in groups of 10 in large (1 m^3) cages containing numerous objects.

Severe undernutrition during suckling followed by several months of refeeding, had no effect on any behavioural measures in open field, dark preference or passive avoidance tests, nor were there any deleterious effects on fertility, sexual or maternal behaviour. Indeed, in sexual behaviour tests with receptive females, males who had been malnourished during infancy, had shorter latencies to mount, intromission and ejaculation and ejaculated more frequently than wellfed

controls. This effect was apparent under both standard and enriched housing conditions.

Differential post-weaning environment produced significant differences in all behavioural measures, irrespective of previous feeding conditions. Enriched animals were more active and exploratory, and less fearful than those housed in standard cages. Females differed from males in the same direction as enriched from standard, the effects being additive.

Nutritional deprivation during suckling resulted in a permanent deficit in body weight (22% in males, 15% in females) accompanied by a smaller deficit in brain weight (10, 12% respectively). In males, four months of living in the enriched cages did not alter the differential in body weight or brain weight attributable to neonatal nutrition. In females, however, enrichment, while not affecting body weight, resulted in a significant increase in whole brain weight as well as in the separate weight of forebrain and cerebellum. The finding of larger adrenals and smaller prostate in enriched males suggests that the crowded social conditions may have been stressful. There were no indices of stress in females.

The absence of permanent adverse behavioural consequences following pre-weaning undernutrition precludes the proposed evaluation of possible remedial effects of environmental stimulation. Brain weight deficit was partially restored in females but not in males, by living in materially and socially enriched surroundings.

V. Biomathematical and computational aspects of neurobiology

The group is responsible for the proceeding of the Institute's data processing, while the scientific direction is inclined towards the theoretical aspects of growth by computer simulation.
Collaboration projects with group I and II were recently started. Much scientific attention has also been paid to the processing of group IV's data.

INSTRUMENTATION

The department uses two computer configurations, the first of which is an IBM 1130 with 16 K words of 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 second installation is a Perkin-Elmer Interdata model 70 system with 64 K bytes of core memory, printer, card reader, papertape reader, console display, 2 local terminals, a dual disk-drive and a magnetic tape transport. The plotter and the graphic display from the IBM can be shared with the Interdata. Several real-time on-line experiments are connected to the Interdata.

COMPUTER SELECTION

The market evaluation as started in the last month of 1979 was continued. A study was made concerning the DIGITAL VAX 11/780, the Perkin-Elmer 3220 and 3240, and the HARRIS 500. As the VAX appeared to be the most flexible system with the possibility of intelligent laboratory I/O using a microprocessor-based concentrator, and also because DIGITAL has the largest experience with instrumentation and is expected to give the best support, this system was proposed to the C.R.I.V.A. (a government committee, taking charge of the approval of computer systems in the field of science and education). After a second report with additional information concerning the advantages and disadvantages of alternatives like the use of a university-computer centre or the set-up of a network configuration grouped around smaller computer systems (DIGITAL PDP 11/44 and Hewlett-Packard 1045F) on the other, the original plan was approved. The new computer is expected to arrive in March 1981.

INSTRUMENTATION RECORDER SELECTION

In the Institute, 9 (PHILIPS ANA-LOG 7) instrumentation recorders are in use, each aged between 10 and 15 years. In view of the urgent need for replacement of these old machines, the computer department was charged with the exploration of the current instrumentation recorder market. Attention was paid to the following machines: Bell and Howell 4020A, Nagra Kudelski TI, Ampex 2230, Honey-

well 101, Honeywell 5600, Racal ST7DS, EMI-SE 3000, EMI-SE 7000 and Hewlett-Packard 3964. From these possibilities, the Racal ST7DS appears to correspond optimally with the needs of the institute.

IN COLLABORATION WITH GROUP I

THEME 1. COMPUTER CONTROLLED DENDRITE MISCROSCOPY

a. *Analysis* of orientation of dendritic fields.

In cooperation with the NIBR, Prof. Ruiz-Marcos, visiting the institute, adapted his method for construction, representation and statistical comparison of matrix neurons to the variety of labels as produced by the dendrite tracking system; subsequently it was programmed on the Interdata system. This method is based upon a 2-dimensional projection of a 3-dimensional structure, obtained by averaging the process density in finite volume elements of a group of nerve cells. Work on orientation analysis will be pursued in 1981.

b. *Acquisition*

The introduction of the orientation analysis as a routine method had severe consequences with respect to the real-time on-line acquisition program. The measuring procedure had to be altered with respect to the tracking of axons and apical dendrites. Moreover the significance of label 12 had to be changed (point on pial surface for cell rotation purposes) which in its turn had intricate consequences for driving the microscope stage. Also the possibility was created to mark multiple cell centres before tracking a single neuron. This caused the correction procedure to be modified, as more than one cell will be written onto a single file.

c. *Metrical analysis*

The analysis procedure has considerably been improved by including the axons into the centrifugal ordering method. An axon may originate either from the soma or from a process. Furthermore, the procedure for unraveling the apical main shaft was corrected. The total number of segments has been added to the items to be statistically analysed, the distance of a cell centre to the pial surface can be computed and the possibility for the analysis of multiple cells per file was created. Finally several corrections in the plotting sections were carried out and some refinements in the execution flow were made. Work on statistical characterization of populations from metrical parameters was initiated.

d. *Topological analysis*

A start was made with quantifying the arborization of dendritic fields. The objective is to arrive at a unique characterization of branching patterns, which will be used for statistical analysis of topologies with a given number of terminal- or cut-segments. This might in future be used for modelling the outgrowth of nerve cells under different growth conditions. As a first result, a program was composed to count the number of branching points that bifurcate into either other branching points, terminal (or cut) points or combinations of these types.
As many actual branching patterns belong to the same topology (due to the invariance with respect to rotations of sub-branches around their bifurcating source branch) each actual topology is transformed into a standardized one and uniquely coded. Furthermore, a start is made in developing an ordering system of these standardized topologies in order to characterize each topology by a definite integer number.

e. *Editing procedures for cell coordinates*

In order to be able to manually alter data in files as created by the dendrite microscope and moreover, to introduce new cell data (measured form camera lucida drawings, made by J.G. Parnavelas) in the same format as the microscope-created dataset, a program was developed with the following features: listing of coordinates and labels within existing files, creating new cells in a dataset, copying from and to datasets; moreover, all kinds of rotations of cells are possible during a copy, and finally incorporation of on-line digitized information of camera

lucida drawings, produced by the MOP-AM-02 tableau (see below).
Using these options (except listing) editing of coordinates (modify, insert, delete) and rescaling (magnification factors for x, y and z) is possible.

f. Digitizing of camera lucida drawings

To reduce time required for entering many cell drawings, an on-line procedure was developed in which the X-Y coordinates were digitized using the MOP-AM-02 tableau. So only the Z-coordinates and labels were to be keyed in manually. These data were stored in a rough format on magnetic tape and converted to microscope format using the above mentioned cell-editor.

THEME 2. PROCESSING OF ELECTRON MICROGRAPHS USING THE DIGITIZER TABLEAU

The analysis of electron microscopical neuromorphometrical structures (like curvilinear length of synaptic contacts or circumference of dendritic segments) is carried out by means of the MOP-AM-02 digitizer. This microprocessor-based system carries out the computation of a set of structural parameters (length, surface, maximum diameter etc.) from discrete coordinate pairs. These 'MOP-functions' can be serially transmitted to a host computer. After the MOP-AM-02 had been connected to the Interdata (current loop) an information processing system was developed, containing the following features: acquisition and decoding of all MOP-functions, building of a dataset, with entries like film- and photo-number, and cell type. Organization into sequential files is based upon slides (film- and photonumber) (within every file, cell type is the sort key). Basic statistical analysis (for slides or for cell types) (currently moment analysis and histogram-representation of population distributions) has been realized. The MOP-package will be gradually extended in the near future.

IN COLLABORATION WITH GROUP II

Recent electrophysiological measurements on dissociated and organotypical tissue cultures put forward questions about correlations between bioelectrical activity and structural properties in growing tissue. Some of these questions could be approached better in a theoretical way. Especially the parametrization and modification of the network structure is experimentally hard to tackle. Therefore, a start has been made with developing a distributed model of a neural network. The network is represented by a matrix of interconnected separate neurons of which the bioelectrical activity is simulated by calculating in discrete time the status of all variables. In this way correlations can be studied between single unit activity and total network behaviour in dependence on structural parameters. Also verification of the output of the model with experimental results on single- and multiple-unit activities will be possible.

IN COLLABORATION WITH GROUP III

In behalf of Radio-Immunoassay

A special purpose editor was written in order to be able to correct RIA-papertape input in an interactive way. This was inevitable because of the frequent malfunctioning of the teletypes, connected to the liquid scintillation counters.

IN COLLABORATION WITH GROUP IV

Analysis of keyboard-scored behaviour: The analysis program for White-system data has been extended with (a) addition of the Friedman two way analysis of variance and (b) the possibility of applying a time window upon all tests in a group. Hence, it is possible tot analyze and test several episodes from the same series of tests.

Skinnerbox package: (a) acquisition: the real-time on-line acquisition program has slightly been modified in order to perform more robustly during multitasking usage. (b) analysis: as a preliminary step to 'error run' vs 'correct run' analysis, a matrix representation of two event types has been incorporated into the program package. This required special routines for virtual storage creation, as the

amount of data can be immense. At this stage, matrices are produced per animal; group-averaged representations will be computable in 1981.

MISCELLANEOUS

Statistics

(a) Analysis of variance: the IBM ANOVA program has been thoroughly revised and supplied with virtual storage scratch arrays, as the number of factors and their levels gave rise to array sizes exceeding the IBM's capacity. Moreover, the possibility was created to analyse experiments with repeated measurements in more than one factor. A straight-forward two-way analysis of variance for unequal group sizes has been realized on the Interdata.
(b) Polynomial fitting: a polynomial fit package has been implemented on the Interdata with the following features: device independent input, batch or interactive; least squares polynomial fit on data consisting of a series of (x, y) coordinates including error analysis. The best fitting polynomial degree can be obtained in a repetitive interactive way; several types of errors in the ordinates of the input datapoints can be accounted for; data points and fitted curve can be plotted in a rectangular coordinate system. More sets of data can be plotted in the same coordinate system.

Systems programming

Some time has been spent in trying to implement the new Perkin-Elmer OS 16 operating system and Fortran IV compiler. However, these products suffered highly from non-performance in a 64 K environment. So the department switched back to the original situation. As the new Interdata tape unit, which arrived in January 1980, showed to be not completely IBM-compatible, almost all programs had to be adapted with respect to tape input. This caused also several conversion steps to be done for the corresponding datasets. This tapedrive problem appeared to be caused by the Interdata interface design. Hardware limitations appeared also to be responsible for a failing effort to implement the '+' control character for the printer.
Aside from these problems, a lot of Fortran-retrievable utilities have been created, intended for relative file-access, record manipulation, logical/physical unit identification and recognition within the user's program, and finally a magnetic tape cracking program.

Hardware developments

See Electronics department and Mechanical workshop for construction of an improved dendrite tracking system.

Production service

All programs have been intensively used. On the Interdata, acquisitions from Skinnerboxes, dendrite system, White-system and Kontron-AM-02, their file-management and analysis were daily - and where possible - concurrently used. This caused many queueing problems.
Statistics and radioimmunoassays were performed on both Interdata and IBM machines.

Monoaminergic mechanisms

The completion of this project concerns the distribution of imipramine (IMI) between rat blood and brain areas and is partly carried out at the Department of Biological Psychiatry, University Hospital, Groningen, under the supervision of Dr. J. Korf.

IMI reached a characteristic distribution in rat brain within two min after its i.v. administration, the highest levels being found in the frontal and occipital cortex, intermediate levels in the striatum, and the lowest in the hypothalamus, cerebellum and brain stem. This distribution was independent of the dose used over a wide range, showing that high-affinity binding was not involved. Since the distribution of IMI was similar to the known differences in cerebral blood flow (CBF) and glucose utilization, drugs that diminish regional differences in glucose

utilization were applied. Gamma-hydroxy-butyric acid or thiopental smoothed the cerebral distribution of IMI, suggesting that the accumulation of the drug was, indeed at least partly, determined by regional CBF.

In order to complete a study on the relation between the IMI levels in blood and brain, ^{3}H-IMI was injected after the administration of unlabelled IMI and the specific activity of IMI was determined in serum and frontal cortex at several time intervals. IMI was purified by HPLC and quantified by UV detection (in collaboration with Drs. D.R.A. Uges and Ing. P. Bouma, Department of Pharmacy, University Hospital, Groningen) and the radioactivity in the eluate of IMI was measured. In rats treated with 25 mg/kg IMI (i.p.) 18 and 2 hours before the injection of 20 ng/kg of ^{3}H-IMI, the specific activity in serum and frontal cortex was no longer different 20 min after the injection of ^{3}H-IMI. The same result was obtained with rats that had in addition been treated with 10 mg/kg IMI 2 times a day for 17 days. Moreover, at 10 min after labelling the ratio of the specific activity in frontal cortex/serum was higher in the latter than in the first experiment, suggesting that subchronic treatment with IMI facilitates the rapid and complete exchange of blood and brain IMI.

Electronics workshop

1. Two *digital multimeters* that meet the high requirements and can, amongst other things, be used as temperature gauges.

2. *Experiment timer.* Behavioural observations of rat social behaviour are made during the rat's dark period under dimmed red lighting conditions. Quite often behavioural interactions are recorded on video tape, and it is necessary to record simultaneously both time and number of the behavioural protocol. Therefore, an experiment timer was developed and built, which displays the protocol number previously selected by the observer, as well as a digital time indication. The observer can start the clock at the same time, when behavioural interactions begin. The red light display of the experiment timer is recorded with a second video camera, simultaneously with the behavioural recording.
The timer has proven to be very useful when analyzing video tapes.

3. *A motor-speed regulator for a micro manipulator.* In the mechanical workshop a micro-drive with a small DC-motor has been constructed for the electrophysiological department. This motor is manipulated by a control, which regulates the speed at which the motor moves the microdrive, by means of pulses, the pulse-width of which can be adjusted.

4. *A spike reset unit* was developed in order to simplify the quantification of neurophysiological data. After a preset number of action potentials the apparatus produces a pulse which is suitable for driving a polygraph pen-writer. In addition, a long cable fitted with a push-button is provided, enabling the user to indicate on the paper record those portions which, due to external disturbances, are unsuitable to be included in the subsequent quantitative analysis.

5. *An alarm-installation* for the buildings in which the institute is located. The purpose of this installation is to secure the various buildings on the premises against burglary. This has been achieved by attaching detectors to the entrance-gates and inside the buildings. Forced entrance during the alarm period results in the sounding of an alarm (from sirens located on the roofs of the buildings) and sets off the revolving lights. Entering is possible with the use of a so-called alarmkey.

6. *Semi-automatic measuring system for neuronal processes.* As reported in Progress Report 1979 fundamental difficulties are experienced with a system using a stepping motor scanning system. Since no good alternative is commercially available, a start has been made to develop a new measuring system which uses DC motors instead of stepping motors, with linear translation via spindles and in which the movement of the microscope stage will be measured on the stage in 3 coordinate axes separately, and not by counting the number of rotations of the DC motor. This new philosophy of measuring necessitates a completely different

design of the electronic circuits. A feedback-system has to be designed between both the DC-motor drivers and the device which measures the movement of the microscope stage. This is necessary for accurate, computer programmed, return to previously measured points (within 0.5 μm). A test-model for this feedback system has been constructed. Furthermore, for the device for measuring the movement of the microscope stage in 3 axes, (a) the SONY schemes are unravelled for the system, in which a part of a SONY device is used, (b) electronic circuits are developed to incorporate that SONY-part and (c) a test-model has been constructed. For the other components of the measuring systems electronic circuits are designed for e.g. counters, motor amplifiers etc. For these components IC circuits on print boards are designed, constructed and tested.

7. For *maintenance and repair* of various electronical apparatuses see Progress Report 1979.

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.

FOR WORKGROUP I

1. *Semi-automatic measuring system for neuronal processes.* As reported in Progress Report 1979, basic difficulties are experienced with a system using a stepping motor scanning stage. However, no good alternative is commercially available. Therefore, a start has been made to design and construct test-models for a system which uses a DC-motor unit for driving the microscope stage with a high accuracy (about 0.5 μm) and with a variable speed with spindle transduction. A new construction has been designed to drive the stage at a high (± 1 mm per second) and a low speed (± a few microns per second). Several systems for measuring the movement of the stage in x, y and z directions with an accuracy of 0.5 μm were explored (e.g. a laser system from the Zeeman Physics Laboratory, University of Amsterdam). Finally, a selection has been made for a device for which some parts are obtained from SONY, Japan, and the stage has to be designed and constructed in our electronics and mechanics department. In view of the accuracy of the measurements, the microscope stage with which the DC-motors will be connected is also currently constructed.
2. The plastic rotating *slide-holder* of the dendrite measurement system has been replaced by an aluminium one.
3. A *projection apparatus* was reconstructed in such a way that projection of both slides and negatives is possible upon the tablet of the MOP-AM-02 digitizer.
4. A *marking stift* for determining x, y coordinates on the tablet of the MOP-AM-02 has been constructed.
5. Several times the *printer system* of MOP-AM-02 has been repaired.

FOR WORKGROUP II

1. *Circulation pumps* modified for use in precision circulation of fluid medium during tissue culture experiments.
2. The *tissue culture set-ups* were improved in several respects.
3. A *micro-electrode bending unit* was constructed for making suitable glass pipettes to be used in tissue culture experiments.
4. A *remote-controlled stepping microdrive unit* was constructed for use with a specially modified stereotaxic unit.
5. Four 8-channel, mercury filled *turning contacts* were constructed for use in recording electrophysiological signals in free-moving rats.
6. A *miniaturized microdrive unit* was built for use in longterm recording of neuronal activities in free-moving rats.
7. A *swivel apparatus* was made which enables an entire nest of rats to be moved about at a variable speed and angle of displacement.

FOR WORKGROUP III

For immunocytochemical incubations of brain sections, PVC staining trays were made. A *perspex electrophoresis bath* was constructed according to Dr. Vandesande (Ghent, Belgium). This apparatus will be used for double immunocytochemical staining of histological material.

FOR WORKGROUP V

As workgroup V's Data-100 lineprinter constantly showed problems with obstruction of the paper advance transport system by improperly folded forms, a *paper pulling device* was developed. This machine consists of a dual pressing-roller assembly, mounted above the forms-stacker, and provided with a slipping clutch and an electromotor.

Paper insertion can be done by pulling the roller apart by a two-lever construction. The paper-puller can be driven constantly (manual) or switched by the motor control from the lineprinter itself.

FOR THE PHOTOGRAPHY DEPARTMENT

1. An electrically driven camera was modified so as to make film exposures possible at varying transport velocities.
2. An adjustable triple-filter holder was constructed.

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, construction of two stainless steel incubation boxes, modification of 150 rats boxes (synthetic fabric), and modifications on a variety of equipment were carried out, *i.e.* microscopes, centrifuges, shakers, y-counters, micromanipulators, micro-scissors, micro-pipettes, microtomes, micro-drivers and photographic equipment.

General technical service

The availability of the building adjacent to ours vacated by the Department of Plant Physiology of the University of Amsterdam, has enabled this service to complete its internal relocation scheme started in 1978. This has resulted, however, in a number of additional repair and maintenance responsibilities previously assumed by the University of Amsterdam.

Apart from the increased routine responsibilities, this department has installed: (1) a ventilation system in the Canteen and Conference Room, (2) a humidification plant in the research animal-house, and (3) an integrated complex and virtually burglar-proof alarmsystem devised by our Electronics Department.

Guestworkers and working visits abroad

Baker, R.E. at Dept. of Anatomy, Medical University of Debreçen, Hungary, July 14-August 1.

Boer, G.J. at Dr. D.M. Gash, Dept. of Anatomy, Medical Center, University of Rochester, Rochester, New York, U.S.A.

Bowden, N. (guestworker from Dept. of Endocrinology, University of Swansea, Swansea, U.K.) for the preparation of a collaborative program and behavioural analysis (Twinning grant subsidized by ETP), December 9-20.

Chouvet, G. (guestworker from Dept. of Experimental Medicine, Claude-Bernard University, Lyon, France) to help adapt automatic sleep-stage detection apparatus for use with developing rats, November 3-5, (Group II).

Drucker-Colin, R. (guestworker from Neuroscience Dept., Autonomous University of Mexico, D.F.) to help initiate collaborative research on the selective suppression of REM-sleep by use of antibodies to brain proteins, July 15-17 (Group II).

Haldar-Misra, C. (guestworker from the University of Varanasi, India) for the

ultrastructural study of the pineal of mammal in organ culture, September 1980-September 1981 (Group III).

McConnell, P. (guestworker from the University of Birmingham, Dept. of Anatomy, England) for research on undernutrition and rehabilitation, until July (Group I).

Ocal, T. (guestworker from the University of Istanbul, Turkey) for the ultrastructural study of the pineal of the mouse 'eyeless', May 1980-May 1981 (Group III).

Pévet, P. at the division of Endocrinology, Hôpital Cantonal, Geneva, Switzerland, June 16-17.

Ruiz-Marcos, A. (guestworker from Cajal Institute, Dept. of Biophysics, Madrid, Spain) has adapted and implemented a program for the analysis of the orientation of neuronal tree structures determined by 3-D coordinates of characteristic points, May and June (Group I and Group V).

Steinbusch, H. (guestworker from Dept. of Anatomy and Embryology, University of Nijmegen) for analysis of dendrite-patterns of serotonergic cells in the rat brain-stem, September and November (Group I and Group V).

Swaab, D.F. at Centre de Neurochimie, Centre National de la Recherche Scientifique, Strasbourg, France, April 18 and at MRC Developmental Neurobiological Unit, London, England, R. Balàsz, May 27.

Swanson, H.H. at Dept. of Zoology, Australian National University, Canberra, Australia, February 19-22.

Uylings, H.B.M. at the Health Science Center, Dept. of Cell Biology, Dallas, Texas, U.S.A., August 25-27.

Van Leeuwen, F.W. at Baylor College of Medicine, Dept. of Cell Biology, Houston, Texas, U.S.A., April 16; at Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins University, School of Medicine, Baltimore, Maryland, U.S.A., April 17 and at Dept. of Electronmicroscopy, University of Ulm, Ulm, F.R.G., October 9-10.

Villanueva, H.H. (guestworker from University Autonoma de Barcelona, Spain) for information on the semi-automatic measuring for 3-D neuronal tree structures, September 9 (Group I and Group V).

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De Bruin, J.P.C. - Agonistisches Verhalten in drei Rattenstammen (Rattus norvegicus). Syllabus Ethologentreffen, Noordwijkerhout.

De Bruin, J.P.C., N.E. van de Poll and H.G. van Oyen - Prefrontal cortex and social behaviour in the rat. Neurosci. Lett., Suppl. 5, S 258.

Karasek, M. and P. Pévet - Aspekty ultrastrukturalne procesów wydzielniczych komorek szyszynki ssaków. Jubileum of the Polish Endocrine Society, Krakow.

Karasek, M. and P. Pévet - Procesów wydzielniczych komorek szyszynki ssaków. 15th Conference of the Polish Society of Electronmicroscopy. Rydzynak Lesna.

Matesz, K. and R.E. Baker - Projection patterns of skin nerves upon dorsal horn of the spinal cord in skin grafted toads. Proc. Satellite Symp. on 'Cellular Analogues of Conditioning and Neural Plasticity' of the 18th Int. Physiol. Congr., Debrecen.

Mirmiran, M. and M.A. Corner - Pharmacological suppression of active sleep in infant rats: effect on adult sleep patterns. Sleep Res. Vol. *8*, in press.

Parnavelas, H.G. and H.B.M. Uylings - Growth and plasticity of cortical dendrites. Proc. Satellite Symp. on 'Cellular Analogues of Conditioning and Neural Plasticity' of the 18th Int. Physiol. Congr., Szeged.

Payman, B.C. and H.H. Swanson - Scent marking and dominance in enclosure colonies of gerbils. Behav. Brain Res., in press.

Pévet, P. - Cytological aspects of indoleamine secretion by the pineal gland. Proc. Int. Symp. on Melatonin, Bremen.

Pévet, P., R.M. Buijs, J. Dogterom, B. Vivien-Roels, F.C. Holder, J.M. Guerné, A. Reinharz, D.F. Swaab, I. Ebels and C. Neaçsu - Peptides in the mammalian pineal gland. Satellite Symp. on Pineal Function. 6th Int. Congr. of Endocrinology, Thredbo.

Pévet, P., F.C. Holder, A. Reinharz, R.M. Buijs, J. Dogterom, B. Vivien-Roels, J.M. Guerné, C. Neaçsu and A. Rochat - The AVT-biological activity present in the pineal is not due to AVT, but to an AVT-like compound. Proc. 11th Int. Congr. of the Int. Soc. of Psychoneuroendocrinology, Florence.

Pévet, P., J. Dogterom, R.M. Buijs and A. Reinharz - Presence of vasopressin, oxytocin and neurophysins in the mammalian pineal gland: failure to demonstrate vasotocin. Proc. 11th Int. Congr. of Endocrinology, Melbourne.

Pévet, P., M.G.M. Balemans, W.C. Legerstee and B. Vivien-Roels - Circadian rhythmicity in the activity of HIOMT in the formation of melatonin and of 5-methoxytryptophol in the retina, Harderian gland and pineal of the male hamster. Proc. Int. Symp. on Melatonin, Bremen.

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. Physiol. *76*, p4B.

Reinharz, A., M.B. Valloton, P. Pévet and J. Dogterom - Neurophysins and neurohormones in the mammalian pineal gland. Satellite Symp. on Pineal Function. 6th Int. Congr. of Endocrinology, Thredbo.

Romijn, H.J., M.T. Mud and P.S. Wolters - Synapse formation in dissociated embryonal rat cerebral cortex in vitro. Acta Morph. Neerl.-Scand., in press.

Romijn, H.J., M.T. Mud and P.S. Wolters - Synapsvorming in gedissocieerd embryonaal hersenschorsweefsel van de rat in vitro. Ned. T. Geneesk. *123*, 2043.

Scholtens, J., N. E. van de Poll and H.G. van Oyen - Gonadal hormones and sexual motivation in the female rat. Proc. of the 21st Dutch Federation Meeting, Nijmegen, 383.

Swaab, D.F. - Synthesis, transport and release of peptides in the brain. Proc. of the 21st Dutch Federation Meeting, Nijmegen, 417.

Swanson, H.H., N.E. van de Poll, J. van Pelt, H.G. van Oyen and J.P.C. de Bruin - Vergleich des Verhaltens zweier Rattenstämme (S3 und WEzob). Syllabus Ethologentreffen, Noordwijkerhout.

Swanson, H.H., N.E. van de Poll, J. van Pelt, H.G. van Oyen and J.P.C. de Bruin - Comparison of the behavior of two strains of rats (S3 and WEzob) in male-female encounters during a normal oestrus cycle. Proc. Europ. Brain and Behav. Society, Annual General Meeting, Louvain-la-Neuve.

Uylings, H.B.M., J.G. Parnavelas and H.L. Walg - Morphometry of 3-dimensional cortical dendritic trees. Proc. Symp. on Morphometry and Stereology, 11th Int. Anatomy Congr., Mexico City.

Van de Poll, N.E. - Steroid hormones and behaviour. Proc. of the 21st Dutch Federation Meeting, Nijmegen, 336.

Van de Poll, N.E., F. de Jonge, H.G. van Oyen and J.P.C. de Bruin - Failure to find sex differences in testosterone activated aggression in two strains of rats. Neurosci. Lett., Suppl. *5*, 323.

Van Leeuwen, F.W. - Light and electron microscopical immunocytochemical localization of neuropeptides in the rat brain. Ad Valvas, Free University.

Van Leeuwen, F.W. - Pitfalls in immunocytochemistry of neuropeptides. Neurosci. Lett., Suppl. *5*, 195.

Van Leeuwen, F.W. - Monospecific localization of neuropeptides, a utopian goal? Proc. of the FEBS Advanced Course Nr. 69 on Biomolecular Electron Microscopy, Schloss Reisenburg.

Van Oyen, H.G., J.P.C. de Bruin, V.D.J. Nolten and S.M. van der Zwan - Behavioral functions of the prefrontal cortex in the rat. Proc. of the 21st Dutch Federation Meeting, Nijmegen, 324.

Van Oyen, H.G., N.E. van de Poll and S.M. van der Zwan - Behavioral and endocrine aspects of sex differences in passive avoidance behaviour. Neurosci. Lett., Suppl. 5, 180.

Van Wijk, M. and J. Korf - Distribution of imipramine in rat brain. Proc. of the 21st Dutch Federation Meeting, Nijmegen, 480.

Van Wijk, M. and J. Korf - Regional distribution of imipramine in rat brain, no relation to receptor binding. Proc. 12th C.I.N.P. Congr., Göteborg, 358.

Vaughan, M.K., L.Y. Johnson, P. Pévet, C. Neaçsu, and R.J. Reiter - Effect of a polypeptide (E5) extracted from bovine pineal glands on plasma and pituitary levels of luteinizing hormone (LH) and prolactin in normal and castrated adult male rats. Proc. 10th Annual Meeting of the Soc. for Neurosci., Cincinnati.

Velis, D.N., R.M. Buijs and D.F. Swaab - Neurosecretoire cellen en hun exohypothalame vezels tijdens de hersenontwikkeling bij de rat. Ned. T. Geneesk. *124*, 855-856.

Vivien-Roels, B., P. Pévet, J. Arendt, G.M. Brown and M.P. Dubois - Immunohistochemical evidence of the presence of melatonin in the pineal gland, the retina and the Harderian gland of mammalian and non-mammalian vertebrates. Proc. 6th Int. Congr. Endocrinol., Melbourne.

Vivien-Roels, B., P. Pévet, J.M. Guerné, F.C. Holder, A. Meiniel, J. Dogterom and R.M. Buijs - On the presence of arginine vasotocin in the pineal organ of non-mammalian vertebrates. Proc. Satellite Symp. on Pineal Function, 6th Int. Congr. of Endocrinology, Thredbo.

Vivien-Roels, B., P. Pévet, J. Arendt, G.M. Brown and M.P. Dubois - Presence of melatonin in the pineal gland, the retina and the Harderian gland: an immunohistochemical study. Proc. 11th Congr. Int. Soc. Psychoneuroendocrinology, Florence.

Papers read

See also 'abstracts' and 'teaching' sections.

Baker, R.E. - Patterns of peripheral reinnervation and topography of dorsal root ganglion cells in skin-grafted frogs. 1st Int. Society of Developmental Neurobiology, Strasbourg, July 1.

Baker, R.E. - Possible mechanisms in the development of misdirected reflexes in skin-grafted anurans. Symposium on 'Cellular Analogues of Conditioning and Neural Plasticity', Szeged, July 21.

Baker, R.E. - Factors affecting the selectivity of synapse formation. Meeting of FUNGO workgroup 'Development and Aging of the Nervous System and Behaviour', Nijmegen, October 17.

Boer, G.J. - Regulation of brain growth by neurotransmitters, hormones and neurohormones. Neurochemie 1980. Ver. Exp. Klin. Neurochemie, Utrecht, June 14.

Boer, G.J. - Vasopressin release from Accurel in vitro and in vivo. AKZO Research Centre, Arnhem, November 28.

Buijs, R.M. - Vasopressine en oxytocine synapsen in het centraal zenuwstelsel. Boerhaave cursus voor postacademisch onderwijs in de geneeskunde, 175-182.

Corner, M.A. - Experimental studies on the possible importance of REM sleep for brain development. Round table Presentation at 13th Annual Meeting of the Association for the Psychophysiological Study of Sleep, Mexico City, April 5.

Corner, M.A. - On the role of REM sleep in development of the central nervous system. Theoretical and methodological considerations. Invited lecture at the 5th Meeting of the European Society for Sleep Research, Amsterdam, September 18.

Corner, M.A. - Longterm sequelae of chronic clomipramine administration in rats during early infancy. 'Anafranil roundtable' at Ciba-Geigy, Basel, November 10.

De Bruin, J.P.C. - Behavioral changes after prefrontal cortex lesions in the rat, Psychonomy, Amsterdam, April.

De Bruin, J.P.C. - Prefrontal cortex and social behaviour in the rat. 4th European Neuroscience Conference, Brighton, September.

De Bruin, J.P.C. - Agonistisches Verhalten in drei Rattenstammen (Rattus norvegicus). Ethologentagung, Noordwijkerhout, October.

De Vries, G.J. - De ontwikkeling van de vasopressinerge neuronen van de nucleus suprachiasmaticus en hun exohypothalame vezels in de rat. Bijeenkomst Nederlandse Vereniging voor Endocrinologie, Utrecht, October 10.

De Vries, G.J. - Ontwikkeling van vasopressine neuronen in de nucleus suprachiasmaticus en het verschijnen van een geslachtsverschil in de vasopressine innervatie van het septum. EM Werkgroep Zenuwstelsel en de Verhaart-groep, Amsterdam, November 11.

De Vries, G.J. - De functie van peptiden in de hersenen. Medisch-fysisch Instituut (TNO), Utrecht, December 1.

Habets, A.M. and H.J. Romijn - Relation between ultrastructure and spontaneous bioelectric activity in developing neuronal networks in tissue culture. Meeting of FUNGO workgroup 'Development and Aging of the Nervous System and Behavior', Nijmegen, October 17.

Pévet, P. - Pineal peptides. University of Geneva, Geneva, June 16.

Romijn, H.J. - Hoe werken onze hersenen? Gemeentelijke Sociale Dienst, Amsterdam, January and October.

Romijn, H.J. - Waarom slapen en dromen we? Volksuniversiteit, Amstelveen, February.

Romijn, H.J. - Contact-neurotrofie en rechtsomgaande uitgroei in gedissocieerd hersenschorsweefsel van de foetale rat in vitro. EM Werkgroep Zenuwstelsel en de Verhaart-groep, Amsterdam, April 22.

Romijn, H.J. - Structuur/functie onderzoek naar de ontwikkeling van foetaal hersenschorsweefsel in vitro. Afd. Biologische Psychiatrie, Groningen, October 27.

Romijn, H.J. - Over de slaap en de droom. Gemeentelijke Sociale Dienst, Amsterdam, November.

Swaab, D.F. - Vasopressin and Oxytocin: hormones, transmitters or modulators? Memorial symposium on 'Brain and Behaviour' in honour of Prof. J.Z. Young (Jan Swammerdam Tri-Centenary), KNAW Trippenhuis, Amsterdam, February 14.

Swaab, D.F. - Vasopressin and oxytocin, neurohormones and neurotransmitters. Centre de Neurochimie, Centre National de la Recherche Scientifique, Strasbourg, April 18.

Swaab, D.F. - Functions of α-MSH and other opiomelanocortins in intrauterine growth, in labour and in brain development. Ciba Foundation Symposium no. 81 'Intermediate Lobe of the Pituitary', The Ciba Foundation for the Promotion of International Cooperation in Medical and Chemical Research, London, June 11.

Swaab, D.F. - Lecture and demonstration 'Specificity problems' at the ENA workshop 'Neuroanatomical techniques in immunocytochemistry', Brighton, U.K., September 15.

Swaab, D.F. - Schade aan de hersenen van ongeboren kinderen, gevolgen voor het latere gedrag en herstel mogelijkheden. Forum 'Hersenwerk', organized by the Dienst Wetenschapsvoorlichting, KNAW, Jaarbeurscongreszaal, Utrecht, November 5.

Swanson, H.H. - Social hormonal factors influencing scent marking, dominance and fertility in the Mongolian gerbil, Monach University, Psychonomy, Melbourne, February.

Swanson, H.H. - Social influences on sexual maturation in the Mongolian gerbil,

Colloquium Endocrinologie, Erasmus University, Rotterdam, May 22.

Swanson, H.H. - Social and hormonal influences on scent marking in the Mongolian gerbil. Amsterdamse Ethologische Kring, September 12.

Swanson, H.H., N.E. van de Poll, J. van Pelt, H.G. van Oyen and J.P.C. de Bruin - Comparison of the behaviour of two strains of rats (S3 and WEzob) in male-female encounters during a normal oestrus cycle. Ethologentagung, Noordwijkerhout, October 6.

Swanson, H.H. and Payman, B.C. - Scent marking and dominance in enclosure colonies of gerbils. European Brain and Behaviour Society, Brussels, November 13-15.

Swanson, H.H. - Social organization in confined colonies of gerbils. BION workgroup Ethology, December 12-13.

Uylings, H.B.M. - Morfometrische studie van de niet-pyramide celontwikkeling in de visuele cortex van de rat. Interuniversitair Oogheelkundig Instituut, Amsterdam, March 21.

Uylings, H.B.M. - Postnatale ontwikkeling van niet-pyramide cellen in de visuele schors van de rat. Verhaart Meeting of Dutch Neuromorphologists, Leiden, June 3.

Uylings, H.B.M. - Development and plasticity of cortical dendrites. Dept. of Cell Biology, Health Science Center, Univ. of Texas, Dallas, August 25.

Uylings, H.B.M. - Morphometrical analysis of growth and plasticity of cortical dendrites. Dept. Physiology and Biophysics, New York Medical Center, New York, August 28.

Van de Poll, N.E. - Steroid hormones and behaviour. Dutch Federation Meeting, Nijmegen, April 11.

Van de Poll, N.E. - Sexuele motivatie in de rat. Vakgroep Fysiologische Psychologie, Utrecht, April 25.

Van de Poll, N.E. - Agressie: de invloed van winnen en verliezen. Psychonomie Werkgemeenschap 'Vergelijkende en Fysiologische Psychologie', Amsterdam, April 29.

Van Leeuwen, F.W. - Immunocytochemistry of neuropeptides in the rat brain. Seminar at the Baylor College of Medicine, Dept. of Cell Biology, Houston, April 16.

Van Leeuwen, F.W. - Valkuilen in immunocytochemisch onderzoek. Netherlands Society of Electron microscopy, Maastricht, April 24.

Van Oyen, H.G. - Respons alternering bij mannelijke en vrouwelijke ratten. Psychonomie Werkgemeenschap 'Vergelijkende en Fysiologische Psychologie', April 29.

Van Oyen, H.G. - Gedragsmatige en hormonale analyse van sex verschillen in vermijdingsgedrag. FUNGO Werkgemeenschap 'Hersenen en Gedrag', November 12.

Teaching

a. Students

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

Blitz, P. (medical student, University of Amsterdam): 'Stage' (Group IV)

Brenner, E. (biology student, University of Utrecht): 'Innervation of spinal cord explants by dorsal root ganglion neurons in vitro' (Group II)

De Jonge, F.H. (biology student, University of Amsterdam): 'Aggressive behavior in male rats: effects of winning and losing on subsequent aggressive responses' (Group IV)

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

Fliers, E. (medical student, University of Amsterdam): 'Immunocytochemical staining of oxytocin and vasopressin producing neurons in the human brain during aging' (Group III)

Gross, L. (medical student, University of Rochester, New York, U.S.A.): 'Immunocytochemical localization of serotonin in relation to the vasopressinergic cells of the suprachiasmatic nucleus' (Group III)

Heinsbroek, R.P.W. (biology student, University of Amsterdam): 'Involvement of the pituitary adrenal system in sex differences in aversively motivated learning' (Group IV)

Hoorneman, E.M.D. (biology student, Free University, Amsterdam): 'The influence of the suprachiasmatic nucleus on passive avoidance behaviour, water balance and temperature regulation' (Group III)

Kleist, M. (biology student, University of Utrecht): 'Factors influencing the specificity of synapse formation in vitro' (Group II)

Koster, A.B. (medical student, University of Amsterdam): 'Immunocytochemistry of vasopressin and oxytocin in mice suffering from renal diabetes insipidus' (Group III)

Partiman, T. (medical student, University of Utrecht): 'Effects of kainic acid lesions in the pontine reticular formation upon sleep/wake patterns in infant rats' (Group II)

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

Schwaggermann, H. (medical student, University of Amsterdam): 'Instrumental methods for selective deprivation of REM-sleep in developing rats' (Group II)

Smeets, J. (psychology student, University of Amsterdam): 'Effects of losing and winning on subsequent aggressive behaviour and avoidance learning in male and female rats' (Group IV)

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

Van den Dungen, H.M. (biology student, University of Utrecht): 'AVT and mesotocin containing systems in the trout brain. Localization of AVP in the human brain' (Group III) and 'Effects of rearing in an 'enriched' environment on sleep patterns in developing rats' (Group II)

Van Pelt-Heerschap, H.M.L. (biology student, Free University, Amsterdam): 'Characterization of oxytocin immunoreactivity in the central nervous system of the pond snail, Lymnea stagnalis (L.), by means of isoelectrofocussing on radioimmunoassay' (Group III)

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

Wilbrink, M. (biology student, Free University, Amsterdam): 'The effects of treatment with chlorimipramine during early postnatal development: the resulting suppression of active sleep and its possible role in behavioral development in the albino rat' (Group II and IV)

b. Lectures and theses

For lectures during the EMBO Practical Course 'Immunocytochemistry and its Applications in Brain Research' see below (*c.*)

Buijs, R.M. - Lecture 'Vasopressinergic and oxytocinergic synapses in the central nervous system', Boerhaave course for postgraduate medical teaching, 'Immunoperoxidase techniques', Leiden, June 6.

Buijs, R.M. - Lecture 'Peptides in the brain', University of Leiden, Medical School, November 24.

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

De Bruin, J.P.C. - Lecture 'Prefrontal cortical lesions and social behaviour' University of Utrecht, Psychophysiology, March.

Pool, C.W. - Lecture and participation panel discussion during course 'Proefdieren, Biologie en Samenleving', University of Amsterdam, Biology, October 2, 6, 9 and 10.

Swaab, D.F. - Integrated lecture 'Neuroendocrine regulation at the menstrual cycle and sexual behaviour', University of Amsterdam, Medical School, January 22.

Swaab, D.F. - Integrated lecture 'Growth and development of the brain', University of Amsterdam, Medical School, January 24.

Swaab, D.F. - Lecture 'Neuroendocrine regulation (vasopressin and oxytocin)', University of Amsterdam, Medical School, February 13.

Swaab, D.F. - Lecture 'Central effects of peptide hormones', University of Amsterdam, Medical School, February 13.

Swaab, D.F. - Promotor for PhD thesis F.W. van Leeuwen, Free University, Amsterdam, 'Light and electronmicroscopical immunocytochemical localization of neuropeptides in the rat brain', March 13.

Swaab, D.F. - Inaugural speech on entry upon the office of Professor extraordinarius of Neurobiology, University of Amsterdam, 'Bederven wij de hersenen van onze kinderen reeds voor hun geboorte?', April 22.

Swaab, D.F. - Member committee for PhD thesis W.L. Lamers, University of Amsterdam, 'Multihormonal control of enzyme clusters in liver ontogeny', May 22.

Swaab, D.F. - Member committee for PhD thesis C.W. Pool, University of Amsterdam, 'An immune- and enzyme-histochemical determination of striated muscle fibre characteristics', October 15.

Swaab, D.F. - Promotor for PhD thesis R.M. Buijs, University of Amsterdam.

Swanson, H.H. - Promotor for PhD thesis B. Payman, University of Birmingham, 'Social factors influencing scent marking and reproduction in the Mongolian gerbil, Meriones unguiculatus', May 23.

Swanson, H.H. - Lecture 'The sociobiology of the gerbil', University of Amsterdam, Biology, course Animal Behaviour, November 28.

Uylings, H.B.M. - Lecture 'Development and plasticity of the nervous system', University of Amsterdam, Biology, course 'Capita Selecta', for peridoctoral students, January 25.

Van de Poll, N.E. - Integrated lecture 'Gonadal hormones and behavior', University of Utrecht, Psychology, May 12, 14, 19 and 21.

Van Leeuwen, F.W. - Lecture 'Principles of Immunoelectronmicroscopy', Boerhaave course for postgraduated medical teaching, 'Immunoperoxidase techniques', Leiden, June 4.

c. EMBO Practical Course: Immunocytochemistry and its Applications in Brain Research

This practical course was held in the Netherlands Institute for Brain Research, May 27-30, 1980. The organizing committee (G.J. Boer, D.F. Swaab and J. Sels) compiled at the request of the EMBO a program dealing with the rapidly developing field of immunocytochemistry and has dealt with the selection of 15 postgraduates out of more than 150 applicants. A teaching staff of 17 international scientists gave introductory lectures, practised immunocytochemistry at the laboratory table and presented demonstrations partially by means of posters. The docents were: J. Barry (France), G.J. Boer (NIBR, The Netherlands), D.M. Boorsma (V.U., The Netherlands), R.M. Buijs (NIBR, The Netherlands), G.D. Burford (U.K.), V. Chan-Palay (U.S.A.), A.C. Cuello (Oxford, U.K.), J.J. Geuze (Utrecht, The Netherlands), J.J. Haayman (TNO, Rijswijk, The Netherlands), C.W. Pool (NIBR, The Netherlands), J.W. Slot (Utrecht, The Netherlands), H.W.M. Steinbusch (Nijmegen, The Netherlands) L.A. Sternberger

(U.S.A.), N.H. Sternberger (U.S.A.), D.F. Swaab (NIBR, The Netherlands), F. Vandesande (Belgium) and F.W. van Leeuwen (NIBR, The Netherlands). In addition, several aspects of the immunocytochemical techniques (e.g. pretreatment of the tissue, raising antibodies, choise of staining procedure, method- and serum specificity, double staining, light- and electronmicroscopical immunocytochemistry) were thoroughly discussed in various informal sessions with the students. Members of workgroup III all have been incorporated in the actual organization of the practical sessions (especially the technicians) and discussions. The practical part of the course was interrupted by a public mini-symposium on 'Recent Immunocytochemical Findings in Neurobiology', held in the building of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, on May 29. A course manual has been prepared containing not only abstracts on the several introductory lectures and demonstrations, but also including full descriptions of the immunocytochemical procedures performed during the course. The course manual has additionally been offered for sale to all applicants who could not participate. The course manual will moreover be used as a model for an IBRO handbook on Immunocytochemistry, edited by A.C. Cuello (Oxford).

Miscellaneous

Baker, R.E. - projectleader in FUNGO workgroup 'Development and aging of the nervous system and behaviour'.

Boer, G.J. - organizer with D.F. Swaab and J. Sels of the EMBO practical course immunocytochemistry and its applications in brain research, Amsterdam, May 27-30; projectleader 'Vasopressin release from Accurel in vitro and in vivo (in cooperation with and sponsored by AKZO Chemie, The Netherlands); supervisor of the radio-isotope laboratory of the Netherlands Institute for Brain Research; chairman of the organizing committee of the VIIIth Dutch British Endocrine Meeting 1982; organizer of the mini-symposium on 'Recent Immunocytochemical Findings in Neurobiology', Royal Netherlands Academy of Arts and Sciences, Amsterdam, May 29.

Corner, M.A. - projectleader in FUNGO workgroup 'Development and aging of the nervous system and aging of the nervous system and behavior'; member of the program committee for the 5th Meeting of the European Society for Sleep Research, Amsterdam, September 1980; organizer of 'roundtable' session at 2nd Meeting of the Int. Soc. for Developmental Neuroscience, New York City, June 1981; regional secretary for the Netherlands of the International Society for Developmental Neuroscience; advisory board of Developmental Neuroscience, Developmental Psychobiology, Developmental Brain Research, Neuroscience and Biobehavioral Reviews and IRCS Medical Science; referee for European J. Pharmacology (1x); research advisor at Dept. of Clinical Psychology, University of Amsterdam; co-editor of 'Adaptive Capabilities of the Nervous System' PBR 53.

Habets, A.M. - projectleader in FUNGO workgroup 'Development and aging of the nervous system and behavior'.

Pévet, P. - secretary-treasurer of the European Pineal Study Group; editor with J. Ariëns Kappers of the book 'The Pineal Gland of Vertebrates Including Man', Progress in Brain Research, Vol. 52; editor of the EPSG Newsletter; projectleader in FUNGO workgroup project 133533; referee for Annales d'Endocrinologie (1x), Cell Tiss. Res. (1x) and Reprod. Nutrition Development (2x).

Pool, C.W. - referee for J. Histochem. Cytochem. (1x).

Romijn, H.J. - referee for a research proposal National Science Foundation (Washington D.C.); co-editor of 'Adaptive Capabilities of the Nervous System', PBR 52.

Swaab, D.F. - member of the advisory boards of Acta Endocrinologica and J. Neural Transmission; member of the editorial boards of J. Developmental Physiology, J. Neuroscience Methods and Peptides; leader of FUNGO-project 133507 and ZWO-project 91106; president of the Dutch Society for Endocrinology; secretary of the Dutch Committee of the Int. Brain Research Organization; member of the Int. Scientific Committee of the European Science Foundation (European Training Programme in Brain and Behaviour Research); organizer with G.J. Boer

and J. Sels of the EMBO practical course 'Immunocytochemistry and its Applications in Brain Research', Amsterdam, May 27-30, and of the mini-symposium 'Recent Immunocytochemical Findings in Neurobiology', Royal Netherlands Academy of Arts and Sciences, Amsterdam, May 29; referee for J. Develop. Physiol. (2x), Acta Endocrinol. (3x), Eur. J. Obstet. and Gynaecol. Reprod. Biol. (2x), J. Neurosci. Meth. (1x), J. Endocrinol. (4x), Eur. J. Pharmacol. (1x) and Peptides (2x).

Swanson, H.H. - member of the advisory council Int. Society of Psychoneuroendocrinology; member of Ethics Committee Int. Soc. for Research on Aggression; invited participant in NATO advanced Study Group Institute 'The Biology of Aggression', Bonas, France, July 21-30; editorial associate of Behav. and Brain Sciences; referee for Physiol. and Behavior (1x), Medical Research Council, London, project grant applications.

Uylings, H.B.M. - co-organizer of the bimonthly meetings of the Dutch Neuromorphologists; workgroup leader FUNGO workgroup 'Development and Aging of Brain and Behavior'; referee for J. Theor. Biol. (1x), Bull. Math. Biol. (2x) and J. Neurosci. Meth. (2x).

Van Leeuwen, F.W. - Chairman of the workshop 'Immunoelectronmicroscopy' of the Dutch Society for Cell Biology.

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 behaviour in rats' of the Psychonomy workgroup 'Comparative and physiological psychology'; advisor of the project 'Hormonal regulation of sexual behavior in the male stumptail Macaca'; leader of projects in FUNGO and Psychonomy; coordinator of the workgroup 'Comparative and physiological psychology' of the Psychonomic Foundation; referee for Behavioral Brain Res. (1x) and Physiology and Behavior (1x); co-editor of 'Adaptive Capabilities of the Nervous System', PBR 53; research grants to study 'Sexual motivation of the rat' (Psychonomy) and 'Is aromatization involved in androgenic stimulation of aggression in rats and mice?' (ETP).

Van Oyen, H.G. - Member of advisory board for the project 'Odor intensity discrimination in the rat' of the workgroup 'Comparative and physiological psychology', Psychonomy, and of 'Genetic analysis of succession discrimination learning'; invited guest of the NATO studygroup on 'Biology of Aggression'.

Seminars given at the Institute in 1980

(organization Dr. G.J. Boer)

January 23 - Dr. A.J. Dunn (Dept. of Neuroscience, University of Florida, Gainesville, U.S.A.): Neurochemical action of ACTH and vasopressin.

February 13 - Prof. J.Z. Young (Marine Biological Association of the United Kingdom, Laboratory, Plymouth, U.K.): Statocysts and cerebellums in Cephalopods.

March 12 - Prof. B. Benson (Dept. of Anatomy, University of Arizona, Tucson, Arizona, U.S.A.): Peptides in the pineal gland.

April 2 - Dr. P.G. Barth (Laboratorium voor Neuropathologie, Vrije Universiteit, Amsterdam): Clinical applications of the Golgi technique in autopsy.

May 14 - Dr. M. Frotscher (Max Planck-Institut für Hirnforschung, Neurologische Abteilung, Frankfurt, F.R.G.): Effects of neonatal lesions and of environmental factors on the structural differentiation of hippocampal neurons.

June 5 - Prof.dr. A. Ruiz Marcos (Instituto Cajal, Madrid, Spain): The use of mathematical modeling in biomedical research. Study of the effect of hypothyroidism on the synaptogenesis of the cerebral cortex.

June 19 - Prof. J.A. Gray (Laboratoire de Physiologie, Hôpital Pitié-Salpêtrière, Paris, France): Septo-hippocampal system, monoamines and non-reward.

June 20 - Prof. D. Criel (Dept. Ophthalmology, University of Utah, Salt Lake City, Utah, U.S.A.): Visual anomalies associated with albinism.

July 2 - Dr. M.L. Feldman (Dept. of Anatomy, Boston University, Boston, Mass., U.S.A.): Neurocytological changes with advancing age in the auditory and visual systems of the rat.

September 23 - Prof.dr. A. van Harreveld (California Institute of Technology, Pasadena, Calif., U.S.A.): L-proline as a glutamate agonist and antagonist.

November 18 - Prof. A.J. Kastin (Endocrinology Section, Dept. of Medicine, Tulane University, New Orleans, U.S.A.): CNS effects of peripherally injected peptides.

December 3 - Prof.dr. E.P. Köster (Laboratory of Psychology, University of Utrecht, Utrecht): Psychobiology of olfaction.

Hubrecht Laboratory
International
Embryological Institute

Progress Report 1980

Edited by J. Faber

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

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vacancy as from November 1979

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(as from August 1979)

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Chief Librarian

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Elze C. Boterenbrood, Ph.D.

Experimental Morphology; Cinematography

K. Hara, Ph.D.

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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. 4).

The Laboratory aims at stimulating international cooperation and understanding by, among other things, organizing International Research Groups in Developmental Biology at more or less regular intervals.

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

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

Introduction

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

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

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

The scientific aims of the Laboratory and the means for their realization are at present the subject of reconsideration by the Royal Netherlands Academy. If changes in the research programme should result from this, they will probably be effected gradually.

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

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

I. Research Group: Embryogenesis in Amphibians

Members: J.G. Bluemink, E.C. Boterenbrood, K. Hara, P.D. Nieuwkoop, G.A. Ubbels, R. Verhoeff-de Fremery, J.E. Speksnijder (res. assoc.), P.A.T. Tetteroo (res. assoc.); E.C.A. Freund, W.J. Hage, C.H. Koster, J.M. Narraway, P. Tydeman, F.J.M. Vervoordeldonk.

Guest workers: K.E. Dixon (Bedford Park, S.A., Australia), J. Paleček (Praha, Czechoslovakia), K. Rzehak (Kraków, Poland), A.W.C. Dorresteijn (Utrecht, The Netherlands), M. Gadenne (Brussels, Belgium).

Collaboration: K.E. Dixon (Bedford Park, S.A., Australia), J.C. Gerhart (Berkeley, CA, U.S.A.), M.W. Kirschner (San Francisco, CA, U.S.A.), J.-C. Beetschen (Toulouse, France), K. Rzehak (Kraków, Poland), E.E. Baulieu (Paris, France), N.H. Verdonk (Utrecht, The Netherlands).

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

of nucleo-cytoplasmic interactions; 5. the structure and function of cell contacts.

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

A. MORPHOGENETIC MOVEMENTS

1. Pregastrular surface movements and duration of cleavage cycles from morula to blastula (Xenopus laevis)

In continuation of the investigation started last year (see previous report, sect. I.A.1) time-lapse films of the animal and vegetal poles of early embryos were used to measure changes in the external surface area of certain lineages. The changes were calculated as percentage differences in surface area of 4 cells chosen from the 6th-cleavage stage and measured in the same phase of successive cleavage cycles up to the 11th cleavage. The changes were found to be linear. The changes at the animal and vegetal pole are not complementary, the percentage enlargement of the surface area at the animal pole being about ten times the reduction at the vegetal pole. The main effect of epiboly may therefore be expected to occur in the equatorial region; this will be measured next year.

Epiboly and reduction of surface area already occur at the earliest time when measurements can be made by this method, i.e., from the 32-cell stage (5th cleavage) at the animal pole and from the 64-cell stage (6th cleavage) at the vegetal pole. Cells dividing perpendicular to the surface of the egg contribute more to epiboly than cells dividing tangentially. A morphometric study has been started of histological sections of the blastocoelic roof made at the same stage in each successive cleavage cycle as that used to measure cell surface areas on the films.

An investigation into the possible relationship between the initiation of gastrulation and the duration of the preceding cleavage cycles is in progress. The cleavage number at which gastrulation starts varies in individual embryos.

In future attention will be focussed on the question whether nuclear or cytoplasmic events determine the beginning of gastrulation. The approach will include the technique of delayed nuclear supply to one half of a temporarily constricted egg; and possibly the study of pseudogastrulation.

2. An electron-microscopical study of the gastrulation defect in embryos of the ac/ac maternal effect mutant (Pleurodeles waltl)

Abnormal gastrulation in the *ac/ac* (*'ascite caudale'*) maternal-effect mutant of *Pleurodeles* has been described by Beetschen[1,2], Beetschen and Fernandez[3] and Fernandez[4]. After normal cleavage all the progeny show the same syndrome at the beginning of gastrulation. The ectoderm of the animal half of the embryo becomes pitted. As gastrulation proceeds the ectodermal pits develop into grooves giving the animal hemisphere a brain-like appearance. Epiboly is disturbed and blastoporal invagination begins normally but remains incomplete. Many embryos exogastrulate but a more or less complete axis may still develop depending on the extent of gastrulation.

Deficient gastrulation in the *ac* mutant was investigated using scanning and transmission electron microscopy to find out whether or not active contraction in the ectodermal pits and grooves was involved. Eggs from homozygous *ac/ac* females were obtained from Prof. J.-C. Beetschen (Université Paul Sabatier, Toulouse, France). Electron-microscopical analysis shows that many ectoderm cells in the bottom of the pits and grooves have narrowed apices and bear many microvilli, while the cortical cytoplasm is dense, filamentous and underlain by a stratum of vesicles. These cells therefore show the characteristic features of contracted cells. Adjacent cells forming the wall of the groove look different in that they have no narrow apical surface, few microvilli, and no stratum of filaments with associated vesicles. Our conclusion is that the syndrome is caused by active contraction of cells at randomly distributed sites in the ectodermal half of the embryo, rather

than by overall expansion. Evidence exists that in cells of amphibian embryos a change in the ion permeability properties of the plasma membrane is often involved in surface contraction. Whether a change in membrane permeability is involved in this case needs to be investigated. In relation with this and other problems we intend to start using the so-called 'vibrating probe' developed by Jaffe and Nuccitelli[5] to investigate topographical and temporal changes in ion transport properties of embryonic surfaces.

The question of the nature and action of the maternal factor(s) causing the syndrome needs further investigation. The above electron-microscopical findings have been accepted for publication (see publ. 14).

[1] Beetschen, J.-C. (1970) - C.R. Acad. Sci. *270*, 855-858.

[2] Beetschen, J.-C. (1976) - Bull. Soc. Zool. France *101*, 57-61.

[3] Beetschen, J.-C. and M. Fernandez (1979) - In: Maternal Effects in Development (Eds. D.R. Newth & M. Balls), Cambridge Univ. Press, 269-286.

[4] Fernandez, M. (1979) - J. Embryol. exp. Morph. *53*, 305-314.

[5] Jaffe, L.F. and Nuccitelli (1974) - J. Cell Biol. *63*, 614-628.

B. UV EFFECTS ON EARLY DEVELOPMENT

1. The effect of UV-irradiation on early embryos: a time-lapse cinematographic study (Xenopus laevis)

UV-irradiation of the vegetal half of anuran zygotes results in disturbance of the germ cell line. In 1977 we started a series of experiments aimed at the question whether this disturbance is due to specific effects on the germinal cytoplasm or to more general effects on embryogenesis (see previous report, sect. V.3).

The experiments were further extended this year. Analysis of time-lapse films of cleaving irradiated zygotes revealed that 1. the 3rd ('horizontal') cleavage planes are situated at a higher level than those in control (non-irradiated) eggs; 2. the animal cap cells show a slight delay of cleavage during the 'synchronous' cleavage period compared with the controls and a stronger delay during the later 'asynchronous' period; 3. the vegetal pole cells show a considerable claevage delay already during the 'synchronous' cleavage period; and 4. the start of gastrulation is also delayed considerably. It is clear that UV-irradiation affects overall morphogenetic processes during early development of the embryo. The findings are being prepared for publication. In how far the development of the primordial germ cells is affected in such irradiated embryos is being examined histologically.

2. The effect of UV-irradiation on the unfertilized egg: a video time-lapse study (Xenopus laevis)

As mentioned under *1.* above, UV-irradiation causes overall morphogenetic effects on early embryonic development. The question arises what is the primary effect of irradiation on the egg, which may ultimately lead to a series of disturbances in later stages. Since it is known that UV activates unfertilized eggs we have made a detailed study of this activation by means of video time-lapse techniques.

The results show that 1. already prior to the occurrence of the 'activation wave' (see D.*1* below) the egg is flattened by gravity, in contrast to normal eggs, and 2. activation can be obtained with a lower dose of UV when irradiation is performed in a Ca^{2+}-free medium. We suggest that the primary effect of UV is to change the surface properties of the egg in such a way that both the surface rigidity and the ion household are modified, which then leads to the initiation of the activation wave. The results are being prepared for publication.

C. NUCLEO-CYTOPLASMIC INTERACTIONS IN EARLY DEVELOPMENT

1. Development of diploid/haploid chimeric embryos (Xenopus laevis)

Nucleo-cytoplasmic interactions are an intriguing aspect of early embryogenesis, particularly with regard to the timing of cleavage cycles and of early morphogenetic processes. Embryos that are chimeric as to the ploidy of their cells are a

particularly useful tool because the cytoplasm of the cells can be expected to be qualitatively the same, the only differences residing in the nuclei.

Bispermically fertilized eggs (see D.3 below and publ. 24) were filmed simultaneously from the top and the side with a double-camera assembly. Analysis of the films revealed that 1. when the two first-cleavage planes determined by the two sperms fuse into one, the egg develops into what is apparently a right/left ploidy-chimeric embryo; 2. in the animal hemisphere, during the asynchronous cleavage period, the blastomeres of one side retain shorter claevage cycles than those of the other side; 3. the shorter-cycle side starts gastrulation later than the other side; and 4. the neural plate is narrower on the shorter-cycle side, ultimately leading to a larva that has the main body axis bent towards that side. Preliminary karyotype analysis indicates that the shorter-cycle side indeed consists of haploid cells and the other side of diploid cells. The findings are being prepared for publication.

D. THE ROLE OF CYTOPLASMIC DISPLACEMENTS AND THE INVOLVEMENT OF THE CYTOSKELETON

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

Upon fertilization the axially symmetrical mature egg becomes symmetrized as a result of pigment shifts and cytoplasmic segregation[1]. The early visible sperm entrance point (SEP)[2] has been shown to be a good marker of the future ventral side (see publ. 5). A wave-like propagation of pigment contraction, visualized by time-lapse cinematography, starts from the SEP between times 0 and 0.1 (fertilization = time 0; 1st cleavage = time 1) and moves over the egg surface (10 µm/sec) to the opposite side[3]. In locally activated eggs a similar wave starts from the 'prick point'. This activation wave (AW) presumably reflects the extrusion of the cortical granules. In fertilized eggs the AW is followed by a second surface wave between times 0.2 and 0.3. This post-fertilization wave (PFW)[4] again proceeds over the egg from the SEP to the opposite side (1 µm/sec). (It is sometimes followed by a second similar PFW, which is, however, much less clearly visible.) Both the AW and the PFW appear to be early expressions of dorso-ventral polarity. The question is whether they actually function in the establishment of the ultimate dorso-ventral polarity or whether they secondarily reflect some other basic processes.

Egg rotation experiments and application of mitotic drugs support the assumption that the asymmetrical PFW(s) and the coincident internal cytoplasmic movements[1] have a common basis in microtubular activity, which is partly associated with the presence of the sperm and the activity of its centriole (see previous report, sect. I.B.2). Therefore, sperm aster growth was studied in detail in 6 µm sections of artificially fertilized eggs fixed at various time intervals determined by video time-lapse recording of the (first) PFW. The following observations were made: 1. The extrusion of the second polar body occurs in the period from time 0.16 to 0.25, concomitantly with the start of the PFW; 2. The histological sections show that the growing tips of the sperm aster rays hit the cortex at the level where the wave front of the PFW is located. They reach the dorsal cortex between times 0.6 and 0.8, at which time the egg shows an increased resistance to rotation and centrifugation treatment[6] (see also publ. 5). This can now be well explained as resulting from the rigid internal structure of the egg due to the presence of the extended sperm aster and additional elements of the cytoskeleton; 3. The fusion of the pronuclei occurs in the same period (0.7-0.8); The pigment granules in the cortical region are found along the astral rays, along other protein fibrils running perpendicular to the cortex, and in the yolk-free cytoplasmic layer under the plasmalemma. The astral rays and protein fibrils react specifically with anti-tubulin serum raised against tubulin isolated from *Xenopus* eggs (see 2 below).

These observations are consistent with the view that grey crescent formation reflects the movement of pigment granules trapped in a contractile matrix of filamentous proteins, carrying with it associated materials of the deeper cytoplasm[5] (see also previous report, sect. I.B.2). It is suggested that the local interaction of the astral rays with the cell membrane or egg cortex initiates the asymmetrical cortical contraction, which at the time of pronuclear fusion expands the grey crescent and probably also causes yolk rearrangements in the vegetal half of the

egg, leading to the formation of the 'vitelline wall' and the 'vegetal dorsalizing centre' (VDC)[6]. The results are being prepared for publication.

We conclude that the PFW reflects sperm aster growth, which in its turn is instrumental in the primary yolk shifts. Both grey crescent formation and the formation of the 'dorsal cytoplasm'[1] reflect rearrangements in the cytoskeleton of the fertilized egg which lead to the ultimate dorso-ventralization of the embryo; in this process neither the grey crescent nor the 'dorsal cytoplasm' act as dorsal determinants. An analysis of the cytoskeleton in the fertilized egg from fertilization till first cleavage is in progress (see also *2* below).

[1] Ubbels, G.A. (1977) - Mém. Soc. Zool. Fr. *41*, 103-116.

[2] Paleček, J., G.A. Ubbels and K. Rhezak (1978) - J. Embryol. exp. Morph. *45*, 203-214.

[3] Hara, K. and P. Tydeman (1979) - Roux' Archiv Enw. Org. *186*, 91-94.

[4] Hara, K., P. Tydeman and R.T.M. Hengst (1977) - Ibidem *181*, 189-192.

[5] Gerhart, J.C. (1980) - In: Biological Regulation and Development, Vol. *2* (Ed. R.F. Goldberger), Plenum Press, 133-316.

[6] Kirschner *et al.* (1981) - Neth. Jl. Zool. *31*, 50-77.

2. Analysis of the cytoskeleton in the fertilized egg (Xenopus laevis)

When egg rotation experiments were made (see previous report, sect. I.B.1) in Ficoll solutions containing vinblastine in a concentration interfering with cleavage, the 'vitelline wall' was found to be considerably extended compared with that in eggs rotated in the absence of vinblastine - as if the coarse yolk could 'slide' more easily with respect to the subcortical yolk. This strongly suggests that in the latter type of rotation experiment as well as in normal development microtubules are involved in the shifting of the yolk relative to the egg cortex.

Together with Dr. J. Paleček (Prague University) we have started an immunocytochemical analysis of the microtubule pattern in the fertilized egg, using antitubulin serum raised against tubulin from *Xenopus* eggs (prepared by J.P.) (see also *1* above). The presence of microtubules in the animal half of the egg was further substantiated by using the indirect immunocytochemical PAP staining procedure[1]. We refrained from using fluorescein- or rhodamine-isothiocyanate as fluorescent labels of antitubulin because of autofluorescence of the yolk at the specific wavelengths used to demonstrate these conjugates, and because the PAP-method is much more sensitive.

Paraffin sections (6 μm) of fertilized eggs fixed at various time intervals showed positively staining fibrillar structures associated mainly with the middle-fine yolk. We assume that they represent bundles of microtubules since they did not stain with appropriate control reactions. The pattern of these structures gradually changed and seemed to become different on the dorsal as compared to the ventral side. However, exact localization needs to be done in semithin and ultrathin sections, for which methods are being elaborated. In serial sections it was shown that fibrillar structures staining blue with our routine azo-fuchsine aniline blue procedure had the same localization as in adjacent sections stained with the PAP procedure. This suggests that the routine staining procedure is an appropriate method for the localization of tubulin structures at the light-microscopical level; this will be checked in semithin and ultrathin sections.

[1] Sternberger, L.A. (1979) - Immunocytochemistry (2nd. ed.), John Wiley, 104-130.

3. Cytochemical analysis of bispermically fertilized eggs (Xenopus laevis)

Bispermically fertilized eggs (see previous report, sect. I.B.3 and this report, publ. 24) are being used for the analysis of the role of the sperm in symmetrization of the uncleaved egg. Such eggs show pigment movements and internal cytoplasmic displacements different from those in the monospermic egg. When visible, the grey crescent forms opposite the point midway the shortest distance between the two sperm entrance points. In sections the 'central cytoplasm' is seen to be

displaced eccentrically, i.e., to the side of the grey crescent (see report for 1977, sect. I.A.i.3 and 4).

The viability of bispermically fertilized eggs is lower than that of monospermic eggs. Nevertheless, a small number of time-lapse films could be made of eggs in which the point midway the shortest distance between the two sperm entrance points was marked by vital staining. These films show that the blastopore appears opposite the marked point. From histological sections it was concluded that a competition occurs between the two sperms. One of them fuses with the female pronucleus in an eccentric position, never in the central region of the egg, as is frequently the case in monospermic eggs. The embryo develops further as a diploid/haploid chimera (see also C.*1* above).

The distribution of the yolk in the bispermic egg is abnormal, which is assumed to result from abnormal rearrangements in the cytoskeleton under the influence of the two sperm centrioles, as reflected externally by the abnormal pigment movements. This abnormal yolk distribution in its turn may cause abnormal gastrulation.

E. REGIONAL DIFFERENCES IN THE CELL MEMBRANE

1. Freeze-fracture analysis of the egg plasma membrane during grey crescent formation (Xenopus laevis)

In addition to the established animal/vegetal polarity of the egg, fertilization brings about the appearance of a dorsal/ventral axis, the side opposite the sperm entrance point (SEP) being the future dorsal side. Earlier experiments have called into question the putative morphogenetic role of the dorsal egg surface (see previous report, sect. I.B.1). The present study was intended to find out whether the establishment of dorsal/ventral polarity brings about regional differences at the level of the plasma membrane (see previous report, sect. I.C.1).

Artificially fertilized eggs were briefly (ca. 4 min) aldehyde-fixed at 30-40 min after fertilization. A piece of cell surface (ca. 0.3-0.4 mm^2) was prepared free either from the pigmented surface of the animal half around the SEP (ventral side) or from the less pigmented equatorial region on the opposite side of the egg in the region of the so-called grey crescent (dorsal side). The isolated pieces of plasma membrane (from 11 eggs of four different batches) were freeze-fractured and replicas were made and analysed. The diameters of the intramembranous particles (IMPs) were measured and the size distribution of IMPs on the dorsal side was compared with that on the ventral side. There were significantly more IMPs of size $\leq$ 8 nm on the ventral side, but the difference found can be explained in terms of the difference between the animal and the vegetal half of the egg found earlier[1]. To conclude: our preliminary results indicate that 30-40 min after fertilization (i.e., at time 0.5-0.6, see D.*1* above) there is no alteration yet in the IMP population of the dorsal plasma membrane. To find out whether the IMP pattern remains unchanged in the period when dorsal/ventral polarity becomes irreversible, further experiments will be carried out between times 0.7 and 0.8.

[1] Bluemink, J.G. and L.G.J. Tertoolen (1978) - Devel. Biol. *62*, 334-343.

2. Dynamic membrane properties in cleaving eggs (Xenopus laevis) (In cooperation with Research Group IV)

It may be presumed that the polarity of the egg exists also as a regional plasma membrane difference between the opposite poles. The maintenance of such a difference then must rely either on a rigid organization of the membrane itself or on anchorages of the cytoskeleton, or both. The technique of fluorescence photobleaching recovery (FPR) (see previous report, sect. VI.2) was used to determine the dynamic membrane properties in the *Xenopus* egg. Eggs were incubated with the fluorescent lipid probe 5-(N hexadecanoyl)-aminofluorescein (Hedaf) either before cleavage or during cleavage, when new membrane is formed.

Upon labelling of the egg plasma membrane the fluorescence appears spotted rather than homogeneous. The distribution of the ca. 1 µm spots conforms to the known distribution of surface protrusions with tips free of intramembranous particles (IMPs)[1]. It is possible that the IMP-free tips represent lipid domains, which are enriched with the fluorescence probe. Comparing the egg plasma membrane with the new membrane formed during cleavage a very large difference was found

in FPR, i.e., in the rate of lateral diffusion of membrane lipids. The diffusion coefficient D for the lipids of the egg plasma membrane was estimated to be less than 10^{-10} cm^2/sec, indicating that the rate of lipid exchange between the tips of the protrusions and the rest of the plasma membrane is low. In contrast, the diffusion of lipids in the new membrane (which is poor in IMPs[2]) is fast: $D = 5.9 \pm 0.9 \times 10^{-8}$ cm^2/sec, which approximates values found for pure lipid systems. These findings indicate that the egg plasma membrane has a relatively rigid organization, suggesting that putative topographical differences will be maintained for longer periods. The new membrane is a more fluid-dynamic system and is therefore presumably more amenable to the expression of surface differences during early cell differentiation. The results are being prepared for publication.

[1] Bluemink, J.G. and L.G.J. Tertoolen (1978) - Dev. Biol. *62*, 334-343.

[2] Bluemink, J.G., L.G.J. Tertoolen, P.H.J.Th. Ververgaert and A.J. Verkleij (1976) - Biochim. Biophys. Acta *443*, 143-155.

II. Research Group: Organogenesis in Mammals

Members: K.A. Lawson; R. Willemsen, W.A.M. van Maurik.

Guest worker: S.A. McDonald (student-visitor).

Collaboration: A.A.W. ten Have-Opbroek (Leiden, The Netherlands).

F. EPITHELIAL-MESENCHYMAL INTERACTIONS IN ORGANOGENESIS

The increasing complexity of the vertebrate embryo can be attributed to the interaction of areas which had been spatially separated earlier and subsequently come into association by morphogenetic movement. In the development of derivatives of the primitive gut, organ rudiments arise as simple outpocketings of epithelium into the surrounding mesenchyme. Final differentiation occurs after growth of both tissue components, of which that of the epithelium is much faster than that of the mesenchyme. Close association of epithelium with mesenchyme, which is essential for rapid epithelial growth, is maintained in organs such as the lung and salivary gland by the epithelium branching as it grows into the mesenchyme. Current work is concerned with the interactions between mesenchyme and epithelium that control cell proliferation, branching morphogenesis and patterning of cytodifferentiation in existing primordia; preliminary work is also being done in an approach to analysing tissue interactions involved in the emergence of organ primordia from the primitive gut.

Recombinates of epithelium and mesenchyme, in normal association, and in heterochronous and heterotypic association, are cultured *in vitro* and their behaviour is analysed at the cell and tissue level using cinematography and histochemical and E.M. techniques.

1. Epithelial branching pattern (Rattus norvegicus)

Earlier work (see previous reports) had shown that the branching pattern of lung epithelium recombined with salivary mesenchyme is salivary in character, and not lung-like. The culture conditions then used, giving the necessary conditions for photo and time-lapse film analysis, were not suitable for the development of the reciprocal recombinate of salivary epithelium in lung mesenchyme. These conditions have been modified to give good development of salivary epithelium/lung mesenchyme recombinates, as well as having reasonable optical properties for photography. The subsequent analysis of branching pattern shows the pattern to be indistinguishable from that of lung epithelium in lung mesenchyme and significantly different from salivary gland, thus confirming the previous conclusion that the mesenchyme determines the position and timing of new epithelial branch points.

On this basis one would expect that a pattern of 'branch point initiating activity' exists in the mesenchyme, and that this would be different in lung and salivary gland. Rapid turnover of extracellular material in the interface between epithelium and mesenchyme (for which mesenchyme is required) occurs in the morphogenetically active regions in the salivary gland[1]; the possibility that catabolic enzymes

for basal lamina components and fibrillar collagen are regulators for morphogenesis should be investigated.

[1] Bernfield, M.R. and S.D. Banerjee (1978) - In: Biology and Chemistry of Basement Membranes (Ed. N.H. Kefalides), Academic Press, New York etc., 137-148.

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

Although lung epithelium from 14-d. fetuses, in which the lungs have started to form bronchial buds, is able to continue branching morphogenesis in salivary mesenchyme, epithelium from the primary bronchi of 13-d. fetuses is unable to do so. However, mechanical removal and replacement of the same piece of heterotypic mesenchyme after the first 2½ days of culture initiates shape changes and subsequent branching morphogenesis of the epithelium within 24 h. (see previous report, sect. II.D.1). Examination of the mitotic activity of the epithelium, using a colchicine block, showed no effect on the colchicine index (CI) within 3-11 h. after removal and replacement of the mesenchyme, but a 50% increase in CI 11-19 h. after treatment. The CI was then not significantly different from that of lung and salivary gland epithelia actively branching in their own mesenchymes. That the mitotic stimulation is mediated by the mesenchyme is shown by the following results: 1. peeled epithelium round which the mesenchyme had not been replaced had a lower CI than the untreated controls; 2. the CI of the mesenchyme was not altered by the treatment. The results suggest that the treatment may rapidly accelerate G_0 or G_1 cells into S. Epidermal growth factor (to which lung epithelium is sensitive[1]) can be used to test whether enhancement of cell division is not only necessary but also sufficient to permit morphogenesis in these heterotypic recombinates.

The ultrastructure of the interface between mesenchyme and epithelium in the reconstituted recombinates shows slight indications of local destabilization of the basal lamina and loss of fibrillar collagen. Quantitative autoradiography will be used to establish whether the treatment results in an increased turnover in basement membrane components (see also *1* above).

[1] Goldin, G.V. and L.A. Opperman (1980) - J. Embryol. exp. Morph. *60*, 235-243.

3. Influence of mesenchyme on lung epithelial cell differentiation (Mus musculus, Rattus norvegicus)

The role of mesenchyme in controlling cell differentiation in lung epithelium was to be investigated using an antigen of the respiratory epithelium[1] as differentiation marker (see previous report, sect. II.D.2). Little progress has been achieved due to technical problems with the antiserum. Some progress has been made in obtaining lung development from primitive guts *in vitro*.

[1] ten Have-Opbroek, A.A.W. (1979) - Dev. Biol. *69*, 408-413.

III. Research Group: Morphogenesis in Cellular Slime Moulds

Members: A.J. Durston, C.J. Weijer (res. assoc.), H. Volman (res assoc.); A.R.J. Bleumink, F. Haring-Vork.

Guest workers: C.D. Stern (London), M.J. North (Stirling, Scotland).

Collaboration: S.K. Brahma (Utrecht), P. Schaap (Leiden), C.D. Stern (London), M.J. North (Stirling, Scotland).

Our general aim is to understand morphogenesis and pattern formation at the biochemical level. We work on the hypothesis, based on findings in the literature, that general principles underly pattern formation. There is much evidence that identical mechanisms are used for constructing different organs (e.g. legs and antennae in insects) during development of an embryo, and there is also limited evidence that identical mechanisms are used to construct different organisms. This hypothesis makes it reasonable to select a simple model system, which shows

some important features of embryogenesis that we wish to understand, and to pose two concrete questions: 1. How does the model system make its pattern, i.e., in a multicellular system: what are the cell-level properties underlying pattern formation and what is their biochemical basis?; 2. Are insights obtained with the model system helpful in elucidating universal principles for pattern formation? Do other organisms make their patterns in the same way? Are there any general parallels?

We have taken this approach, using the cellular slime mould *Dictyostelium discoideum* (Dd) as a model system. This choice was for three main reasons: 1. Dd development leads to formation of a simple pattern of two cell types (stalk cells and spores). At an earlier stage (slug stage), this final pattern is foreshadowed by a 2-zone embryonic pattern (of prestalk and prespore cells), which is regulative. If it is perturbed, e.g. by removing cells to disturb the cell type ratio, it returns homeostatically to the normal situation. This sort of behaviour occurs in many or all embryos, and presumably reflects the existence of homeostatic control systems which ensure reliable pattern formation irrespective of environmental perturbations. Dd offers a simple paradigm for studying these; 2. Dd has many practical advantages, including easy culture and bulk production, fast development, growth in synthetic media and availability of developmental mutants; 3. Much was known already about Dd development although not about its pattern formation. Most important, from our point of view, it was known that during the first stage of Dd development (aggregation), cell migration is controlled by a chemotactic system involving chemotaxis and relaying in response to 3,5, cyclic AMP (c-AMP).

Our work on this system, over the last few years, has led us to the conclusion that Dd pattern formation and pattern regulation up until the slug stage consists of two aspects: cell sorting, via c-AMP chemotaxis, which arranges prestalk and prespore cells in an axial pattern; and a homeostatic mechanism, probably working via negative feedback, which maintains the correct ratio of these two cell types (for details, see the Progress Reports 1977-1979, and relevant publications[1-4]).

[1] Durston, A., F. Vork and C. Weinberger (1978) - In: Biochemical and Biophysical Information Transfer in Recognition, (Eds. J. Vassileva-Popova and E.V. Jensen), Plenum, New York, 693-708.

[2] Durston, A. and F. Vork (1979) - J. Cell Sci. *36*, 261-279.

[3] Matsukuma, S. and A. Durston (1979) - J.E.E.M. *50*, 243-251.

[4] Durston, A. and C. Weijer (1980) - Vakblad voor Biologen *16*, 320-327.

G. MORPHOGENESIS AND PATTERN FORMATION IN *DICTYOSTELIUM DISCOIDEUM*

1. Cell type separation

To study properties of the Dd cell types, with a view to understanding cell sorting and cell type homeostasis, we required a method for purifying them in bulk. We used density gradient centrifugation, which has been reported as a cell type separation method for Dd by several previous workers [1,2] and chose Percoll (Pharmacia) as a dense, non-toxic, iso-osmotic centrifugation medium. We then worked out a separation method, with the following main results:

i. Most previous investigators have used high-speed centrifugation. Cells are layered on top of a suitable concentration of Percoll (or other medium) in a fixed-angle centrifuge head and spun at about 40,000-80,000 x g (average) for 20 min. The outcome is that the Percoll (or other centrifugant) spins to a sigmoidal density gradient and two (or sometimes more) bands of cells separate within the gradient. Experiments performed at the slug stage (where differentiation markers are available) show that the bands of cells can be enriched for Dd cell types. Typically, more prespore cells are found in the lower, denser band. This phenomenon has led others to claim that the Dd cell types differ sharply in density. Our investigations show that the two-band separation formed in this method is an artifact, due to the fact that centrifugation on a suitable given Percoll concentration (with given density) immediately splits cells with a distribution of densities into two populations. Cells having lower density than the Percoll stay up (top band). Cells having higher density go through the Percoll (bottom band). We find that Dd cells spun at high speed on the usual Percoll concentrations first split to the top and bottom of the centrifuge tube, then spread both towards the middle, as the gradient becomes sigmoidal.

ii. The fact that high-speed density-gradient centrifugation involves high-speed centrifugation of cells against the bottom of the centrifuge tube suggests that this method might damage cells. This proved to be so, since the densities of high-speed-spun cells were found to be unstable. If a high-speed-spun centrifuge tube containing two bands is respun at low speed (ca. 2,500 x g) for 30 min, or even allowed to stand for a time, this fails to change the pre-spun gradient (as shown by density marker beads), but does affect the positions of the bands of cells. The most dense cells (which had originally moved downward) travel up to join the top band. Their density is evidently unstable. This consideration makes it desirable to use another method. We find that reasonable cell-type separation can be obtained using low-speed centrifugation on pre-made gradients (see below) and that stability of cell density is better using low-speed gradients.

iii. Separation of the Dd cell types was found to be critically dependent on the osmolarity of the centrifugation medium. It depends on osmotic differences between the cell types, not density difference *per se*. NaCl (0.5-0.7%) was added to give a hypertonic external medium in our standard separation. Separation was found also to be enhanced by Ca^{2+} depletion (inclusion of EGTA or EDTA) and cell density was also affected by prior growth conditions (e.g. by the brand of protease peptone used in the culture medium).

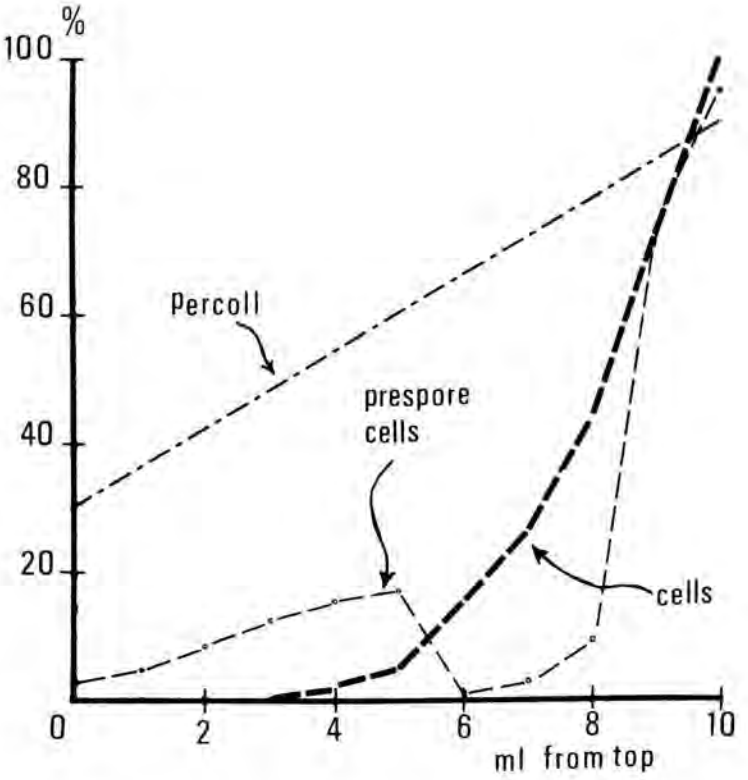

Fig. 1. Percoll density-gradient centrifugation of Dd slug cells. The figure shows the typical result from centrifugation on a 30%-90% Percoll gradient, under the conditions given in the text. As will be seen, there are typically two zones of prespore cells (at 9-10 and at 5) and one zone of prestalk cells (at 6-8). (Prespore cells are characterized by 'prespore vacuoles', which are not present in prestalk cells.)

percoll: percentage percoll
cells: total cell density
prespore cells: cells carrying the prespore vacuole

iv. We find that the most reliable procedure for separating Dd cells is low-speed centrifugation (ca. 2,500 x g) on an appropriate pre-made continuous gradient (typically linear 30-90% Percoll in an appropriate buffer). Fractionation of slug-stage-cells on such a gradient shows that prestalk and prespore cells have different, but overlapping, density distributions (Fig. 1). Harvesting standard regions from such a gradient gives reasonably pure cell types (purity > 95% for prespore, > 90% for prestalk). The method does not appear to be very deleterious, since, for example, optical density oscillations (see below), which are critically dependent on metabolic state, are unaffected by it.

[1] Miller, Z., J. Quance and J. Ashworth (1969) - Biochem. J. *114*, 815-818.

[2] Maeda, Y. and M. Maeda (1974) - Exp. Cell. Res. *84*, 88-94.

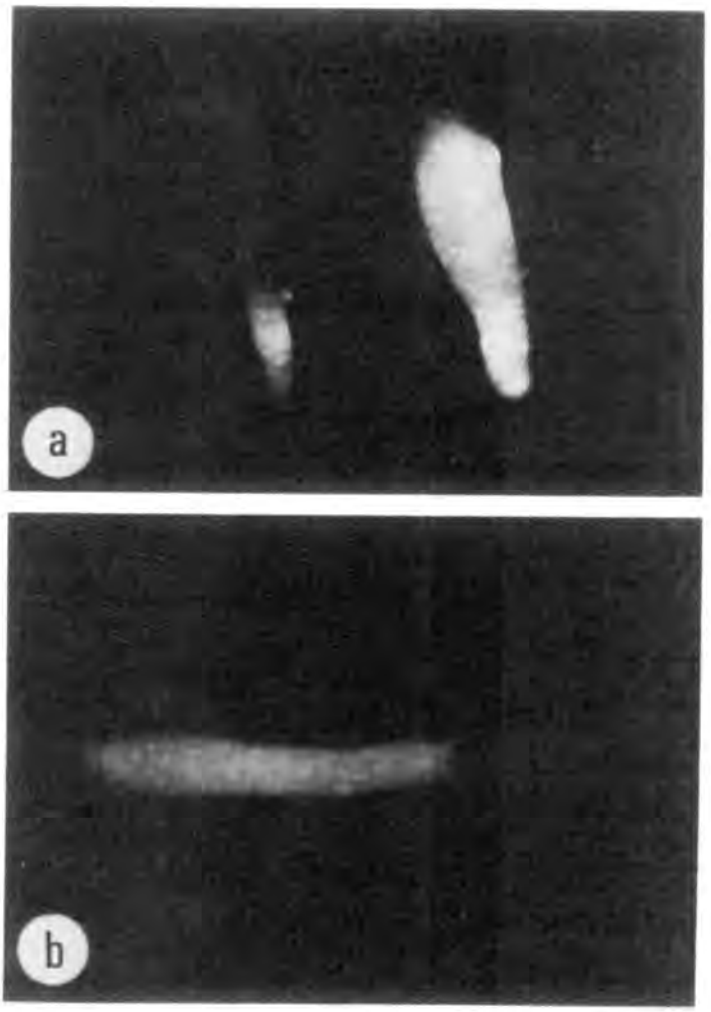

Fig. 2. Sorting of Dd cells. Density-gradient centrifugation was used to fractionate Dd cells after 4 hr of development. Various combinations were made between fluorescent (TRITC)-labelled and unlabelled density fractions and the mixtures were allowed to form slugs.

a: (left) a mixture of labelled low-density and unlabelled high-density cells gives labelled cells in the anterior (prestalk) zone of the slug; (right) labelled low-density cells mixed with unlabelled low-density cells give non-localized fluorescence.

b: labelled high-density cells mixed with unlabelled low-density cells give labelling in the rear (prespore) zone of the slug.

2. Origin and properties of the prestalk and prespore cell types

i. Previous workers have reported that density-gradient-separated prestalk and prespore cells from slugs or earlier stages sort out when mixed. Our own previous cell marking experiments and films have also shown that prestalk and prespore cells sort out during slug formation and during regulation of slug pieces (see reports for 1978 and 1979). Using the density gradient method described above, we confirmed that (TRITC-)labelled and unlabelled cell density classes from the slug stage sort out from each other. Less dense (prestalk) cells return to the front of the slug. We were also able to confirm via TRITC marking that cell density classes isolated as early as 4 hr of development also sort out. Less dense cells end up in the front of the slug (Fig. 2).

ii. We have started to examine the c-AMP-dependent properties of purified density classes from various stages, in order to investigate the nature of cell sorting and the origin of the two cell types. To date, we have detected a difference in the chemotactic response in early (5 hr) cells. Less dense cells make a stronger and faster response to c-AMP than to denser cells, but to the same range of c-AMP concentrations. Preparatory work has been done, and preliminary results obtained, on autonomous signalling and the relaying response in the two cell types, by examining optical density and pH in aerated cell suspensions. Both observables oscillate autonomously due to c-AMP oscillations. Both also show well-defined fast and slow responses to c-AMP, which are known to reflect the c-AMP chemotactic and relaying responses respectively. In future, we shall also look at c-AMP content of the cell types and at c-AMP-related enzymes (c-AMP phosphodiesterase, adenylate cyclase).

iii. An hypothesis alternative to a chemotactic difference as the basis of cell sorting is that the Dd cell types differ in competence for cell contact. Together with the technical workshop (see section VI.*1*), we have constructed an agglutinationmeter, which will be used to make quantitative studies on cell adhesion; on effects of reagents such as anti-contact site Fab fragments on adhesion (see report for 1979); and on contact differences between the purified cell types.

iv. Certain developmentally regulated enzymes (glycogen phosphorylase, alkaline phosphatase) were measured in the cell types, in connection with our intention to use these as markers in differentiation experiments.

v. Transmission EM studies were carried out in collaboration with Ms. P. Schaap, M.Sc. (University of Leiden) to look for differences between the cell types. It was found that two classes of electron-dense and less electron-dense cells, which had been detected by Ms. Schaap in pre-aggregation Dd populations, separate on the basis of density. Less electron-dense cells tend to be less dense.

vi. The main aim of purifying the Dd cell types is to study the proportionality mechanism governing the cell type ratio, and in particular to look for morphogens regulating the transition prestalk $\rightleftarrows$ prespore. To do this, we need to follow cell type homeostasis (i.e. (purified) prestalk → prestalk + prespore and (purified) prespore → prestalk + prespore) *in vitro*. We were able to obtain such cell-type regulation in our *in vitro* system (cell suspension culture in microtest trays under pure oxygen - see report for 1979). Pure slug-stage cell types incubated under these conditions progress to an equilibrium ratio of about 50% prespore to 50% prestalk (as checked using prespore-specific antibody and glycogen phosphorylase). This ratio differs from the cell-type ratio found in slugs during development on a solid surface. It is the same ratio as we obtain during normal development of Dd cells *in vitro* and is also typical of ratios obtained by other workers who use *in vitro* suspension culture.

3. TRITC marking experiments

The TRITC marking experiments started last year (see previous report, sect. E.3a.i) were finished. Experiments to follow cell movement showed that prespore or prestalk cells grafted into the correct zone of a slug move readily within their own zone, and with some difficulty into the zone of the other cell type. However,

prespore cells grafted into a regulating prespore piece split into two populations, one of which goes to the future prestalk, the other to the future prespore zone. Prestalk cells grafted into a regulating prestalk piece also have a distinct movement pattern. Experiments to follow differentiation of grafted cells showed that marked prespore and prestalk cells remain stably determined if placed anywhere in a slug or regulating piece except in a regulating piece of the same type. These findings have led us to a model for Dd cell-type homeostasis which says that prestalk $\rightleftarrows$ prespore transitions are stabilized by feedback inhibition.

4. Differentiation of the Dd cell types: effects of EACA

A previous study[1] suggested that proteolysis was important for later Dd development, probably via regulation of the supply of free amino acids, which is known to act as developmental signal in Dd. These authors suggested that proteolytic activity might be implicated in determining the ratio of the Dd cell types. We studied the effects of the anti-proteolytic agent E-amino caproic acid (EACA). North (unpublished) had shown that this agent might have an effect on the cell type ratio in Dd, since in mixtures of EACA-treated and untreated cells the latter make the majority of the spores produced.

Time-lapse films showed a delay in development of EACA-treated cells: formation of larger aggregation territories and an ultimate block at the early aggregation stage. Chemotactic tests showed that the c-AMP chemotactic response of these cells is delayed, and possibly impaired compared to control cells. Immunofluorescent staining showed that EACA-treated cells never make the prespore antigen typical of later cells. Mixing TRITC-labelled EACA-treated cells and unlabelled control cells (or *vice versa*) showed that the former cells are simply left behind at post-aggregation stages; they are not shunted toward either cell type. All of these findings are consistent with the idea that EACA blocks development at an early stage. None suggests that EACA has any effect on the cell-type ratio mechanism.

A further effect of EACA was noted when examining the centrifugation of Dd cells in Percoll gradients. With normal cells this reveals sub-populations of cells even at very early stages of development (see *1* above). EACA-treated cells, in contrast, formed only one band. However, this was not apparently related to the effect of EACA on development since glycine, which is not an inhibitor, had the same effect. The significance of this observation is not yet known.

[1] Fong, D. and J. Bonner (1979) - P.N.A.S. *76*, 12, 6481-6485.

H. THE ROLE OF c-AMP IN AVIAN EMBRYOGENESIS (*GALLUS DOMESTICUS*)

A recent series of papers[1-3] state 1. that low c-AMP concentrations disturb chick embryogenesis; 2. that chick embryo cell suspensions (ca. stage 4) amplify an added c-AMP pulse, just as do *Dictyostelium* cells; and 3. that chick embryo cells probably have other responses (chemotaxis and increased cell adhesion) to c-AMP. We have started investigations to check and follow up these findings.

[1] Robertson, A. and A. Gingle (1977) - Science *197*, 1078-1079.

[2] Robertson, A. *et al.* (1978) - Science *199*, 3, 990-991.

[3] Gingle, A. (1977) - Devl. Biol. *58*, 394-401.

1. Search for a chemotactic response by early chick embryo cells

Cell suspensions and small pieces of tissue were isolated from early chick embryos (st. XIII-XIV (Eyal-Giladi & Kochav) or st. 3-4$^+$ (Hamburger & Hamilton)). Methods for dissection, cell dissociation and culture have been published previously[1]. The cell suspensions were made from whole blastoderms (upper and lower germ layers). Pieces of tissue were excised from the mesoderm, primitive streak, endoderm or epiblast. A variety of substrates were tested for their suitability for chemotactic tests of this material. These include: the surface of an agar gel (0.35-3%) in Tyrode's solution; the surface of various types of plastic tissue culture dish under a 2% agar slab; various concentrations of gelatine; a collagen lattice[2] supported in a well in 2% agar; a plasma clot supported in 2% agar. The intention was to find a medium which would permit relatively unimpeded cell movement in

two or three dimensions, while minimizing convection (and facilitating establishment of chemo-attractant gradients) either via increased viscosity or restricted volume. We were most successful with plasma clots (which permitted multidirectional migration of cells out of tissue pieces, along the plasma fibres), or using the surface of a plastic dish for migration of dissociated cells. These conditions and others were used to examine chick cells and pieces of tissue for a chemotactic response to 10^{-2}-10^{-8}M c-AMP and to large, freshly dissected pieces of chick tissue (usually stage 3-4^{+} primitive streak) as attractants. The c-AMP was administered 1. via addition to medium surrounding the cell population or piece; 2. via diffusion from micropipettes of siliconized glass (ca. 10-20 µm tip) containing a c-AMP solution; 3. iontophoretically via delivering ca. 50 nA negative pulses through a glass microelectrode containing a c-AMP solution. Results were analysed by observation at appropriate intervals and, in sample experiments, by filming. The result from more than 500 experiments was that we found no noticeable distortion of the distribution or behaviour of a population of dissociated cells (250 expts.) or of the extent or directionality of spreading of an isolated piece of tissue (300 expts.) in relation to any of the chemotactic sources tested.

[1] Stern, C. (1980) - J.E.E.M. (in press).

[2] Davis, E.M. (1980) - J.E.E.M. 55, 17-31.

2. Search for a relay response by early chick embryo cells

Cell suspensions were prepared from chick embryos, usually at stage 3-4^{+}, but in some cases at later stages (up to st.7) and tested for a c-AMP amplification response. An aliquot of cell suspension was mixed with an aliquot containing c-AMP or lacking it (control), incubated at 37°C, boiled to inactivate c-AMP phosphodiesterase and then assayed for c-AMP, using a competitive binding assay. We looked for a net increase over added c-AMP and the basal (unstimulated) control level. This basic plan was used while varying relevant parameters including stage of tissue (st.4-7); part of the embryo taken (whole embryo or area pellucida only); number of cells used (2-6 x 10^{6}); cell dissociation method (pipetting in Ca^{2+}, Mg^{2+}-free Ringer or Tyrode with or without (0-2 mM) EDTA for various times); stimulating c-AMP concentration (10^{-6}-10^{-8}M); presence or absence of the phosphodiesterase inhibitor IBMX (10^{-3}-10^{-5}M); time course of the experiment (time for equilibration at 37°C before addition of the c-AMP stimulus and time for a response after addition); medium used for the experiment (Tyrode or Ringer) and its pH. To date, the treatments tried give a consistent result. Under favourable conditions added c-AMP is destroyed within about one min in the absence of IBMX, but not in its presence, presumably due to native c-AMP phosphodiesterase. Control cells make a basal level of about 0.4 pM c-AMP/10^{5} cells in the presence of IBMX, and no measurable c-AMP in its absence. We have not, so far, seen a relay response (i.e., an increase in c-AMP over stimulus and basal level). These experiments are being continued to look at the effect of dibutyryl c-AMP (which should enter the cell) and to optimize ionic concentrations for adenylate cyclase activity.

IV. Research Group: Membrane regulation (previously named: Regulation of the cell cycle)

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

Guest workers: J. Aldenhoff, J.A.M. van den Biggelaar, A.W.C. Dorresteijn, M. Gadenne, C.J.P. Grimmelikhuijzen, R. Hill, D. Louvard, W.H. Moolenaar.

Graduate students: A. Bierman, B. Defize, H. Spanjer.

Collaboration: Dr. J.G. Bluemink, Hubrecht Laboratory, Utrecht: Ultrastructure of the cell surface - Dr. R. van Wijk, Molecular Cell Biology, Utrecht: Hyperthermy; Membrane regulation of protein synthesis - Dr. G. Rijksen, Medical Enzymology, AZU, Utrecht: Isoenzyme patterns of pyruvate kinase as a test for the

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I. MEMBRANE REGULATION IN GROWTH AND DEVELOPMENT

In this research group attention is focussed on the role of the plasma membrane in 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. As the programme of differentiation is associated with alterations in the growth behaviour of the cells, or even with growth arrest (terminal differentiation), understanding of the regulation of the cell cycle is a major part of our research. The key role ascribed to the plasma membrane in the regulation of cell multiplication and differentiation is almost self-evident if one realizes that:

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

From the work of numerous investigators on a variety of cell types there are now considerable data on dynamic changes in various membrane properties and components which have been correlated with the cell cycle, growth, differentiation and transformation: expression and dynamic properties of membrane receptors, properties of membrane lipids, ion metabolism and electrical membrane properties, transport of nutrients, ultrastructure of the plasma membrane. Despite all these studies, the causes and consequences of the molecular events at the plasma membrane level are as yet obscure. Although we realize that these interactions have to be complex and interdigitating, we strongly believe that they can only be resolved by coordinated multidisciplinary research in which the various aspects of the possible molecular interactions are investigated in parallel in a limited number of suitable experimental systems.

Embryonal tumour cells lines provide such systems. An important feature of these cell lines is their capacity *in vitro* to undergo a specific process of cellular differentiation evoked by external stimuli. In the absence of differentiation-inducing stimuli such cells therefore resemble the embryonic cells in various states of determination and differentiation from which they originate. Upon induction of differentiation their proliferation programme is modified or arrested in a particular phase of the cell cycle, and the cells lose their malignant properties. Embryonal tumour cells can originate in different periods of embryogenesis and organogen-

esis. Those originating or inducible from early embryonic cells or from germ cells (embryocarcinoma cells) are capable of participating fully in normal embryonic development and can give rise *in vitro* to a large variety of differentiated cell types. During organogenesis embryonal tumour cells can be isolated which are more limited in their differentiation capacity, as are their normal embryonic counterparts.

As specific developmental processes can be monitored *in vitro* in isolation and in nearly unlimited numbers of (synchronous) cells by using embryonal tumour cell lines, the study of the underlying molecular regulation at the plasma membrane level is not technically limited by the availability of material, nor is the process to be studied obscured by other events which occur simultaneously in the intact embryo. For a number of years this research group has in particular exploited murine neuroblastoma cell lines, and more recently rat pheochromocytoma cell lines, both originating during organogenesis from neural crest cells, to study the role of the plasma membrane in the control of cell multiplication and (terminal) neuronal differentiation.

In the immediate future research will be extended to murine embryocarcinoma cells. The characterization of these cells during the last years has offered revolutionary new possibilities for the study of the molecular control of early development and transformation, and especially of their interrelationships. Their capacity to undergo an extensive programme of determination and differentiation *in vitro*, as well as to participate in normal development, provides the tools to study early development and the processes which lead to embryonic tumour formation with a broad technological approach ranging from molecular biology to experimental embryology.

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 ((Na^+-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.

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

By means of time-lapse cinematography a family tree analysis of Neuro-2A cell proliferation was performed. Variation in intermitotic time between sister cells is such that the mean difference in intermitotic time equals the sample standard deviation in this parameter and also the reciprocal of the slope of the curve obtained by plotting the logarithm of the fraction of sister cells having a difference in intermitotic time larger than or equal to an indicated time t (β-curve). This observation indicates that the intermitotic times of sister cells differ only because of the occurrence of a single random transition in the cell cycle (A-state)[1]. Cells which are less closely related, e.g. cousin, second cousin and unrelated cells, show progressively more variation in intermitotic time characterized by the observation that the mean difference in intermitotic time is increasingly larger than the reciprocal of the corresponding β-curve. From these data it has been concluded

that Neuro-2A cells in culture show variation with respect to the length of the determinate B-phase[1], such that the further cells have deviated from a common ancestry the larger the mean difference in length of the B-phase between these cells will be. By means of computer simulation data a method has been worked out to quantify the amount of variation in length of the B-phase within a cell culture (submitted for publication, see publ. 30).

[1] Smith, J.A. and L. Martin (1973) - Proc. Natl. Acad. Sci. USA *70*, 1263-1267.

2. Potassium transport in the neuroblastoma cell cycle

Cell cycle-dependent alterations in the electrical membrane properties and in passive K^+ transport across the plasma membrane have been studied in detail in synchronized neuroblastoma cells (clone Neuro-2A). It was concluded that K^+ transport is regulated on at least two levels: 1. intrinsic modulations of the passive permeability properties of the plasma membrane, and 2. modulations in active, (Na^+-K^+)-ATPase-mediated ion translocation (see previous report, sect. IV.1a; this report, publ. 15, 16). This year further attention was given to the role of (Na^+-K^+)-ATPase during the cell cycle. The functional activity of this enzyme was determined through the cell cycle from the ouabain-sensitive K^+ influx in intact cells, as measured by $^{42}K^+$ tracer flux analysis, and compared with the enzyme activity under optimal substrate conditions as determined by measuring ouabain-sensitive ATP hydrolysis in cell homogenates.

The functional activity decreases more than four-fold on transition from mitosis to the early G_1 phase, shows a rapid transient six-fold increase upon entry into S-phase, after which it increases steadily during further progression through the cell cycle. The ouabain-insensitive K^+ influx component, which is not mediated by (Na^+-K^+)-ATPase, increases linearly through the cell cycle, as does cellular protein content. The optimal enzyme activity shows a modulation during the cell cycle similar to that of the functional activity, with one important difference: the transient activation upon entry into S-phase is not detectable under optimal substrate conditions. Combining total K^+ influx values with efflux data showed that net loss of K^+ occurred with transition from mitosis to G_1-phase, while net accumulation occurred with entry into S. Throughout mid S-phase net K^+ flux was virtually zero, but a large net influx occurred again just before the next mitosis. This net flux was of the correct order of magnitude to account for the changes in intracellular K^+ content.

Modulations in the functional (Na^+-K^+)-ATPase activity could result from: 1. intrinsic membrane changes resulting in conformational changes of the enzyme or alterations in the number of enzyme copies per cell; 2. changes in the availability of intracellular substrates. Clearly only changes of the first category would be detectable when assaying the enzyme activity in cell homogenates. We therefore concluded that the G_1/S transition is associated with a rapid transient increase in the functional (Na^+-K^+)-ATPase activity due to an enhanced availability of intracellular substrate, probably Na^+. The relevance of these changes for cell cycle progression was determined by studying the effects of ouabain on ^{3}H-thymidine incorporation and cell cycle kinetics. The latter was assayed by time-lapse cinematography (see *1.* above and publ. 30).

Complete inhibition of the (Na^+-K^+)-ATPase-mediated K^+ influx by 5mM ouabain prevents the cells from entering S-phase, while partial inhibition by lower concentrations of ouabain (0.2 and 0.5 mM) causes partial blockage in G_1 and, to a lesser extent, a reduced rate of progression through the remainder of the cell cycle. It was concluded that the transient increase in (Na^+-K^+)-ATPase-mediated K^+ influx at the G_1/S transition is a prerequisite for entry into S-phase, while maintenance of adequate levels of K^+ influx is necessary for the normal rate of progression through the remainder of the cell cycle. The results have been submitted for publication (see publ. 21).

3. Sodium transport in the neuroblastoma cell cycle

The indication for a possible critical role for Na^+ entry at the G_1/S transition (see *2* above) as well as the similarity between the sequence of ionic events at this crucial period in the cell cycle and those occurring at growth stimulation of quiescent cells (see previous report, sect. IV.1b; this report, publ. 20) led us to

a more detailed study of Na^+ transport during the cell cycle, employing $^{24}Na^+$ tracer analysis. The large variety of Na^+-transporting systems, the relatively low intracellular Na^+ content, and fast transport kinetics pose relatively large technical difficulties for such studies. As a preliminary to determining the unidirectional Na^+ influx during the cell cycle it was shown that Na^+ influx rates can be determined by the uptake of $^{24}Na^+$ during a 3-5 min incubation in a medium containing ouabain to block (Na^+-K^+)-ATPase-mediated back-flux.

Undirectional Na^+ influx decreases rapidly during the early G_1-phase, increases transiently about two-fold at the onset of S-phase, after which it remains nearly constant during the remainder of the cell cycle. Since 1. the transient increase in Na^+ influx coincides with the transient activation of (Na^+-K^+)-ATPase at the G_1/S transition, 2. these ionic changes are electrically silent and therefore probably not of electrodiffusional origin, and 3. both phenomena can be blocked by the diuretic amiloride, it is concluded, in analogy with earlier work on the ionic changes involved in serum stimulation of quiescent cells (publ. 20), that the transient activation of (Na^+-K^+)-ATPase is due to enhanced availability of intracellular Na^+, probably through the activation of a Na^+-K^+ antiport system. Prior to the G_1/S transition no amiloride-sensitive component of the Na^+ flux is detectable. We also studied the effects of amiloride on the initiation and progression of DNA synthesis, as measured by ^{3}H-thymidine incorporation, and on cell cycle kinetics, as determined by time-lapse film analysis (see publ. 30). When applied in the G_1-phase amiloride particularly lowers the probability for the G_1/S transition in a dose-dependent manner, and thus interferes with the initiation of DNA synthesis, such that the cells accumulate in the G_1-phase. It is concluded from these results that the activation of this amiloride-sensitive Na^+ influx, which in its turn results in the earlier observed transient activation of (Na^+-K^+)-ATPase, plays a crucial role in the regulation of the G_1/S transition. It could provide a molecular basis for the restriction point in the cell cycle proposed earlier[1]. The results are being prepared for publication.

[1] Pardee, A.B. (1974) - Proc. Natl. Acad. Sci. U.S.A. *71*, 1286-1290.

4. Growth factor-membrane interaction during the neuroblastoma cell cycle

The similarity of the sequence of ionic events evoked by the addition of serum growth factors to serum-deprived, quiescent N1E-115 cells (see publ. 20) to that occurring at the G_1/S transition of synchronized Neuro-2A cells (see *2* and *3* above) has led to the hypothesis that the observed alterations in cationic transport are due to the interaction of growth factors with their appropriate receptors on the plasma membrane in the late G_1-phase. We therefore studied the effect of serum on cell cycle kinetics and cation transport during the early phases of the cell cycle in synchronized Neuro-2A cells. Upon removal of serum in early G_1-phase neither the increase in (amiloride-sensitive) Na^+ influx nor the stimulation of (Na^+-K^+)-ATPase activity is observed, while the probability for the G_1/S transition is strongly reduced. If serum is present for the first 3-5 hr after mitosis, cell cycle kinetics are identical with those of control cells grown in the continuous presence of growth factors. Further, experiments in which serum was added in a pulse of 1 hr to synchronized cells otherwise in serum-free medium, demonstrated that the presence of serum at the normal time of the G_1/S transition is a prerequisite for a normal level of DNA synthesis. These preliminary results suggest that functional interaction between growth factors and membrane receptors, manifested in the triggering of rapid changes in cation transport properties, is only possible in the late G_1-phase, indicating that the cell modulates the expression of these receptors during the cell cycle. In the absence of these interactions the cells are arrested in the G_1-phase, and in the case of committed cells such as neuroblastoma cells they will be switched from a proliferative programme to a differentiating pathway. It is tempting to speculate that the alterations in the dynamic state of membrane lipids and proteins during the G_1-phase observed earlier[1] (see also publ. 7) are involved in the regulation of the expression of the receptor sites for serum growth factors during the cell cycle.

Further, we have recently shown that the two neuroblastoma clones Neuro-2A and N1E-115 both have functional receptors for a purified growth factor, i.e., epidermal growth factor (EGF), and we are in the process of characterizing both

the ion flux and mitogenic responses with a view to repeating the above experiments using EGF in place of serum.

[1] de Laat, S.W., P.T. van der Saag and M. Shinitzky (1977) - Proc. Natl. Acad. Sci. U.S.A. *74*, 4458-4461.

5. Regulation of (Na^+-K^+)-ATPase at the molecular level

To discriminate between the various modes of regulation of the activity of (Na^+-K^+)-ATPase during the cell cycle (number of copies per cell, conformational changes, substrate availability) further experiments have been carried out.

External ATP (4 mM) is capable of stimulating the pump activity of Neuro-2A (Na^+-K^+)-ATPase. This 1.7-fold stimulation is accompanied by an increase in enzyme sensitivity for ouabain. Experiments with radioactively labelled ATP have demonstrated that externally added ATP is not taken up by the cell, while cation flux and electrophysiological measurements have indicated that external ATP specifically enhances the plasma membrane permeability for Na^+ compared to K^+. It was concluded that external ATP induces the (Na^+-K^+)-ATPase to assume a more active conformation, in addition optimizing the pump requirement for internal Na^+. Direct evidence for phosphorylation of external sites on the cell membrane due to treatment with 4 mM ATP could not be obtained (paper submitted). At present experiments are being carried out to determine the ouabain sensitivity of Neuro-2A cells during the cell cycle in the presence and absence of external ATP. These measurements can give information both on protein conformation changes during the cell cycle and, in combination with direct ^3H-ouabain binding studies, on the modulation of the number of (Na^+-K^+)-ATPase copies during the cell cycle.

Experiments have also been performed to determine the ouabain sensitivity and the Na^+ concentration required for half-stimulation of the (Na^+-K^+)-ATPase in 27,000 x g pellets of cell homogenates. It was shown that the rate of ATP hydrolysis by (Na^+-K^+)-ATPase is inhibited by ouabain under conditions of optimum substrate concentration, with $K_D = 80 \pm 10$ µM. Stimulation of the ATP hydrolysis rate by Na^+ obeys Hill kinetics, with half-stimulation at 20 ± 2 mM Na^+ and an apparent number of cooperative sites equal to 1.7 ± 0.1. Both the ouabain affinity and the Na^+ affinity of the (Na^+-K^+)-ATPase turned out to be independent of cell density (20,000 - 400,000 cells/cm^2).

Experiments to characterize possible cell cycle-dependent conformational changes of the (Na^+-K^+)-ATPase, as manifested by altered substrate affinities, are in progress.

In cooperation with Dr. D. Louvard (Heidelberg) we are at present testing whether the surface distribution and mobility properties of (Na^+-K^+)-ATPase can be monitored after labelling with a rhodamine-conjugated antibody (Fab'-fragment) against the enzyme, using image-intensified fluorescence microscopy and fluorescence photobleaching recovery measurements. This would make it possible to study the possible causal relationships between alterations in membrane dynamic properties and the functioning of the enzyme.

6. Membrane potential and neuroblastoma cell proliferation

The membrane potential of Neuro-2A cells in sparse cultures shows significant modulation during the cell cycle (see publ. 16). In order to investigate to what extent these modulations are required for progression of the cell through the cycle, Neuro-2A cells were grown in media containing high K^+ concentrations (in exchange for Na^+). Under these conditions the membrane potential is strongly reduced, while gradients for Na^+ and K^+ ions across the plasma membrane are almost neutralized. Neuro-2A cells can be grown in media containing 100 mM K^+ for several generations with doubling times only slightly longer than in media with normal K^+ concentrations (5.4 mM). Cell cycle analysis of cultures freshly placed in media containing 100 mM K^+ revealed an increase in transition probability (see *2* and *3* above) from 2.0 to 2.9 hr, together with an increase in length of the determinate B-phase (see *1* above) from 7.1 to 7.6 hr as compared with control media. This latter observation agrees with the slightly lower rate of thymidine incorporation in synchronized cell cultures at high K^+ concentrations. The above data together confirm the idea that a low membrane potential favours the transition from the G_1 to the S-phase, while a high membrane potential stimulates progres-

sion of the cell through S-phase. It is well-known that the rate of uptake of a number of nutrients required for progression of the cell through S-phase depends on the steepness of the Na^+ gradient. Experiments on the uptake of the amino-acid analogue α-aminobutyric acid showed a strong reduction in the presence of 100 mM K^+.

Previous studies (see publ. 7) have demonstrated that modulations in the lateral mobility of membrane lipids and proteins during the cell cycle of Neuro-2A cells are correlated in time with changes in membrane potential: in the G_1-phase membrane depolarization is accompanied by an increased mobility of membrane components. Preliminary experiments indicate that the electrical field across the plasma membrane can in itself control the dynamic properties of membrane components and suggest a direct control of the membrane potential over other membrane functions such as the expression of receptor sites. Through such mechanisms the membrane potential could exert a regulatory role in the cell cycle.

7. Surface architecture during the cell cycle of Neuro-2A neuroblastoma cells (in collaboration with P. van Maurik and J.G. Bleumink)

During their division cycle mammalian cells in culture necessarily have to increase their surface area in order to keep their phenotype constant upon cytokinesis. It is believed that the net production of surface membrane during the cell cycle occurs through asynchronous insertion of the various membrane constituents, which in turn could provide a basis for understanding the observed modulations in membrane structure and function (see [1] for review). Using scanning electron microscopy (SEM) we have now analysed the concurrent alterations in cell shape and surface architecture through the cell cycle. Mitotic cells obtained by selective detachment were plated on plastic cover slips at a density of 20,000 cells/cm^2. At twelve time points in the cell cycle these synchronized cultures were fixed and prepared for SEM. The SEM analysis revealed marked changes in cell shape and surface architecture during the cell cycle: 1. mitotic cells, which are round and loosely attached to the substratum through fine processes, show a highly irregular cell surface with abundant blebs and microvilli; 2. in the G_1-phase the cells start to flatten against the substratum, the gradual loss of surface irregularities apparently compensating for the concomitant increase in surface area/volume ratio; 3. through the S-phase and most of the G_2-phase the cells are very flat, have a stretched cell surface and gradually occupy more area on the substratum; 4. just prior to mitosis the cells suddenly round up through a retraction process, anchorage to the substratum being maintained through fine processes, and the cell volume probably increasing rapidly. Although quantitative measurements have yet to be made, the SEM analysis made so far indicates that cell surface growth is predominant during a short period around mitosis, at which time a large store of cell surface material becomes located in surface blebs and microvilli, to be used through surface stretching during the remainder of the cell cycle.

[1] de Laat, S.W. and P.T. van der Saag (1981) - In: Genetic Expression in the Cell Cycle, Vol. II (Eds. G.M. Padilla & K.S. McCarthy, Sr.), Academic Press, New York.

8. Junctional communication in the cell cycle of Neuro-2A neuroblastoma cells

In an initial series of experiments we have found that exponentially growing Neuro-2A cells show gap junctions in freeze-fracture electron microscopy, as well as ionic coupling and dye coupling as revealed by electrophysiological measurements and intracellular Lucifer Yellow CH iontophoresis. During cell division the functional membrane junctions will be physically disrupted, after which gap junctions have to be reformed. Most likely the process of formation and disruption of junctions will involve a lateral reorganization within the membrane of the molecular junctional components. In view of the cell cycle-dependent alterations in the mobility of membrane lipids and proteins reported earlier we started to investigate the establishment of junctional communication through the cell cycle, using intercellular transfer of Lucifer Yellow CH as a criterion for the presence of functional gap junctions.

In a first series of experiments dye coupling was followed during the cell cycle in cultures synchronized by selective detachment of mitotic cells. Junctional com-

munication is not detectable till mid G_1-phase, after which the cells are dye-coupled through the remainder of the cell cycle. In a second series of experiments co-cultures of synchronized S-phase cells, showing junctional communication, and mitotic cells were tested for intercellular dye coupling. These experiments demonstrated that mitotic and early G_1 cells are not only incapable of forming functional gap junctions among themselves, but also fail to do so with S-phase cells which are already coupled. As both membrane lipid fluidity[1] and the lateral mobility of membrane lipids and proteins (see publ. 7) are specifically reduced during mitosis and early G_1-phase, these results suggest that gap junction formation in Neuro-2A cells is controlled by the dynamic properties of the plasma membrane.

[1] de Laat, S.W., P.T. van der Saag and M. Shinitzky (1977) - Proc. Natl. Acad. Sci. U.S.A. *71*, 1286-1290.

9. Direct evidence for Na^+/H^+ exchange in N1E-115 neuroblastoma cells

Studies of the early ionic events occurring upon growth factor/membrane interaction (see publ. 17, 20) have prompted further research to obtain direct evidence for the existence of a Na^+/H^+ exchange mechanism in neuroblastoma cells. The sudden addition of Na^+ to N1E-115 cells suspended in Na^+-free medium causes a rapid but transient increase in the rate of H^+ release from the cells. Li^+ can substitute for Na^+, but addition of choline, K^+ or Ca^{2+} has no effect. This process has the following properties: it is distinct from metabolic acid production and is not inhibited by azide; it is saturable with respect to external Na^+ (half-maximal response at about 10 mM); it is independent of membrane potential and can be mimicked by addition of the Na^+/H^+-exchanging ionophore monensin to cells in Na^+-containing media. Na^+-induced H^+ extrusion is accompanied by a rise in intracellular pH, as inferred from an enhanced uptake of weak acids using the flow dialysis assay. Conversely, Na^+ uptake by the cells is stimulated upon lowering the intracellular pH with externally applied acetate. Na^+-induced H^+ efflux, intracellular alkalinization and acetate-stimulated Na^+ transport are completely inhibited by amiloride and do not occur in digitonin-permeabilized cells. It is concluded that the plasma membrane of neuroblastoma cells contains an electroneutral Na^+/H^+ exchange system which may contribute to the regulation of intracellular pH.

10. The determination of intracellular pH

The indications that a Na^+-H^+ antiport system is involved in the action of growth and differentiation factors (see publ. 17, 20) have led us to investigate the possible role of intracellular pH (pH_i) in the regulation of growth and differentiation in more detail. Such studies require the availability of methods to monitor changes of pH_i in intact cells. To this end pH-sensitive glass microelectrodes with tip diameters smaller than 1 μm and insulated to the very tip were developed; these enable us to record pH_i continuously in tissue culture cells such as N1E-115 cells. The microelectrodes are prepared by simultaneous pulling of two coaxial capillaries (inner: Corning 0150 glass; outer: lead insulating glass). Their response is pH-sensitive in the order of 58 mV per pH unit, as expected. Their practical use was tested by measuring pH_i in N1E-115 cells induced to differentiate by 3-4 days' incubation in culture medium supplemented with 2% fetal calf serum and 1.5% dimethyl sulfoxide. The pH_i in these cells was found to be 6.95 ± 0.04 ($N = 26$) and could be altered by addition of ammonium chloride and sodium acetate. As an alternative approach, we have started to determine pH_i by using the fluorescent indicator 6-carboxy fluorescein diacetate[1]. If this method can be applied to surface-attached cells its use will not be restricted by the size of the cells.

[1] Thomas, J.A., R.N. Buchsbaum, A. Zimniak and E. Racker (1979) - Biochem. *18*, 2210-2218.

11. Role of the lipid composition of the plasma membrane

i. *Compositional changes during differentiation.* Earlier experiments (see previous report, sect. IV.1e.i) provided evidence for significant changes in the lipid composition of the plasma membrane upon differentiation of Neuro-2A cells. For reasons as yet unclear these data appeared not to be reproducible in recent studies.

We therefore decided to reinvestigate this matter and the necessary experiments are in progress.

ii. *Fatty acid supplementation.* The lipid composition of the plasma membrane can be modified by growing cells in lipid-defined media. Under these conditions the growth characteristics of Neuro-2A cells as well as the function of the membrane-bound enzymes (Na^+-K^+)-ATPase and adenylate cyclase can be modified by prolonged supplementation of fatty acids to the medium (see previous report, sect. IV.1e.ii). The availability of suitable chemically defined media (see *14* below) prompted investigation of the more immediate effects of fatty acid supplementation on growth behaviour, lipid composition of the plasma membrane, and cation permeability properties of Neuro-2A cells. As a standard experimental procedure the cells were grown at high density for 24 hr in defined media and subsequently treated for 2 hr by addition of stearic, linoleic or oleic acid (resp. at 40, 40 and 20 μgr/ml). All three fatty acids induced an increase in ^{3}H-thymidine incorporation into both the TCA-soluble and insoluble fraction, stearic acid having the most pronounced effect. In addition they all produced a similar small but significant decrease in the cholesterol: phospholipid ratio (Chol/PL) of crude membranes. Despite this similarity significant differences were found in their effect on the physico-chemical behaviour of the plasma membrane, as determined by fluorescence polarization measurements of lipid microviscosity or fluorescence photobleaching recovery measurements of the lateral mobility of a fluorescent lipid probe (Hedaf). Stearic acid reduced lipid mobility and increased microviscosity, whereas oleic and linoleic acid had the reverse effect.

Preliminary experiments indicated pronounced effects of these supplementations on the cation permeability properties. Oleic and linoleic acid caused a 15 mV depolarization of the membrane potential, which could be attributed to a specific increase in Na^+ permeability leading to an increased Na^+/K^+ permeability ratio. Stearic acid induced a non-specific increase in Na^+ and K^+ permeability, leaving their permeability ratio unchanged. These results suggest that part of the cell cycle-dependent modulations in membrane permeability properties can be mimicked by, or might be due to alterations in the degree of saturation of the phospholipids.

iii. *Lipid synthesis.* To investigate whether a change in lipid biosynthesis accompanies differentiation of Neuro-2A cells, biosynthesis was measured by ^{14}C-acetate incorporation into different lipid classes, which were then separated by thin-layer chromatography. Cells were grown for 24 hr on defined medium, after which differentiation was induced by raising the internal c-AMP level.

After 40 hr of differentiation a 50% decrease as compared to control cultures was observed in the ratio of acetate incorporated into cholesterol to that incorporated into phospholipids (Chol/PL incorporation ratio). A transient increase in acetate incorporated into cholesterol ester was also found. These changes resulted in a steady increase in the cholesterol ester: free cholesterol incorporation ratio over the 40 hr period.

Measurements on crude membranes also revealed a drop in Chol/PL, though less pronounced than the Chol/PL incorporation ratio into lipids measured in the whole cell.

Apparently the combined action of a decrease in cholesterol synthesis and an increase of its esterification enables the cells to reduce the availability of cholesterol for incorporation into the plasma membrane during neuronal outgrowth. These results are being prepared for publication.

12. Isolation and characterization of plasma membrane from N1E-115 neuroblastoma cells

For various reasons the necessity was felt to isolate purified plasma membranes from a murine neuroblastoma cell line other than Neuro-2A. Although the cell line N1E-155 has some disadvantages from the practical point of view, e.g. longer cell cycle times (ca. 24 hr), lower saturation density and lower adhesiveness to the substratum - factors which hamper economical mass cultivation and mechanical collection of mitotic cells to establish synchronous cultures - recent studies on changes at the plasma membrane level accompanying differentiation and growth stimulation in this cell line[1-4] (see also publ. 20) have prompted new interest. Because of the disadvantages mentioned only relatively simple methods that will lead to rapid isolation of the plasma membrane with relatively high yield can be

applied. Two such methods were used. The first procedure[5] employs a discontinuous sucrose gradient in which the sample in 33% (W/V) sucrose is placed in the middle ('sandwich' technique). The second method[6] also consists of a single centrifugation step but employs a viscosity barrier of dextran T-500 to separate plasma membranes from mitochondrial membranes and endoplasmic reticulum. Using (Na^+-K^+)-ATPase as a plasma membrane marker enzyme, membrane preparations from growing cells were obtained which were purified 12 to 25-fold with respect to the original homogenate, with a yield ranging from 15-20%. These encouraging results will be followed up by a more detailed analysis of the lipid and protein composition of these membranes, while similar procedures will be applied to whole differentiated cells and/or isolated neurites of differentiated cells.

[1] Moolenaar, W.H. and I. Spector (1978) - J. Physiol. *278*, 265-286.

[2] Moolenaar, W.H. and I. Spector (1979) - J. Physiol. *292*, 297-306.

[3] Moolenaar, W.H. and I. Spector (1979) - J. Physiol. *292*, 307-323.

[4] Littauer, Y.Z., M.Y. Giovanni and M.C. Glick (1980) - J. Biol. Chem. *255*, 5448-5453.

[5] Jakoi, E.R. and R.B. Marchase (1979) - J. Cell Biol. *80*, 642-650.

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13. The action of nerve growth factor on rat pheochromocytoma cells (PC12)

The studies on the mode of action of nerve growth factor (NGF) as an inducer of neuronal differentiation in PC12 cells were continued (see previous report, sect. IV.1i). Flux experiments using $^{86}Rb^+$ as a tracer for K^+ transport demonstrated that NGF induces a rapid stimulation of (Na^+-K^+)-ATPase activity in PC12 cells. This stimulation is amiloride-sensitive and can be mimicked by the Na^+-ionophore monensin, indicating that the activation of the enzyme by NGF is due to an increased amiloride-sensitive Na^+ influx (see publ. 17). Subsequent $^{24}Na^+$ influx measurements confirmed this hypothesis. So far this sequence of NGF-induced early ionic events, which precedes PC12 cell differentiation, resembles that evoked upon growth stimulation of quiescent N1E-155 neuroblastoma cells by serum (see publ. 20).

The finding that mitogen/membrane interaction and differentiation factor (NGF)/membrane interaction are followed by very similar early events raises the important question at what level the discrimination between the ultimate biological effects is then made.

14. Growth of embryonal tumour cells in defined media

The development of culture media of defined composition capable of sustaining growth of cells without serum is of vital importance for a better understanding of growth, differentiation and hormone action. In the last few years great progress has been made in this respect; for a number of established cell lines and primary cell cultures defined serum-free media have been developed which sustain growth to the same extent as serum-containing media and also permit induction and maintenance of the differentiated state[1,2].

Our efforts to find defined culture conditions for embryonal tumour cells started last year (see previous report, sect. IV.1f) were continued. Neuroblastoma cells (neuro-2A) can now be cultured permanently in a medium consisting of Dulbecco's modified Eagle medium and Ham's F12 medium (1:1) supplemented with sodium selenite and transferrin only. In contrast to the conditions described for most cell types insulin is not required for Neuro-2A cells, neither for better attachment to the culture substratum nor for better growth.

For further analysis of growth and differentiation in defined medium it will be necessary to use synchronized cell populations. Since it became clear that it is impossible - for unknown reasons - to obtain mitotic cells from cultures in defined medium by the selective detachment method, other methods have to be developed. Mitotic accumulation can be induced by a number of drugs primarily affecting microtubules. A new drug of this type is nocodazole[3]. First results obtained with Neuro-2A cells in defined medium indicate that nocodazole causes total mitotic arrest but permits re-entry into the cell cycle upon withdrawal of the drug and

replating of the treated cells in fresh defined medium. At present further research is under way exploring the possibilities to culture other neuroblastoma cell lines and embryonal tumour cell lines in defined media.

[1] Barnes, D. and G. Sato (1980) - Anal. Biochem. *102*, 255-270.

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15. Retinoblastoma cells in vitro

In collaboration with the Wilhelmina Kinderziekenhuis (University Children's Hospital, Utrecht; Dr. F.A. Beemer) and the University Clinical Genetical Center, Utrecht (Dr. J.O. van Hemel) a primary retinoblastoma cell line, established from a patient biopsy in 1977, was obtained and successfully cultured in the Hubrecht Laboratory since 1979. In 1980 a second (permanent) retinoblastoma cell line, Y-79, was obtained in collaboration with the Institute for Radiotherapy (University Hospital, Utrecht; Ir. J. Schippers). This cell line has been described earlier[1].

Retinoblastoma cells are another example of embryonal tumour cells since they originate during development from a rod cell of the visual receptor layer in the retina. Retinoblastoma is the most common malignant eye tumour of childhood. There are two forms, hereditary (40%) and non-hereditary (60%). The hereditary form is transmitted as an autosomal dominant trait with 90% penetrance and has been connected with an interstitial deletion of the long arm of chromosome 13.

Under *in vitro* conditions retinoblastoma cells can undergo morphological differentiation, as was also observed in the original tumours in the formation of rosettes and fleurettes, cell arrangements typical for photoreceptor elements. Virtually nothing is known in this system about the effects of differentiation triggers that are effective in other embryonal tumour cell systems (e.g. c-AMP, DMSO or serum starvation). This will only be the case when markers (preferably biochemical ones) become available for a better assessment of the degree of differentiation.

Preliminary observations on the cell clone isolated in Utrecht have led to the hypothesis that this particular cell line originates from, or even represents a primitive stem cell, which may have the capacity to differentiate either into a pathway leading to neuroblasts and neuronal cells or into one leading to spongioblasts and glial cells. This was suggested earlier by Taylor *et al.*[2]. Further research will be carried out to explore this possibility with the aid of specific neuronal and glial markers.

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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)

This joint project (see previous report, sect. V.1) was continued during a stay of P.D. Nieuwkoop of six weeks at the Institut Teknologi Bandung (Indonesia). Laboratory culture methods for sea turtle embryos were elaborated, and a beginning was made with the development of operation techniques for experimental embryology of reptiles. These consist of 1. implantation of reptilian organ anlagen into the coelomic cavity of 2½-day chick embryos cultured at slightly lowered temperature (34-35°C), and 2. intracoelomic grafting of anlagen in sea turtle host embryos cultured at optimal temperature (27-30°C).

In Utrecht P.D. Nieuwkoop started with the histological analysis of the origin and development of primordial germ cells in the sea turtle, using material collected last year in Indonesia.

2. The origin of primordial germ cells in anurans (Bombina orientalis) (P.D. Nieuwkoop)

In view of the demonstrated mesodermal origin of the primordial germ cells in urodeles, which arise along with the induction of the mesoderm by the endoderm in the blastula stage (see previous reports), the question arises whether in anurans, apart from their demonstrated endodermal origin, primordial germ cells may perhaps also originate secondarily by induction. Pilot experiments to test this possibility were initiated.

3. Electron-microscopical investigations of intercellular contacts during early cleavage stages in a mollusc (Limnaea stagnalis) (A.W.C. Dorresteijn, J.A.M. van den Biggelaar, Zoological Laboratory, Utrecht; J.G. Bluemink, W.J. Hage)

Determination of the different cell lineages in the molluscan embryo partly depends upon differential segregation of the ooplasm and partly upon cellular interactions[1]. This study is a continuation of that started last year (see previous report, sect. V.4) and was undertaken to identify by freeze-fracture electron microscopy the structures that might be involved in cellular interaction between the blastomeres at successive cleavage stages. Since gap junctions are generally assumed to function as channels for intercellular communication we wanted particularly to analyse the time of their appearance and their distribution between pairs of blastomeres in relation to the determination of dorsoventrality in the embryo.

In early cleavage stages (up to the sixth cleavage) three kinds of intercellular junctions could be distinguished: intermediate, septate and gap junctions. The first two form 'junctional belts' located on the cell border at the periphery of the embryo. Gap junctions first appear at the 4-cell stage. Up to the sixth cleavage no difference in the distribution pattern could be found between and within each of the four quadrants of the embryo. Some of the cell tiers along the animal-vegetal axis lack gap junctions either within the tier or between adjacent tiers. All gap junctions observed in freeze-fracture replicas show plaques with an irregular pattern of intramembranous particles (IMPs). The average IMP diameter is 12 ± 2 nm. In embryos fixed after the fifth cleavage gap junctions are found between the micromeres at the animal pole and the central 3D macromere. This is in agreement with the postulated interaction between these cells at this stage. The results have been prepared for publication.

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4. Junctional communication in early molluscan development (Patella vulgata) (J.A.M. van den Biggelaar, A.W.C. Dorresteijn, Zoological Laboratory, Utrecht; L.G.J. Tertoolen, S.W. de Laat)

In the 32-cell *Patella* embryo the macromeres of each quadrant still have the capacity either to develop into the mesentoblast or to form endodermal structures. At this stage the macromeres protrude into the embryo and one of them (then denoted as 3D) transiently contacts the overlying animal micromeres. This event determines this macromere to form the mesentoblast and establishes dorso-ventral polarity. As an extension of our previous electrophysiological experiments (see previous report, sect. V.4) we have determined the possible involvement of junctional communication patterns in this determinative event by monitoring the transfer of the fluorescent dye Lucifer Yellow CH (MW 457) after its iontophoretic introduction into various macromeres during the early cleavage stages. Up to the 16-cell stage no intercellular dye transfer was observed. After fifth cleavage the spreading of the dye demonstrated the establishment of a particular communication pattern within the embryo: dye transfer is possible between each vegetal macromere and its immediate neighbours, with the exception of the junctional membrane between the 3D cell and its neighbour on the future dorsal side ($2d^2$). Furthermore, it appeared that dye transfer can occur from the 3D macromere to the overlying micromeres. These results are the first demonstration that the formation of specific intercellular communication patterns is involved in cell determination during early development. The results have been published (publ. 8) or submitted for publication (publ. 13).

5. The organization of the plasma membrane of the molluscan egg in relation to the localization of morphogenetic determinants (J.E. Speksnijder, Zoological Laboratory, Utrecht)

This project started in October and is partially carried out at the Hubrecht Laboratory, using the facilities for freeze-fracture electron microscopy. The results will be reported next year.

6. Junctional communication between epithelial cells in hydroids (Hydra attenuata) (C.J.P. Grimmelikhuijzen, Max-Planck Institut für Medizinische Forschung, Heidelberg; L.G.J. Tertoolen, S.W. de Laat)

As reported earlier (see previous report, sect. V.5), electrophysiological measurements showed the absence of electrotonic coupling between epithelial cells in intact animals and under various conditions of regeneration. This finding has now been extended to nerve-free animals. To confirm this suprising result we extended this study by following possible intercellular transfer of the fluorescent dye Lucifer Yellow CH (MW 457) upon intracellular application through iontophoresis. Dye location was also monitored in tissue sections. No indications for dye transfer could be detected. It was concluded that 1. gap junctions between epithelial cells, observed by electron microscopy, do not function as communicative intracellular junctions; 2. morphogenetic substances probably diffuse via the extracellular space; 3. nerve-free *Hydra*, which are still able to contract on electrical or mechanical stimulation, cannot conduct the contraction pulse along electrotonically coupled epithelial cells. The results have been published (publ. 3, 9).

7. Feasibility study of the use of cell culture systems to test for teratogenicity (C.L. Mummery, S.W. de Laat)

In our initial approach to developing a screening method for potential teratogens *in vitro* we are making use of culture systems suitable for studying either early differentiation (embryocarcinoma cell lines EC, PCC4 and OG15-51) or terminal differentiation (neuroblastoma clones N1E-115, Neuro-2A). Integral to the use of these systems for teratogen testing is the development of a defined serum-free medium (see section IV.*14*), since cell-foreign proteins present in serum in the usual nutrient media are known to become strongly associated with the cell surface and/or the extracellular agents to be tested[1,2]. Aspects of cell behaviour which are being used as 'markers' to indicate possible teratogens include 1. changes in proliferation rate (Neuro-2A) and 2. inhibition or induction of cellular differentiation (extension of neurites in N1E-115 cells) and cell-cell interactions (aggregation in EC cell lines). At present a suitable list of teratogens is being drawn up which are being systematically tested for their ability to interfere with the usual behaviour of the above cell lines in culture. In the long term, assuming useful correlation of behaviour is found between these or some other cell lines, the project will be extended to develop a rapid assay for the effects observed. This research is supported by Shell International Research Corporation.

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8. Dynamic properties of the plasma membrane in early amphibian development (Xenopus laevis) (M. Gadenne, Univ. de Bruxelles; E.J.J. van Zoelen, S.W. de Laat)

Fluorescent lectins show capping behaviour on dissociated cells from early *Xenopus* embryos which depends on the stage of development. To establish whether this is due to changes in the intrinsic dynamic properties of the plasma membrane during early development we examined the lateral mobility of a fluorescent lipid probe (Hedaf) by fluorescence photobleaching recovery measurements in dissociated cells obtained from the early blastula to the neurula, making a distinction between cells from different germ layers. So far the results indicate a gradual, significant increase of about a factor two in the diffusion coeffficient of membrane lipids during this period of development, without significant differences between

the various germ layers at any particular stage. This study will be continued by measuring the diffusion coefficient of membrane proteins under the same conditions.

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

Last year (see previous report, sect. V.6) a study was initiated of the usability of the determination of the apparent viscosity of amniotic fluid lipids by fluorescence polarization measurements as a diagnostic tool for fetal lung maturity. Parallel measurements of the lecithin/sphingomyelin ratio and the apparent viscosity of amniotic fluid samples of various gestational ages gave comparable diagnostic prediction values but the fluorescence polarization method has the advantage of being less time-consuming. The results are in qualitative agreement with published data[1].

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

10a. The regulation of reproduction and spawning (Bombina orientalis, Pleurodeles waltl)

The method elaborated last year (see previous report, sect. V.7a) to procure fertilized eggs of *Bombina* by injection of a pituitary suspension has now been tried out in animals outside the reproductive season. Fertilized eggs were obtained but the percentage of fertilization was quite low and the timing of oviposition imprecise.

Pleurodeles (wild type and *ac* mutant) were injected intraperitoneally out of season with HCG in physiological saline. The females were induced to deposit eggs but the injection was ineffective in the males.

10b. Recording of characteristics of egg batches from individual females (Xenopus laevis)

This is now possible thanks to the method of permanent marking of adult females elaborated last year (see previous report, sect. V.7b). Certain important egg characteristics of batches from a number of females (four batches per female) were recorded. The data indicate that this is a suitable way better to meet the requirements of research and of breeding for colony maintenance.

10c. Prevention of rickets in anurans (Rana pipiens, R. lessonae)

The method of UV treatment used last year for *Discoglossus* (see previous report, sect. V.7c) proved to be successful in *Rana* species as well. A variety of live food in the diet is also important.

10d. Tumours and other diseases

Three greatly enlarged spleens of *Xenopus laevis* were sent to the Registry of Tumors in Lower Animals, National Museum of Natural History, Washington D.C. and were identified there as splenitis with bacteremia (2) and splenomegaly (1).

No diagnosis could be made of the contagious disease in axolotl (see previous report, sect. V.7e). The bacteria *Escherichia coli*, *Aeromonas hydrophila* and *Pseudomonas spec.* were all present in large numbers in the spleen, the intestine and the cloaca.

VI. Projects carried out in cooperation with the Technical Workshop

1. Construction of an agglutination-meter for quantitative studies on cell adhesion (H.R. Reitsma, H.L. Krielen, C.J. Weijer, A.J. Durston)

We have constructed a agglutination-meter for use in studies on cell adhesion in *Dictyostelium discoideum* (see section III.F.*2*). This a spectrophotometer, set up

to measure time-dependent optical density (O.D.) or 0-10° light scattering in aliquots of a cell suspension. Aliquots are continuously mixed and subjected to a standard shear force by being placed in one or more of 20 special micro-cuvettes positioned at the edge of a 22 cm-diameter disc rotated at up to 30 r.p.m. by a DC-motor. Illumination (from a conventional Tungsten lamp with a current-stabilized power supply) is via adjustable angled mirrors, to enable O.D. or scattering measurements. The cuvettes were modified from previous designs to enable accurate measurements on very small aliquots (< 100 µl) and to facilitate cleaning and handling. The machine is operated and data are handled and stored via a machine language (BASIC) programme run in an ITT 2020 Apple microcomputer with two specially designed interfaces (containing two programmable timers, analog-digital and digital-analog converters and 16 digital inputs and outputs). Data are stored and processed using flexible discs or else plotted directly on a chart recorder. Data are collected in parallel from each of up to 20 cuvettes during each rotation cycle.

VII. Miscellaneous

1. Prof. P.D. Nieuwkoop devoted much time to the completion of the text and figures for the monograph on 'Primordial Germ Cells in the Invertebrates' to be published with Dr. L.A. Sutasurya (see publ. 23).

2. Prof. A.G. Johnen and Ms. B. Albers used the library facilities for a period of one week in preparation for a book to be written jointly with Prof. Nieuwkoop (see publ. 22).

3. Prof. R. Chandebois used the library facilities for one month.

4. Prof. J.-C. Beetschen visited the Laboratory for a week to discuss a joint project involving the *ac* mutant of *Pleurodeles waltl* (see sect. I.A.2).

5. Dr. P. Cardellini stayed at the Laboratory for two months to collect and analyse material from various anuran species in relation to the problem of the 'haemoglobin shift' during amphibian ontogenesis.

6. Six scientists worked in the Central Embryological Collection for periods longer than a few days:
a. Dr. R.L. Hughes (five weeks): preparation of a review on monotreme embryology; study of the reproductive tract of the extinct marsupial *Thylacinus cynocephalus* and of the fetal membranes of some other marsupials; survey of human material available.
b. R. Lorente (one week): study of neural tube development in early primate embryos.
c. Prof. W.P. Luckett (2½ months): study of fetal membrane development in *Ctenodactylus* (Rodentia); study of dental development in *Tarsius* (Primates); cooperative studies of early dental development in several insectivores, marsupials, rodents and bats.
d. Dr. S.L. Ullmann (one month): study of the sexual differentiation of the gonad in the pouch young of *Isoodon* and *Perameles* (Marsupialia).
e. J.R. Wible (one week): checking the possibilities of using material from the Collection for a dissertation on the ontogeny and phylogeny of the carotid artery in mammals.
f. Dr. D.T. Yew (five weeks): comparative study of eye development in *Agnatha* and *Chondrichthyes*.

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Institute for Ecological Research

Progress Report 1980

Edited by J.W. Woldendorp

Instituut voor Oecologisch Onderzoek,
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The Netherlands

CONTENTS

The protection of the Isle of Voorne against the sea requires the heightening of the outer dune ridge.
The Department of Dune Research "Weevers' Duin" is involved in experiments to fix the sand by promoting vegetation development.

1. Task and function of the Institute

The Institute was founded in 1954 by the Division of Sciences of the Royal Netherlands Academy of Arts and Sciences 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. A new seat for experimental research is situated at Heteren, near Arnhem. Field work is carried out in various parts of The Netherlands (see Fig. 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 and the Coot.

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 inform-

Fig. 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)

ation 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, where 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.

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)

Dune Research 'Weevers' Duin'

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)
A.J. Smit (Guest worker, BION/ZWO)

3. General summary

The multidisciplinary research projects on characteristic adaptions of birds and grassland plants to their specific environment were continued. These long-term studies on the population level were carried out in close collaboration with the universities of Groningen and Utrecht.

At the end of 1980 the Proceedings appeared of the symposium on 'The integrated study of bird populations', which was organized to celebrate the twenty-fifth anniversary of the Institute. In these Proceedings much of the Institute's research of the previous period on the Great Tit was summarized. Special attention was given to the effects of territory occupancy, feeding conditions and local survival in the winter on the population fluctuations. Also the population genetics and the energy requirements for incubation were studied in detail. A number of additional papers on territorial behaviour, on the characteristics of natural nesting sites and on the nutrition of the Great Tit are in preparation or have been submitted for publication. The results obtained so far give a good picture of the events occurring in the breeding and the winter season and of the effects of these on the population fluctuations. Also the energetics of breeding Great Tits and their nestlings are now fairly well understood. In the near future attention will be focussed mainly on the processes occurring in the period which follows immediately upon the breeding season and on the energetics of free-living birds. The latter aspect will be studied, in collaboration with the University of Groningen, using the D_2O^{18} technique.

Here a report is only given of the research on the production of second broods. In breeding populations of the Great Tit a varying number of breeding pairs starts a second brood. The hypothesis that the percentage of second broods started depends on the availability of food, could not be confirmed. In an experiment in which Great Tit pairs had access to a virtually unlimited food supply, of which they made ample use to feed the young of the first brood, no more second

broods were produced by the experimental group than by a control group which had no extra food source at their disposal.

The Bird Migration Department has now finished the analysis of recoveries of ringed birds. In the previous report the use of these recoveries in establishing the distribution pattern over the year of a number of species of ducks and geese was described. In the present report the same recovery data have been used to analyze the relationship between the survival of the Grey Heron and the severity of the winter. It was shown that the survival rates of both first-year and after-first-year birds varied with the severity of the winter. No relationship was found between the reporting rate of dead birds and the severity of the winter. The same methods of analysis were used to study the relationship between the survival rate of the Purple Heron and the drought conditions in the wintering area in tropical West Africa. Particularly the survival rate of the Dutch after-first-year birds appeared to be related to drought in the Sahel area. The techniques which were developed in previous years to establish the spatial distribution of bird populations and to determine their survival rates in relation to fluctuating environmental conditions, can in principle now be applied to the recovery data of other bird species. Together with programmes from other investigators, they form a set of tools which can be applied by users of the Euring Data Bank to studies on fundamental ornithology or on nature conservation.

In the project on the characteristic properties of grassland plants in relation to the local environmental conditions, special attention was given to a detailed description of the *Plantago* species occurring in The Netherlands. The occurrence of these species in the Dutch plant communities was studied in cooperation with the University of Nijmegen. The wide ecological amplitude of *P. lanceolata* was confirmed. Of the two *Plantago* species of salt-plant communities, *P. coronopus* clearly has its optimum on the higher-lying and *P. maritima* on the lower-lying salt marshes. It is intended to order the plant communities in a multidimensional pattern and to also include in this ordination our own data of 115 selected habitats in which the *Plantago* species were found to be present.

When characterizing the microclimate of these 115 habitats *P. lanceolata* was found to occur over a wide range of microclimatical conditions. In a synoptic representation of the microclimate of the various types of grasslands the temperature and the humidity conditions at a height of 1 cm in the vegetation were compared with those at 1 meter. Both parameters are related to the height and the density of the vegetations. The state of the soil surface proved to be decisive for the microclimate above it.

The wide ecological amplitude of *P. lanceolata* also became apparent from the soil-chemical analysis of the habitats of the *Plantago* species. The species occurs over a wide range of pH values but more often at relatively lower than at higher pH values. Habitats of *P. media* and *P. major* generally have high pH values. These species apparently have a preference for the nitrate over the ammonium ion. The hypothsesis that *P. major* is a species of nutrient-rich habitats was not confirmed from a purely soil-chemical point of view. The higher nutrient availability can possibly be ascribed to better soil-physical conditions and to the absence of competition.

The survival of *P. lanceolata* in vegetations of varying structure was correlated with the light transmission in the summer period. Generally, the population density increased in open vegetations but decreased considerably in the densest vegetations. The smallest individuals were most susceptible to the effects of dense vegetations. Large plants can compete adequately for light on high vegetations because their upward-growing leaves reach lengths of 40-60 cm or more.

The demographic properties of *P. maritima* were studied on a beach plain on the island of Goeree, where seeds were sown in four series of plots in different sites. Germination was possible immediately but the numbers of seedlings were low. Burrowing and heavy trampling were important causes of mortality as was the daily inundation by sea water. In order for this species to become established many seeds have to be released.

On trampled sites the rate of leaf turnover of *P. major* plants was higher than on untrampled sites, but the standing crop, the number of spikes and seed weight were also higher. Apparently, the species is well-adapted to trampled, compact soil.

The development of daughter rosettes in *P. lanceolata* in the first growing

season was higher than that of the other species. The numbers of daughter rosettes formed during the first and second growing seasons were influenced by the availability of nutrients and water. The other *Plantago* species formed daughter rosettes in the first growing season only when the apex was injured. It was concluded that the apical dominance in the first growing season is weaker in *P. lanceolata* than in the other species. External influences, therefore, have a great impact on this species.

The results obtained so far have confirmed the hypothesis of the previous report that *P. lanceolata* is more versatile than the other species.

4. Effect of the addition of food during the breeding season on the production of second broods by the Great Tit, *Parus major* (J. den Boer-Hazewinkel)

INTRODUCTION

In a breeding population of Great Tits a varying number of breeding pairs start a second brood after having successfully raised a first one. The occurrence of second broods can considerably enlarge the production of young in a population. This makes second broods an interesting object of investigation from the point of view of population dynamics.

The following considerations led to the hypothesis that the condition of the breeding pair is an important factor in the production of second broods:

1 The percentage of Great Tit breeding pairs starting a second brood decreases with increasing numbers of breeding pairs of both Great Tit and Blue Tit, *Parus caeruleus*. This could point to competition for food, both intra- and interspecific (see Dhondt 1977).
2 Analysis of van Balen's (1973) data showed a positive relationship between the availability of food and the percentage of second broods started.
3 Kluyver (1963) found a negative relationship between clutch size or number of fledglings in the first brood and the production of second broods.

To test this hypothesis, part of a breeding population of Great Tits was supplied with extra food during the breeding season, the other part serving as control.

EXPERIMENTAL DESIGN

Oosterhout, a mixed forest which represents an optimal habitat for Great Tits and has an area of 11.4 ha, was chosen for the experiments in 1979 and 1980. For a detailed description of the area, see van Balen (1973).

Breeding densities of the Great Tit in 1979 and 1980 were the highest recorded for Oosterhout since 1957. The number of breeding pairs registered for the Blue Tit was intermediate for 1979 and very high for 1980. Inspections of nestboxes provided information about clutch size, number of eggs hatched, hatching date, number of young fledged, and fledging date. Young were ringed and weighed at the age of 7 days and weighed again at 14 (1980) or 15 (1979) days. Parents were caught, identified, and weighed when their young were 7 days old.

Food was offered inside the nestboxes and consisted of a mixture of deep-frozen insects. Food supplied in the 1979 experiment consisted of larvae of *Galleria mellonella*, larvae of *Tenebrio molitor*, and larvae and pupae of *Adoxophyes orana* (hereafter referred to as G, T, A, and Ap, respectively). These insects were offered and consumed in the approximate weight-ratio of 17G : 4.5T : 4.5A : 4.5Ap. The water content ranged between 60 and 70 per cent, and the dry mass contained 25 to 50 per cent fat and 40 to 55 per cent protein. The digestibility of these insects is unknown. In the 1980 experiment food consisted of: G, T, and larvae of caterpillars (mostly Geometrids, to be referred to as C) collected on oaks in June and July of 1979. The insects were supplied in an approximate weight-ratio of 2.5G : 2.2T : 1C.

Feeding was done such that food was almost continuously present in the nestboxes during the experiment (maximum number of feeding times/day = 4, maximum amount of food offered/feeding = 20 grams).

In 1979, observations on one female in a glass-backed nestbox yielded some information on the behaviour of Great Tits with respect to the use of the artificial food source inside the nestbox. The frequency of visits to the young was recorded by chronographs for three breeding pairs with and three breeding pairs without

an artificial food source inside the nestbox. Feeding in the nestboxes was started when the young were 7 days old and continued until fledging. Breeding pairs were divided into two groups (fed and unfed) comparable as to location of the nestbox in the area, birth date of young, clutch size, mean weight of 7-day-old young, and the age of the female.

In 1980, food was supplied inside the nestboxes starting about 9 days before hatching and continued until fledging. The Oosterhout area is divided into two parts separated by a house and lawn and a small part of the forest without nestboxes. It is assumed that in the breeding period the Great Tits restrict foraging to the part in which they have their nest. In one part all Great Tit breeding pairs were supplied with extra food, in the other part none. Besides the food supplied in the nestbox, extra food was provided outside the nestbox in two food containers situated not more than 20 metres from the nestbox-tree. After initial problems with Jays (*Garrulus glandarius*), which gulped the contents of the containers down in no time, had been solved, these external containers were kept filled with (mainly) live mealworms during the period from May 18th to June 12th.

OBSERVATIONS ON THE USE OF THE ARTIFICIAL FOOD SOURCE INSIDE THE NESTBOX

A glass-backed nestbox in which deep-frozen insects were supplied was kept under observation for about five hours. The nest contained 8 young. The observations were done in the period when these were 10 to 18 days old. The male of the breeding pair had probably been disturbed by the manipulations with the nestbox prior to the observations, and did not show up.

The female never entered the nestbox without food, which she had either caught herself or taken with her from the internal container the last time she left the nestbox. After feeding the young, she seemed to wait for faeces. If a faecal sac was produced, she left the nestbox with it; if not, and if young were still begging for food, she fed them with food from the container one or more times. Sometimes she carried food with her when she left the nestbox, usually when young no longer begged for food. She then usually returned with the same food item to feed it to the young. On a few occasions the female was seen swallowing food taken from the container, mostly small items (i.e., *Adoxophyes orana*).

The carrying of food from a container out of the nestbox was observed in other breeding pairs as well.

CONSUMPTION OF FOOD FROM THE ARTIFICIAL FOOD SOURCE INSIDE THE NESTBOX

In 1979, twelve breeding pairs which successfully raised a first brood, used on average 55 g food per young from the containers in the nestboxes (range 39-68 g).

In 1980, eleven breeding pairs consumed on average 80 grams during the incubation period (range 46-110 g); during the nestling period they used 64 grams per young (range 53-125 g).

According to calculations by Mertens (1977), one Great Tit nestling requires about 142 grams of larvae of *Tortrix viridana* during a nestling period of 18 days. Calculations made with values given by van Balen (1973) gave only 85 grams per nestling for a nestling period of 17 days. Van Balen obtained his values from observations on a glass-backed nestbox in otherwise natural surroundings, and estimated the size of the preys fed to the nestlings. The fat and protein contents per gram of the food used in our experiments proved to be double those given by Mertens (1977) for *Tortrix viridana*.

It is concluded that the food taken from the supply inside the nestboxes in the present experiments must have formed a substantial proportion of the total amount of food required by the young during the nestling period.

CONSUMPTION OF FOOD FROM THE ARTIFICIAL FOOD SOURCES OUTSIDE THE NESTBOXES

A total of 3214 grams of food disappeared from the containers outside the nestboxes during a 24-day period. The area concerned was found to contain 17 breeding pairs of Great Tits (11 raising a successful first brood plus 4 raising a repeat brood in nestboxes, 2 pairs breeding in natural holes).

It is unlikely, however, that Great Tits had consumed a large part of this food. In the first place, it is estimated that a considerable part was lost to what was probably some predatory insect, since mealworms were found that had been cut into pieces about two segments long, the contents of many of which had been removed. Secondly, observations near 7 pairs of containers showed that Great Tits used this food infrequently. During 8 hours of observation they were seen taking food from the containers 29 times (14 of them concerning the same male in about one hour of the observation period). Apart from the Great Tits, a robin (*Erithacus rubecula*) and a wren (*Troglodytes troglodytes*) were seen taking food twice and four times, respectively. Thirdly, the rate of disappearance of food from containers near Great Tit breeding pairs in natural holes (which had no food supply inside the hole) was not any higher compared to other containers.

VISITING FREQUENCY

Figure 4 shows that, compared to the controls, the visiting frequency of three breeding pairs with an extra food supply inside the nestbox was considerably lower. The control broods were comparable to the experimental broods as to location of nestbox in the area, hatching date, and, approximately, the number of young.

It should be kept in mind here that in some of the recorded visits the parent had not collected the food itself but merely returned with food taken from the internal container.

The data in Fig. 4 give no information about the share taken by the male and the female in feeding. Observations from a hide placed near 6 nests with extra food, showed that both the male and the female fed the nestlings.

WEIGHT OF FEMALES, MALES, AND NESTLINGS

In 1980 the females (weighed when their young were 7 days old) from nestboxes with an artificial food supply had a mean weight of 18.7 g ($n = 11$), which was significantly higher ($ts = 4.6969$, $p<.001$) than that of the control females ($n = 20$), which was 17.5 g. Females breeding in both 1979 and 1980 were on average 0.8 g ($n = 7$) heavier in 1980 due to the extra food, whereas those not fed in either year (before weighing!) were on average 0.1 g ($n = 7$) lighter in 1980 ($ts = 2.8854$, $.01<p<.02$).

In the males, the extra food had no significant effect on weight.

In both years, young from nests supplied with extra food were about 1 gram heavier than those from nests without extra food.

PERCENTAGE OF SECOND BROODS

No significant difference in percentage of second broods started was found between breeding pairs with and without an extra source of food during the first brood. In 1979, the percentages of second broods started by breeding pairs with ($n = 12$) and without ($n = 15$) access to extra food were 33 and 47, respectively. In 1980, the respective percentages were 9 ($n = 11$) and 14 ($n = 24$).

CONCLUSIONS

On the basis of the present findings, the hypothesis that the physical condition of a breeding pair of Great Tits that has reared a first brood successfully, is an important factor in the production of second broods is highly unlikely, at least for Great Tits in an optimal habitat.

Great Tit pairs with access to a virtually unlimited food supply of which they made ample use to feed the young of the first brood, did not produce more second broods than those which had no extra food source at their disposal. From the reduced frequency of visiting the young it can be concluded that the former required considerably less energy to raise their young. Since the frequency of visiting of male plus female was registered the share taken in feeding by each member of the pair is not known. Theoretically, either the male or the female could have profited exclusively from the extra food. Females showed significantly higher weights when they had access to extra food, whereas males did not. Casual observation of 6 nests showed clearly that both male and female fed the young while an extra food supply was present in the nestbox. A more likely explanation

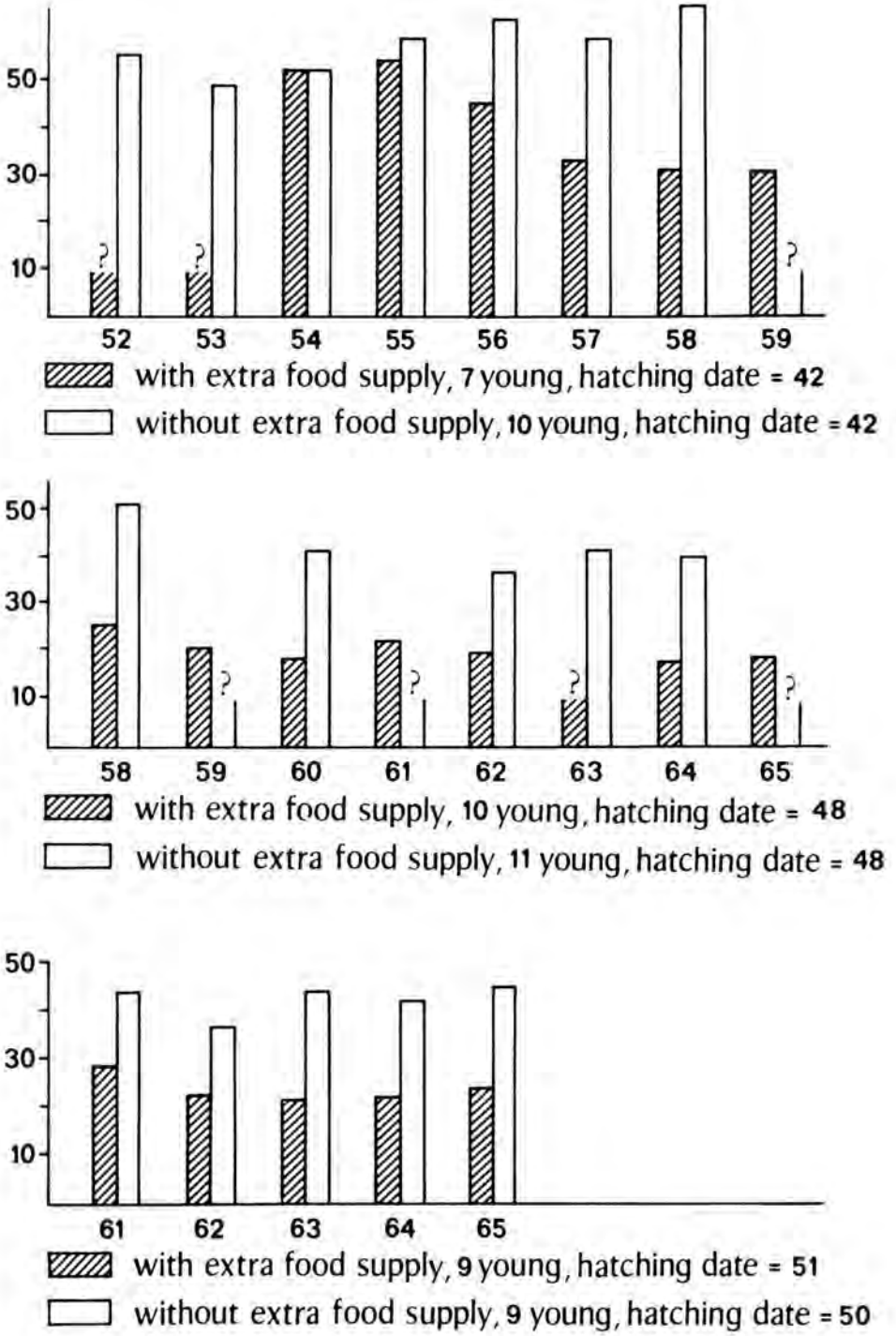

Fig. 4. Effect of supplementary food on the visiting frequency of three breeding pairs of Great Tits (data from 1979). Due to failures of the chronographs, some data (indicated by question marks) are lacking.
Date is given as number of days counted from the first of April (for example, 46 = 16th of May).

of the sex divergence in this respect seems to be that both parents profited equally while rearing the young and that the high weights of the females resulted from the extra food consumed during the incubation period.

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5. Grey Heron survival and winter severity (A.J. Cavé)

INTRODUCTION

The Grey Heron (*Ardea cinerea*) is, as far as the Dutch population is concerned, a short-distance migrant. The great majority do not travel more than a few hundreds of kilometres, although recoveries made more than 4000 km away are on record (Perdeck 1977).

The main food of the Grey Heron - fish and small mammals - is difficult to obtain in periods with ice and snow. This suggests that the winter is a critical part of the year for obtaining food, and on this basis survival rates of the Grey Heron can be expected to vary according to the severity of the winter. North (1979) analysed survival rates, estimated from recovery data, of British Grey Herons in relation to winter temperatures, and found such a relation only for the first-year birds. It seemed interesting to consider the relation between winter severity and survival rates for the Dutch population as well.

MATERIAL

The recoveries of nestlings ringed in The Netherlands between 1957 and 1969 and recovered before June 1st, 1979, were used, and were limited to dead birds killed unintentionally. For annual numbers ringed, use was made of the data of the Dutch Ringing Centre. For the analysis, no initial date was chosen for the first year, i.e., this date differed according to the date of ringing. The second and following years of life were assumed to start on June 1st.

The analyses cover 4,409 nestlings ringed and 428 recoveries of birds found dead.

METHODS

The basic model used in this study to calculate survival rates is the one proposed by Cormack (1970) and reconsidered by Cavé (1977). With this model age-specific survival rates, i.e., survival rates for specific age groups, e.g. first-year and after-first-year birds, can be calculated. This model includes a reporting rate which is assumed to be independent of age and year of recovery. The reporting rate is the proportion reported of the ringed birds dying in a given year.

It is possible to extend this model such that the survival rates can be estimated specifically for both age and year; the reporting rate can be estimated year-specifically. For instance, the first-year survival rate can be estimated for each year of the study (running from one initial date to the next).

This makes it possible to detect relations between year-specific estimates of reporting and survival rates and a particular environmental factor that varies between years, e.g. winter severity. For this purpose it is not necessary to calculate the year-specific estimates. In the age-specific model the reporting rate and the survival rates are estimated as constants. These constants can be replaced by some expression representing the relation between the survival rate or the

reporting rate and the given environmental factor. North (1979) used $[1 + \exp(a+bx)]^{-1}$ for this purpose. In this expression x represents the environmental factor and a and b are parameters to be estimated. For instance, if the first-year survival rate of the age-specific model is replaced by $[1 + \exp(a+bx)]^{-1}$, parameters a and b are estimated instead of the first-year survival rate. This method was employed for the present analysis.

With the age-specific model a first-year survival rate, a second-year survival rate, and so on can be estimated. The survival rate is assumed to be independent of age from some age onward. The minimum number of age groups that must be distinguished to describe the age-dependency in the data is determined by the outcome of a chi-square test applied to the data classified according to age. This test will be called chi-square age. Chi-square tests were also used to compare the annual numbers of recoveries from each cohort (the recoveries of birds ringed in one calendar year) with the numbers expected according to the model. These tests will be called chi-square total. For chi-square tests it is necessary to pool small expectations. Here, 2 was chosen as minimum expectation.

The extent to which one model gives a better description of the data than another was tested by likelihood-ratio tests. For these tests pooling is not needed.

If, in the expression $[1 + \exp(a+bx)]^{-1}$, b is zero, there is no relation between the survival or reporting rate, which this expression represents, and the environmental factor. The probability that b will differ from zero is given by the ratio b/standard error (s.e.). In a model where more than one parameter (reporting rate and survival rates) has been replaced by $[1 + \exp(a+bx)]^{-1}$, the rate having the smallest absolute value of b/s.e. has the weakest relation with factor x. This has been used in a procedure applied to simplify complicated models by assuming this rate to be constant, as will be made clear below.

The analysis was started by choosing the appropriate age-specific model with the use of chi-square age (model I). A chi-square total was used to decide whether the age-specific model is adequate to describe the data. When this was not the case, the reporting rate and the survival rates of the age-specific model were replaced by $[1 + \exp(a+bx)]^{-1}$ (model II). Comparison of the two descriptions of the data (model II versus model I) was based on a likelihood-ratio test. The adequacy of model II was tested with a chi-square total. When the results of the likelihood-ratio test (model II versus model I) was significant, an attempt was made to simplify model II by using b/s.e.. The rate (reporting rate or one of the survival rates) having the smallest b/s.e. was assumed to be constant (model III). Model III was tested against model II with a likelihood-ratio test. The data fit to model III was tested with a chi-square total. When the likelihood-ratio test result was significant, the procedure was not continued and model II considered to be appropriate; otherwise, the procedure was continued.

To simplify notation, no distinction is made in this report between the notation of the true parameter value and its maximum likelihood estimator. The meaning in which a symbol has been used is always clear from the text.

For the Grey Heron, the reporting rate and the survival rates have been related to the severity of the winter, measured as the number of days with freezing temperatures (max. temp. < 0°C; mean of 5 weather stations) in The Netherlands in December, January, and February. This factor is called ICE.

RESULTS

Model I. This is an age-specific model in which ICE is not taken into account, including a reporting rate (REP), a first-year survival rate (SURF), and a survival rate for the after-first-year birds (SURA). The following estimates were obtained:

REP = 0.106 ; s.e. = 0.005

SURF = 0.30 ; s.e. = 0.02

SURA = 0.52 ; s.e. = 0.04

chi-square age : P = 0.572

chi-square total : P = 0.006

Conclusions: chi-square age shows that age-dependency is sufficiently described (a single survival rate is insufficient). The survival rate of the first-year birds

is distinctly lower than the survival rate of the after-first-year birds. The chi-square total shows that the annual recoveries of the separate cohorts are poorly described by the model.

Model II. REP, SURF and SURA are replaced by $[1 + \exp(a+b\ ICE)]^{-1}$. The following estimates were obtained:

REP : a = 2.229; s.e. = 0.103
b = -0.006; s.e. = 0.006; b/s.e. = -1

SURF : a = 0.566; s.e. = 0.223
b = 0.032; s.e. = 0.015; b/s.e. = 2.1

SURA : a = -0.910; s.e. = 0.306
b = 0.058; s.e. = 0.017; b/s.e. = 3.4

likelihood-ratio test model II versus model I: $P < 0.001$

chi-square total : $P = 0.189$

Conclusions: this model gives a better description of the annual recoveries of the separate cohorts than model I does (likelihood-ratio test), and these data are sufficiently described (chi-square total). This result shows that the rates are related to ICE.

Model III. In model II the absolute value of b/s.e. is smallest for REP. Model III is a simplification of model II, made by assuming REP to be a constant. The following estimates were obtained:

REP = 0.105; s.e. = 0.005

SURF : a = 0.445; s.e. = 0.185
b = 0.038; s.e. = 0.013; b/s.e. = 2.9

SURA : a = -1.056; s.e. = 0.276
b = 0.065; s.e. = 0.016; b/s.e. = 4.1

likelihood-ratio test model II versus model III: $P = 0.317$

chi-square total : $P = 0.205$

Conclusions: model III describes the annual recoveries of the separate cohorts as well as model II (likelihood-ratio test) and gives a good fit (chi-square total). This result shows that there is no significant relationship between REP and ICE.

Model IV and final model. A fourth model can be made by assuming both REP and SURF to be constants. A likelihood-ratio test of model III against model IV shows that the latter fits the data less well ($P = 0.002$) and a chi-square total shows that model IV gives an inadequate fit of the data ($P = 0.022$).

From all this it is concluded that model III is the appropriate model and that both SURF and SURA are related to ICE.

Survival rates with minimum and maximum ICE. It is not easy to see from the estimates of model III how SURF and SURA vary with ICE. To visualize this point, SURF and SURA were calculated for the year with the minimum value of ICE (1966-1967: 2.6) and for the year with the maximum value of ICE (1962-1963: 46.6). The estimates were as follows:

ICE min : SURF = 0.37; s.e. = 0.04
SURA = 0.71; s.e. = 0.05

ICE max : SURF = 0.10; s.e. = 0.05
SURA = 0.12; s.e. = 0.06

In severe winters the survival rates for both the first-year and after-first-year birds are low.

DISCUSSION

It has been shown that the survival rates of the first-year and after-first-year birds vary with the severity of the winter, measured by the number of days with freezing temperatures in December, January and February. In hard winters survival rates are low. The reporting rate shows no relationship with winter severity.

Using a similar method of analysis, North (1979) found an effect of winter severity on the survival rate of the first-year birds in Britain. However, he did not estimate a reporting rate, and it cannot be concluded from his results whether the relation he found is correct or is in fact a relation with the reporting rate. In the present study it was found that the reporting rate is unrelated to winter severity, which suggests that North's (1979) conclusion is correct.

North (l.c.) found that in the British Grey Herons only the survival rate of the first-year birds shows a relation with winter severity. In the Dutch birds the survival rates of both the first-year and after-first-year birds are affected by winter severity. This divergence might be due to more severe winters on the Continent as compared with Britain.

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6. Purple Heron survival and drought conditions in the wintering area in tropical West Africa (A.J. Cavé)

INTRODUCTION

The Purple Heron (*Ardea purpurea*) is a long-distance migrant. Moreau (1972) mentions for the wintering area of the West Palearctic birds the semi-arid zone south of the Sahara. Recovery data from tropical Africa suggest that the Dutch Purple Herons winter even further south, i.e., mainly south of 10°N (den Held 1981; van der Kooij 1976).

In the semi-arid zone south of the Sahara (the Sahel area) rainfall is highly variable. Den Held (1981) found a negative correlation between the size of the Dutch Purple Heron colonies and the drought conditions in this area. As a measure of drought conditions, den Held (l.c.) used the sum of the maximum monthly discharges of the rivers Niger and Senegal in the preceding year. This correlation suggests that survival rates of the Purple Heron are related to the drought conditions in this area. To check this point, survival rates were estimated from ringing and recovery data and related to the discharge figures used by den Held (l.c.) as mentioned above.

The results of this study will be published in detail elsewhere, and only the main conclusions will be given here.

MATERIAL

The recoveries of nestlings ringed in 1960-1977 and recovered before May 1st, 1978, were used, and were restricted to dead birds divided into two categories: 'shot' (killed intentionally) and 'found' (killed unintentionally). The annual numbers ringed used in the analysis were those of the Dutch Ringing Centre. No initial date was chosen for the first year, and the initial date for the second and subsequent years of life was May 1st.

The analysis covers 8,489 nestlings ringed, 163 of which were recovered as 'found' and 168 as 'shot'.

METHODS

The methods used for the analysis of survival rates are described in detail in section 5 of this Progress Report, and will only be briefly outlined here.

Age-specific survival rates were obtained from the 'shot' and 'found' birds separately, with Cormack's (1970) method, which also yields a reporting rate. To relate the discharges of the Niger and Senegal to the reporting rate and the survival rates, these were replaced by $[1 + \exp(a+bx)]^{-1}$ as proposed by North (1979). In this expression x represents the discharge figures used by den Held (l.c.) and a and b are parameters to be estimated.

The elimination procedure described in section 5 was used to find the appropriate model.

RESULTS

Before the results of the survival analysis are considered, some remarks must be made about the North-South distribution of the recoveries in the course of the year.

In the first place, a remarkably high proportion of the winter recoveries (December, January, February) come from Europe ('found' 100%; 'shot' 70%), which shows that not all of the birds winter in Africa. Secondly, among the 'shot' birds significantly ($P<0.01$) more recoveries are made south of the Sahara than among the 'found' group, which suggests that the 'shot' birds represent the part of the population that migrates to this area better than the 'found' birds do. Thirdly, among the recoveries of 'shot' birds there is a remarkable difference between the first-year and after-first-year birds as to the time of recovery. Most of the first-year birds were recovered before December 1st, whereas a much smaller proportion of the after-first-years were recovered in that period of the year. This suggests that a considerable part of the first-year mortality occurs before December, i.e., before the birds arrive in the wintering area, which is not the case for the after-first-year birds.

When Cormack's (1970) method is used to estimate age-specific survival rates from the recoveries of 'shot' birds, it is sufficient to distinguish a first-year survival rate, an after-first-year survival rate, and a reporting rate. Consideration of the river discharges in relation to the first-year survival rate, the after-first-year survival rate, and the reporting rate, showed no significant relationship between these discharges and the first-year survival rate, but a significant relation between the discharges and the after-first-year survival rate and the reporting rate. When the discharges are large, the reporting rate and the after-first-year survival rate are large.

When Cormack's (l.c.) model is used to estimate age-specific survival rates from the recoveries of 'found' birds, it again suffices to distinguish a first-year survival rate, an after-first-year survival rate, and a reporting rate. No significant relations were found between the river discharges and these rates.

DISCUSSION

Den Held (1981) found that the size of the Dutch Purple Heron colonies is positively correlated with the discharges of the rivers Niger and Senegal in the preceding year, which serve as a measure of the drought conditions in the wintering area in the winter preceding the breeding season. This drought is thought to affect the survival of the breeding birds in the wintering area. The relation found between the river discharges and the survival rate of the after-first-year birds calculated from the recoveries of 'shot' birds is in line with this view. No relation was found between the river discharges and the after-first-year survival rate calculated from the recovery category 'found'. This can be explained by the poor representation of 'found' birds in tropical Africa.

Den Held's (l.c.) finding that the Dutch Purple Heron colonies fluctuate in relation to drought conditions in the Sahel area implies that a substantial part of the breeding birds spend the winter in that area. Nevertheless, it has been found that a large proportion of the winter recoveries ('shot', later years, 64%) come from Europe. This indicates that a dead bird has a much greater chance of being recovered in Europe than in tropical Africa. When mortality is high in Africa in a given year, a small part of the total mortality in that year occurs in Europe, and when mortality is low in Africa a larger part of that mortality occurs in Europe.

Consequently, a low reporting rate is to be expected in years with high mortality in Africa and a high one in years with low mortality in Africa. Mortality is high in Africa south of the Sahara when the discharges of the Senegal and Niger are low, and vice versa. Consequently, a low reporting rate for the year as a whole is to be expected when the discharges are small and a high reporting rate when the discharges are large. This is exactly what has been found. It is therefore clear that the North-South distribution of the recoveries in winter leads to an overestimation of the part of the population wintering in Europe.

The first-year survival rate calculated from the 'shot' birds is not significantly related to the river discharges. This is an unexpected finding, because the survival rate of the after-first-year birds is related to these discharges and both first-year and after-first-year birds winter in tropical Africa. However, the first-year birds were mainly recovered before December 1st, which is not the case for the after-first-year birds. This suggests that a larger part of the mortality of the first-year birds occurs before December and before the birds arrive in their wintering area, which would explain why the relation between the first-year survival rate and the river discharges is weaker than the relation between the after-first-year survival rate and these discharges.

It is concluded from ringing and recovery data that the survival rate of the Dutch after-first-year birds is related to drought in the Sahel area. This supports the findings of den Held (1981).

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7. The occurrence of *Plantago* species in Dutch plant communities (J. Haeck)

Studies on the relationships between the demographic, physiological and genetic characteristics of plant species and the characteristics of their habitat require knowledge of these habitats. Of course, for the *Plantago* species we already had a qualitative impression, and a detailed description of various habitat characteristics is now being prepared (see, for instance, the contributions of Stoutjesdijk and Troelstra in this report). But a broad, more quantitative survey of the incidence of the *Plantago* species in different habitats or types of vegetation is lacking in the Dutch literature (as is the case for virtually all other species). Therefore, we did some field observations (in connection with measurements on soil properties and microclimate made by Troelstra and Stoutjesdijk, respectively), and attempted to supplement this information with data from the literature.

In the Survey of Dutch Plant communities (Westhoff and den Held 1969) the occurrence of 'character species and differential species' is mentioned for each syntaxonomic entity. Thus, this book provides a first impression of the ecological amplitude of many plant species in The Netherlands, but no mention is made of more or less constant 'companions' and no quantitative information is given.

To obtain such data we had to go back to the original phytosociological literature, i.e., the tables giving the description of the various communities. For this purpose we used an existing collection of tables at the Department of Geobotany of the University of Nijmegen (see van der Maarel 1979) as well as many other sources. Our collection comprises about 180 of the 280 associations described in Westhoff and den Held (1969). The total number of coena amounted to 350, because for many associations the data for various entities of lower syntaxonomical rank are given, or tables prepared by various authors for different regions could

be used. Moreover, we included tables for several coena not described in the book of Westhoff and den Held.

For each coenon we noted for each species the percentage of relevés in which it was present. For the first and simplest quantification we used only the presence in a relevé, and no consideration was given to abundance values. The mean number of relevés per coenon is 26.1, and coena with less than 10 relevés were not used.

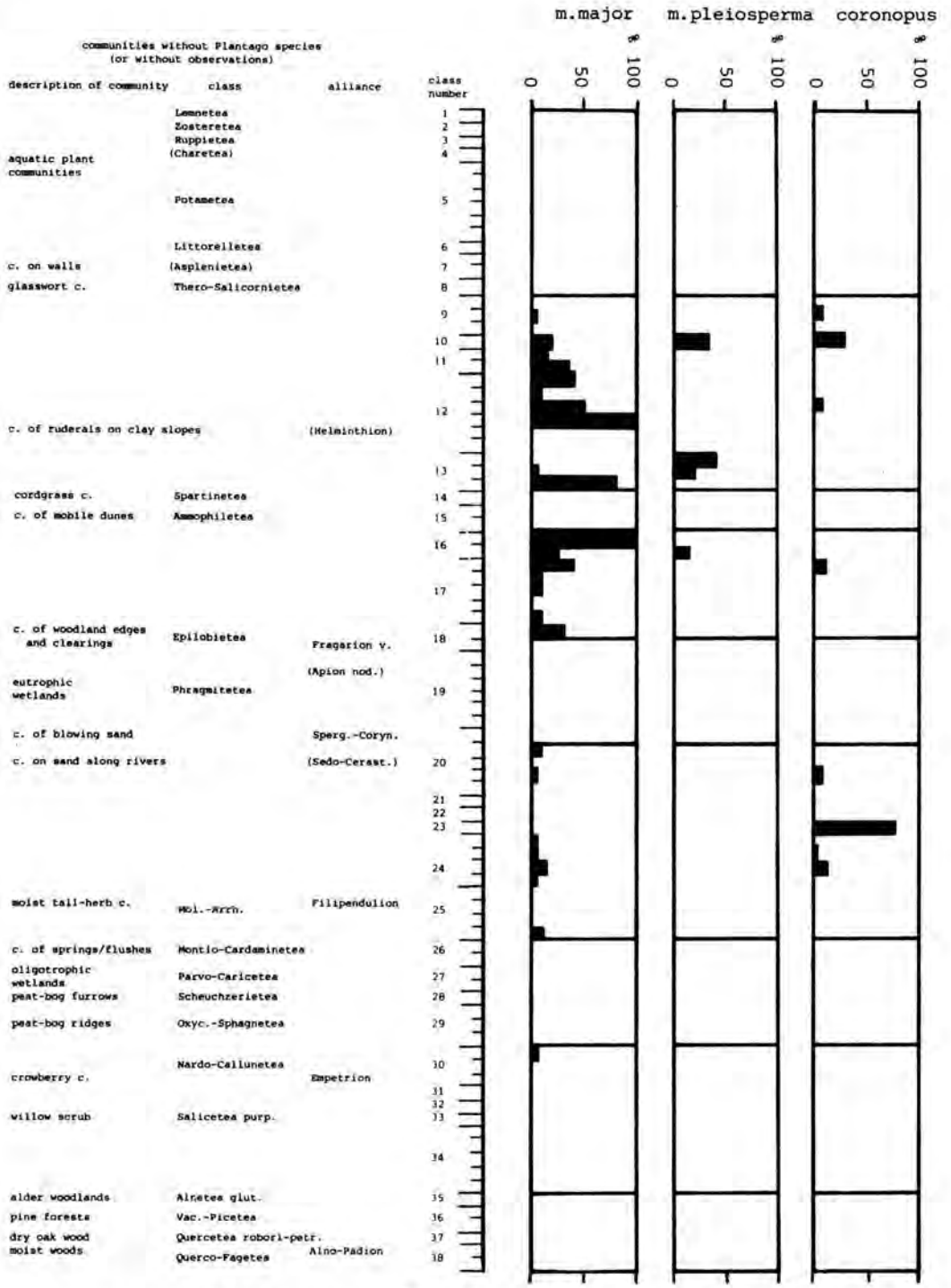

Fig. 7. Mean per cent occurrence of *Plantago* species in vegetation units at the alliance level in The Netherlands.

In this way we obtained a (semi)quantitative survey of the occurrence for most plant species over the total range of communities. As an example, the data for the *Plantago* species are summarized, in Fig. 7, which shows the mean presence value per alliance. The classes are numbered and arranged according to Westhoff and den Held (1969). On the left side of this figure the syntaxa without *Plantago* species are mentioned (and briefly characterized), on the right side the syntaxa with *Plantago* species present.

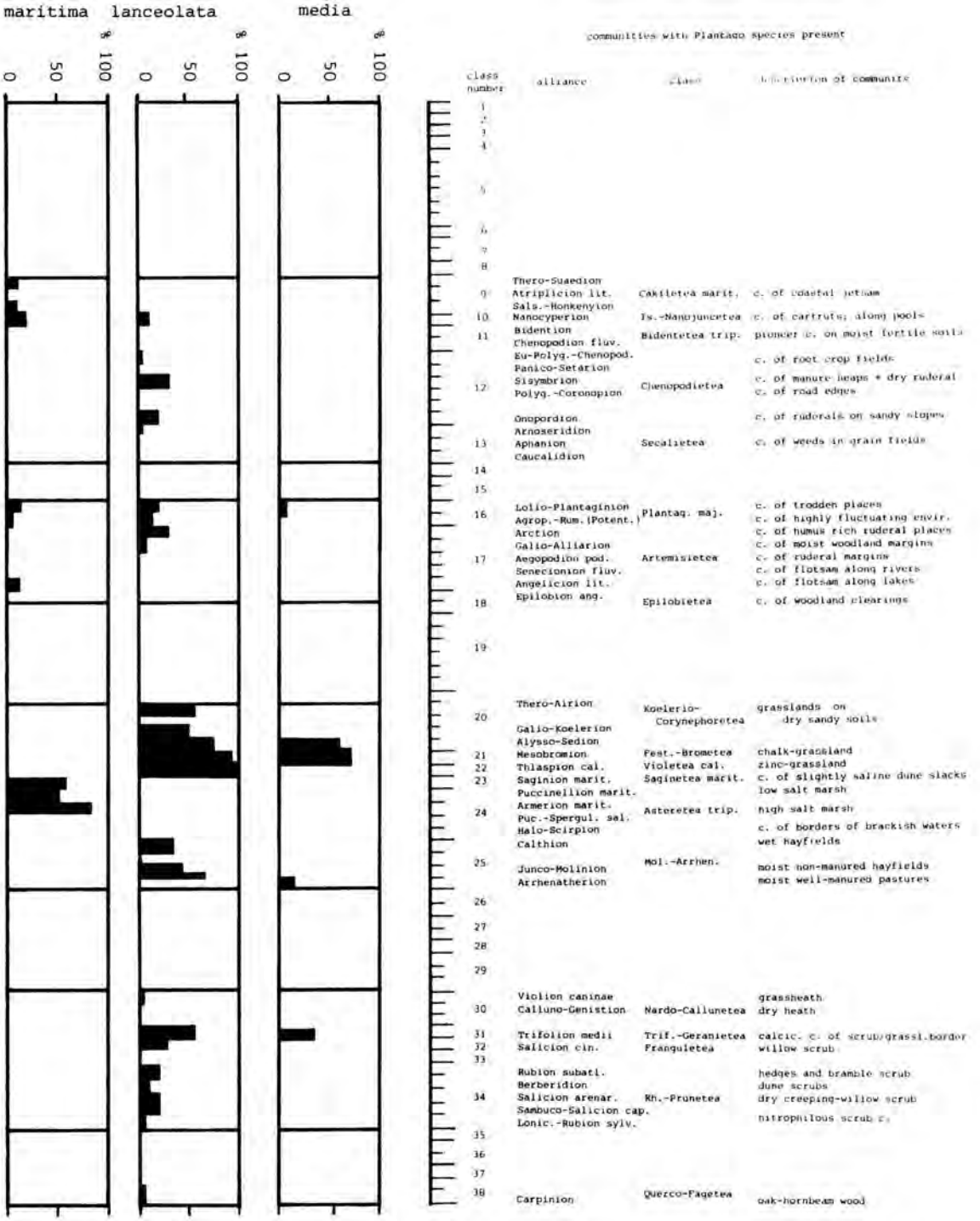

Fig. 7. (continued)

We can see from this figure that *Plantago media* occurs in only a very few communities, i.e., the *Mesobromion* (comprising two associations: those of the chalk grassland of the southern part of the province of Limburg and a type of grassland found on dunes along the major rivers), the borders of such communities adjacent to woods: *Trifolion medii*, and in two subassociations of the *Arrhenatherion* (not too heavily manured grassland near or on the dikes along rivers).

P. lanceolata is found in a wide variety of plant communities ranging from more or less ruderal vegetations to shrub and wood. According to Westhoff and den Held, *P. lanceolata* is a character species of the class *Molinio-Arrhenatheretea*, found on wet or moist not too heavily manured meadows and hayfields, but in our figure we can see that this species is even more common in other types of grassland on dry sandy and calcareous soils.

Of the two *Plantago* species of salt-plant communities, *P. coronopus* clearly has its optimum on the higher-lying and *P. maritima* on the lower-lying salt marshes, but both species are also found in some contact and disturbance communities near the sea and *P. coronopus* also on (not too dense) grassland and in a particular very open ephemeral inland community.

The two subspecies of *P. major* are commonly not distinguished in the phytosociological literature, but it is certain that *P. major* ssp. *pleiosperma* does not occur in the heavily trodden communities of the *Polygono-Coronopion* and the *Lolio-Plantaginion* where *P. major* ssp. *major* has its optimum, *P. m. pleiosperma* is found in ephemeral vegetations along the shores of our large rivers or on moist small paths.

From Fig. 7 we can see the difficulties posed by a one-dimensional arrangement (based roughly on an increase in the complexity of the vegetation structure, i.e., from open water and pioneer communities to woodland). We see, for instance, how difficult it is to place the fresh water or salt-marsh communities along this line. In fact, of course, the communities should be ordered in a multidimensional pattern. For our material this is possible, and this procedure is now being applied in collaboration with the Department of Geobotany at Nijmegen. We hope to be able, after classification and ordination of the communities, to show for many species - not only those of *Plantago* - the performances along various factor axes.

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8. *Plantago lanceolata* and its habitat: Micrometeorological aspects (P. Stoutjesdijk)

Plantago lanceolata is found in a wide range of habitats (see Haeck, this report, section 7) corresponding with the range of the micrometeorological conditions in the vegetations in which it occurs. A synoptic representation of the differences in this respect between the various types of grassland in which *P. lanceolata* occurs is given in Fig. 8. In this diagram the temperature and humidity conditions at a height of 1 cm in the vegetation can be compared with those at a height of 1 m. All measurements were done under comparable conditions, i.e., strong solar radiation (600-900 W/m^2), in the months from May to August.

The diagram shows how the temperature and humidity in the vegetation are related to the height and density of the vegetation. The density is characterized by the percentage of solar radiation that is transmitted down to the level of 1 cm. This radiation was measured with small Silicium cells with a diameter of 1 cm and a peak sensitivity at 700 mm. These cells proved to give a good estimate of the thermally effective radiation in situations where a truly caloric instrument like the Kipp solarimeter was too bulky to use. Especially where transmission is low, say less than 5 per cent, the percentage of photosynthetically active radiation that is transmitted is much lower, because absorption is selective for these wavelengths. Comparative measurements showed that a reduction factor of 3-4 would be appropriate. For the same reason there must be a high ratio far red/red radiation when

transmission is low, which is of interest in relation to seed germination (Stoutjesdijk 1972).

The diagram gives a good impression of the conditions under which seedlings of *P. lanceolata* live, and also, but to a lesser extent, of those for the full-grown plants. Full-grown leaves extend mainly above the level where the differences between vegetations are the largest. This means that in a dense, high vegetation the environment of the leaf is a few degrees warmer and the humidity lower than the diagram indicates. In a warm, dry, open vegetation both the air temperature round the leaf and the saturation deficit are lower than at the 1 cm level. The actual temperature reached by the leaf itself also depends on the amount of sunshine it receives, on the thermal radiation from the surroundings, which is much higher in dry open vegetations with high surface temperatures than in denser grassland, and on the transpiration intensity of the leaf.

The leaves of *P. lanceolata* are capable of strong transpiration. From measurements of leaf temperature and energy considerations it is possible to characterize the transpiration power capacity of the leaf relative to that of a free water surface and independent of the physical conditions in the environment (Stoutjesdijk 1970). The water loss of *P. lanceolata* leaves can be one-fifth to one-half of that of a free water surface of the same temperature and dimensions and under the same conditions. Comparison of these values with those obtained for a wide range of species showed that *P. lanceolata* belongs to those species with the strongest capacity for transpiration. That the leaves are amphistomatous is of importance in this connection.

In a closed grassland vegetation, leaves exposed perpendicular to the direct solar radiation had temperatures that were 1-5°C above the air temperature close to the leaf. Compared with the air temperature at a height of 1 m, the temperature excess amounted 5-7°C. The highest excess temperature measured so far on *P. lanceolata* leaves was 14°C above the air temperature at a height of 1 m. In the centre of rosette the excess temperature was up to 15°C. Of course, there can be

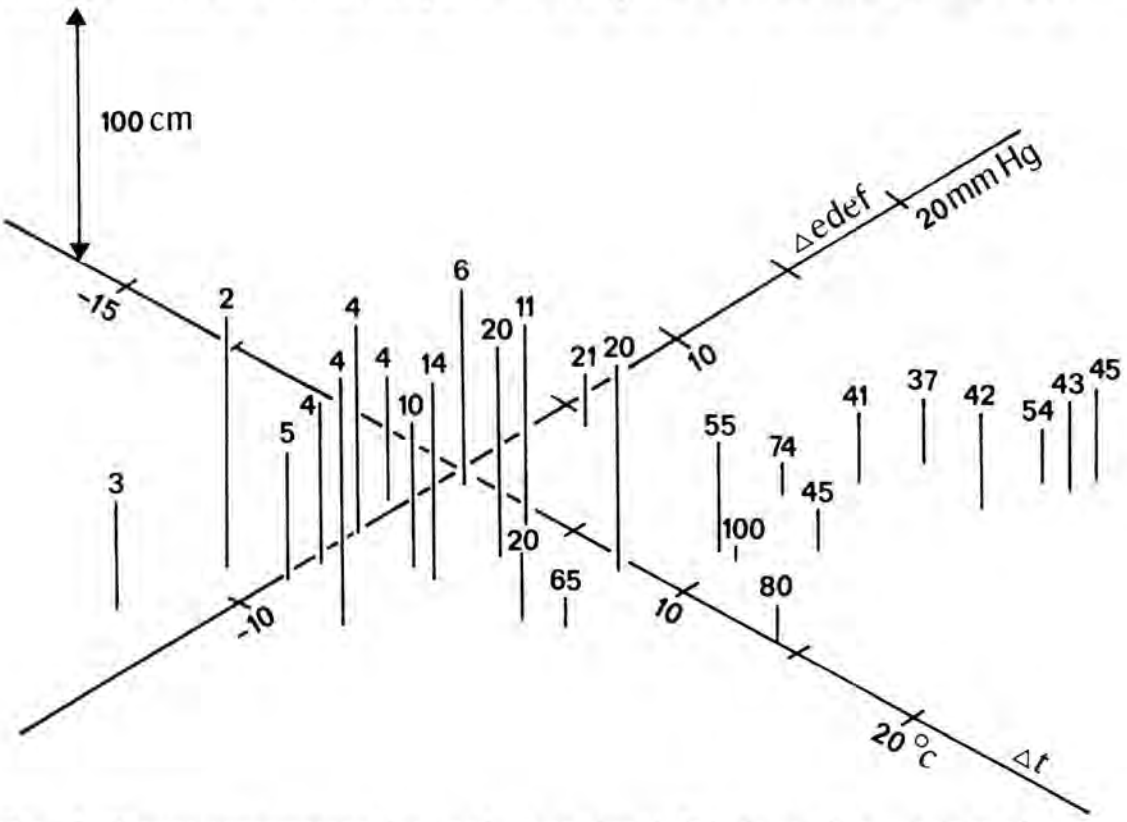

Fig. 8. Excess temperature (Δt) and saturation deficit (Δe_{def}) at a height of 1 cm in grasslands where *P. lanceolata* occurs. The lengths of the bars indicate the height of the vegetation and the numbers at the top of each bar the percentage of radiation transmitted.

considerable differences between the leaves of one rosette, because leaves in a position parallel to the direction of solar radiation have temperatures that are only slightly above ambient temperature.

The stability or degree of instability of the habitat conditions deserves attention, but as yet we have little information on the biological importance of this factor.

In the dry-warm habitat there is strong fluctuation of the air temperature at the 1 cm level, even when the flux of solar radiation is stable. This is due to the to the nature of the convection process. Strong overheating of the ground surface or of the lower air layers in close contact with it created an unstable situation. Parcels of hot air rise continuously and are replaced by cold air from the surroundings. This can result in extremely rapid temperature fluctuations, measured on a time scale of seconds. Up to a height of 10 cm temperature changes over a range of 5-10°C can occur in a few seconds. Leaves, which have a rather high thermal inertia, cannot follow these rapid temperature fluctuations, but anthers certainly can.

Fluctuations on a larger time scale, e.g., half an hour, may occur under partially cloudy weather when clouds obscure the sun. This can result in temperature changes of more than 10°C in both plants and the air temperature near the soil. These temperature changes are coupled with humidity changes, since a drop in temperature is accompanied by a lowering of the saturation deficit. The intensity of these fluctuations is related to the magnitude of ΔT in the diagram, because when the sun is obscured, temperatures near the surface fall to a level which is close to the air temperature at a height of 1 m.

There are also the diurnal variations, and these too are greater where ΔT is higher. This applies to both temperature and humidity variations.

Over still longer periods there is, furthermore, a difference between the changes that occur in various types of vegetation, for instance during a period of drought.

In a dense high vegetation the stability is high, not only for humidity, which stays relatively close to the saturation point, but also for the temperature, i.e., the temperature inside the vegetation differs by only a few degrees from the air temperature at a height of 1 m. This situation seems to change radically only after a prolonged period of drought.

The state of the soil surface proved to be decisive for the microclimate above it, and in this respect there is a difference between high lush vegetations and open sparse vegetations. The former use much more water, but this water is extracted rather evenly from the whole root zone, and the soil surface, being protected from direct solar radiation, dries out slowly. In an open sparse vegetation water loss from the surface is rather rapid and once the surface has become dry, little water vapour passes through it from lower strata and the microclimate above it is dry-warm regardless of the water content of the soil below. This situation can occur even when the soil is water-logged if it is covered with a dry moss or litter layer. During the drying process there is a change from a warm-humid to a warmer dry microclimate near the surface.

With a very thin vegetation on a soil with a low water-holding capacity this change is very rapid, taking about a day, but is slower where the soil has a higher water-holding capacity and the vegetation is less sparse.

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9. On the soil chemistry of natural habitats of *Plantago* species and *Hypochaeris radicata* in various parts of The Netherlands in relation to the chemical composition of the plants (S.R. Troelstra, L. Sluimer, W. Smant, R. Wagenaar, M.A. van der Meulen)

INTRODUCTION

Very few comparative data on the nutrient status of natural habitats of different

Plantago species are available (e.g., Kruijne *et al.* 1967). Since opinions on the differences within the group of *Plantago* species with respect to the soil-chemical environment and such related factors as nutrient availability are often largely a matter of either common knowledge (e.g., the occurrence of *P. media* and *P. maritima* in chalk grasslands and saline habitats, respectively) or hypotheses without substantial support (e.g., the postulated occurrence of *P. major* in 'nutrient-rich' and 'stable' environments), the current study was undertaken as an initial attempt to provide investigators of *Plantago* species working in different research areas (e.g., plant physiology, plant demography, plant genetics, and plant microorganism relationships) with relevant data pertaining to the natural habitats. Moreover, the present study has the advantage that the analyses were performed on all samples at the same time and with the same techniques, which means that the data set is highly suitable for purposes of comparison.

MATERIALS AND METHODS

In the May-June period in 1979, a soil- and plant-sampling program was carried out in various parts of The Netherlands, covering a total of 115 natural habitats, primarily of *Plantago* species. In general, three successive 10 cm soil-depth increments were sampled, giving a total of 310 soil samples; i.e., 115, 108, and 87 for the soil depths of 0-10 cm, 10-20 cm, and 20-30 cm, respectively.

After drying (*ca.* 35°C) and sieving (2 mm), bulk samples were mechanically subdivided (Retsch subsampler type PTZ). Part of the sample was ground in a mortar mill (Retsch type RMO), and analyses were performed on either ground or unground samples, as indicated.

The pH of the soil was measured potentiometrically in 1:2½ (w/v) suspensions of unground soil in H_2O or 1 *N* KCl (pH-H_2O and pH-KCl). Carbonates were measured according to Scheibler by treating ground soil with 4 *N* HCl. Organic matter was determined as loss-on-ignition, i.e., weight loss of ground soil samples after ignition at 430°C for 24 h (Davies 1974).

Total P was measured by digesting ground soil samples with Fleischmann's acid (Houba *et al.* 1974). Organic P was determined by an ignition method applied to ground soil samples (Saunders and Williams 1955). Olsen-P and labile organic P were determined after shaking unground soil with 0.5 *M* $NaHCO_3$ (Olsen *et al.* 1954; Watanabe and Olsen 1965; Bowman and Cole 1978). Total N was measured in digests of ground soil samples either by a distillation/titration method (Houba *et al.* 1974) or with an ammonia electrode Orion 95-10 (e.g., Bremner and Tabatabai 1972).

Exchangeable cations were determined by atomic absorption spectrophotometry after shaking unground soil with neutral ammonium acetate. Cation-exchange capacities (CECs) were measured in non-calcareous soils only by a percolation procedure applied to unground samples (Houba *et al.* 1974). Chloride and electrical-conductivity analyses were carried out on 1:5 water extracts of unground soil.

The granular composition (soil texture) of the samples was determined by dry sieving (fractions > 53µ; Retsch Vibro sieve equipment) and a pipette method (fractions < 53µ; Houba *et al.* 1974).

To obtain an impression of the mineralization potential and the levels of the respective forms of mineralized N (NO_3 and/or NH_4) soil material was aerobically incubated in polypropylene centrifuge tubes in the laboratory for three successive 2-week periods at 30°C and 10-45% moisture (soil dry weight), the latter depending on soil texture and organic matter content. Clay and peat samples were mixed with ignited and acid-washed quartz sand. After incubation, extraction was performed with 1 *N* KCl, and ammonium and nitrate were determined separately with the steam distillation method (Bremner 1965), using MgO and Devarda alloy. Ammonium was determined in the distillate either by titration with 0.01 *N* potassium bi-iodate (high concentrations) or by the very sensitive indophenol-blue method (low concentrations; Kempers 1974). Nitrogen concentrations were calculated as ppm N (soil dry weight).

As a first approximation of comparison on a soil-volume basis instead of a soil-weight basis, some values obtained on a weight basis were transformed into volume-basis values with the use of bulk density estimates for the pretreated soil material. Comparison of values on a weight basis may give a rather distorted picture (e.g., the occurrence of *P. lanceolata* on peaty locations).

RESULTS AND DISCUSSION

For the sake of brevity, only the results obtained for the surface soil layers (0-10 cm) will be discussed here.

Table 9.1 summarizes the observed ranges (weight basis) for several soil properties of the natural habitats of *Plantago* species and *Hypochaeris radicata*; Table 9.2 shows mean values (volume basis where relevant) only for the same properties. It is evident that there is a considerable degree of overlapping except for very low pH values (*P. lanceolata, H. radicata*), very high $CaCO_3$ contents (*P. media, P. lanceolata*), very high organic-matter or total-N contents (*P. lanceolata*), and (very) high chloride levels and electrical conductivities (*P. maritima, P. coronopus*).

Table 9.3 summarizes the preliminary results of the textural analyses (grain-size distribution). Here too there is a high degree of overlapping except for relatively coarse (*H. radicata*) and relatively fine textures (*P. maritima*).

In general, the results are in line with data reported by Kruijne *et al.* (1967) for the 0-5 cm soil layer of (semi-)natural habitats of three *Plantago* species and *Hypochaeris radicata* (Table 9.4).

The most important differences between habitats of the various *Plantago* species concern the pH and chloride content (salinity). In the field *P. lanceolata* occurs over a wide range of pH values but more often at relatively low than at high pH values. This wide pH range together with the wide variety of textural classes is indicative of the high ecological flexibility of this species.

Habitats of *P. media* and *P. major* generally have relatively high pH values, which might be related to the higher organic-anion content (C-A) of these species (Fig. 9; see also Troelstra and Smant 1979, 1980). After incubation in the laboratory at 30°C and 10-45% moisture, the accumulated mineral N in soils from natural habitats of *P. media* and *P. major* was predominantly in the nitrate form(Tables 9.5 and 9.6). It was therefore postulated that the higher organic-anion levels in *P. media* and *P. major* reflect an adaptation to the natural environments of these species (i.e., nitrogen becomes more available in the nitrate form), which might mean a more or less obligatory preference of these species for nitrate-nitrogen. Waterculture experiments are in progress to test this hypothesis, but results of preliminary experiments have already suggested that *P. media* and *P. major* do not require a specific C-A level or N source (NH_4/NO_3) for normal growth (Troelstra *et al.* 1981).

P. coronopus and *P. maritima* also occur frequently at relatively high pH values, but here salinity leads to relatively low C-A levels. The chloride contents mentioned in Table 9.2 correspond with NaCl concentrations in the soil solution (at 25 vol. % moisture) of *ca.* 200 m*M* and 25 m*M* for natural habitats of *P. maritima* and *P. coronopus*, respectively. In the case of *P. maritima*, uptake of ammonium-nitrogen may also be a factor involved in the relatively low organic-anion level of this species (Tables 9.5 and 9.6).

The frequently encountered statement that *P. major* is a species of nutrient-rich habitats does not hold from a purely soil-chemical point of view. Mean values for soil-chemical properties of habitats of *P. major* are in most cases slightly higher, which is due mainly to the rather incidental occurrence of some extreme values. As to the natural habitats of *P. major*, it is probably important to recognize that the occurrence of this species tends to be more solitary and on more compacted sites than is the case for the other *Plantago* species. It must be kept in mind that 'nutrient availability' is a rather complex function representing factors related to soil chemistry, soil physics (transport of nutrients), competition, plant species (rooting pattern, presence of root hairs), and so on.

Hypochaeris radicata is a species of habitats with low levels of nutrients and a relatively low soil pH.

Besides the range of values, classification of values is also important from an ecological point of view. These classifications are given in Tables 9.7 (bulk density), 9.8 (pH, % $CaCO_3$), 9.9 (organic matter, total N, exchangeable K), and 9.10 (total P, total organic P, Olsen-P, labile organic P, total $NaHCO_3$-P). These tables show some of the above-mentioned aspects in more detail.

Some linear correlation coefficients of plant and soil parameters are presented in Table 9.11; since in most instances the relationships will not be linear, these coefficients must be seen as rather tentative. *P. maritima* has been omitted from

Table 9.1. Ranges of some selected properties of the 0-10 cm soil layer of natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species	pH-H_2O	pH-KCl	% $CaCO_3$	% org. matter	chloride (mg/100g)	conductivity 1:5 extract ($\mu S\ cm^{-1}$)
P. media (15)*	5.8-7.8	5.2-7.5	0-52.6	2.75-12.6	0- 3.6	55- 285
P. major (17)	5.3-8.4	4.4-8.0	0- 9.9	0.94-11.3	0- 4.5	46- 310
P. lanceolata (72)	4.3-7.8	3.7-7.5	0-52.6	1.25-74.0	0- 9.6	16- 428
P. coronopus (4)	5.1-8.4	4.2-7.4	0- 4.7	4.28-12.9	0-106	58-1044
P. maritima (2)	7.8	7.2-7.3	4.7- 9.9	12.9 -14.2	106-353	1044-2535
H. radicata (8)	4.5-5.9	3.7-4.9	0	1.39-5.67	0- 0.4	29- 116

	$\mu g\ g^{-1}$				
	total N	total P	total org. P	Olsen-P	labile org. P
P. media (15)	750- 3950	233- 796	68- 311	4.0- 38	4.2-22
P. major (17)	280- 4380	229-2184	49- 344	3.1-110	3.5-39
P. lanceolata (72)	350-23590	105-1367	20-1115	2.8- 44	3.7-70
P. coronopus (4)	1470- 4460	193- 862	130- 326	4.1- 32	11-23
P. maritima (2)	4460- 4970	862-1099	326- 524	32- 62	19-34
H. radicata (8)	390- 1760	73- 517	31- 242	2.2- 38	6.5-41

	meq/100g				
	K	Na	Ca**	Mg	CEC** (NH_4OAc pH7)
P. media (15)	0.12-0.78	0.01-0.89	5.31-24.2	0.43-1.76	6.19-21.1
P. major (17)	0.08-1.81	0.02-0.47	1.82-10.2	0.20-2.88	3.58-11.7
P. lanceolata (72)	0.03-0.81	0.01-0.90	0.73-49.7	0.10-5.18	0.97-52.9
P. coronopus (4)	0.11-1.59	0.10-22.7	1.50	0.18-10.3	4.83
P. maritima (2)	1.59-1.82	22.6 -22.7	-	10.3 -12.7	-
H. radicata (8)	0.08-0.22	0.01-0.21	0.39-6.35	0.10-0.60	1.46- 9.23

* number of samples analysed
** calcareous soils not included

Table 9.2. Some selected soil properties (mean values) of the 0-10 cm soil layer of natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species	pH-H_2O	pH-KCl	% $CaCO_3$	% org. matter	chloride (mg/100 cc)	conductivity 1:5 extract ($\mu S\ cm^{-1}$)
P. media (15)*	7.1	6.6	8.8	6.7	1.4	155
P. major (17)	6.9	6.2	2.9	5.0	0.9	134
P. lanceolata (72)	6.1	5.4	2.3	11.3	0.9	130
P. coronopus (4)	7.1	6.5	1.9	7.0	25	360
P. maritima (2)	7.8	7.3	7.3	13.5	212	1790
H. radicata (8)	5.1	4.2	0	4.2	0.1	55

	$\mu g\ cc^{-1}$				
	total N	total P	total org. P	Olsen-P	labile org. P
P. media (15)	2720	528	230	10	15
P. major (17)	2220	837	229	49	28
P. lanceolata (72)	3480	506	276	13	24
P. coronopus (4)	2720	497	210	16	20
P. maritima (2)	4360	908	393	44	25
H. radicata (8)	1770	345	196	15	33

	meq/100 cc				
	K	Na	Ca**	Mg	CEC** (NH_4OAc pH7)
P. media (15)	0.41	0.13	12.1	1.23	15.6
P. major (17)	0.49	0.16	4.73	1.42	7.40
P. lanceolata (72)	0.28	0.17	10.3	1.30	13.1
P. coronopus (4)	0.58	5.77	1.86	3.67	6.00
P. maritima (2)	1.58	21.0	-	10.7	-
H. radicata (8)	0.17	0.08	2.51	0.33	6.17

* number of samples analysed
** calcareous soils not included

Table 9.3. $CaCO_3$, organic matter, and grain-size distribution for soils (0-10 cm layer) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species	% (105°C basis)						
	$CaCO_3$*	organic* matter	>53µ **	16-53µ **	2-16µ **	<2µ **	clay-humus** factor
P. media	9.0	6.7	5-91(53)	1-37(13)	1-16(8)	1-18(10)	5-20(11)
P. major	2.3	5.0	13-93(53)	1-56(18)	1-27(10)	1-34(10)	2-37(12)
P. lanceolata	2.3	11.3	0-95(58)	1-58(11)	0-31(8)	0-54(10)	2-77(16)
P. coronopus	1.9	7.0	38-90(72)	1-17(8)	1-12(5)	1-15(6)	6-20(10)
P. maritima	7.5	13.5	12-38(25)	17-22(19)	12-18(15)	15-23(19)	20-25(22)
H. radicata	0	4.2	47-97(81)	1-22(7)	1-15(4)	1-15(4)	2-12(6)

* mean
** range (mean)
clay-humus factor = % humus + ¼ of fraction <16µ

Table 9.4. More or less optimal values for some soil properties (0-5 cm depth) of grassland habitats of three *Plantago* species and *Hypochaeris radicata*, as reported by Kruyne *et al.* (1967). To facilitate comparison, data for P and K have been transformed into the units used in the present study

species	pH-H_2O	P-citric acid ($\mu g\ g^{-1}$)	K- value (meq/100 g)	clay-humus factor*
P. media	>7.00	0- 140	0.31- 0.60	11- 20
P. major	6.05- 7.00	>150	>0.60	<20
P. lanceolata	5.05->7.00 (no distinct preference)	< 80(140)	<0.31(0.42)	<40 and >60
H. radicata	<5.05	< 80	<0.31 (still also at relatively high values)	<20 and >60

* clay-humus factor = $\frac{2}{3}$ humus + $\frac{1}{4}$ of fraction <16μ

this table (only two locations investigated) and the data set for *P. coronopus* is rather small.

In general, expression of the data on a soil-volume instead of a soil-weight basis leads to only a minor improvement of the correlations. Plant-P contents show fairly good correlation with Olsen-P values, which is a rather remarkable finding for these widely divergent locations. Inclusion of the labile organic P pool generally gives rise to only a slight increase of the correlation coefficient. This might

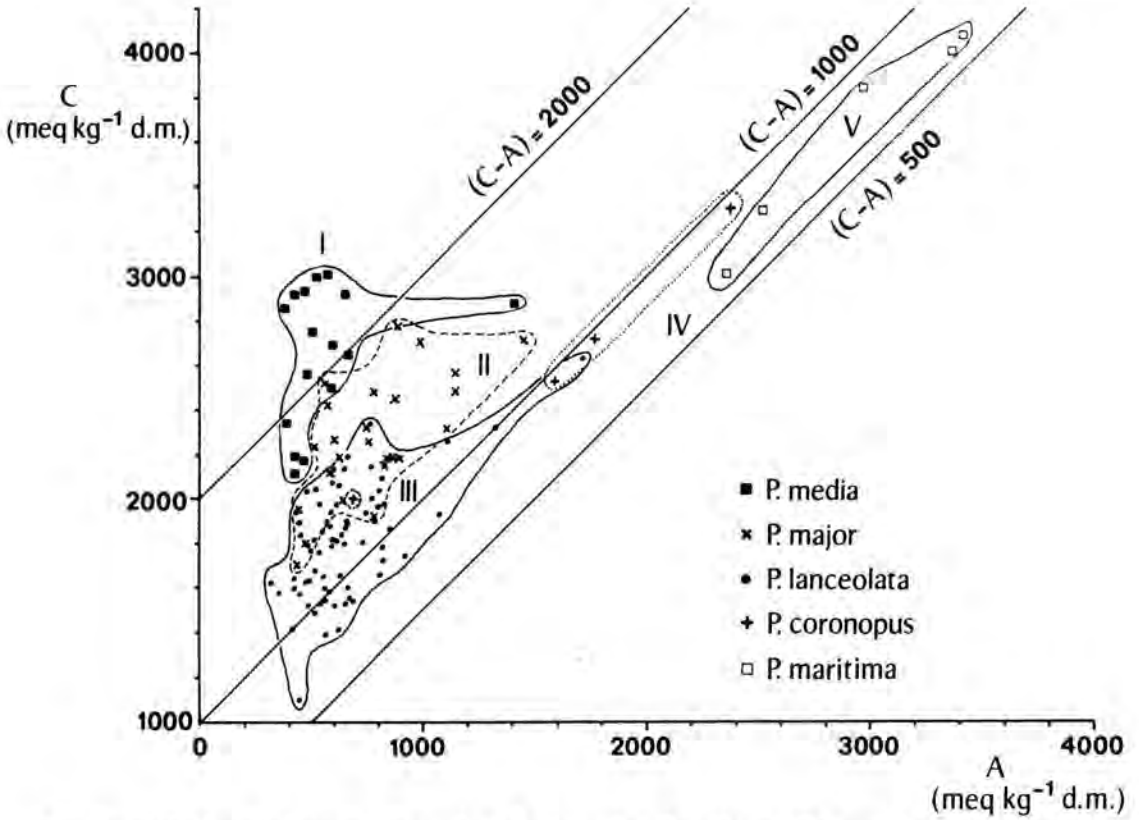

Fig. 9. Relation between accumulated amounts of cations (C) and inorganic anions (A) in field samples of *Plantago* species collected in different locations in The Netherlands.

Table 9.5. Accumulation of mineral N in soils (0-10 cm layer) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands, during incubation at 30°C and 10-45% moisture (soil dry weight), the latter depending on soil type.

species	µg cc^{-1}							
	NH_4-N				NO_3-N			
	period of incubation (weeks)				period of incubation (weeks)			
	0	2	4	6	0	2	4	6
P. media	6-23 (12)*	0-167 (28)	0-102 (15)	0- 49 (6)	0.6-28 (7)	24-198 (82)	49-223 (116)	75-238 (140)
P. major	3-23 (13)	0-143 (25)	0-154 (20)	0-170 (16)	0.7-52 (8)	2-154 (64)	4-252 (100)	11-268 (121)
P. lanceolata	5-43 (14)	0-569 (88)	0-628 (100)	0-626 (99)	0.2-53 (7)	0.6-198 (44)	0-223 (68)	0-238 (88)
P. coronopus	8-18 (13)	0- 66 (25)	0- 53 (20)	0- 41 (11)	0.9- 3 (2)	10- 96 (50)	28-152 (93)	59-196 (128)
P. maritima	18-25 (21)	66-197 (131)	28-174 (101)	4-136 (70)	2- 3 (3)	6- 19 (12)	16- 70 (43)	74-113 (93)
H. radicata	9-43 (19)	9-133 (53)	13-140 (63)	2-123 (58)	0.9- 2(0.9-53)** (2) (8)**	2- 49 (17)	0.6- 84 (29)	0-106 (47)

* mean

** if one extreme value is included

Table 9.6. Nitrification-degree classes (per cent NO_3 in total N mineralization) of Ellenberg (1977) applied to net-production data of incubation experiments in the laboratory (0 < 5%, I 5-25%, II 25-50%, III 50-75%, IV 75-90%, V > 90%)

species	nitrification degree		
	period of incubation (weeks)		
	2	4	6
P. media	IV	V	V
P. major	III	III	IV
P. lanceolata	II	II	II
P. coronopus	IV	V	V
P. maritima	I	II	III
H. radicata	I	II	III

Table 9.7. Bulk-density classes of soils (0-10 cm layer; dried at *ca.* 35°C and 2 mm-sieved) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species		g cc^{-1}			
		< 0.75	0.75-1.00	1.00-1.30	> 1.30
P. media	(15)*	-	1	13	1
P. major	(17)	-	1	9	7
P. lanceolata	(72)	6	5	49	12
P. coronopus	(4)	-	1	3	-
P. maritima	(2)	-	2	-	-
H. radicata	(8)	-	-	4	4

* number of samples

Table 9.8. pH and carbonate classes of soils (0-10 cm layer) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species		pH-H_2O				
		< 5.00	5.00-5.50	5.50-6.00	6.00-7.00	> 7.00
P. media	(15)*	-	-	1	4	10
P. major	(17)	-	4	2	1	10
P. lanceolata	(72)	8	22	8	15	19
P. coronopus	(4)	-	1	-	-	3
P. maritima	(2)	-	-	-	-	2
H. radicata	(8)	2	5	1	-	-

		pH-KCl						
		<4.00	4.00-4.50	4.50-5.00	5.00-5.50	5.50-6.00	6.00-7.00	>7.00
P. media	(15)*	-	-	-	2	3	2	8
P. major	(17)	-	5	1	-	1	3	7
P. lanceolata	(72)	5	20	11	5	10	6	15
P. coronopus	(4)	-	1	-	-	-	-	3
P. maritima	(2)	-	-	-	-	-	-	2
H. radicata	(8)	2	5	1	-	-	-	-

	% $CaCO_3$						
	< 0.1	0.1-0.5	0.5-1.0	1-5	5-10	10-20	> 20
P. media (15)*	5	1	1	5	-	-	3
P. major (17)	7	-	-	6	4	-	-
P. lanceolata (72)	52	2	3	10	2	-	3
P. coronopus (4)	1	-	1	2	-	-	-
P. maritima (2)	-	-	-	1	1	-	-
H. radicata (8)	8	-	-	-	-	-	-

* number of samples analysed

mean that the Olsen-P value already includes some of the 'most labile' organic P (hydrolysis during the period of extraction), which would make this method particularly suitable for the investigation of the P status of more natural environments. Only in habitats of *H. radicata* (low nutrient level, low pH) did total P, total organic P, and total $NaHCO_3$-P show better correlation with plant-P.

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Table 9.9. Organic matter, total nitrogen, and exchangeable-potassium classes of soils (0-10 cm layer) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species	% organic matter (loss-on-ignition)						
	< 2.5	2.5-5	5-10	10-15	15-20	20-30	> 30
P. media (15)*	-	5	8	2	-	-	-
P. major (17)	1	10	4	2	-	-	-
P. lanceolata (72)	3	23	30	5	4	1	6
P. coronopus (4)	-	2	1	1	-	-	-
P. maritima (2)	-	-	-	2	-	-	-
H. radicata (8)	2	4	2	-	-	-	-

	total N (g cc^{-1})						
	< 500	500-1000	1000-2000	2000-4000	4000-8000	8000-16000	> 16000
P. media (15)	-	-	3	11	1	-	-
P. major (17)	1	-	6	8	2	-	-
P. lanceolata (72)	-	2	17	40	6	6	-
P. coronopus (4)	-	-	1	2	1	-	-
P. maritima (2)	-	-	-	-	2	-	-
H. radicata (8)	-	1	2	5	-	-	-

	exchangeable K (meq/100 cc)						
	< 0.1	0.1-0.2	0.2-0.3	0.3-0.5	0.5-0.8	0.8-1.2	> 1.2
P. media (15)	-	4	1	4	6	-	-
P. major (17)	-	1	4	7	3	1	1
P. lanceolata (72)	5	29	14	14	9	1	-
P. coronopus (4)	-	2	-	-	1	-	1
P. maritima (2)	-	-	-	-	-	-	2
H. radicata (8)	-	6	2	-	-	-	-

* number of samples analysed

Table 9.10. Phosphorus classes of soils (0-10 cm layer) collected from various natural habitats of *Plantago* species and *Hypochaeris radicata* in The Netherlands

species	total P ($\mu g\ cc^{-1}$)					
	< 100	100-200	200-400	400-800	800-1600	> 1600
P. media (15)*	-	-	3	11	1	-
P. major (17)	-	-	1	9	6	1
P. lanceolata (72)	-	3	23	37	8	-
P. coronopus (4)	-	-	1	3	-	-
P. maritima (2)	-	-	-	1	1	-
H. radicata (8)	-	1	5	2	-	-

	total organic P ($\mu g\ cc^{-1}$)					
	< 50	50-100	100-200	200-400	400-800	> 800
P. media (15)	-	-	5	10	-	-
P. major (17)	-	2	5	10	-	-
P. lanceolata (72)	1	2	22	36	11	-
P. coronopus (4)	-	-	3	1	-	-
P. maritima (2)	-	-	-	1	1	-
H. radicata (8)	1	-	3	4	-	-

	P ($\mu g\ cc^{-1}$)								
	< 3	3-6	6-9	9-12	12-15	15-30	30-60	60-120	> 120
	Olsen-P								
P. media (15)	-	2	7	5	-	-	1	-	-
P. major (17)	-	2	2	-	-	2	5	4	2
P. lanceolata (72)	-	17	19	16	2	13	5	-	-
P. coronopus (4)	-	1	-	1	-	2	-	-	-
P. maritima (2)	-	-	-	-	-	1	1	-	-
H. radicata (8)	-	1	4	-	-	2	1	-	-
	labile organic P								
P. media (15)	-	-	1	2	3	9	-	-	-
P. major (17)	-	1	2	-	1	3	10	-	-
P. lanceolata (72)	-	1	2	7	4	38	19	1	-
P. coronopus (4)	-	-	-	-	1	3	-	-	-
P. maritima (2)	-	-	-	-	-	1	1	-	-
H. radicata (8)	-	-	-	1	-	1	6	-	-
	(total $NaHCO_3$ - extractable P)								
P. media (15)	-	-	-	-	1	13	-	1	-
P. major (17)	-	-	-	1	2	-	3	9	2
P. lanceolata (72)	-	-	-	1	3	32	23	13	-
P. coronopus (4)	-	-	-	-	-	-	4	-	-
P. maritima (2)	-	-	-	-	-	-	1	1	-
H. radicata (8)	-	-	-	-	1	-	6	1	-

* number of samples analysed

Table 9.11. Linear correlation coefficients of some plant parameters (ionic contents; meq kg^{-1} d.m.) and soil parameters of natural habitats of *Plantago media*, *P. major*, *P. lanceolata*, *P. coronopus*, and *Hypochaeris radicata* (coefficients < 0.50 not shown). Values between parentheses refer to a soil-volume basis instead of a soil-weight basis. Soils and plants were sampled in May-June of 1979.

	leaves/shoots				
soil/plant	*P. media* (n=15)	*P. major* (n=18)	*P. lanceolata* (n=72)	*P. coronopus* (n=4)	*H. radicata* (n=8)
K/K	0.71(0.73)	0.67(0.76)	- (0.53)	- (-)	- (-)
Na/Na	- (-)	0.74(0.82)	0.61(0.67)	0.78(0.79)	0.92(0.91)
Mg/Mg	- (-)	- (-)	- (-)	0.76(0.85)	0.66(0.64)
total P/H_2PO_4	- (-)	- (-)	- (-)	- (-)	0.78(0.77)
total org. P/H_2PO_4	- (-)	- (-)	- (-)	- (-)	0.64(0.68)
Olsen-P/H_2PO_4	0.53(0.57)	0.50(0.54)	0.67(0.69)	- (-)	0.59(0.55)
labile org. P/H_2PO_4	- (0.50)	- (-)	- (-)	-0.62(-0.90)	- (0.53)
total $NaHCO_3$-P/H_2PO_4	0.52(0.59)	- (0.56)	- (0.61)	- (-)	0.79(0.76)
Cl/Cl	- (0.50)	- (0.59)	- (-)	0.74(0.74)	0.51(0.50)

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10. Survival of *Plantago lanceolata* in vegetations of varying structure (J.H. Mook, J. Haeck, J. van der Toorn)

The demographic approach to the study of local adaptations of plant populations to their environment as part of a large-scale research program (van der Aart 1979), has led to two lines of research. Some results of a comparison of different species of *Plantago* have been given in a previous report (van der Toorn, Haeck and Mook 1980). Because three of these species, *P. coronopus*, *P. major* ssp. *major*, and *P. media* , have different habitat preferences, they could not be compared directly. The fourth species *P. lanceolata*, occurs in such a wide range of grassland vegetations that habitats where this species grew together with one of the other species could be chosen. Thus, in each habitat *P. lanceolata* and one of the other species could be compared, which means that demographic data on populations of *Plantago lanceolata* from grasslands with widely different properties with respect to abiotic environment and management became available at the same time. These grassland cases were supplemented with a few in which *P. lanceolata* was present alone. Thus, this second approach permitted comparison of demographic qualities of eight populations of one species. These populations were studied during two years, 1978/1979 and 1979/1980. Some preliminary results will be given here.

Table 10 shows characteristic properties of the environments. To characterize

As part of the Grassland Research Project the genetic variability within one plant species (*Plantago lanceolata*) is investigated at the experimental field in Heteren. Plants from different localities are grown in an uniform environment.

Table 10. Survey of investigated populations and description of habitats.

Location	Soil type	Management	Structure of vegetation	Maximum height of vegetation in 1979 (cm)	Light transmission May-August 1979 (%)
Achterberg meadow	sand	lightly and infrequently grazed by cattle	short and relatively open	11	50
Achterberg hayfield	sand	mown once a year (June/July)	moderately high with dense tussocks of grass	27	29
Bruuk hayfield	peaty loam	mown once a year (August/September)	high and dense grass and *Juncus*	77	4
Bruuk path	peaty loam	foot path, heavily trodden, mown once a year	short and dense grass with few open spots	20	17
Uddel	sand	roadside, moderately trampled	short and moderately dense grass	9	66
Westervoort	sand and some gravel-rich clay	old railway embankment, lightly grazed by rabbits	moderately high, but open spots present	39	17
Pannerden	sandy clay	small river dike, lightly grazed by ponies	moderately high and dense grass	28	17
Heteren hayfield	heavy clay	mown once a year (early July)	very high and dense grass	73	6

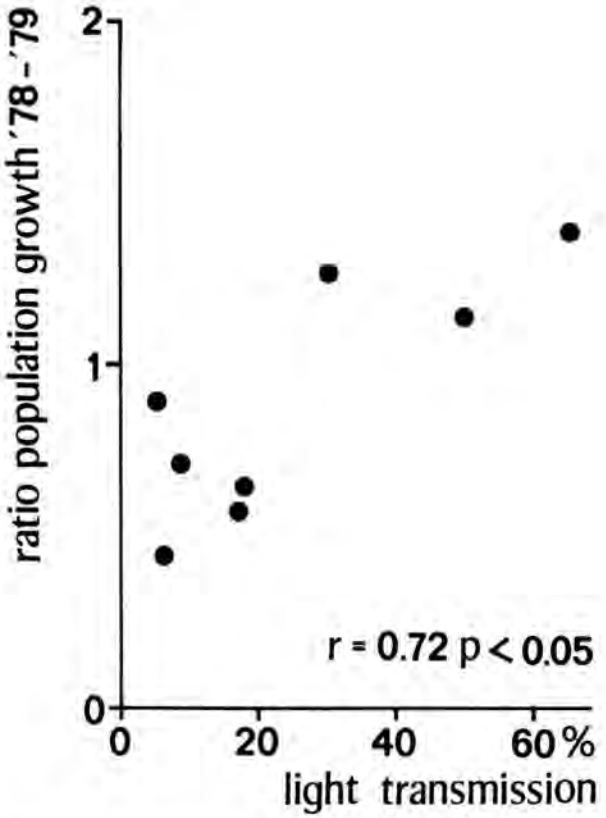

Fig. 10.1. Ratios of population growth of *Plantago lanceolata* in relation to light transmission of the vegetation in summer (May-August).

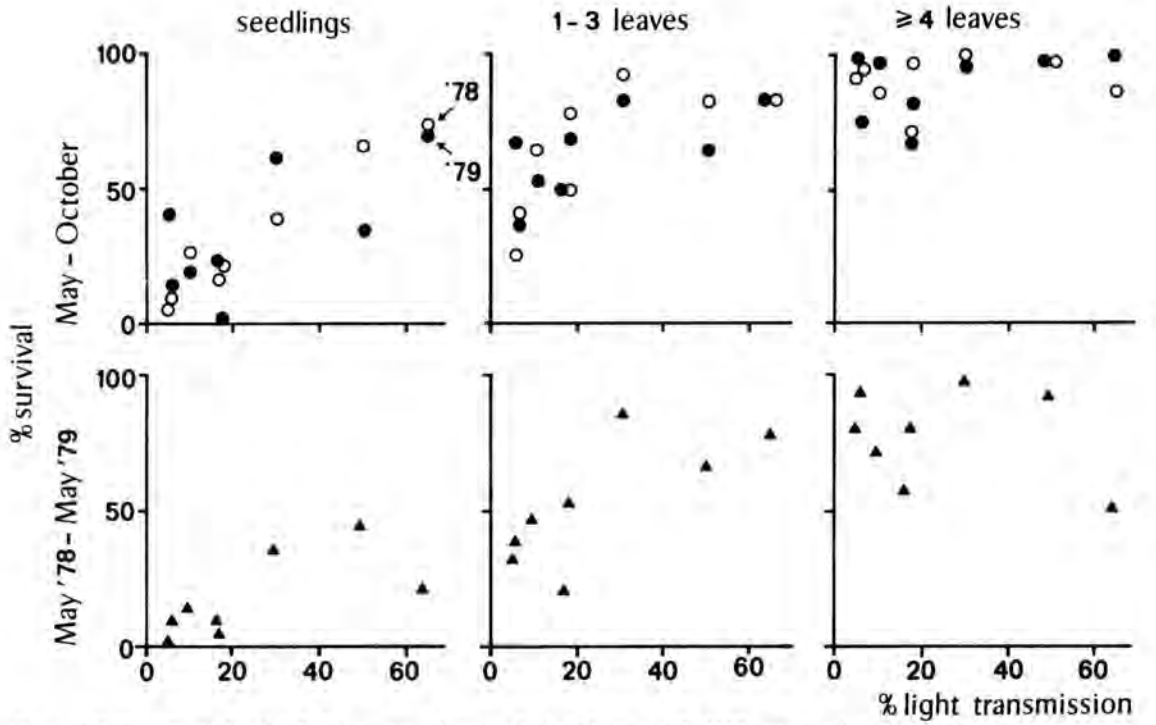

Fig. 10.2. Survival of *Plantago lanceolata* plants of different size in relation to light transmission in the vegetation.

the large differences in structure and density of the vegetations, a quantitative measure was needed. We chose light transmission for this purpose, i.e., the percentage of light penetrating to the soil level in the summer period (May-August 1979).

Comparison of the densities of one population in October of 1978 and 1979 showed that relatively wide differences in positive or negative population growth occurred. These differences were correlated with light transmission in the summer and thus in some way with the density of the vegetation. As can be seen from Fig. 10.1, the population density increased in open vegetations, but decreased considerably in the densest vegetations.

Such trends in population growth can of course be caused by trends in either natality or mortality, or both. There are indications that differences in the occurrence of seedlings also contributed to this trend. Secondary rosettes were not taken into account in the calculations. Further analysis of the data is needed, however, before more details can be given.

More information is available on survival. No correlation was found between survival during the winter and the density of the vegetation, but in the summer period (May-October) survival was correlated with light transmission.

Because survival was also correlated with size, the plants were divided into three categories: seedlings (with cotyledons), plants with one to three leaves, and plants with four or more leaves. Fig. 10.2 (upper graphs) shows the survival from May to October for these categories of plants in relation to the light transmission. Apart from the differences in the general level of survival, a positive correlation was found for the two groups with the smallest individuals. This points to an important contribution by survival in the summer to the trend in population density. Even when survival is calculated for a period of a whole year (May 1978-May 1979), the correlation is still present.

Light transmission is used here as an indicator for the general structure of the vegetation. However, most of the mortality occurred in the period during which the biomass of the vegetation is greatest, e.g., before the hayfields are mown. It is therefore possible that light had a direct influence on survival, but other factors that vary simultaneously may have been involved or been more important. Large plants can compete adequately for light in high vegetations because their upward-growing leaves reach lengths of 40-60 cm or more.

These findings raise the question of how populations of *Plantago lanceolata* maintain themselves in dense vegetations. This is also relevant with respect to the finding that populations of this species in some situations (e.g., after disturbance) show a dramatic explosion followed by a decrease in numbers (van den Bergh 1979). To investigate these problems, further demographic studies will be concentrated on a few selected populations in which more attention will be given to the spatial distribution of individuals in the population.

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11. Experimental ecology of *Plantago maritima* (C.W.P.M. Blom and J. van Heeswijk)

INTRODUCTION AND METHODS

Some hypotheses originating from the comparison of demographic properties of *Plantago* species in relation to their environment are now being tested in exper-

imental studies. In this report an experiment concerning *Plantago maritima*, a species growing on mud flats and beach plains, is described. At Kwade Hoek (island of Goeree, The Netherlands) seeds of *P. maritima* were sown in four series of three plots each. Series A was situated on the edge of the inner dunes, an area influenced considerably by rabbits, series B on an intensively trampled cattle path, and series C and D near creeks. Series C was flooded during each high-tide period, series A, B, and D were flooded only during extremely high tides. On each plot 100 seeds (collected in the preceding year) were sown in a regular pattern. All these areas were natural habitats of *P. maritima*. In the zones of A, B, and C the naturally occurring numbers of *P. maritima* plants were relatively low (0-5 individuals a square metre). In series D higher numbers were observed, and sometimes more rosettes per individual plant were found.

Table 11. Vegetation analysis of the *Plantago maritima* sowing plots at Kwade Hoek, Goeree (The Netherlands). Per species the percentages of soil covering are given

Series	A	B	C	D
Total cover	60 %	20 %	80 %	100 %
Cover of droppings	15 %	-	-	5 %
Maximum height of herb layer	5-15 cm	5 cm	5 cm	5 cm
Date of record	Sept.1980	Sept.1980	Sept.1980	Sept.1980
Plantago maritima	5-10 %	< 5 %	-	10 %
Plantago coronopus	5-10 %	-	-	< 5 %
Carex arenaria	10 %	-	-	-
Honkenya peploides	10-15 %	< 5 %	-	-
Festuca rubra	< 5 %	-	-	90 %
Atriplex hastata	5-10 %	-	-	< 5 %
Spergularia media	< 5 %	5 %	< 5 %	-
Poa pratensis	10-15 %	< 5 %	-	-
Elytrichia repens	< 5 %	-	-	-
Salicornia europaea	-	< 5 %	-	-
Puccinellia maritima	-	-	40 %	-
Glaux maritima	-	-	40 %	25 %
Aster tripolium	-	-	-	< 5 %

Table 11 shows the species composition of the plots. *P. maritima* seedlings were mapped once a month. After emergence of the seedlings the sowing pattern could generally be recognized quite well. In each zone *P. maritima* seedlings were also counted in reference plots to obtain an impression of spontaneous germination.

RESULTS AND DISCUSSION

The percentages of emerged seedlings and the mortality rate are given in Fig. 11.1 - 11.4. In all plots the first seedlings were visible three weeks after sowing. In the highest-lying zone (series A) germination took place throughout the season. In series B few seedlings emerged except for a small germination wave in August. In series C germination took place soon after sowing. The mortality in series B and C was high, although the plants in series C lived longer than those of series B (plots on a heavily trampled cattle path). The mortality in series C occurred mainly in August. In the dense vegetation layer of series D, relatively many seedlings germinated. Compared with series B and C, series D had low mortality rates. In the reference plots the emergence of *P. maritima* seedlings was very low, the highest numbers being 4 in series A, 2 each in series B and C, and 3 in series D. Frequent observation provided a rather good impression of the causes of seedling mortality in *P. maritima*. In the sandy zone of series A, especially in plots A-1 and A-3, seedlings were regularly excavated by burrowing rabbits. The

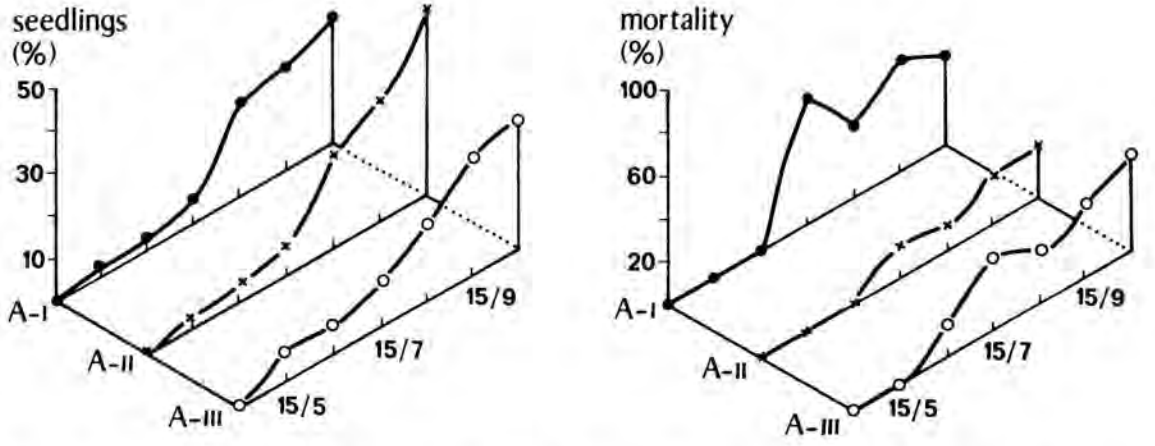

Fig. 11.1. Series A. Germination and mortality of *Plantago maritima* sown on the edge of the inner dunes at Kwade Hoek (Goeree, The Netherlands). The percentages of living plants are given in relation to the numbers of seeds sown. The percentages of dead individuals were calculated with reference to the numbers of seedlings which emerged in the preceding period.

results show clearly that in this relatively unfavourable environment the emerged seedlings were able to maintain themselves by rapid growth. In series B the seedlings died off rather soon. This can be ascribed to the intense tramplings by cows. Apparently, seedlings of *P. maritima* cannot stand this trampling.

Due to the daily flooding, the soil of series C was marshy. The roots of many seedlings were evidently knocked loose repeatedly. This phenomenon was reinforced by the influence, albeit of low frequency, of the trampling by cows. In the grassy plots of series D the plants were able to maintain themselves relatively well. Just as for series A, a low flooding frequency was apparently not disastrous: in series A and D, plants originating from this sowing experiment were still present at the end of the season. To obtain an impression of the influence of winter conditions and the rate of survival, observation of the plots will be continued in the spring of 1981.

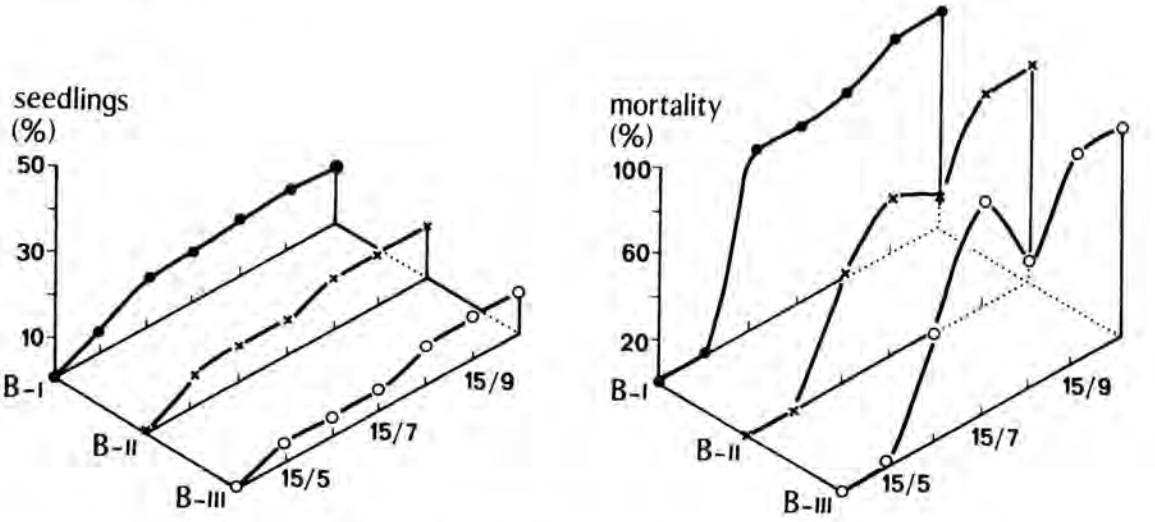

Fig. 11.2. Series B. Germination and mortality of *Plantago maritima* sown on a cattle path.

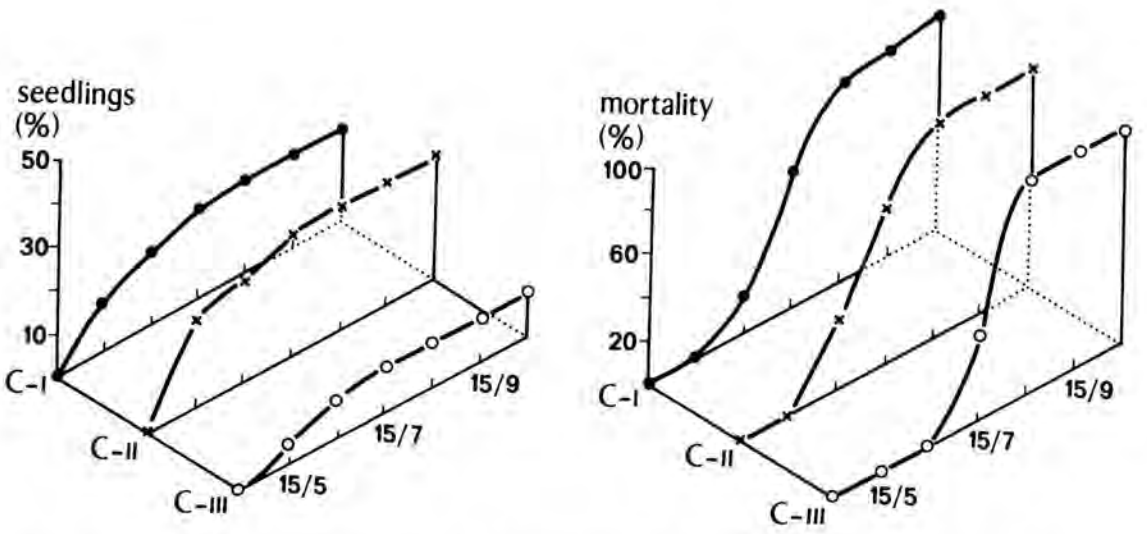

Fig. 11.3. Series C. Germination and mortality of *Plantago maritima* sown near a creek, flooded during each high-tide period.

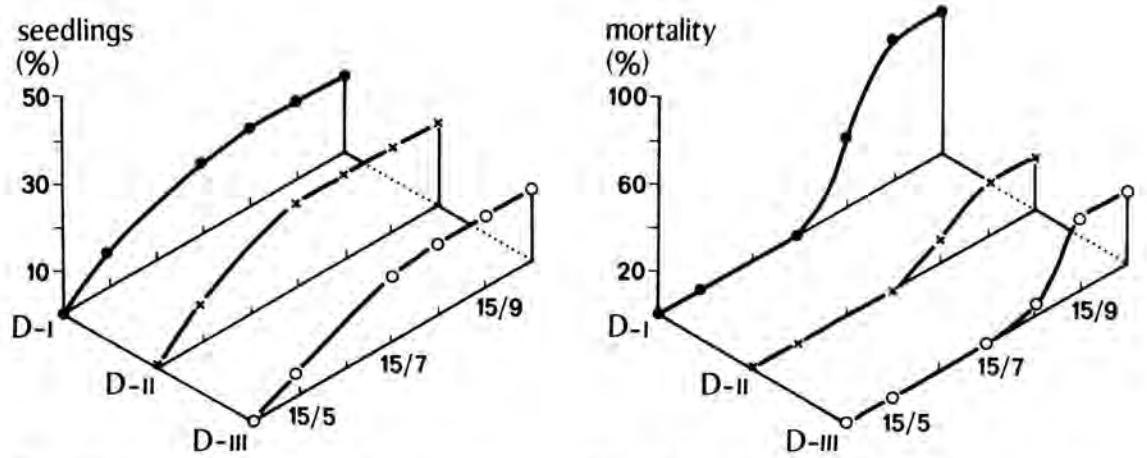

Fig. 11.4. Series D. Germination and mortality of *Plantago maritima* sown near a creek, flooded only during extremely high tides.

From the results, it may be concluded that the emergence of *P. maritima* seedlings from artificially sown seeds can occur immediately, but the numbers of seedlings are low. Germination can occur in a sandy soil (series A) as well as in muddy soils (series B, C and D). Burrowing and heavy trampling are important causes of mortality, as is daily inundation by seawater. To obtain establishment of *P. maritima*, many seeds have to be released. A seed-bank study might provide information about the amounts of seed necessary for establishment.

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12. The leaf demography of trampled and untrampled plants of *Plantago major* ssp. *major* (J. van Heeswijk and C.W.P.M. Blom)

INTRODUCTION AND METHODS

In 1980, the leaf demography of *Plantago major* was studied in experimental plots. Trampling was carried out by means of a trampling machine (see Blom 1979). The plants were four years old when the measurements described below were started, and the trampling treatments had been applied since 1976. Ten untrampled plants on a loose soil and ten heavily trampled plants on a strongly compacted soil were selected. The leaves of each plant were marked in order of appearance. This method made it possible to follow the fate of individual leaves. Each fortnight, the number of living leaves, the length of the longest leave, and the age of the leaves of each plant were determined.

RESULTS AND DISCUSSION

The effects of trampling on the number of living leaves in the course of the year are shown in Fig. 12.1 (left). From May to September the heavily trampled plants formed considerably more leaves than the untrampled individuals did. Fig. 12.1 (right) shows that *P. major* plants react to trampling: trampled plants had a considerably greater length of the longest leaf than untrampled individuals. The product of the number of leaves and the length of the longest leaf gives a good measure of the shoot biomass (Noë and Blom 1981). Trampled plants produced considerably more shoot biomass than untrampled *P. major* plants did.

The mean age of the successive leaves is shown in Fig. 12.2. Few differences in leaf age were observed between the two treatments. It is clear, however, that the turn-over rate of leaves of trampled plants is considerably higher than that of untrampled individuals; during the growing season, trampled plants formed more leaves than untrampled individuals did. *P. major* ssp. *major* reacts to trampling both by forming a greater standing crop and by the formation of many spikes and heavy seeds (Blom 1979). Since the ability of the roots to penetrate a trampled, compact soil is also high, the species is well adapted to these conditions. For purpose of comparison, a similar experiment will be carried out with young single-rosetted *P. lanceolata* plants in 1981.

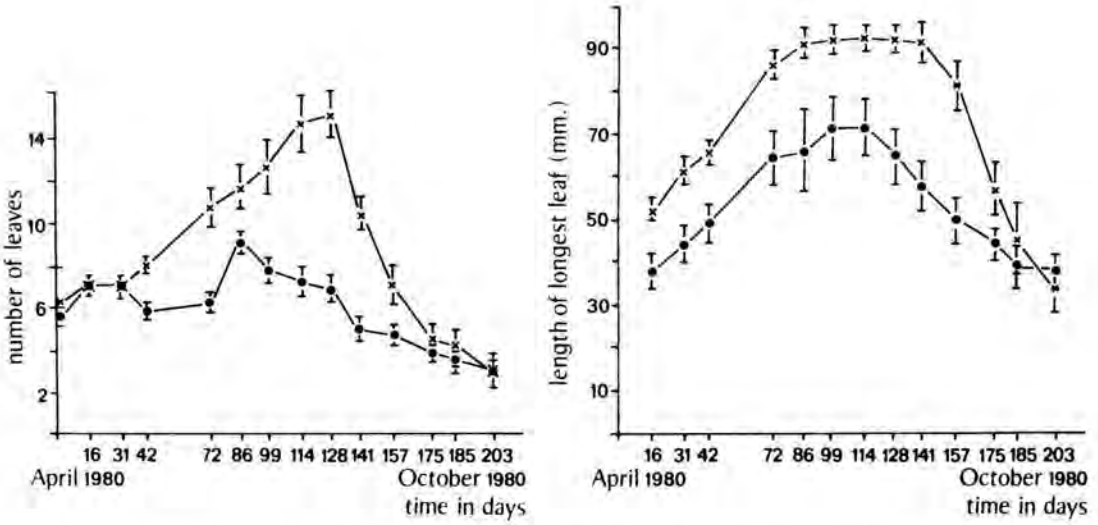

Fig. 12.1. Mean number of living leaves (left) and mean length of longest leaf (right) per *Plantago major* plant growing in an experimental plot. Crosses: strongly compacted soil, heavily trampled. Dots: loose soil, untrampled. Vertical bars: two standard errors.

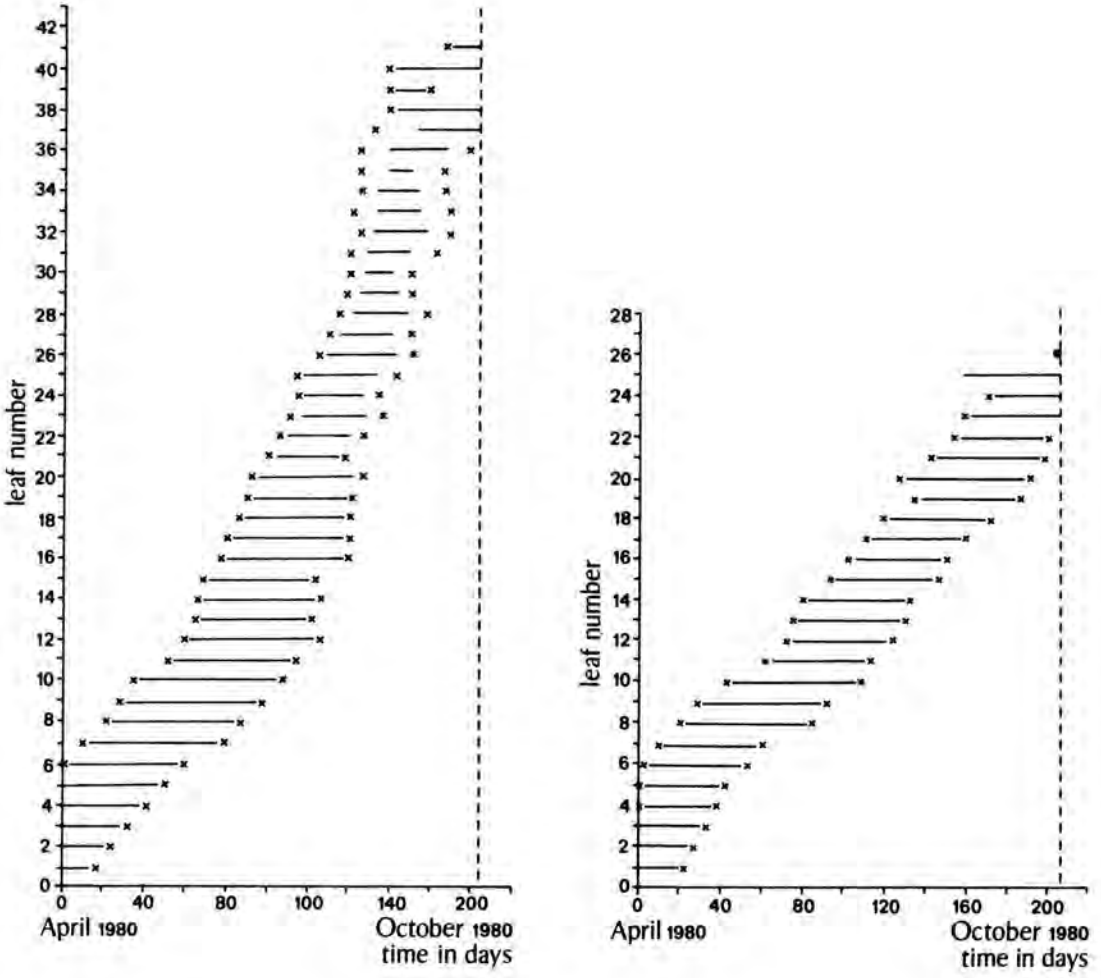

Fig. 12.2. The mean age (length of lines) with 95 per cent confidence outer limits (marks) of successive leaves per *Plantago major* plant on strongly compacted soil (left) and on loose soil (right).

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13. Effect of the supply of water and nutrients on vegetative regeneration in *Plantago* species (R. Soekarjo)

INTRODUCTION

The importance of the vegetative regeneration of *Plantago* species in relation to the ability of a species maintaining itself in areas that are heavily trodden upon, has been put forward earlier (Soekarjo 1979, 1980). However, no difference in regeneration potential was found between the species investigated. Vegetative regeneration proved to be strong, but similar in the four species studied. The

tolerance to treading and grazing should be explained by taking into consideration both the regenerative capacity and the degree in which the apex of the plant is injured. Only in *P. lanceolata* the extent of the root-contraction is insufficient to pull the apex below ground level. It is also the only species of which adult plants are very susceptible to treading, as has been shown earlier (Blom 1979). On the other hand, only *P. lanceolata* readily forms daughter rosettes in the first growing-season without mechanical injury to the apex (Soekarjo 1980).

Experiments have been done to examine the effect of an ample supply of water and the administration of nutrients on the development of daughter rosettes in *P. coronopus*, *P. major*, *P. media*, and *P. lanceolata*.

THE DEVELOPMENT OF DAUGHTER ROSETTES IN *P. LANCEOLATA* IN THE FIRST GROWING-SEASON

Plants were grown separately in pots in the greenhouse on capillary mats to ensure water supply to satiety. After six months, during which time a small amount of nutrients was given to the plants growing in the sand, the experiment on the effect of added nutrients was actually started. This same low dosage was continued for the control plants. The amount Q used as a unit in the experiments reported here, was a mixture of macro-nutrients derived from Steiner's soilless culture solution (Steiner 1968), supplemented with macro-nutrients.

The unit value of Q is:

42.2	mg	$Ca(NO_3)_2.4H_2O$
21.5	mg	KNO_3
6.5	mg	KH_2PO_4
22.1	mg	$MgSO_4.7H_2O$
15.0	mg	$FeSO_4.7H_2O$ as macro-nutrients,

with 0.4 mg NA_2EDTA Komplexon III) and 0.3 mg $FeSO_4.7H_2$=, and:

0.96	mg	$MnSO_4.4H_2O$
0.21	mg	H_3BO_3
0.024	mg	$ZnSO_4.7H_2O$
0.0037	mg	$CuSO_4.5H_2O$
0.0037	mg	$Na_2MoO_4.2H_2O$ as micro-nutrients,

contained in 12 ml of tap water, given to the plants twice a week. Nutrition was given at two levels. The effect of the treatment was already significant after 8 days (Fig. 13.1).

The number of scions developed after treatment with the higher amount was twice as great as that obtained with the lower amount. After the initial bud break, further development of scions in both treatments was approximately the same. As there are more scions in the plants treated with the higher dosage of nutrients, these plants will need more nutrients for the further development of the larger

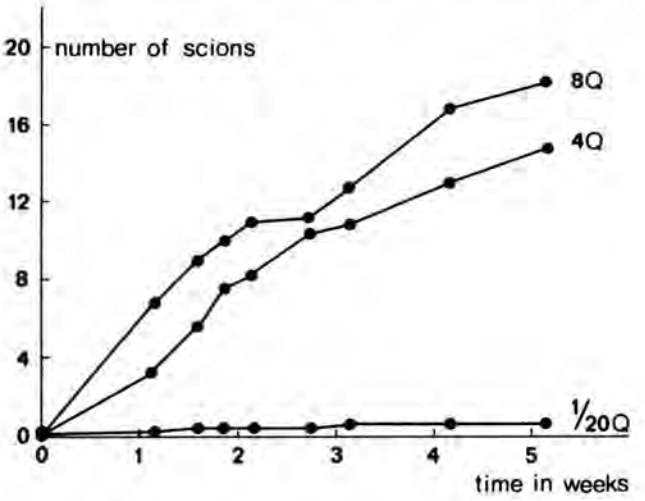

Fig. 13.1. Influence of nutrition on the formation of daughter rosettes in young, vigorously growing plants of *P. lanceolata*. (For the amount Q see text.) Mean values of 5 plants per treatment.

number of scions already present than the plants treated with the lower dosage. As the result of this intra-individual competition for nutrients, apparently the amount of nutrients remaining available for the development of more new scions in the two treatments is approximately the same.

THE DEVELOPMENT OF DAUGHTER ROSETTES IN *P. LANCEOLATA* IN THE SECOND GROWING-SEASON

Without further administration of nutrients, one year old plants were grown for six months in separate pots on inverted dishes for good drainage (dry) and on capillary mats (wet), respectively. The dry-grown plants were small and had formed no daughter rosettes, whereas the wet-grown plants had long leaves in an upright position and had developed a mean number of 2.5 ± 1.1 (n = 20) daughter rosettes. Under the same conditions, while growing on sand, the ample supply of water was crucial for the development of daughter rosettes.

After this period, the plants were all put on capillary mats, and nutrition was given at two levels. From 8 days onwards, there was a clear and differentiated response (Fig. 13.2).

The dry-pretreated plants did not develop any daughter rosettes when given an ample supply of water only. The wet-pretreated plants were still able to develop daughter rosettes, as they had done during the pretreatment period. When the higher dosage of nutrients was given, no difference between dry and wet pretreatment was noticed. The lower dosage elicited different responses in the plants. The plants with the dry pretreatment responded in the same way as the plants had responded to the higher dosage, only starting later and reaching lower values. The wet pretreatment resulted in a different response. These plants showed a more gradual increase in the number of scions during the first three weeks. Later on, the increase became stronger and the number of scions approached the value for the plants which had received the dry pretreatment. The development of new scions in the 'wet' plants that were given the lower dosage of nutrients was impeded by the presence of daughter rosettes formed during the pretreatment period. The response of these plants illustrates the intra-individual competition between the various growing points, in other words the competition for a limited amount of nutrients by metabolic sinks of different activity determines the number of scions.

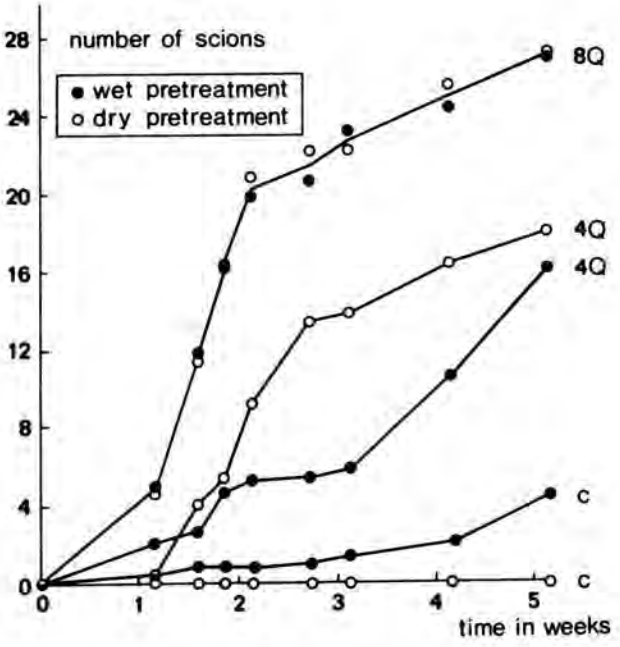

Fig. 13.2. Influence of nutrition on the formation of daughter rosettes in the second year by *P. lanceolata* plants after dry and wet pretreatment. Mean values of 5 plants per treatment.

The slow increase of new scions in the 'wet' control plants must be attributed to a low nutrient supply by mineralization of leaf material.

THE DEVELOPMENT OF DAUGHTER ROSETTES IN *P. MAJOR*, *P. MEDIA*, AND *P. CORONOPUS* IN THE FIRST GROWING-SEASON

Although a dose-dependent increase in fresh weight as well as in dry weight of the plants was obtained after administration of nutrients, *P. major*, *P. media*, and *P. coronopus* did not respond by forming new scions, in contrast to *P. lanceolata*. *P. coronopus* incidentally developed some scions, but in no correlation with the amount of nutrients given. *P. major* and *P. media* did not form any scions at all. The occasional occurrence of daughter rosettes in these two species and possibly also in *P. coronopus*, should therefore be attributed to mechanical or climatological injury to the plant apex. Any factor which causes a decrease in the metabolic activity of the apical meristems (including temporary drought), will diminish the degree of apical dominance and cause the development of axillary buds, when growing conditions are restored.

THE DEVELOPMENT OF *P. MEDIA* PLANTS AFTER A LONG PERIOD OF STARVATION

When seeds are germinating in close proximity to one another, the normal development of the seedling is retarded by an insufficient supply of water and nutrients due to mutual competition. Often the seedling habitus is retained for a long period. Many seedlings are reported to die under these conditions (Harper 1977; Cook 1979). A surviving plant, being ontogenetically older, may have an advantage over young seedlings germinating in the next growing-season. These two types of plants, that look alike, are hard to tell apart in the field.

To investigate the development of this kind of plants, *P. media* seeds were sown on sand and kept in the greenhouse for a period of one year and given tap water only. Under these conditions most of the plants survived and still had the habitus of young plants. These plants were put separately in pots with sand on capillary mats. Nutrition was given at 4 levels. The control plants were given tap water only.

As the plants showed a dose-dependent increase of dry weight of the parts above the ground, it may be concluded that even the highest nutrient dosage is still sub-optimal (Fig. 13.3).

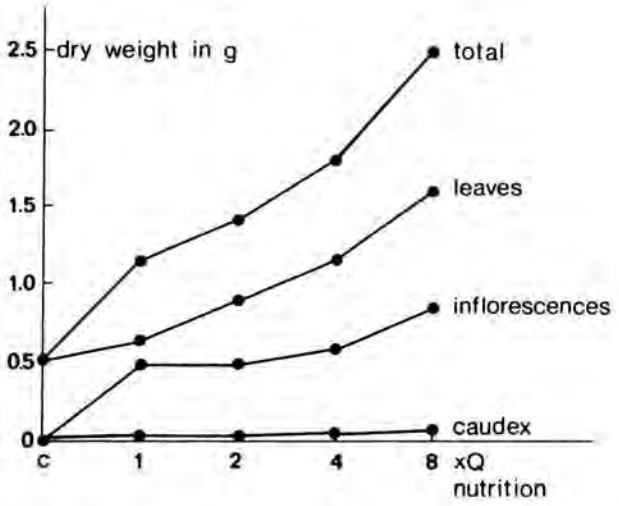

Fig. 13.3. Dry weight of parts above the ground and the caudex 10 weeks after the start of the administration of nutrients, in *P. media* plants starved for one year.

Three weeks after the start of the experiment the difference between the effects of the treatment was evident. The number of newly developed leaves showed a dose-dependent increase. After six weeks the plants that had been given nutrition had all formed more than twice the number of leaves when compared with the control plants. However, the difference between the effects of the doses had become relatively smaller. The cause of this phenomenon becomes clear when the concomitant development of inflorescences and scions is taken into consideration.

Unexpectedly, the plants that received nutrition started very soon to develop inflorescences and scions from axillary buds. In the fifth week the first inflorescences and scions became visible (Fig. 13.4), accompanied by a decrease in the rate of leaf appearance.

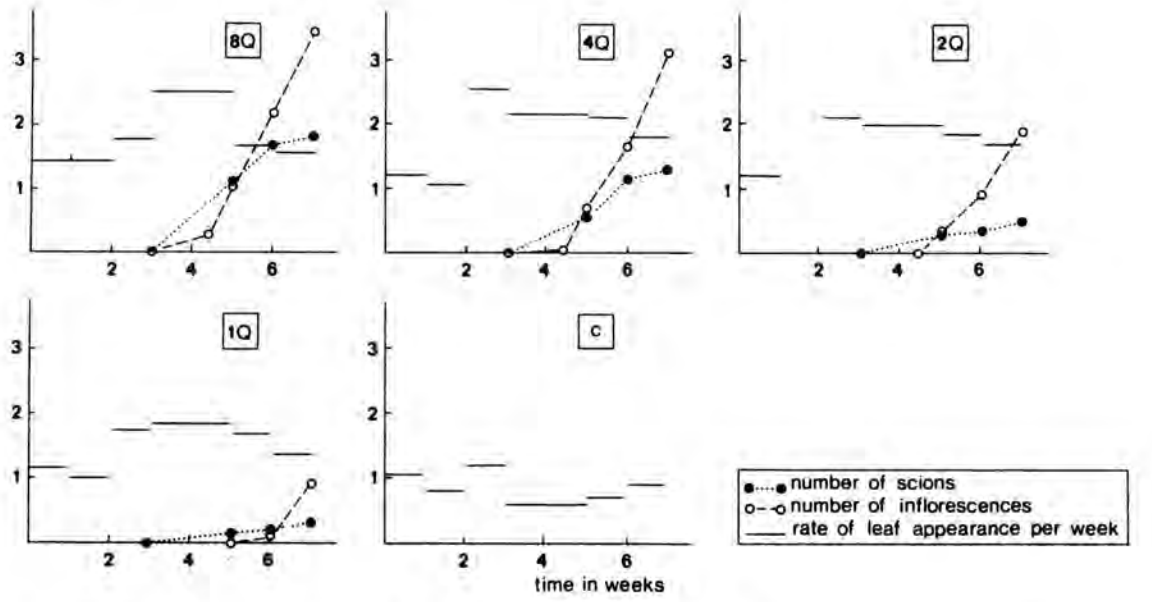

Fig. 13.4. Influence of supplied nutrients on the recovery of the development in *P. media* plants after one year's starvation. Mean of 5 plants per treatment.

It may be concluded that the apical dominance in these plants is not as strong as that in young plants growing normally as described in the preceding section. In these plants in their second year, the apical meristem - due to the prolonged starvation period - was evidently incapable of monopolizing the nutrients offered. The strong competition by the inflorescences and scions developing, manifests itself in the decreasing rate of leaf appearance from the apical meristem. It should be noted, that the number of scions developed after five weeks is doubled for every step in the dosage range (Fig. 13.4). In this stage of development there is a linear correlation between the nutrient dose and the number of scions developed. No correlation exists between the number of leaves and the nutrient dose.

DISCUSSION

The intra-individual competition phenomena described in this report stresses the importance of the nutrient status of plants in the correlative regulation processes. This is in agreement with the findings of McIntyre, who has demonstrated the importance of the availability of water and nutrients for the regulation of apical dominance (McIntyre 1977). However, these results should not be regarded as evidence for the idea that hormonal regulation is not important in the processes which underly apical dominance. It is the level of metabolic activity of a particular sink which determines its competitive power. As hormones influence the rate of incorporation of metabolites as well as the uptake of water by cells, they regulate

the over-all metabolic activity. In the cases where non-limiting amounts of nutrients were given, competition between different metabolic sinks did not occur. But even when limiting amounts are given, the fact that apical dominance is overcome, may only be an expression of the degree of metabolic activity of the central apical meristem. The behaviour of the *P. media* plants after a long period of starvation as compared to the behaviour of young vigorously growing plants can be explained along these lines. For the situation in the field the inference of the results obtained is that only *P. lanceolata* can benefit directly from an increased supply of nutrients in the first growing-season by making more daughter rosettes. In the other three species only injury to the apex, mechanical or otherwise, will cause the development of daughter rosettes in the first growing-season.

The apical dominance in the first growing-season is weaker in *P. lanceolata* than it is in the other species (Soekarjo 1980). External influences therefore have a great impact on this species. In the second and following growing-seasons the apical meristem in the three other species is less active in early spring, which makes it possible that axillary buds present can escape from apical dominance. Later in the growing-season the apices are all active, including those of the daughter rosettes. The number of daughter rosettes then will stabilize and generally no more scions will develop. When, however, there are influences which decrease the activity of the meristems present, such as drought periods and mechanical damage, additional scions will develop. *P. lanceolata* on the other hand, continues to form daughter rosettes during the whole growing-season, if the supply of water and nutrients is sufficient. When damaged, an extra outburst of new scions occurs. As mentioned before (Soekarjo 1980), *P. lanceolata* also forms shoots on roots, particularly when they are injured and the supply of water is adequate. Therefore, although *P. lanceolata* is the species that is most sensitive to treading, it is also the species with the largest range of regenerative potential and the species that develops the largest number of daughter rosettes when the apex is undisturbed.

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14. The effect of disintegration on the infective potential of the root-nodule endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* (P.A.I. Oremus, A.D.L. Akkermans, L. Nijholt)

INTRODUCTION

Recently, a number of *Frankia* strains have been isolated in pure culture that are able to induce N_2-fixing root nodules on non-leguminous plants, *i.e.*, *Alnus*, *Comptonia*, *Elaeagnus*, and *Myrica* spp. (Callaham, del Tredici and Torrey 1978; Baker and Torrey 1979, 1980; Berry and Torrey 1979; Lechevalier and Lechevalier 1979; Quispel 1979; Quispel and Tak 1978). Although this progress has greatly

increased insight into actinomycete-plant symbiosis, a large number of actinorhizal plants, including *Hippophaë* ssp., are known whose N_2-fixing endophyte has not yet been cultivated *in vitro*. For studies on the nodulation of these plants, seedlings have to be inoculated with a suspension of crushed nodules or with soil on which nodulated plants have grown. In all such experiments it is essential to have the disposal of standardized inoculation methods and to know the infective potential of the inoculum. The infective potential, or infectivity, is defined as the number of infective particles per unit of inoculum. This number is calculated as the amount of inoculum needed to induce one nodule per plant. Although this method has given reliable information on the infective potential of different types of nodule homogenate of *Alnus glutinosa* (Akkermans and van Dijk 1975, 1981; van Dijk 1978, 1979; Houwers and Akkermans, in press) and *Hippophaë rhamnoides* (Oremus 1980), little attention has been paid to the effect of disintegration of the nodule material on the infectivity. During disintegration of the nodules, the number of endophyte particles released from the tissue increases. Under prolonged homogenization, the size of the endophyte particles gradually decreases until the viability of the cells is lost by damage of the endophyte particles.

Until now, no information has been available about the effects of the size and physiological stage of the endophyte particles on viability and infectivity. In view of the information in the literature on the viability of *Rhizobium* bacteroids in leguminous nodules, it seems likely that the viability and thus the infectivity of the non-growing, N_2-fixing phase of the endophyte, *viz.*, the vesicles, will be much lower than that of the growing phase, the *hyphae*. We therefore investigated the effect of prolonged sonication on the infectivity of vesicle clusters of the nodule endophyte of *Hippophaë rhamnoides*, in an attempt to determine the degree of disintegration of the nodule tissue needed to obtain an inoculum with a maximum and constant infective potential. As already mentioned, pure cultures of the endophyte, *Frankia* sp., of *Hippophaë* nodules are not available yet. Preliminary attempts to isolate this micro-organism (Akkermans, Nijholt and Dickhoff, unpublished results) failed because of the presence of large numbers of contaminating micro-organisms in and on the nodules. If, however, the ratio between viable *Frankia* particles and contaminants could be increased by enhanced disintegration of the nodule homogenates, it would be easier to isolate this *Frankia* strain by the dilution-series method. The possibilities offered by this technique will be discussed.

MATERIALS AND METHODS

Root nodules were collected from field-grown plants. Healthy lobes were cut off and washed in tap-water containing a drop of teepol. A ten-gramme portion of lobes was then homogenized in sterile 0.05 M phosphate buffer (pH 7.0) for 2 min at 30,000 rpm in a Virtis homogenizer (S45) and the homogenate was filtered through a sterile 100-µm nylon filter. The 100-µm residue was homogenized (1 min at 30,000 rpm) and filtered again, and the filtrate passed through a sterile 20-µm filter as described elsewhere (Akkermans, van Straten and Roelofsen 1977). The 20-µm residue, which contained the vesicle clusters of the endophyte, was washed with 300 ml sterile phosphate buffer on a larger 20-µm filter fixed in a funnel, and the residue collected in the tip of the filter was taken up in 100 ml sterile phosphate buffer. The suspension was divided into 10 samples of 10 ml each, brought into sterile tubes, and sonified with a Branson sonifier (B12) for 0, 5, 10, 15, 20, 30, 40, or 50 seconds. Next, the same phosphate buffer was used to make series of seven ten-fold dilutions of the 20-µm filtrate and of each of the sonified 20-µm residues. A sample of each dilution series was taken for microscopical counts of vesicle clusters, *hyphae* particles, and detached vesicles.

The quantity of inoculum was expressed indirectly per gramme nodule (fresh weight) or directly per mg total organic carbon (TOC) in the cell material. The latter was determined with a Beckman TOC-analyzer (model 915A). The total number of micro-organisms in the different fractions was determined by plating the suspensions on agar media containing either yeast extract-glucose (7 g yeast extract and 10 g glucose per litre) or Tween 80-NH_4Cl (Blom, Roelofsen and Akkermans 1980). The latter medium was used to isolate *Frankia* strains. The results were expressed per gramme nodule material of the starting material.

Endophyte-free seedlings of *Hippophaë rhamnoides* L. ssp. *rhamnoides* were cultivated in a phytotron on perlite (Oremus 1980) and were transferred just

before inoculation to 450-ml glass jars (3 plants per jar) provided with sterilized Hoagland solution without inorganic nitrogen. Each pot received one ml of inoculum of a given dilution. The experiment was performed in triplicate. The culture solution was renewed every ten days. To avoid cross-infections, forced aeration of the solutions was not applied. The nodules were counted five weeks after inoculation. The number of nodules per pot in each dilution series were used to calculate the MPN (most probable number) (Brockwell 1963) of infective particles per gramme nodule fresh weight.

RESULTS AND DISCUSSION

The amount of TOC collected from the 20-µm filtrate and the 20-µm residue of the nodule homogenate were 56 and 4 mg TOC/g nodule (fresh weight), respectively. The nodules used in this particular experiment had an average dry weight of 20 per cent (w/w) and a TOC content of 55 per cent w/w (based on dry weight). From these data it can be calculated that only 55 per cent of the nodule tissue was collected in the 100-µm filtrate; the remaining 45 per cent, which comprised mainly woody parts of the nodules, was retained on the 100-µm filter and discarded.

From these data it can be concluded that only 7 per cent of the TOC was collected on the 20-µm filter. The latter fraction consisted mainly of vesicule clusters, as described previously (Akkermans, van Straten and Roelofsen 1977; van Straten, Akkermans and Roelofsen 1977). The remaining 93 per cent, of the 20-µm filtrate consisted of a mixture of plant and endophyte material.

From previous studies with *Alnus* nodules it was concluded that the 20-µm filtrate and the 20-µm residue contained about 40 and 30 per cent of the endophyte material, respectively. These values were based on the distribution of diaminopimelic acid, a specific marker of the endophyte (Akkermans 1978; van Dijk 1979). A similar distribution of endophyte material over the two fractions can be expected for *Hippophaë* nodule homogenates (data not shown). This indicates that the 20-µm filtrate of the nodule homogenate consists mainly of plant material (almost 80 per cent of the TOC in the fraction).

When examined under the microscope, the 20-µm residue fraction proved to contain 2×10^6 vesicle clusters per gramme of nodule (fresh weight). With increasing sonication time the average diameter of the vesicle clusters decreased (Table 14.1) and the number of detached vesicles released from the clusters increased (Table 14.2). In addition, there was a slight increase in the number of small *hyphae* fragments. After 50 seconds of sonication, about 16×10^6 vesicles were detached.

Table 14.1. Average diameter of the vesicle clusters after various periods of sonication of the 20-µm residue

sonication time (sec)	diameter of vesicle clusters	
	mean	S.D.*
0	79	24
5	51	23
15	32	18
50	15	8

* S.D. = standard deviation

Since each vesicle cluster contains about 200 vesicles and 2×10^6 vesicles clusters were counted per gramme nodule material, it is evident that only 4 per cent of the vesicles were released from the clusters. These observations indicate that the clusters are slowly peeled during sonication, resulting in a steady increase in the number of detached vesicles and *hyphae* fragments of various sizes. The total number of endophyte particles in the 20-µm residue (*viz.*, the sum of the numbers of *hyphal* particles, detached vesicles, and vesicle clusters) increased ten-fold,

viz., from 2.0×10^6 to 18.6×10^6 - 20.6×10^6 per gramme nodule (fresh weight) (Table 14.2) within 50 seconds after the start of sonication. This increase was mainly due to the larger number of detached vesicles. This number increased further with longer sonication times (data not shown).

Table 14.2. Effect of duration of sonication of nodule material on the number of *hyphae* particles and the number of detached vesicles

sonication time (sec)	number of *hyphae* particles ($x10^6$) per g nodule (fr.wt.)		number of detached vesicles ($x10^6$) per g nodule (fr.wt.)	
	mean	S.D.*	mean	S.D.*
0	<< 0.1	n.d.**	<< 0.1	n.d.**
5	1.0	0.3	7.5	0.9
20	2.0	0.8	10.7	2.6
50	2.8	0.7	15.8	1.5

* S.D. = standard deviation
** n.d. = not determined

In addition to these microscopical observations, the viability of the endophyte particles was measured indirectly by assessing the infective potential. As shown in Table 14.3, the 20-µm filtrate and the 20-µm residue together contained 3×10^5 infective particles per gramme nodule (fresh weight). This value is in good agreement with previous findings concerning the infective potential of *Hippophaë* nodules (Oremus 1980). Most of the infective particles were found in the 20-µm filtrate; the infective potential of the 20-µm residue was almost 300 times lower than that of the filtrate (based on nodule fresh weight). When the number of infective particles are calculated per mg TOC in the fractions, the two fractions differ less strongly, *viz.*, by a factor of only 23.

Since about 80 per cent of the TOC in the 20-µm filtrate is of plant origin (see above), the infective potential of the 20-µm filtrate will (on the basis of the estimated TOC content of the endophyte) be about five times greater than that of the 20-µm residue. These preliminary observations suggest that the infective potential of endophyte particles does not differ greatly between these two fractions of the homogenate, but further experiments are needed to validate this hypothesis. To assess the effect of disintegration of the vesicle clusters on the infective potential of this nodule fraction, plants were inoculated with various dilutions of the 20-µm residue. As shown in Table 14.3, the infective potential had increased ten-fold after 10 seconds of sonication. This initial increase in the infective potent-

Table 14.3. Number of infective particles per g fresh nodule weight

fraction	20-µm filtrate	20-µm residue						
sonication time (sec)	-	0	5	10	15	20	30	40	50
number of infective particles	290,000	918	4,240	9,190	4,240	918	918	918	424

Factor for 95% fiducial limits (x,÷) approx. 3.5

ial was in accordance with the increase in the number of infective particles (Table 14.2). The decrease of the infective potential after prolonged sonication must be due to damage of the endophyte cells, *e.g.*, denaturation of proteins (A. Burggraaf, pers. comm.). Although these results indicate that disintegration of vesicle clusters by sonication leads to an increase in the number of *Frankia* particles as well as in the infective potential, the effects are small. The data on the infective potential suggest that prolonged disintegration will only result in a further decrease of the infective potential. As to the extent to which sonication of vesicle clusters contribute to the isolation of the endophyte of *Hippophaë* nodules, the following can be said. When nodule homogenates are diluted and plated on agar medium, many contaminants, including non-infective actinomycetes, overgrow the plates. As shown in Table 14.4, the 20-µm residue was found to contain 0.2×10^6 bacteria per gramme nodule fresh weight when plated on yeast extract-glucose agar. The number of colonies found did not increase after sonication of the vesicle clusters.

Table 14.4. Total number of micro-organism in nodule homogenates of *Hippophaë rhamnoides*

fraction	sonication time (sec)	number ($\times 10^6$) per g nodule (fr.wt.) A*	B**
20-µm filtrate	0	7.9	129
20-µm residue	0	0.2	0.8
20-µm residue	5	0.4	1.4
20-µm residue	10	0.5	2.8
20-µm residue	15	0.3	2.7
20-µm residue	20	0.6	4.3
20-µm residue	30	0.5	7.4
20-µm residue	40	0.4	1.8
20-µm residue	50	0.6	2.4

* A = on yeast extract-glucose medium

** B = on Tween 80-NH_4Cl medium

Suspensions plated on Tween 80 - NH_4Cl medium, which was developed to obtain rapid growth of *Frankia* Avc I1 (Blom, Roelofsen and Akkermans 1980), gave somewhat higher values, and a small increase in the number of micro-organisms was found after sonication for up to 30 seconds. So far, no *Frankia* colonies have been observed on these media.

From these results we conclude that it is unlikely that the small increase in the ratio between the numbers of viable endophyte particles and the total number of micro-organisms in the sonicated residue will be large enough to contribute to the improvement of the isolation procedure of the endophyte of *Hippophaë* nodules solely by the use of the dilution method. Other methods will have to be developed to decrease the numbers of contaminants in the homogenates, and further studies on the nutrient requirements of this particular *Frankia* strain will have to be performed.

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Limnological Institute

Progress Report 1980

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CONTENTS

1.1. HISTORY OF THE INSTITUTE

The concerted effort of the Division of Natural Sciences of the Royal Netherlands Academy of Arts and Sciences and the Ministry of Science and Education, to strengthen the position of ecological research in the Netherlands, resulted in the establishment of three institutes: two involved in aquatic ecology, namely, the 'Hydrobiological Institute' at Nieuwersluis and the 'Division Delta-Research of the Hydrobiological Institute' at Yerseke and of the 'Institute for Ecological Investigation' at Arnhem, concentrating on problems of a terrestrial ecological character. The 'Hydrobiological Institute' founded in 1957 filled a gap that existed in biological and chemical research of the abundantly present freshwater systems in the Netherlands. Due to the separate development of the two institutes 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 (see photograph).

Dr. Miss M.F.E. Nicolai (1957-1960) was the first director of the Institute. After her death Dr. H.L. Golterman succeeded her and was in 1972 joined by Dr. R. Soekarjo. Both directors resigned in 1978 in which year Dr. S. Parma took over as director.

Aerial view of the Tjeukemeer Laboratory at Oosterzee. Our research vessel is lying at the background on the Tjeukemeer.

1.2. ORGANIZATION OF THE INSTITUTE

The Institute is financed primarily by the Ministry of Science and Education by means of the funds allotted to the Royal Netherlands Academy of Arts and Sciences. Moreover, research is done on a contract base, financed by other ministries and organizations.

The permanent strength of the Institute in 1980 was 41 full time places, (i.e. 45 persons). Additionally, one employee was financed via the Ministry of Public Health and the Environment and two by the Ministry of Economic Affairs. Twenty-eight persons were working at the 'Vijverhof' Laboratory at Nieuwersluis and 17 at the Tjeukemeer Laboratory at Oosterzee, both including the staff members. Also, 14 guestworkers and 25 students took part in the scientific programme during a part of 1980 (see Table 1). The general service departments, covering the administration, library, photography, ships and workshop, consisted of 13 members.

1.3. RESEARCH PROGRAMME

The research programme at the 'Vijverhof' Laboratory is split up into two work-groups, viz. 'Primary and Secondary Production' and 'Mineralization of Organic

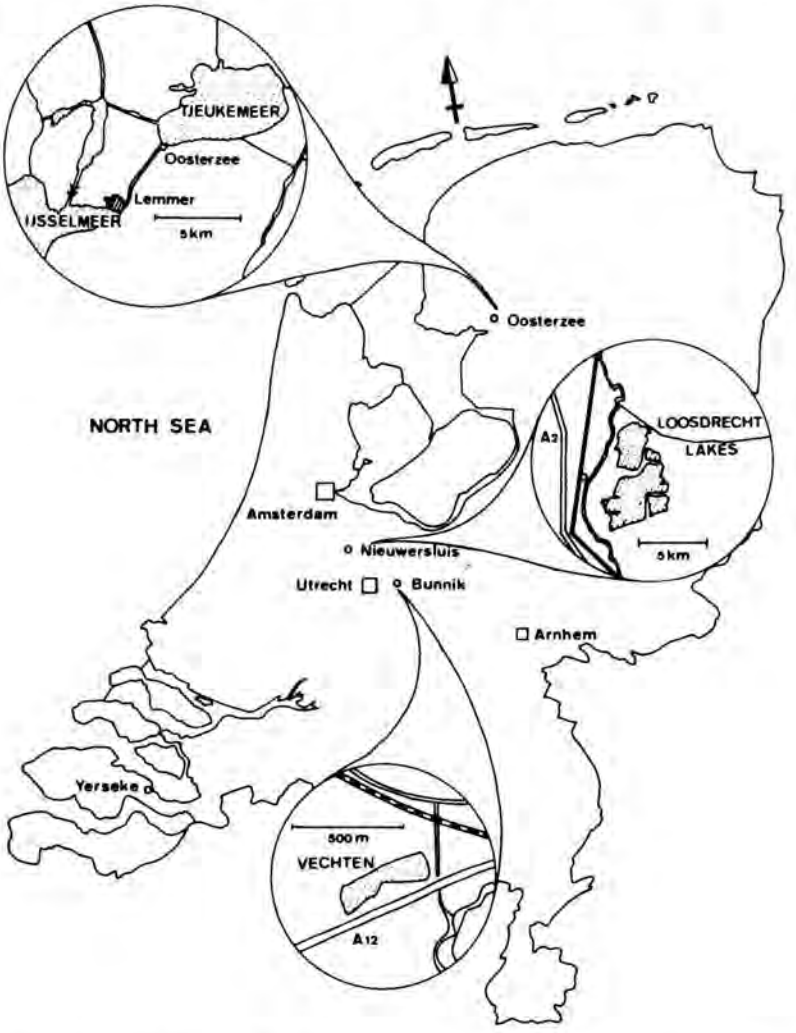

Fig. 1. Map of The Netherlands showing the study areas of the Limnological Institute. Lake Vechten and Loosdrecht Lakes research at Vijverhof Laboratory, Nieuwersluis (province of Utrecht). Tjeukemeer research at Tjeukemeer Laboratory, Oosterzee (province of Friesland).

Matter', and a research group, 'Ecophysiology of Macrophytes'. Besides their own research topics (see 2.2 and 2.3) the workgroups cooperate in two projects. The first one is entitled 'Carbon cycle in Lake Vechten', and is focussed on several aspects of the carbon budget of this lake. The second, a joint research project, started recently and is entitled 'The restoration of the Loosdrecht Lakes'. A third more or less independent research project - 'Oxygen budgets of ditches' - is financed by the Ministry of Public Health and the Environment. It should be a basis for the management of polder waters - typical Dutch freshwater systems.

The Tjeukemeer Laboratory has also two workgroups, viz. 'Algology' and 'Food Chain and Production Studies'. Both groups are working mainly on the Tjeukemeer, but also other Frisian lakes are included in their research programmes.

Table 1. Research scheme in 1980: s = student, g = guest worker

"VIJVERHOF" LABORATORY, NIEUWERSLUIS

Workgroup 'Primary and Secondary Production'

Dr. R.D. Gulati (leader)	R. van Keulen	D. de Vries (s)
Dr. H.J. Gons	G. Postema	W. van Houten (s)
Drs. W.A. de Kloet	K. Siewertsen	P. van der Linden (s)
Drs. J.T.A. Vulto (till March)	P.J. Boesewinkel-de Bruyn(g)	J. Werkhoven (s)

Workgroup 'Mineralization of Organic Matter'

Dr. Th.E. Cappenberg (leader)	M.J. Bär-Gilissen	P.J. Boesewinkel-
Drs. H. Verdouw	M.D.M. Trommel	de Bruyn (g)
Dr. C.L.M. Steenbergen	H.R. Kwist (g)	G.J. Jonkheer (s)
Drs. J.J. Olie	Ir. E.F. van der Heide (g)	H. Stam (s)
Ing. A.G. Wisselo	Drs. M.A.C.I. Blaauboer-	A.P.M. Lauwen (s)
C.A. Hordijk	Wiercx (g)	J.W. Bos (s)
E.M.J. Dekkers	Drs. P.C.M. Boers (g)	A.J. Moons (s)
H.J. Korthals	A.M.M. Jansen (g)	R. Bergsma (s)

Researchgroup 'Ecophysiology of aquatic macrophytes'

Dr. P.H. Best (leader)	G. Wiegers (g)	M. Kramer (s)
J.H.A. Dassen	J.T. Meulemans (s)	E. Jagtman (s)
Drs. M. Priem (g)		

Project 'Polder Research'

Dr. S. Parma (leader)	Ir. R. Veeningen (g)	H.R. Kwist (g)

"TJEUKEMEER" LABORATORY, OOSTERZEE

Workgroup 'Algology'

Dr. J.R. Moed (leader)	Drs. J.H. Coers (g)	R.J.V. Mevissen (g)
Dr. H. de Haan	Drs. A.C. Arkesteijn-	O. Jethoe (g)
J. Voerman	Dijksman (g)	D.J. Wijbenga (s)
Th. de Boer	Ing. G.J. Schrotenboer (g)	M.J.W. Veldhuis (s)
H.A. Kramer	Ing. P.J. Timmer (g)	S. Kromkamp (s)
H.L. Hoogveld	R.T.P. Hoekstra (g)	J. Schreurs (s)
N. van Benthem		

Workgroup 'Foodchain and Production Studies'

Drs. J. Vijverberg (leader)	A.G. Frank-Landman	H. Bakker (s)
Drs. W.L.T. van Densen	J. Swart	P. Bremer (s)
Drs. H.W. de Nie	Drs. A.F. Richter (g)	A. Kroon (s)
Drs. E.H.H.R. Lammens	E.G. de Boer (g)	J. Meischke (s)
P.J. Mac Gillavry	C. Fortgens (g)	J.H.M. Stöpetie (s)
Th.H. Frank	A. Ypma (g)	

The work of the Institute is based on five year plans of studies which are carried out after approval of the Board of Trustees installed by the Royal Netherlands Academy of Arts and Sciences.

The Institute organizes training programmes for students from the Dutch universities. Training and research facilities are also offered to students and scientists from abroad.

1.4. SET-UP OF THE PROGRESS REPORT

The set-up of this report is transitional. Unlike in the previous years the individual reports of the workgroups will be in future based on five year plans of studies. So, the separate projects will be easy to recognize. This system is already adapted for the two workgroups at the Tjeukemeer Laboratory for which the 5-year plans are at present available.

2. 'Vijverhof' Laboratory

2.1. GENERAL INTRODUCTION (S. Parma)

A great deal of the work at this establishment is confined to Lake Vechten (Fig. 1). This lake 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 to the 5-6 m isobath. The phytoplankton composition points to a slightly eutrophic environment.

The lake has formed a permanent research object of the Limnological Institute since 1960. The limnological studies up to 1975 were aimed mainly at understanding the physical, chemical and biological characteristics of the lake. Evidently the processes, involving the three main properties of an ecosystem, i.e. the energy flow, the mineralization and nutrient recycling and the population regulation, cannot proceed in isolation. In view of this there was a strong desirability for both a coordinated and 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.

A small part of the work of the 'Vijverhof' Laboratory is since 1968 situated in the 'Vechtplassen' (see Progress Report 1979, par. 4.1). Recently a more intensified study started in this area. The whole lake system (see Fig. 1) has been formed as a result of excavation for peat. Dredging of sand resulted in a few deep and thermally stratified lakes, but the majority is shallow. The lakes differ limnologically, particularly due to their hydrological regimes, location, morphology and 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.

One of the lakes, the middle Loenderveen Lake serves as a source of drinking water supply for the city of Amsterdam. Water from the surrounding lakes is used for supplementation, and if a shortage is expected highly eutrophic and polluted water from the River Vecht is admitted into the lake system through locks. A majority of the lakes has become eutrophic by the inlet of waste water and Vecht water.

In the near future a gradual restoration of the original biotic communities in this part of the lake area (water surface 1830 ha, depth 1-2 m) is to be expected since recently the waste water treatment was intensified, and from 1982 onwards supplementation will occur with dephosphatized water from the Amsterdam-Rijn Canal. Since a decreased phosphorus input will cause eventually lower P-concentrations in the sediments and overlying waters, resulting in an improvement of the light climate, it is expected that this will be reflected in the species composition and primary production of algae and macrophytes.

Coordinated studies have been started to investigate the biotic and abiotic changes in this area accompanying the decrease in phosphorus loading. In this project five institutes and authorities are participating, namely, the Municipal

Waterworks, Amsterdam; the Provincial Water Authority, Utrecht; the Research Institute for Nature Management, the Microbiological Laboratory of the University of Amsterdam and the Limnological Institute. They are cooperating in the workgroup 'Water quality research Loosdrecht' (W.O.L.).

2.2. WORKGROUP 'PRIMARY AND SECONDARY PRODUCTION'

2.2.1. Introduction (R.D. Gulati)

The studies on primary and secondary production were focussed mainly on the aspects which have so far not received adequate attention but are important to get a better insight into the functioning of the ecosystem of Lake Vechten.

The work on the primary production of phytoplankton in the littoral and in the open water, as well as on the comparison of production rates in the eastern and western basins was completed. The contribution of photoautotrophic bacteria and algae in the anoxic layers to the areal primary production of the lake was investigated in collaboration with C.L.M. Steenbergen of the workgroup 'Mineralization of Organic Matter'.

The work in the littoral region concerned mainly the structural, metabolic and production aspects of seston, and the communities in the benthos.

The zooplankton research was concentrated mainly on the following aspects: a comparison of the capturing efficiencies of different plankton samplers; horizontal differences in the densities of zooplankton; metabolic studies of zooplankton, i.e., respiration and nitrogen and phosphorus excretion rates, and the role of the regenerated nutrients in meeting the demands of phytoplankton.

The group also participated in the diurnal study of the carbon cycle of Lake Vechten on September 9 (see 2.5.3).

2.2.2. Primary production (W.A. de Kloet)

The studies on the transect in the eastern basin of the lake in progress since 1979 to investigate the spatial differences in the phytoplankton production rates, chlorophyll, seston mass and zooplankton grazing were concluded. The emphasis of studies this year was to accurately assess the energy input via the autotrophs on areal basis for the whole lake.

Comparison of the rates in the two basins

The horizontal and vertical distributions of phytoplankton (chlorophyll a) were investigated *in situ* simultaneously in the eastern and western depression. Also underwater light climate, water temperature and dissolved oxygen concentration were measured on a number of the sampling dates.

The maximum differences in the primary production rates in the two basins did not exceed 20%. The means of nine measurements for the period May 21 - October 15, when the lake was stratified, were, however, comparable (Table 2). Variance ratio ($F_{8,8} = 1.49$) $S_w^2 : S_e^2$ (where S_w^2 and S_e^2 are, respectively, the variances of the rates measured in the western and the eastern station) did not differ significantly ($P < 0.05$).

Table 2. Means with S.D. of primary production, chlorophyll-a and activity coefficients at the stations in the eastern and western basin of Lake Vechten during the period May 21 - October 15, 1980.

	east	west	N
Primary production mg $C.m^{-2}.h^{-1}$	35.5 ± 19.0	34.7 ± 23.2	9
Chlorophyll-a $mg.m^{-2}$	121 ± 55	151 ± 103	10
Activity coefficient mg $C.mg^{-1}$chl-$a.h^{-1}$	0.48 ± 0.51	0.53 ± 0.64	9

Though chlorophyll concentration and its seasonal variations were higher in the western than in the eastern part, the variance ratio ($F_{9,9} = 3.5$) did differ not significantly ($P < 0.05$). The relatively higher amount of chlorophyll in the western basin per unit area was due to a higher concentration in the strata between 6 and 8 m in this part of the lake. The concentrations in the epilimnion (strata above 5 m) in the two parts were comparable. Despite the areal differences in the amount of chlorophyll, the activity coefficients (mg C fixed.m^{-2}.hr^{-1} per mg chlorophyll) were similar (see Table 2).

It may be broadly concluded that the phytoplankton primary production rates measured in the eastern basin are representative of the whole lake; consequently, the available production data of the eastern station can be used to compute the energy balance and carbon cycle of the lake.

Contribution of phototrophic populations (W.A. de Kloet and C.L.M. Steenbergen)

The primary production (^{14}C-uptake rate) was measured extensively, particularly during summer stratification when the water below the depth of 6.5 m was anaerobic. The main purpose of this study was to accurately estimate the carbon-fixation rates of photoautotrophic populations in metalimnion and hypolimnion. These populations consisted of *Mallomonas caudata*, having a maximum density at depth 6 m, chroococcal Cyanobacteria peaking at a depth of 6.5 m, and filamentous green sulphur bacteria (Chlorobiaceae) and *Chromatium* cells having maximum densities at, respectively, 6.5-7 m and 7-7.5 m. Both Chlorobiaceae and Chromatiaceae are strictly anaerobic and carry out anoxygenic photosynthesis, using sulphide as electron donor in their photosynthetic reaction. In order to measure their photosynthetic activity care must be, therefore, taken to avoid contamination of the anoxic water with oxygen. The samples were thus incubated under anaerobic conditions, taking care to exclude air bubbles. Oxygenic, i.e., algal photosynthesis was distinguished from the anoxygenic one using DCMU (3-3,4-dichlorophenyl-1,1-dimethyl-urea). DCMU specifically inhibits oxygenic photosynthesis, without affecting anoxygenic photosynthesis. In addition, meta- and hypolimnetic samples were incubated under aerobic conditions, leaving an air pocket inside the bottles. Results for epilimnion (0-5 m) and for meta- and hypolimnion (5-9 m) together were calculated on annual basis taking into account the total volumes of the respective strata (see Table 3).

Depending on the incubation conditions - aerobic or anaerobic - during the stratification period, the primary production for the 5-9 m depth stratum differed. Aerobic incubation of samples resulted in complete elimination of anoxygenic photosynthesis and in strong inhibition of the oxygenic photosynthesis by *Mallomonas* and Cyanobacteria (see also 2.3.4). Thus, the annual primary production of meta- and hypolimnion, measured under aerobic conditions, was 343 kg C, whereas, measured under anaerobic conditions, it was 634 kg C (= 507 + 127) (see Table 3). This is an increase of 7.3% on basis of total pelagic primary production. The share of anoxygenic photosynthesis in the total annual primary production was 3.6%

Table 3. Range (mean in brackets) of daily and annual primary production for two strata of Lake Vechten. Data expressed as kg C per respective volumes of the strata.

stratum	kg C day^{-1}	kg C $year^{-1}$	%	type of photosynthesis
0 - 5 m	3.585 - 27.511 (11.410)	2876	81.9	oxygenic by algae
5 - 9 m	0.126 - 4.400 (1.360)	343*		oxygenic by algae and Cyanobacteria
	0.126 - 10.576 (2.010)	507	14.4	oxygenic by algae and Cyanobacteria
	0.204 - 2.197 (0.504)	127	3.6	anoxygenic by green and purple bacteria

* Total carbon fixation of the stratum obtained by aerobic incubation of anoxic samples during summer stratification.

(Table 3). The photosynthetic sulphur bacteria thus accounted for only a minor fraction of the total primary production in the lake, although, particularly in late summer their maximum primary production rate equalled that of the epilimnetic phytoplankton.

2.2.3. Periphyton studies (H.J. Gons and R.J. van Keulen)

In 1979, submerged macrophytes no longer occupied most of the littoral zone. Therefore, more attention was paid to the benthic communities in the littoral. From July to December, the depth distribution of particulate organic matter (POM) was measured along a transect in the SE part of the lake (Fig. 2). Using a specially developed sampler the organic matter from 18.5 cm^2 of bottom area was removed quantitatively. Five such replicates were taken at every station. Moreover, *Ceratophyllum* plants were taken to the laboratory, where the periphyton was separated from them. Also, water was collected between the plants and at the sediments, using the above mentioned sampler, while underwater light was measured between the plants. Oxygen exchange of the samples was determined in the laboratory after incubation in the dark and in a range of irradiances and at temperatures prevalent in the lake.

Structural aspects

Although cell counts and pigment analyses are not yet complete, some general remarks are made. Compared with the sediments, the material from the upper plant portions was more densely populated by algae, as in previous years mainly pennate diatoms and filamentous green algae, and by microfauna. The periphyton from the lower plant portions, however, usually had the lowest chlorophyll concentrations (Table 4), and the highest proportion of chlorophyll breakdown products. The predominant algae in the sediment samples were often similar to those dominant in the open water. These species were also present upon the plants, but especially in summer, their biomass was low compared to that of the pennate diatoms and filamentous green algae.

The different materials were further characterized by gravimetric and chemical analyses. In summer ash content of the sediment samples was higher than that of the plants, but towards the end of the year the values became similar. Carbonates contributed two to three times more to the material from the plants than to that from the sediments. Carbon content of the organic matter did not differ significantly, nor did the organic C:N ratio, which had values around 9.

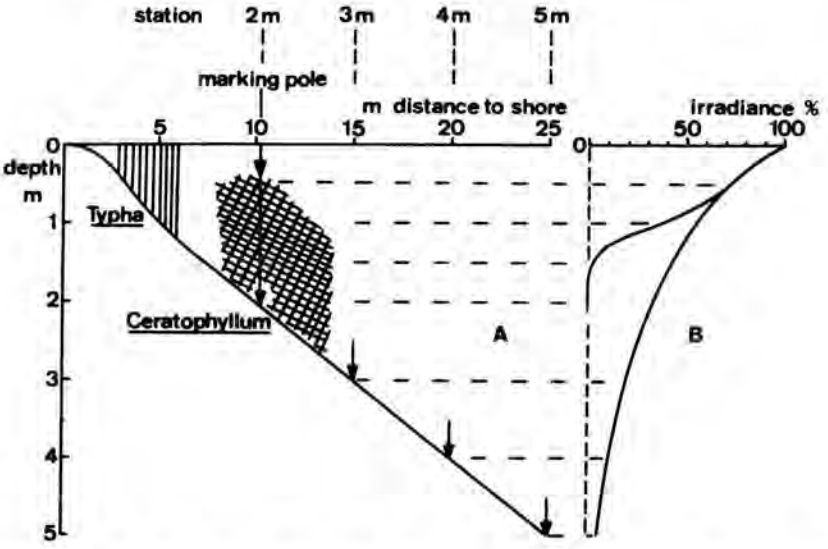

Fig. 2. A. Sampling stations along a transect in the littoral zone of Lake Vechten. B. Light distribution at station 2 m, i.e., with dense *Ceratophyllum* stand, and at station 5 m, on September 9, 1980.

Table 4. Some characteristics of community metabolism along a transect of the littoral zone of Lake Vechten on September 9, 1980. See Fig. 2 and text for further explanation.
R = respiration of autotrophs and heterotrophs.
P_G^{max} = light saturation value of the gross primary production.

station	chlorophyll a content of organic matter (%)	P_{max} mg $O_2.mg^{-1}$chl a. h^{-1}	P_G^{max}/R
2 m; plant top sections	0.17	1.7	1.33
2 m; plant middle sections	0.26	1.2	1.65
2 m; plant lower sections	0.02	0	0
2 m; plant integrated	0.19	1.2	1.21
3 m; sediment	0.05	4.0	1.80
4 m; sediment	0.08	3.4	1.98
5 m; sediment	0.04	1.4	0.65

Particulate organic carbon (POC) and biological oxygen demand (BOD)

A characteristic feature of the littoral zone is its predominantly sandy substrate. This allows to measure POC which takes part in the cycle of the lake (Table 5). The value for the area grown with *Ceratophyllum* refers to the maximum population density of the plants. This could not be established accurately after summer, since by this time the plants got fragile and partly decomposed. The POC values were the highest in summer, and decreased steadily through autumn and winter (Table 5). This was also true for the biological oxygen demand per unit area. Turnover times of POC, assuming 1 mol oxygen to be equivalent to 1 mol carbon, were rather constant throughout the study period, and were comparable for the

Table 5. Vertical and seasonal changes in the biological oxygen demand (BOD) of particulate organic carbon along transect of the littoral zone of Lake Vechten. See Fig. 2 and text for further explanation.

station	months	particulate organic carbon $g.m^{-2}$	BOD $mg.m^{-2}.h^{-1}$	turnover time days
2 m; plant samples	VII + VIII	57	190	43
	IX + X	-	-	30
	XI + XII	-	-	30
2 m; sediment	VII + VIII	-	-	-
	IX + X	15	27	63
	XI + XII	7	13	76
3 m; sediment	VII + VIII	9	16	67
	IX + X	6	14	59
	XI + XII	6	10	71
4 m; sediment	VII + VIII	19	26	82
	IX + X	9	21	63
	XI + XII	5	9	59
5 m; sediment	VII + VIII	37	46	93
	IX + X	12	30	51
	XI + XII	6	10	58

different sediment samples. The periphyton associated with *Ceratophyllum*, however, had always a shorter turnover time than was the case with the sediment associations. This may reflect the greater autotrophic participation in community metabolism. As reported earlier (Progress Report 1979, par. 3.3.3), the attenuation of light in dense stands of *Ceratophyllum* results in a gradient in the distribution of algae upon the macrophytes leading to considerable differences in the turnover times. Periphyton collected from the plant tips had a mean turnover time of 26 days, and that from the lower plant parts, where live algae were scarce, had values similar to those for the organic matter from the sediments.

Since autotrophic activity was minor, the BOD of sediments would for the greater part be due to mineralization of sedimenting particles. When the BOD values are converted to decrease rates of organic matter they are of the same order of magnitude as the sedimentation rates measured by Verdouw (personal communication) during 1979.

Oxygen exchange in the light

Response to light depended on the origin of the samples with respect to the *in situ* light conditions (Fig. 2). Thus, the oxygen exchange rates of periphyton collected from the lower plant parts did not, or only poorly at best, vary with illumination, and the reaction of the sediment samples depended on the depth they were taken from. In Table 4 characteristics are given for the samples of September 9, 1980, when the lake's carbon cycle was subject to an integrated study and the littoral development was at its maximum. Using the oxygen method, only the value of gross primary production (P_G) can be established since the oxygen consumption in dark (R) is due to respiration of both the algae and heterotrophic organisms. Light saturated rates expressed per unit chlorophyll (P_{max}) were within the range of values known for phytoplankton, but for those for the periphyton from the lower parts of the plant.

The ratio of autotrophic to heterotrophic activity in the communities, P_G^{max}/R, can be used as an index, where P_G^{max} is the light satured value of P_G. Should the algal respiration rate equal 10% of the maximum gross photosynthesis, no heterotrophic organisms would be expected when $P_G^{max}/R = 10$. However, the found values of the index were less than 2, implying that oxygen consumption in the dark due to heterotrophic organisms exceeded algal respiration by at least factor 4. Over a 24 hours period the heterotrophic oxygen consumption was always higher than the gross primary production

Laboratory studies (J. Werkhoven)

Photo-heterotrophy of pennate diatoms may explain their predominance in the periphyton of the littoral zone of Lake Vechten. This hypothesis is being tested using continuous cultures of *Nitzschia palea*. Good progress has been made on the nutrient requirements of this alga and on the prevention of algal growth upon the walls of the culture vessels.

2.2.4. Phytoplankton - zooplankton interactions (R.D. Gulati, K. Siewertsen and G. Postema)

The abiotic factors, namely, underwater light climate and water temperature play an important role in controlling the population dynamics and production rates of phytoplankton in winter and early spring. The nutrients, N and P, particularly the latter, may limit production in the spring period when the first maximum of primary production is recorded. The biotic factors, grazing by zooplankton in particular, progressively become more important as the season advances.

The zooplankton grazing studies in Lake Vechten during the years 1971-1978 have repeatedly demonstrated that in May and June the herbivores remove a significant proportion of seston mass (Ø 33 µm), i.e., between 8 and 62% per day in this period. Closely linked to grazing is the process of nutrient regeneration by zooplankton. Besides the egestion process which perhaps regenerates detritus rather than nutrients directly, the biochemical oxidation of assimilated material during metabolism releases excretion products such as NH_3-N, dissolved org-N, PO_4-P and dissolved org-P.

The present study concerns the community metabolism, i.e., the rates of respiration and recycling of nitrogen (NH_3-N) and phosphorus (PO_4-P). The rates were measured in simultaneous laboratory experiments during the course of 1980,

Table 6. Means with S.D. of oxygen, nitrogen and phosphorus ratios calculated from the biochemical composition of zooplankton and their food and those found in the excretion products. Theoretical maxima of these ratios for the biochemical constituents are also given for comparison.

	O:N	N:P	O:P
Zooplankton	14.5 ± 1.0	13.4 ± 1.1	193 ± 3.5
Food	17.9 ± 1.6	11.6 ± 0.4	207 ± 14
Metabolic products	21.3 ± 5.0	5.1 ± 1.3	112 ± 31
Carbohydrates	∞	–	∞
Fats	475	0.3	138
Proteins	7.7	25.4	197

using a range of water temperatures prevalent in the lake and the entire community of zooplankton, excluding microzooplankton. The data on zooplankton and on phytoplankton primary production of 1978 are used to compute the excretion rates and the phytoplankton requirements of nutrients in relation to supply by zooplankton.

Specific excretion rates

The increase in NH_3-N excretion rates with that of temperature (4-24.3°C) was markedly higher than that in PO_4-P (Fig. 3). The former increased from 7.6 to 55.5 µg N mg^{-1} zooplankton carbon, and the latter from 1.2 to 5.6 µg P.mg^{-1} zooplankton carbon as the temperature rose from 4 to 24°C. In both cases there was a significant correlation between temperature and excretion rate ($P < 0.05$). The Q_{10} calculated from the regression formulae (Fig. 3) was respectively 2.5 for N and 2.1 for P. It is likely that relatively more fats are metabolized at lower temperature but more proteins at higher temperature. It follows that N:P ratios of the excretion products will increase with the rise in temperature. Moreover,

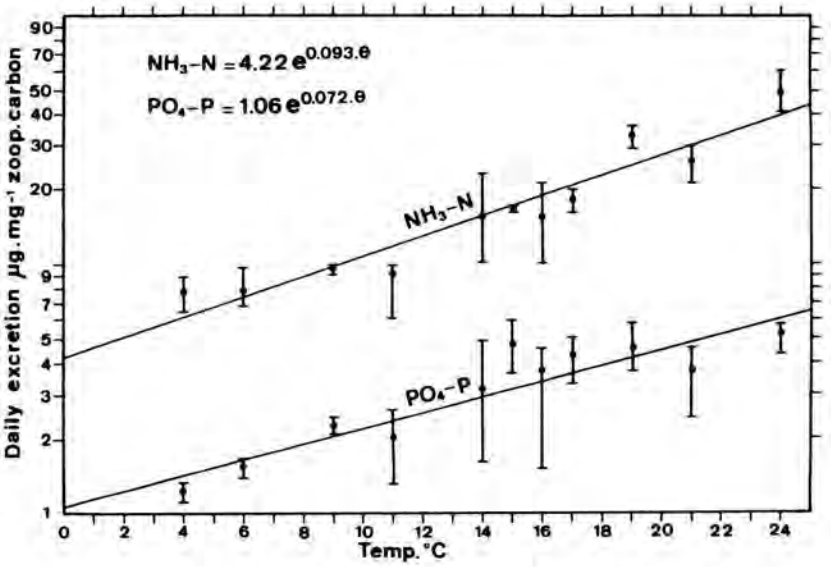

Fig. 3. NH_3-N and PO_4-P excretion rates (Y) of zooplankton as exponential a, function ($Y = ae^{b\theta}$) of water temperature (θ). Shaded circles and horizontal bars, are, respectively, the means and standard deviation of the rates.

changes in the amount of carbohydrates oxidized will not effect the N:P ratios but influence O:N and O:P ratios. The changes in these ratios with increase of temperature are discussed in the light of the seasonal variations in the biochemical composition of zooplankton organisms and their food.

The O:N:P ratios

The average O:N, N:P and O:P ratios in the metabolic products, for the range of temperatures employed, are compared with those obtained from the biochemical composition of zooplankton organisms and of their sestonic food in Lake Vechten (Table 6). Nitrogen in lipids and proteins was assumed to be, respectively, 0.61 and 17.80%, and phosphorus 2.1 and 0.70%; oxygen required to oxidize 1 g each of carbohydrate, fats and protein was assumed to be 1.17, 2.90 and 1.38 g O_2 (obtained from textbooks of comparative physiology).

The O:N ratios calculated from oxygen consumption and nitrogen excretion rates are higher than those in the food assimilated and in the zooplankton, indicating that carbohydrates and fats are metabolized at a higher rate than they are taken in with food. Moreover, the relatively lower O:N ratio of the animals is due to proportionally higher protein in their contents than in the part metabolized. The ratio in the excretion products decreased as the temperature increased indicating a relative increased participation of proteins in the metabolism.

The N:P ratios exhibited a trend opposite to that noticed in the case of O:N ratios, viz., a decrease in the metabolic products compared to the ratios in the food and in the animals. This is, nevertheless, expected if assimilated proteins are retained by zooplankton at the cost of fats which, when metabolized, will release three times more phosphorus per unit mass and tend to lower the N:P ratios. The rise in the N:P ratios with increased temperature confirms the decrease in the case of O:N ratios. Though the changes in N:P ratios of the excretion products give information about the substrates - fats and proteins - being metabolized, little can be said about the carbohydrate metabolism. Since the carbohydrate content of the food assimilated is relatively the highest in the summer period, their role in metabolism may not be ignored. Lower N:P ratios in winter (Fig. 4) are due not only to the increased fat metabolism but also to the decrease in the role of proteins. Considering the theoretical N:P ratios of 0.3 in fats and 25.4 in pro-

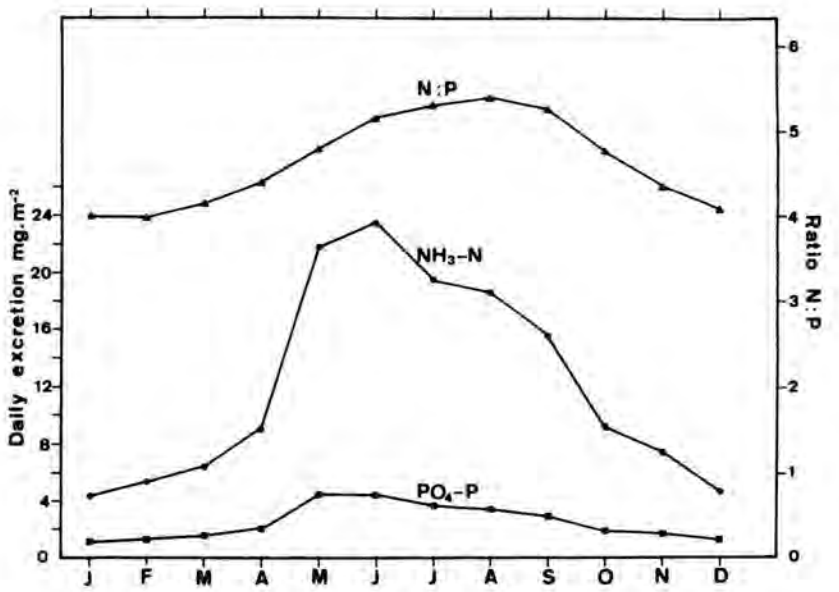

Fig. 4. The daily N and P excretion rates and their N:P ratios of zooplankton in Lake Vechten during 1978. The areal computation based on the regressions in Fig. 3 and zooplankton mass and water temperature in the strata between surface and 3-6 m and 3-6 m and oxygen zero depth.

teins and 5.1 ± 1.3 in excretion products (Table 6), it would require approximately 4 parts, or 80% fats and 1 part or 20% proteins in the substrate to be oxidized to produce a ratio of 5.1 (0.8 x 0.3 + 0.2 x 25.4 = 5.3). It is therefore clear that even a 1% decrease in protein in the metabolic substrate will produce a marked change, i.e., a decrease of 0.25 (0.01 x 25.4) in the N:P ratio, the role of fats in influencing the ratio being less important. Lastly, the relative proportions of proteins and fats in zooplankton, 63:19% or 3.3:1, are in sharp contrast with their respective proportions of 1:4 in the excretion products. From the above discussion it can thus be deduced that metabolic turnover of fats in zooplankton is 13.2 times (4 x 3.3) faster than that of the proteins.

The mean O:P ratio of 112 ± 31 in the metabolites is the lowest compared to those of zooplankton organisms and their food. It is striking that the range of this ratio is quite close to the theoretical ratio for the fat metabolites, and thus reflects the dominant role of fats in the zooplankton metabolism. However, considering the contribution of carbohydrates and proteins the found O:P ratios are rather high, and can only be explained if one assumes that phospholipids may be involved in the metabolism more actively than non-phosphate lipids.

Primary production and zooplankton nutrient regeneration

Both from the zooplankton excretion studies on Lake Vechten and from the relevant literature on other lakes it has become increasingly evident that N and P regeneration by zooplankton may play a significant role in meeting the nutrient demands of phytoplankton. The specific N and P excretion rates (μg N or P mg^{-1} zooplankton-C.d^{-1}) were converted to real rates for Lake Vechten using the seasonal zooplankton biomass data (mg zooplankton-C.m^{-3}) for 1978 of the two depth strata 0-3.6 m and 3.6 m - oxygen zero depth. This was integrated to get the areal excretion rates (mg N or P $m^{-2}.d^{-1}$).

The temperature correction was applied using the temperature-excretion rate relationship in Fig. 3 and the mean temperatures of the two depth strata. The nutrient demand of the phytoplankton was computed from the primary production (^{14}C uptake rates) of 1978 using a C:N:P ratio of 40:7.5:1. The results are summarized in Table 7.

The supply of nutrients by zooplankton is related closely to the water temperature and zooplankton mass, as well as with the grazing and assimilation pattern. The supply of N and P by zooplankton increases sharply in May when it fully meets the phytoplankton demand. From June onwards the supply covers substantially the N (36-162%) and P (49-239%) demands of phytoplankton (Table 7). It may

Table 7. Mean daily demand of N and P by phytoplankton in Lake Vechten during 1978, the daily supply of these nutrients by zooplankton, and supply as percentage of demand.

Period	Demand ($mg.m^{-2}.d^{-1}$)		Supply ($mg.m^{-2}.d^{-1}$)		Supply as % of demand	
	N	P	N	P	N	P
January	30.5	4.1	3.9	0.9	12.7	22.0
February	41.8	5.6	7.2	1.7	17.3	30.0
March	35.4	4.7	8.9	1.9	25.1	41.7
April	42.1	5.6	10.8	2.9	25.7	39.4
May	26.0	3.4	25.1	4.8	96.7	142.0
June	58.4	7.8	28.9	5.1	49.5	64.9
July	27.6	3.6	9.9	1.8	35.9	48.8
August	31.7	4.2	22.9	3.8	72.1	91.4
September	13.0	1.7	11.5	2.2	88.1	127.0
October	8.0	1.1	4.9	0.9	61.0	84.5
November	5.0	0.7	8.1	1.7	162.0	239.0
December	8.5	1.1	5.6	1.2	66.1	110.0

be remarked that the nutrient supply via zooplankton is based on minimal excretion rates since the experiments to measure excretion were carried out in filtered (0.7 µm) lake water and the animals were starving. Moreover, data on dissolved org.N and P fractions are not included in the calculations because of their poor reproducibility. Also a C:P ratio of 40:1 employed to calculate the phytoplankton P demands is one of the lowest encountered in the published literature; a C:P ratio of 60:1 in algae is perhaps not an overestimate but may mean that P supply via zooplankton would more or less completely meet the daily demands of phytoplankton. The nutrients mineralized by zooplankton via their egestion products and autolysis on death, though not included in the above estimates, may not be unimportant as a source of supply.

In summary, the role of zooplankton in nutrient regeneration and supply may be all the more significant during the stratification period (May-September). During this period there is a net loss of nutrients to the anaerobic hypolimnion because of sedimentation of the particulate material. In the absence of a 'fluvial' input in Lake Vechten the nutrients supplied by zooplankton, coupled with microbial breakdown of algae in the surface waters and autolytic release from dead or dying algae, should to a great extent prevent their becoming limitative in the upper productive layers.

2.2.5. Zooplankton sampling techniques (D. de Vries and W. van Houten)

The routine zooplankton sampling in Lake Vechten has been carried out employing a 5 l Friedinger sampler (length 60 cm). The main purpose of the present investigation was to confirm if the capture efficiency of this sampler was comparable with other samplers in use, namely: Ruttner (1 l, 28 cm), Van Dorn (3 l, 45 cm), Haney (3.7 l, 26 cm), and Schindler (34 l, 50 cm).

The lake was sampled on four occasions, including once during night, in the period March 12 - June 26 at 1.5 and 5.3 m depths. Five replicates were taken with each sampler on the first two dates and three each on the subsequent ones at both the depths. The samples were sieved through a filter mesh of 33 µm so that, besides the crustacean zooplankton, rotifers were also included in the comparison. In the case of the Schindler sampler, the samples, because of the large volume, were subdivided into 10 equal parts using Kott's apparatus. This was also employed in case of samples taken with other samplers when the zooplankton concentrations were high. The replicate counts of each sampler were first tested for normality. Bigger volumes gave lower numbers of significant departures from normality and lower values of dispersion.

The relative capture efficiencies of the samplers were calculated by using Student's t-Test for significant differences ($P < 0.05$) in the mean densities per litre for each species caught by a pair of samplers, say *Keratella cochlearis* mean for Schindler was compared with Haney, and that of Haney with Ruttner and so on. This was repeated for all the species and all sampler combinations. The number of times a sampler had a significantly higher or lower capture (nos. l^{-1}) for a species than the other sampler it scored, respectively, positive or negative; in the remaining cases the means were not different, and the capture efficiencies comparable. The Schindler sampler was compared to all other samplers, but ex-

Table 8. A comparison of significantly ($P < 0.05$) low and high catch percentages and intensity indexes of these catches for different samplers.

Sampler	Low catches		High catches	
	%	index	%	index
Ruttner	7.5	0.50	17.5	0.46
Van Dorn	15.0	0.75	5.0	1.00
Haney	15.0	0.75	12.5	0.83
Friedinger	15.0	0.86	12.5	0.83
Schindler	70.0	0.37	10.0	0.57

cluded for the comparison of the other four samplers. This, because the Schindler departing the most from the others in having the lowest relative capture efficiencies, could bias the comparison of the others.

Considering the 3 sampling dates (thus excluding night samples), 2 sampling depths, and 6 or 7 species encountered, 40 is the maximum score (March 12, 2 depths x 6 species = 12; April 15, 2 depths x 7 species = 14; and June 25, 2 depths x 7 species = 14). The Schindler caught in 28 out of the 40 cases significantly lower than the other samplers and thus had 70% low catches; only in 4 out of 40 cases, i.e., in 10% of the cases, had it catches higher than the other samplers (Table 8). The index of significant low or high catch was obtained from x/y where x is number of cases a sampler caught low or high out of the 40 cases as above and y is the sum of low or high catches for a sampler compared with all the other samplers. The Schindler caught in 75 cases significantly lower when compared with the other samplers, and has thus the lowest catch index of 0.37 (28/75). The Ruttner and Friedinger appeared to be better than the others, though the former had, besides relatively high capture efficiencies for rotifers, one of the lowest for the crustacean zooplankton. The Haney and Van Dorn, comparable in the sampling volume, had somewhat lower efficiencies than the two mentioned already.

The samplers differed the most with regard to *Keratella cochlearis*. The data are being analysed further to compare the avoidance reaction of animals with different samplers in light and dark. The depth related differences in the capture efficiency were qualitative rather than quantitative.

In addition to the above studies the catch efficiencies of two versions of the Schindler sampler were compared. One of these samplers has an opening on the top lid, covered with 33 µm nylon gauze, to allow passage of air to facilitate filtration of zooplankton on the other end, and the other has no such opening. The mean zooplankton counts of the five samples taken with each of these versions of the sampler did not differ significantly ($P < 0.05$).

The zooplankton sampled with a Friedinger sampler at surface to 3 m and that with a closing-net sampler at this depth when compared did not reveal any significant differences for the species encountered. However, in the 5.4-8.4 m strata, the closing-net caught significantly less than the Friedinger in the case of 5 out of 13 species found. Lastly, zooplankton concentrations based on a haul with the closing-net from surface to 8.4 m, and those on three hauls in the column of water (surface - 3 m; 3-5.4 m and 6-8.4 m) did not differ. The conclusion derived from this is that the turbulence caused by net haul over the entire column at one stretch was not more than that by three separate hauls in the column.

2.3. WORKGROUP 'MINERALIZATION OF ORGANIC MATTER'

2.3.1. Introduction (Th.E. Cappenberg)

As part of the coordinated and integrated ecosystem research in Lake Vechten the main goal of the studies has been to investigate the dynamics of the cycling of carbon, nitrogen, phosphorus and sulphur in the limnetic region and in the sediments. Emphasis was laid on aerobic and anaerobic mineralization of organic compounds, produced during primary production processes, to their inorganic states. Particular attention was paid to the kinetic aspects of mineralization, using labelling and chromatographic techniques. Also, ecological interrelations between micro-organisms observed in the field, are being tested in the laboratory. The organisms are cultured under defined conditions using chemostats. Their kinetic relationships are studied using labelled compounds.

Besides in the diurnal study (see 2.5.3) all members of the group were involved in a bi-monthly monitoring of physical, chemical, and biological parameters, carried out at 1.2 m depth intervals in the lake and in the sediments.

The main conclusions from these observations are:

a. The concentration of dissolved organic matter (TDOC, filtrate of 0.45 µm membrane) shows no appreciable seasonal changes during the year (mean values of 4.5 to 6.5 mg $C.l^{-1}$ in the water-phase, and of 12.4 to 15.3 mg $C.l^{-1}$ in the sediment), in contrast to the pool of particulate organic matter (POC, size fraction between 104 and 0.45 µm). Both in the epi- and in the hypolimnion there was an

increase of POC-pool during the summer stratification from 0.5 to 1.6 mg $C.l^{-1}$. After the autumn turnover POC-values declined sharply to about 0.2 mg $C.l^{-1}$. So this pool (consisting of about 20% of living material, see 2.3.2) has a highly dynamic behaviour, in contrast to the TDOC-pool, which mainly consists of refractory organic material. Only a relative small portion (about 5%, see 2.3.2) shows high turnover rates in supplying carbon and energy sources for heterotrophic bacterial growth.

b. The atomic ratios of C:N:P, calculated for the various chemical fractions of the water layers of 0 to 6 m (epilimnion) and of 6 to 9.6 m (hypolimnion) were, respectively, 573:42:1, and 138:35:1, compared with 414:43:1 during the circulation period. The atomic ratios of C:P are extremely high in the upper layers, indicating limiting phosphorus conditions for phytoplanktonic growth. Further, a decreasing ratio of C:N was noted during the stratification in lower layers, since C-carbon compounds are more rapidly mineralized than N-carbon ones. These observations are contrary to those in the sediments, where the C:N ratio increased from about 11:1 to 14:1 in the deeper mud layers, indicating a relative faster mineralization rate of N-compounds (proteins) in comparison to C-compounds (cellulose).

c. From the field data on oxygen and sulphate the mineralization of carbon in the metalimnion (6 to 8 m depth) and hypolimnion (8 to 11 m depth) was calculated, taking in account the relative importance of each microbial reaction to total organic carbon oxidation. For O_2, SO_4^{2-} and CO_2 reduction the amount of carbon mineralized was taken to be the stoichiometric relationship between electron acceptor reduced as predicted by the oxidative reactions of organic matter. Vertical diffusion corrections were necessary across the 6 m depth contour and were made using the equation $F = -D.d_c/d_x$, where F is the flux rate in $moles.cm^2.sec^{-1}$, D is the average eddy diffusion coefficient ($1x10^{-2}$ $cm^{-2}.sec^{-1}$), and d_c/d_x is the concentration gradient in $moles.cm^{-3}.sec^{-1}$. The measured consumptions and fluxes were calculated for the metalimnion and hypolimnion on a molar basis and compared with the input of carbon calculated from ^{14}C-incubations of phytoplankton and anaerobic photosynthetic bacteria (see Table 9).

At the onset of stratification in May the amount of mineralization of carbon for O_2-reduction was 6%, for SO_4^{2-}-reduction was 2%, for CO_2-formation over the dissolved inorganic carbon pool in the hypolimnion was 7.1%, and for CO_2-reduction was 12.8% of the primary production in the pelagic zone. Summing up, this means a mineralization of about 28%, the rest must be attributed to mineralization and respiration processes in the aerobic zone, and partly burried more or less permanently into the sediments. In August, when the stratification is optimal, the percentages were, respectively: 5.7, 1.2, 18 and 14.4, giving a value of 39.3% anaerobic mineralization of the limnetic production. These are necessarily the theoretical, maximal values. More accurate data are needed to quantify *in situ* these different mineralization processes. Special attention is also needed to the aerobic mineralization in the lake but no method is yet available.

Table 9. Range (mean in brackets) of primary production of photoautotrophs inhabiting various strata in Lake Vechten during summer stratification (period June 16 - October 23, 1980). Values are given as daily C-fixation in kg for the whole volume of the lake.

depth	range in kg $C.d^{-1}$	percentage (%)	type of photosynthesis
0 - 5 m	3.585 - 21.100 (9.857)	45.6 - 91.3 (67.3)	oxygenic by eukaryotes
5 - 9 m	0.152 - 10.576 (3.667)	1.1 - 38.6 (20.3)	oxygenic by eukaryotes and prokaryotes
	0.204 - 2.197 (0.979)	1.5 - 14.1 (7.8)	anoxygenic by prokaryotes
	0.399 - 4.203 (1.846)*		
0 - 9 m	4.266 - 33.873 (15.359)		oxygenic + anoxygenic

* Photosynthetic rate as measured under aerobic conditions.

In Fig. 5 a general scheme of the interrelationships between the C-, N-, P- and S-cycling processes is given, and the successive involvement of oxygen, nitrate, sulphate and carbon dioxide as electron acceptors in mineralization of the photosynthetically formed organic matter is illustrated.

2.3.2. Aerobic mineralization (J.J. Olie, C.L.M. Steenbergen, H.J. Korthals, M.J. Bär-Gilissen, H.R. Kwist, M.A.C.I. Blaauboer-Wiercx and H. Stam)

General

The field study on the relationship between the release rates of extracellular organic products of the phytoplankton and numbers and activity of the heterotrophic bacteria was finished. This year the carbon production of a spring bloom of phytoplankton and its subsequent mineralization was quantified. The photosynthetic carbon fixation, excretion of photosynthetic products, respiratory turnover, and uptake and mineralization of the DOC and POC pools were measured simultaneously in the laboratory. Also the activity and total numbers of heterotrophic bacteria during the season were measured. The complex relationships between extracellular release of carbon compounds by autotrophic growth and its subsequent uptake by the bacterial flora was also studied by isolating relevant bacterial species and culturing them under chemostat conditions.

Bloom studies

Parameters concerning the fixation and mineralization of carbon were measured weekly from February to April. Seston (< 125 µm) was concentrated and incubated with $NaH^{14}CO_3$ to obtain ^{14}C-labelled POC and DOC fractions. In experiments with lake water we used filters with living and dead seston $PO^{14}C$ material for studying the respiratory turnover and mineralization. The uptake of $DO^{14}C$ was measured by submerging these filters in lake water with and without addition of ^{14}C-bicarbonate. The variation of the duplicates did not exceed 10% of the mean. Corrections were made for label adsorption and for dark fixation of $NaH^{14}CO_3$. The pH was maintained constant throughout the incubation period.

The correlation between the concentration of chlorophyll-a and primary production, as well as that between the numbers of the dominant algal species (*Chlorella* and *Asterionella* species) and primary production, was good. Also a good cor-

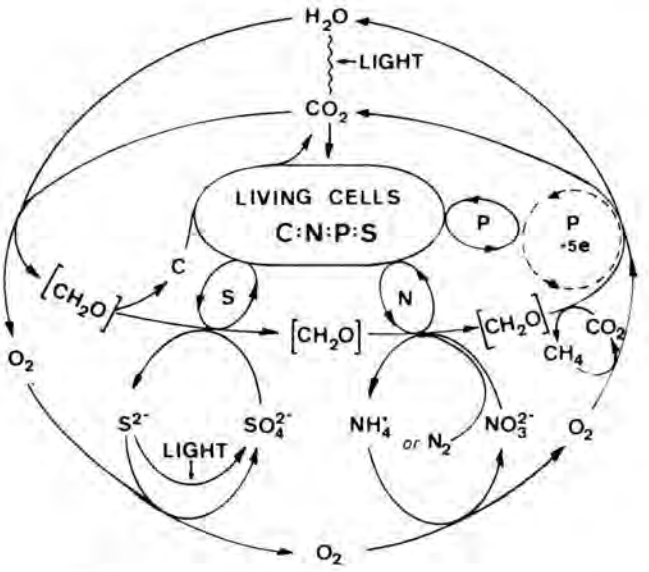

Fig. 5. General scheme of the interrelationships between the C-, N-, P- and S-cycling processes in aquatic ecosystems, and the successive involvement of oxygen, nitrate, sulphate and carbon dioxide as electron acceptors in mineralization of the photosynthetically formed organic matter.

relation was found between the primary production and extracellular release of carbon; the latter never exceeded 7%. In the mineralization experiments a reverse correlation was noted between the DO^{14}C produced and primary production. The DO^{14}C produced did not exceed 4% of the net primary production, indicating that the mineralization rates in these laboratory studies were extremely low. A similar but yet unexplained reverse correlation existed between the respiratory turnover and net primary production, although the respiratory rates found (17 and 27% of the primary production) are not unrealistic. More precise data on mineralization of the DOC- and POC-pool and their respiratory turnovers are needed. These may be obtained by concentrating seston from the lake not by centrifugation or filtration, but by sedimentation for short periods in a sedimentation trap, and subsequently incubating this material in laboratory diffusion systems using double labelling techniques in order to know more precisely the diverse pool sizes of carbon fractions and their conversion rates into carbon dioxide.

Bacterial activity

The flow of DOC into heterotrophic organisms can be studied in several ways. An approach to investigate the mineralization of extracellular products is to measure the metabolic activity of heterotrophic bacteria in the field. Thus, the total numbers of bacteria were counted using epifluorescence microscopy, combined with Electron Transport System staining. The ETS-system can be used, by reducing INT (2-(p-iodophenyl) 3-(p-nitro-phenyl) 5-phenyl-tetrazoliumchloride) to a red formazan for which reduction equivalents are required, as a measure of actual activity of the bacterial population. A combination of the field observations and tests with pre-cultured bacterial isolates did not give reliable figures, since chemical and biological reduction on the filters interfered with the results. So no correlation was found between the ETS-activity and bacterial numbers.

For studying the uptake of the excreted DOC-pool by bacteria, the antibiotic agent gentamycine was used as an inhibitor of bacterial activity during the incubation of field samples with ^{14}C-labelled bicarbonate. The inhibition of bacterial activity was incomplete and the variation considerable. The lack of response to the antibiotic could be due to the relatively high concentration of Ca^{2+} in the lake water. Employing a chelating agent (EDTA) to reduce the Ca^{2+} concentration produced undesirable side effects. Consequently, gentamycine as a bacterial inhibitor was a failure under natural conditions in Lake Vechten.

The use of differential filtration of the algal and bacterial fractions in lake water using Nucleopore filters after incubation of ^{14}C-labelled bicarbonate gave promising results. About 93 to 97% of the radioactivity associated with bacteria passed through a 5 µm filter, and was retained on a 0.2 µm filter. When the controls for both algae and bacteria (bacteria in algal fraction and vice versa) are included, differential filtration seems a useful method to separate phytoplankton from bacterioplankton. The technique will be employed in studies on the flow of carbon compounds from one component to another.

Microbial biomass indicators

Besides to activity studies much attention was paid to the development of useful methods to determine microbial biomass. The relation between the phytoplankton chlorophyll and adenosine triphosphate (ATP) was investigated during a year cycle in Lake Vechten. Simultaneously amounts of POC (> 0.5 µm) and dissolved carbon were separated and determined. Chlorophyll concentration has long been used as an index of algal biomass. ATP assays have offered some promise in discriminating living from dead or detrital material, because of the rapid break-down of ATP in extracellular solution or dead microbial cells. POC contains both living and dead organic carbon and may be expected to follow the productivity and biomass distribution of total microbial populations rather closely in oligotrophic to mesotrophic lakes.

In general, samples from the mixed layer (0 to 6 m depth) showed good consistency of chlorophyll:ATP ratios (mean, 18 ± 4.6; n = 23). Thus, over a year, the ratio fluctuated by not more than 25% around the mean. During the summer stratification extensive fluctuation of the chlorophyll:ATP ratio was found in the meta- and hypolimnion layers, with 10 to 20 fold increase in the ratio at 8 to 9 m depth. Consequently, the applicability of chlorophyll and ATP concentrations as quantitative biomass indicators in these strata seems very doubtful. The correlation

between the POC values and chlorophyll concentrations was poor. In contrast, a positive and significant correlation ($r = 0.8$; $P < 0.001$; $n = 23$) was found between the POC values and ATP concentrations. This is in agreement with the above expectation. Thus, the ATP concentration may be considered a rather precise indicator of total microbial populations including phytoplankton, bacteria, protozoa and zooplankton (< 104 µm).

Over the year the POC values exceeded by about four times (mean of 3.9 ± 1.0; $n = 23$) the amount of living carbon, calculated using a carbon to ATP ratio of 276:1 which is considered to be a reasonable good estimate. So, on the average, the POC values consisted of 20% of living and the rest of dead organic carbon. Finally, the linear regression equation between ATP and chlorophyll concentrations, found over the year, was calculated. From this equation a rough estimate of algal biomass versus non-algal biomass was derived. It seems that on the average 18.7% of total ATP originated from non-algal sources. These microbial biomass indicators will be used in future studies on the cycling of various elements in diverse habitats.

2.3.3. Nitrogen and phosphorus cycle (H. Verdouw, E.M.J. Dekkers, P.C.M. Boers, A.G. Wisselo and A.J. Moons)

General

For the second year in succession the P-cycle in Lake Vechten was investigated. The main purpose is, like in the case of the C and N cycles, to quantify the mineralization of organic P-compounds to inorganic ones, and their subsequent uptake in autotrophic and heterotrophic growth.

Nitrogen cycle

The studies on the concentration patterns of various nitrogen compounds and sedimentation measurements have been concluded. The main research subject at present is the study of the role of hypolimnetic and sedimentary ammonia in the nitrogen cycle. Transport of hypolimnetic ammonia must be an important factor in the nitrogen supply of the epilimnion during the stratification period, because of the existing strong concentration gradients below the thermocline. Calculations of ammonia flux through the 8 m contour, using the measured concentration gradients, indicate that transport coefficients in the order of 4×10^{-2} $cm^2.sec^{-1}$ in the hypolimnion have to be assumed, in order to balance the downward nitrogen flux connected with the sedimentation of particulate matter (see Fig. 6).

Calculations of the coefficient of eddy diffusion, using observed heating rate and temperature increase as a function of depth during the heating period of the lake, gave values of about 1×10^{-2} $cm^2.sec^{-1}$ in the waterlayers from 5 to 9 m. Apparently, more detailed knowledge on transport processes in the hypolimnion is required.

The work on ammonia turnover rates in the sediments is in progress. Results of the methodological work on the $^{15}NH_3$ labelling and recovery in sedimentary ammonia would enable the start of experiments on Lake Vechten cores in the spring of 1981. Detailed determinations of sediment composition have been carried out in intact cores taken by SCUBA diving at several places in the deepest area (10 m) of the eastern depression. The percentages dry weight, total carbon, carbonate, nitrogen, organic matter and exchangeable ammonia, all as a function of depth in the sediment (in slices of 2 cm, up to 44 cm depth), were determined. Organic-rich sediment was present in the upper 20 to 30 cm, depending on the sampling station. Below these depths a firm layer of clay mixed with sand was found. The gradients of the measured parameters were confined to the upper 20 cm, suggesting that this is the active layer involved in nutrient exchange studies with the overlying water.

Phosphorus cycle

The changes in the amount of different P-compounds (PO_4-P, P_{diss}, P_{tot}) were measured monthly at four sampling stations in the eastern depression of Lake Vechten in 1.2 m water strata from surface to bottom. The concentration patterns did not significantly differ from those in the preceding year. The mean concentrations in the photogenic zone were: PO_4-P, 5 $mg\text{-}m^{-3}$; P_{diss}, 9 $mg.m^{-3}$; and P_{tot}, 11 $mg.m^{-3}$. From these data, together with those on primary production and on

excretion products of zooplankton (see 2.2.4), a scheme of the P-cycle in Lake Vechten is given (Fig. 7). The daily uptake of phosphate can be estimated by comparing the concentration in the water and the rate of uptake associated with the primary production. From calculations based on the primary production rates, assuming a C:P ratio of 50:1 in phytoplankton, a daily phosphorus-uptake of about 0.7 $mg.m^{-3}$ can be calculated. Given a mean seston concentration of 3.72 g $C.m^{-2}$ or 74 mg $P.m^{-2}$, a mean depth of the photogenic zone of 5.1 m and a C:P ratio of 50:1, a phosphorus turnover time of about 20 days can be derived (74 x 1/5.1 x 1/0.7 = 20). Next year this turnover rate will be calculated more precisely, together with studies on the rates of seston-P to organic dissolved P, and of organic dissolved P to PO_4-P using the determination of exchange processes between ^{31}P and ^{32}P.

During a 24-h field experiment, the diurnal change in the PO_4-P concentrations in the lake was followed (see Table 10). In the upper water layers the PO_4-P concentration pattern followed a sinusoid curve, with the relatively high values at night and noon. In the water layers near the bottom (7.2 to 9.6 m) concentrations remained more or less constant over the whole 24-h period. These remarkable changes in ortho-phosphate values indicate that the conclusions regarding the P-cycle based on analyses of concentrations of P-compounds in one single water sample, taken at a fixed time of the day, should be viewed with caution. These 24-h experiments will be repeated to get more information about these exchange processes.

This summer an investigation was started on the exchangeable amounts of total phosphorus in sediments of the Loosdrecht Lakes, in cooperation with the W.O.L.-workgroup (see paragraph 2.1). After surveying the lake bottom area, three representative sampling-stations in the open lake area (in the so-called first, third

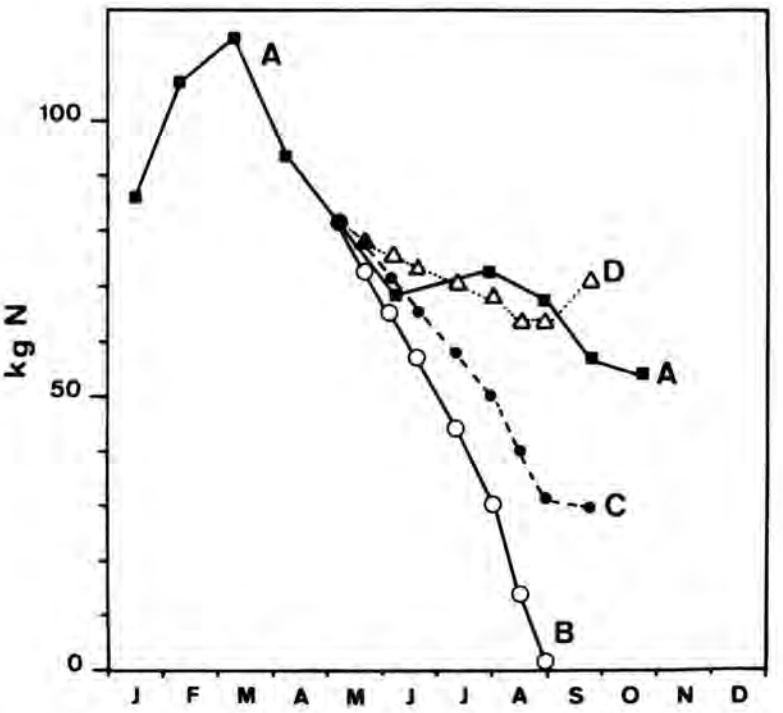

Fig. 6. Nitrogen contents of the water-layers 0-8 m in Lake Vechten, eastern depression.
A. Directly from N-determinations.
B. Calculated from N-content on May 9, 1980 and sedimentation measurements in the following periods.
C. = B, with correction for ammonia flux through the 8 m plane, based on measured concentration gradients, with a diffusion coefficient of 2 x 10^{-2} $cm^2.sec^{-1}$.
D. = C, with diffusion coefficient 4 x 10^{-2} $cm^2.sec^{-1}$.

Table 10. Change in the mean PO_4-P concentrations ($\mu g.l^{-1}$) in Lake Vechten during a 24-hour period (September 8-9, 1980).

Time	Open water (0 - 7.2 m)	Littoral
20.00 h	2	5
1.30	17	15
7.00	4	5
13.00	14	10
20.00	8	9

and fifth Lake) were selected for future studies. The sediments appeared to be rather homogeneous in character with a high humic content (50 to 60% of the sediment could be burnt at 550°C). The total phosphorus content in the sediments was 0.06% on dry wt. basis; roughly 70 per cent was exchangeable.

2.3.4. Anaerobic mineralization and production (Th.E. Cappenberg, C.L.M. Steenbergen, C.A. Hordijk, H.J. Korthals, M.J. Bär-Gilissen, G.J. Jonkheer and A.P.M. Lauwen)

General

In the anaerobic metabolic processes in lake sediments, like in Lake Vechten, organic substrates will be broken down ultimately to the most reduced form (CH_4) and to the most oxidized form (CO_2). These processes involve many microbial

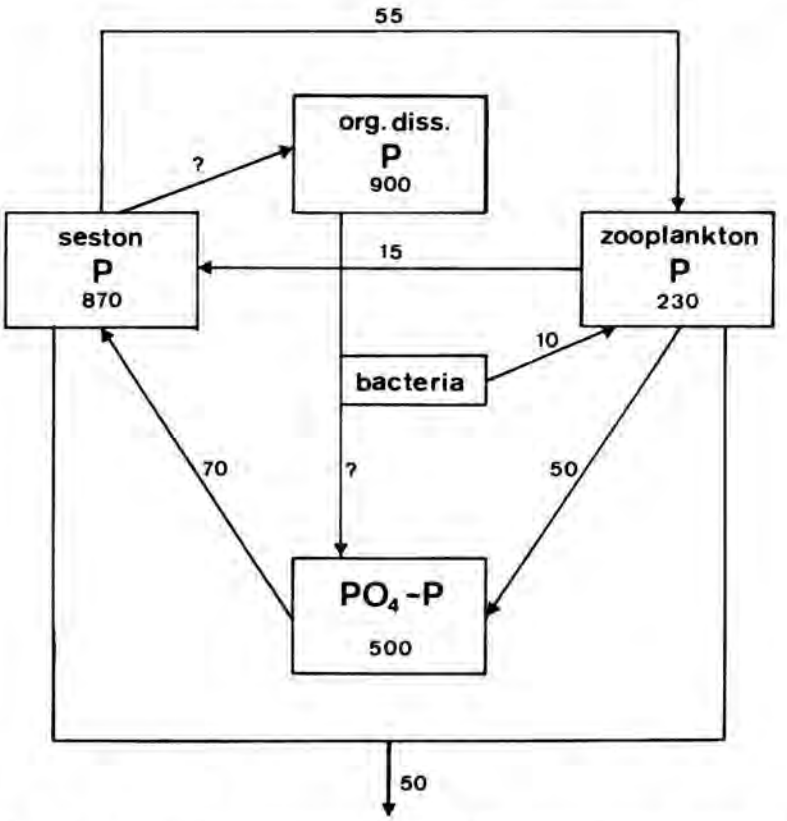

Fig. 7. Schematic representation of the phosphorus cycle in the eastern depression of Lake Vechten (0-6 m depth, volume = $1.02 \times 10^5 m^3$). Annual average in g $P.day^{-1}$ (values x 10^{-2} = $\mu g\ P.l^{-1}$).

species. This microbial food chain starts with hydrolysis of complex organic material such as algal cell wall debris, which settles through the anaerobic hypolimnion to the sediment. It is nowadays well-known that acetate is the main precursor for methanogenesis in these environments. Approximately 70% of the methane produced is derived from acetate which, therefore, is one of the key intermediates in the conversion of organic matter.

Besides CO_2 as electron-acceptor in these processes, also SO_4^{2-} is an important acceptor in the anaerobic layers of Lake Vechten. Below the depth of 8 m, in the absence of light and oxygen, low-molecular-weight breakdown products, such as lactate, are metabolized by anaerobically respiring bacteria. One of the results of these mineralization processes is the formation of H_2S by sulphate-reducing bacteria. In the water layers above, where sunlight can penetrate, this H_2S is used as an electron donor in the anoxygenic photosynthesis carried out by green and purple bacteria (Chlorobiaceae and Chromatiaceae).

Break-down studies

More evidence was obtained that the break-down of algal cell walls is the rate-limiting step in the break-down into low-molecular-weight intermediates, as lactate, acetate and propionate. From a number of isolates obtained from mud samples incubated with algal cell wall components of *Chlorella*, the rates of production of lower fatty acids were recorded (Table 11). Evidently acetate was the most abundant organic compound formed.

Table 11. Production rate of *Chlorella* cell wall material.

Composition of cell walls of *Chlorella* : hemicellulose 59%, cellulose 33%, lignin 2.6%, polysaccharides 2.8%, pectin 1.8%.
Production rate of $\mu mol.hr^{-1}$ from the isolates (ratio 6:3:1).

type of acid	sporeforming rods (gram$^+$)	non-sporeforming rods (gram$^-$)	cocci (gram$^+$)
acetic	2.8	3.2	1.2
propionic	0.08	1.1	2.3
isovaleric	-	-	0.006
butyric	0.003	0.04	-
lactic	0.5	0.6	-

(production of the gases CO_2 and H_2, traces of capronic and isobutyric acid)

With uridine-^{14}C-labelled *Chlorella* cell wall material the kinetic aspects were studied. The samples from various depths of the mud core were incubated under *in situ* temperature. The magnitude of the break-down is given by the disappearance rate from mud samples of ^{14}C-labelled *Chlorella*, and by rate of break-down calculated using first order kinetic reactions. The time (in days) versus ^{10}log of radioactivity plot gave a straight line with a correlation coefficient of r - 0.955. From the regression equation the turnover rate constants (k-values) were calculated for the mud at different depths. Also the pool size of algal cell walls in the different mud layers was determined using extraction and ^{14}C-algal cell wall recovery methods. At the 1 cm depth layer in Lake Vechten sediments a k-value of 0.18 day^{-1} and a pool size of 66 $\mu g.g$ wet mud was found, giving a turnover rate (turnover rate constant times pool size) of 11.8 $\mu g.day^{-1}.g^{-1}$ of algal cell walls. For the 6 cm and the 12 cm layer k-values and pool sizes were, respectively, 0.32.day^{-1} and 80 $\mu g.g^{-1}$, and 0.21.day^{-1} and of 37 $\mu g.g^{-1}$, giving turnover rates of 25.6 and 7.7 $\mu g.day^{-1}.g^{-1}$. On an areal basis the turnover rate is about 25 $\mu g.day^{-1}.cm^{-2}$ and for the whole sediment area in Lake Vechten (below the 8 m contour) about 400 kg C.$year^{-1}$ (calculated from the gross formula $(C_6H_{10}O_5)_n$,

with 40% C). This annual break-down of 400 kilo C compares favourably to the input data of carbon obtained by sedimentation measurements (about 800 kilo C. $year^{-1}$), for the eastern depression of Lake Vechten below the 8 m depth contour.

From the preceding it is clear that the main products from algal cell wall material in the sediment are lower volatile and non-volatile fatty acids. To follow their break-down kinetics accurately measurements of their respective pool sizes are needed. These were performed using high pressure liquid chromatographical (HPLC) techniques. Several derivatization procedures were tested for the determination of C_1-C_5 volatile and non-volatile fatty acids. The resulting p-bromophenacyl esters were separated via either the HPLC or GLC techniques depending on the desired selectivity and sensitivity. For catalytic properties crown ether compounds were used to form p-bromophenacyl ester derivates of the fatty acids. The method is based on the capability of the crown ether, dicyclohexyl-18-crown-6, to effect a solid aprotic solvent transfer of the potassium carboxylates and cause the carboxylate anions to become unusually reactive nucleophiles. HPLC separation was performed successfully on reverse-phase RP-18 columns using acetonitril/water mobile phases. A UV-detection system and a photo-fluorescence detection system were used, respectively. Good results were obtained using 4-bromomethyl-7-methoxycumarine esters with the fluorescence system, determining in the same sample the lactate, formate, acetate, glycolate and propionate esters in the mud. Similarly, these nanomolar determinations with the proper sample treatment and ion-exchange procedure proved to be possible in water samples, and will be used in our kinetic studies to examine the pathways and rates of transformation of organic carbon compounds to their inorganic forms.

Anaerobic photoautotrophs and the sulphur cycle

In the micro-aerophilic metalimnion of Lake Vechten Cyanobacteria find their ecological niche, since they prefer low oxygen tension. Also many of them possess the property of facultative anoxygenic photosynthesis to carry out CO_2 photoassimilation under both aerobic and anaerobic conditions, using, respectively, H_2O and H_2S as electron donors. In the uppermost zone of the metalimnion eukaryotic algae may have their population maximum, e.g., the motile cells of *Mallomonas caudata* (Chrysophyceae), which prefer low light conditions. In a series of experiments the *in situ* rates of oxygenic and anoxygenic photosynthesis were estimated at various depths in meta- and hypolimnion, using the ^{14}C-technique. Light and dark bottles were incubated under aerobic and anaerobic conditions. In the latter case anoxygenic photosynthesis was distinguished from the oxygenic one, using DCMU that specifically inhibits oxygenic or algal photosynthesis.

Based on the shape of photosynthesis-depth curves the stratification period can be roughly divided into two periods, separated by the onset of the metalimnetic bloom of Cyanobacteria at the end of July. Typical results are depicted in Fig. 8. In the early summer period oxygenic photosynthesis showed a maximum CO_2 fixation rate at 1 m depth, but was negligible in meta- and hypolimnion. The shape of the photosynthetic profile is rather common and depends on the relationship between algal photosynthesis and the vertical gradient of light intensity. In contrast, a DMCU-insensitive photosynthetic maximum appeared at 8 m depth. The CO_2-fixation rate at the border line between oxygen and sulphide concentrations was conspicuous. Gasvacuole-containing filamentous Chlorobiaceae, sulphur granules-containing *Chromatium* cells and colonial forms resembling *Thiopedia* were observed at this depth.

The mid and late summer period was characterized by a bloom of chroococcal Cyanobacteria. Based on the vertical distribution of the specific pigments, phycoerythrin and phycocyanin, the population maximum of Cyanobacteria was found at 6.5 m depth. In the same period *Mallomonas caudata* peaked at a depth of 6 m, with densities of about 2×10^5 cells.l^{-1}. The filamentous Chlorobiaceae had their maximum density (about 10^7 filaments.l^{-1}) at a depth of 6.5 to 7 m. The Chromatiaceae dominated at a depth of 7 to 7.5 m. This typical vertical distribution of groups of organisms was reflected in the shape of the photosynthesis-depth curves (Fig. 8).

The algae from the euphotic zone showed a light dependent photosynthetic maximum at a depth of 1 m. The metalimnetic maximum of photosynthesis, which was severely inhibited by DCMU, consisted largely of oxygenic photosynthetic activity carried out by *Mallomonas* and Cyanobacteria. In addition, the photosynthetic rate

in the metalimnion was inhibited upon incubation under aerobic conditions (see Fig. 8 and Table 9). Photosynthetic rate measured under aerobic conditions amounted on the average to only 34% of that measured under anaerobic conditions. Obviously, *Mallomonas* is very sensitive to relatively high oxygen concentrations since maximum inhibition was observed at a depth of 6 m. It is likely that the 'addition' of oxygen to the samples enhanced photorespiration and excretion of soluble organic products of photosynthesis. In the previous years excretion rates amounting from 40 to 60% of total fixation rates were not uncommon during August in samples (containing *M. caudata*) taken from the metalimnion (see Progress Report 1979, par. 3.4.2).

Finally, anoxygenic photosynthesis took place throughout the meta- and uppermost zone of hypolimnion, its maximum was usually found at the oxygen-sulphide borderline. It is most unlikely that the Cyanobacteria contributed to the anoxygenic fixation of CO_2 since sulphide could not be detected in the 6-6.5 m stratum and since Cyanobacterial sulphide-dependent anoxygenic photoassimilation occurs only at relatively high sulphide concentrations.

In summary, taking the total volumes of the various strata of the lake into account, on the average 28% of the daily photosynthetic carbon fixation, during summer stratification, takes place in meta- and hypolimnion (see Table 9). The green and purple bacteria contribute on the average 8% to the total daily photosynthetic production. Assuming H_2S to be the only electron donor in their photo-

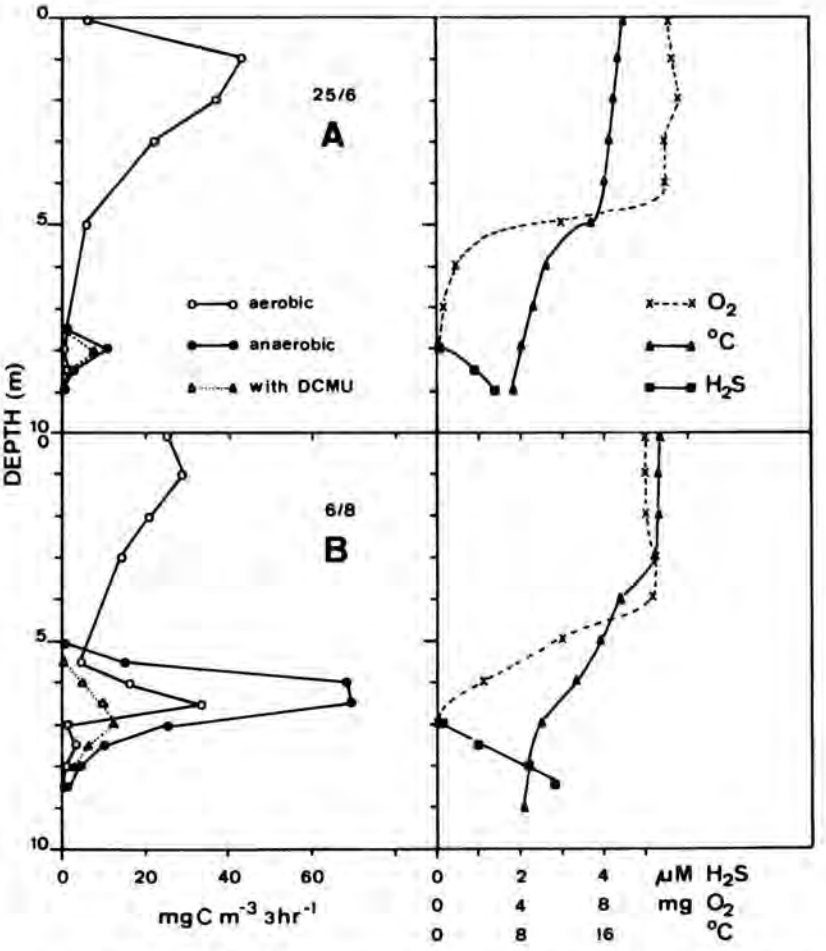

Fig. 8. Profiles of photosynthetic rates in Lake Vechten, measured under aerobic and anaerobic conditions with and without DCMU, and some relevant limnological characteristics at early stratification (A, June 25, 1980) and at maximum stratification (B, August 6, 1980).

synthetic reaction, carbon fixation of 0.2 to 2.2 kg $C.day^{-1}$ is equivalent to oxidation of 1.1. to 12.5 kg $H_2S.day^{-1}$ (average of 5.7 kg $H_2S.day^{-1}$). The total amount of H_2S, detected in the photosynthetically active uppermost zone of the hypolimnion never exceeded 2.7 kg. Although rates of photosynthetic oxidation of sulphide will vary with time and depth, the calculations do give some idea of the order of magnitude of this process. ^{35}S-labelled sulphate and sulphide are being used to obtain more data in the anaerobic zone and in the sediments, together with the quantification of diffusion and turnover rates of acetate and lactate as a possible link between the sulphur and the carbon cycle in the lake.

2.3.5. Water balance of Lake Vechten (J.W. Bos and A.G. Wisselo)

For computing the mass-balance of several chemical elements in Lake Vechten one needs to know the volume of water that moves through the lake. Besides the storage of water in the lake the following components have been studied so far: 1) the rates of groundwater in- and outflow were calculated, using an electrical simulation model, from measurements of the water level in observation wells and in the lake; 2) a relation between rainfall and the level of the lake was determined; and 3) the evaporation was calculated as a function of the available radiant energy and a term combining saturation deficit and wind speed. These data are being analysed further to obtain a water balance of the lake for the year 1980.

2.3.6. Characterization of sedimented organic particulate material (M. Suijker, J.W. de Leeuw, D. van de Meent, Th.E. Cappenberg, H. Verdouw and J.J. Olie)

In cooperation with the Organic Geochemistry Unit, Delft University of Technology, organic material from water and sediment samples during the summer stratification was separated into various fractions by ultra-filtration. The fate of this material during its sedimentation in the lake was examined by comparing the chemical composition of the particulate material. For this, pyrolysis mass spectrometric, pyrolysis gas chromatographic, and CHN analyses were performed, together with microscopic observations and particle distribution studies of the fractions. The data were statistical analysed by computer-multivariate analysis using non-linear and factor mapping.

Preliminary results showed significant correlations between the chemical and structural analyses, and the microscopical observations on the fractions in the various layers. The distributions of particle-fractions did not significantly change with depth in the lake; also, no correlation was found between the chemical and microscopical observations of similar fractions at different depths. Although further analysis of the data as well as observations in other ecosystems are needed, pyrolysis mass spectrometry seems to be a useful tool to characterize the particulate organic matter fractions in an aquatic ecosystem.

2.4. RESEARCHGROUP 'ECOPHYSIOLOGY OF AQUATIC MACROPHYTES'

2.4.1. Introduction (P.H. Best)

Several aspects of the growth of aquatic macrophytes were investigated. Although at first the attention was directed mainly on the predominant submerged species, the floating-leaved and emergent plants were also studied. Field observations on standing crop and related growth rates are supplemented by experiments under controlled environmental conditions; the interpretations are facilitated by the use of simulation models.

As part of the integrated ecosystem studies in Lake Vechten the role of the macrophytes in the cycling of carbon, nitrogen and phosphorus is being assessed.

2.4.2. Studies in Lake Vechten (P.H. Best, J.H.A. Dassen, M. Priem and G. Wiegers)

Distribution and production

Like in 1979 the distribution of the main emergent, floating-leaved and submerged macrophytes was recorded. There is a distinct tendency for the submerged and emergent species to decline. This, in the case of submerged plants, is due to decreasing transparency of the lake water and to shadowing by the surrounding

trees; in the case of emergent plants also cattle grazing is an important factor. The number of plant species and their abundances have declined also with time. Only 43% of the emergent and floating-leaved species found in 1963 is still present. A similar comparison for the submerged species is not possible since they were not recorded in 1963. Total primary production, estimated by harvesting peak biomass, decreased by about 60%.

A comparative study has been started on the production characteristics and nutrient recycling of *Elodea* sp., *Polygonum amphibium* and *Phragmites australis*.

Macrophyte-periphyton community

Although growth and overall production of *Ceratophyllum* sp. is approximated quite well by the simulation model (see 2.4.3), the functional aspects and interactions within the higher plant-periphyton community are not yet properly understood, and data on respiration, in particular, are scanty. A start was made to fill these gaps.

In September a 24 hour observation was made on the oxygen regime in a *Ceratophyllum* community at a rooting depth of 2.5 m (this work was done in cooperation with K. Kersting, Research Institute for Nature Management). Continuous registration of the oxygen concentrations showed fluctuations between 10 and 11 $mg.l^{-1}$ during the afternoon and early evening. At night the O_2-concentration dropped to 6 $mg.l^{-1}$ close to the lake bottom and in the water layers with most plant material. The specific respiration rate (expressed on organic weight basis), however, was highest in the tips of the higher plants. The oxygen changes in the macrophyte-periphyton community were also measured *in situ* in closed containers. Oxygen consumption at night varied between 1.2 and 2.5 $mg.g^{-1}$ $org.wt.hr^{-1}$, whereas oxygen production during the day varied between 1.5 and 5.8 $mg.g^{-1}$ $org.wt.hr^{-1}$. Since this community has a standing crop of about 82 g $org.wt.m^{-2}$ in September (consisting for at least 50% of macrophytic material), this indicates respiration rates at night between 114 and 155 mg $O_2.m^{-2}.hr^{-1}$ and production rates during the day of 164-337 mg $O_2.m^{-2}.hr^{-1}$.

The variations in O_2-concentration in the different water layers are relatively small despite substantial oxygen production by the community during the day and consumption during the night as demonstrated by the closed container experiment, indicating considerable horizontal transport of oxygen in the lake possibly caused by water movements and wind action.

The 24 hour observations will be repeated in other crucial periods of the year, and respiration will be studied in more detail.

Decay of Ceratophyllum

At the end of the growing season the submerged macrophytes are degraded to a large extent by microbial action. A combination of laboratory and field experiments was made to investigate the interactions between decaying plant material, ambient water and sediment. Decomposition of lyophilized *Ceratophyllum* plant material was monitored in the laboratory under aerobic as well as anaerobic conditions at temperatures ranging from 5° to 18°C. A parallel experiment starting in December was done *in situ* in closed containers in Lake Vechten. In the laboratory series the initial stages of decomposition (0-17 days) were followed, whereas the *in situ* series lasted much longer (127 days).

The temperature greatly influenced the decomposition rate and the conversion of particulate to dissolved organic matter. Loss of organic matter from the decomposing plant material occurred faster under aerobic than under anaerobic conditions, e.g., at 18°C only 63% remained after 17 days. During the initial leakage phase (2 days) P and N were released from the plant tissue. The C:N ratio changed during this period from 10.3:1 in the lyophilized plant material to 12.0:1. The P:N ratio did not change and remained 0.25:1. Subsequently N was constant, P tended to decrease and C remained fairly stable under anaerobic conditions but increased under aerobic conditions. After 17 days the C:N ratio decreased to 8.4:1 (which ratio is close to that in sedimented organic matter in Lake Vechten) and the P:N ratio to 0.11:1.

Most organic matter from the plants was recovered in the sediment (see Fig. 9), although the decay processes, particularly during the leakage phase, caused an enormous rise in the N, P and C concentrations in the water phase. The *in situ*

decomposing plant material showed a tendency similar to that in the 5° and 10° laboratory series.

2.4.3. Growth and photosynthesis of Ceratophyllum demersum (P.H. Best, M. Priem, J.T. Meulemans and M. Kramer)

The data on growth and photosynthetic rate of *Ceratophyllum* in previous years collected are used to develop a preliminary growth model. In processing the data it became clear that growth of submerged macrophytes in relatively shallow eutrophic waters, e.g., in Lake Vechten, is mainly light limited. The light quantity reaching the photosynthetic tissues of the submerged plants is absorbed by plankton and detritus, and the macrophytes. In spring, the *Ceratophyllum* turions, lying on the lake bottom, start elongation growth using the reserve substances. Several plants die in this stage due to lack of light and consequent exhaustion of their reserve stock. In summer, when the plants are buoyant, a decrease in water transparency limits production in general, and the formation of propagules in particular. Since light is an important driving variable more attention is paid now to a) the light climate in the lake, b) factors affecting the light absorption by the plants' photosynthetic tissues, and c) survival strategy in relation to the net carbon balance.

Several photosynthetic characteristics of *Ceratophyllum* were studied. Although the plant showed mainly C_3 features also indications for C_4 activity were found.

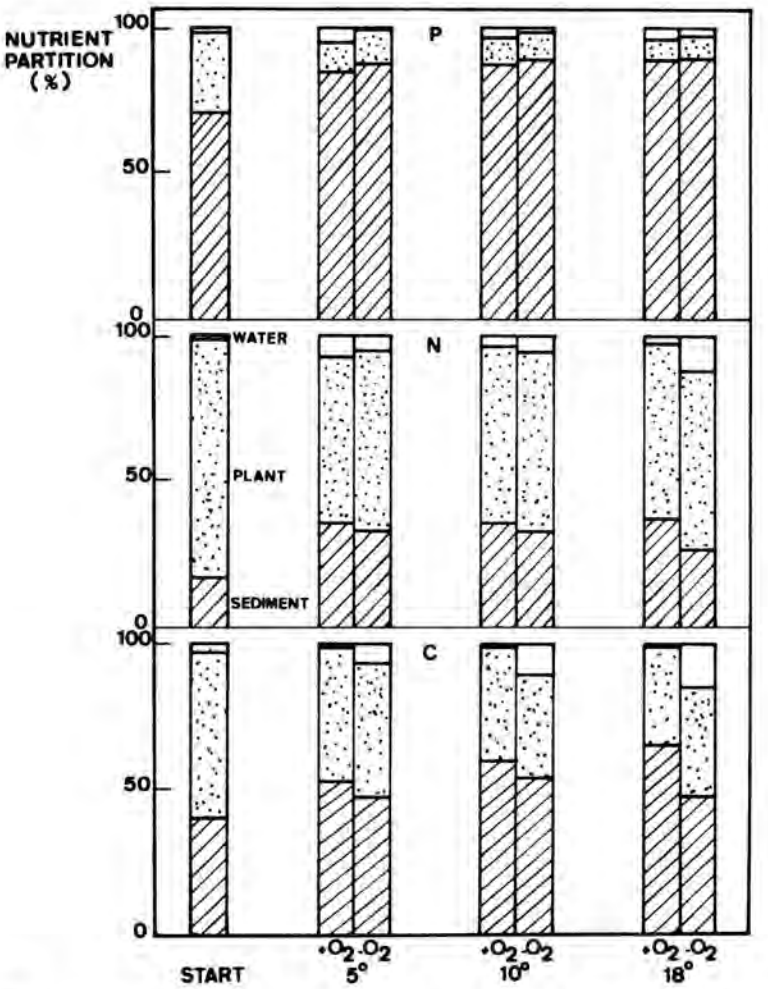

Fig. 9. Percentages of nutrients in, respectively, the sediment, water and plant material at the beginning of the experiment and after 17 days of decomposition under aerobic or anaerobic conditions at temperatures of 5, 10 and 18°C.

Typical Kranz-anatomy was absent. No light saturation was proven in a statistically significant way, although it was approximated at intensities higher than 36 $W.m^{-2}$. Photosynthetic activity was only slightly depressed by enhancement of the O_2-concentration. Malate was a major part of the early photosynthetic products.

The significance of the macronutrients nitrogen and phosphorus for growth of *Ceratophyllum* is in study. A primitive continuous culture system for the application of nutrients in low concentrations was attempted. In this system the effects of combinations of different N- and P-levels on the growth of *Ceratophyllum* were tested at several light intensities. The data are being processed.

Some progress was made in the development of methods for the quantitative determination and separation of the different nitrogenous compounds in the plants.

2.4.4. Vechtplassen research (P.H. Best)

As part of the recently started studies in this area (see par. 2.1) an initial survey was made of the submerged and floating-leaved macrophytes. This work was partly financed by the Beyerinck-Popping Foundation. Physico-chemical data are provided by Gemeentewaterleidingen, Amsterdam.

The presence of submerged macrophytes depended strongly on the light climate in the water, whereas no correlation with P-concentrations of the water was found. The annual average phosphorus concentrations in this area varied between 0.02 and 0.29 mg l^{-1} PO_4- P and 0.06 and 0.52 mg l^{-1} total P.

In the eastern and western Loenderveen Lakes the species diversity is still very high. In particular the eastern lake shows a great similarity to the original situation. Submerged dense growth of several *Characeans*, *Elodea* sp., *Najas marina* and several *Potamogetonaceae* is locally found, and the floating species are represented by *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata*. Secchi disc-readings vary between 0.94 and 1.12 m, and turbidity between 2.4 and 3.5 FTU. The waterworks' lake (middle Loenderveen Lake) is relatively poor in macrophytes, despite its high transparency (Secchi > 3 m). Only single plants of *Zannichellia palustris* and *Elodea* sp. are present. Reason for this might be the high NH_4-N concentration (annual average of 0.44 $mg.l^{-1}$).

The Loosdrecht Lakes are scarcely colonized by submerged plants; only the submerged seedlings of *Rorippa amphibia* occur in all these lakes. The floating-leaved plants found are the same as those in the Loenderveen Lakes. Here blooms of blue-greens are present in August - September, the transparency is much lower and the turbidity higher than in the Loenderveen Lakes, respectively, 0.24-0.69 m and 5.2-8.5 FTU.

In the Breukelerveen Lake and the Vuntus, both of which are separated from the others by dykes, no submerged or floating-leaved macrophytes are detected, but the blooms of blue-greens are present the year round.

This survey will be repeated after some years. One would expect that the decreased phosphorus loading (see par. 2.1) will result in less algal blooms, improvement in the light climate and increase in the growth of submerged and floating-leaved macrophytes.

2.5. PROJECT 'CARBON CYCLE IN LAKE VECHTEN' (H.J. Gons)

A part of the research efforts of the two workgroups and the research group is focussed on an integrated and coordinated study of the carbon cycle in Lake Vechten. A number of studies has already been reported in the workgroup reports (see e.g. 2.2.2; 2.3.1; 2.3.5).

2.5.1. Introduction

Main characteristics of Lake Vechten, besides its slightly eutrophic state, are: the absence of surface in- and outflowing streams and small area to relatively great depth. The result is a largely autotochthonous input of organic matter into a cycle with quantitatively important branches in the de-oxygenated hypolimnetic water in summer and in the extensive littoral zone (Fig. 10).

The carbon cycle as central theme of research has developed gradually. At first, the attention was devoted to the species composition and production processes in the limnetic zone and to mineralization in the anaerobic sediments; since 1973 macrophytes, aerobic mineralization, periphyton and phototrophic bacteria of the

hypolimnion have been investigated successively. Thus, the carbon flow studies of the various compartments were not carried out simultaneously, while some studies have only recently been started. Because of this, the construction of an annual carbon budget is only possible by extrapolating the results of previous years and the recent preliminary investigations.

A fairly good integration of the inputs and outputs of organic carbon may show whether a system is balanced or is changing, or reveal the gaps in the research. Comparison with the budgets of other lakes may improve our insight into the role of morphological, hydrological, as well as environmental factors. In such a budget, however, most interrelationships between the compartments will be concealed, e.g., it is difficult to assess the extent to which the organic matter produced during a phytoplankton bloom, say in spring, enters either the grazer or detritus food chain or both. It was decided, therefore, to try and establish the system's carbon flow during characteristic periods of the year, after simultaneous measurements on the relevant compartments. We expect that this approach will provide adequate information to interprete the *in situ* changes in the organic carbon pools of the lake.

2.5.2. Annual carbon budget

The budget was computed for 1979, the first year for which data for most compartments were available. The input, i.e. the net carbon fixation by the various compartments of primary producers, and the output of organic carbon, i.e. the net carbon dioxide production by the compartments of heterotrophic organisms, are distinguished. Imports and exports were not taken into account.

For detailed information concerning the compartments, see other sections on Lake Vechten research in this report and in the Progress Reports 1978 and 1979.

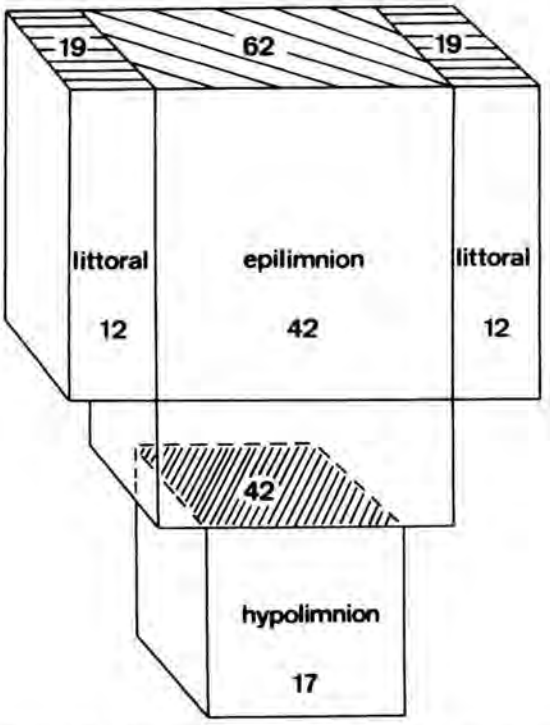

Fig. 10. Contribution of the major regions of Lake Vechten to its area (4.7 ha) and volume (0.28×10^6 m^3) during summer stratification. The numbers in the hatched parts of the boxes give percentage of area, the other percentage of volume. Hypolimnion represents the de-oxygenated water below the 7 m contour; for the littoral zone 5 m depth was taken as lower limit. Between depths of 5 and 7 m, water and sediments exhibit seasonal changes of oxygen from zero to 100% saturation.

Carbon fixation (Fig. 11A)

Primary production of the phytoplankton, mainly by centric diatoms and dinoflagellates, constituted more than half of the total autotochthonous input of organic carbon. This included about 5% extracellular carbon, but excluded the carbon fixation in the hypolimnion. The latter occurred under anaerobic conditions and was due to a distinct community of Chroococcaceae, Chlorobiaceae and Chromatiaceae, which in summer represent the bulk of the chlorophylls in the system. This completed limnetic production to almost two thirds of the total.

In the littoral zone five groups of producers can be distinguished. Emergent macrophytes occupied only 2.2% of the lake area, but their input was considerable: two thirds of all macrophytes together with a stand area of 17%. The submerged macrophytes declined markedly during the last years (see 2.4.2). This also affected the production by epiphytic and epipelic algae, respectively, by decreased and increased substrate availability.

Although epiphytic algae were by far the most productive locally, depending on the population density of the macrophytes, epipelic algae contributed the most to the production by the algae of the littoral zone. Both epiphytic and epipelic communities are characterized by pennate diatoms and filamentous green algae, but sedimented planktonic species may also be important.

Carbon dioxide production (Fig. 11B)

Aerobic mineralization occurs in the epi- and metalimnetic layers, where excretion products and suspended particles from the various sources form the substrates. Nearly a quarter of the lake's carbon output (equal to half of the phytoplankton production) was due to breakdown by aerobic bacteria in the open water. Also particulate organic matter sedimented on the macrophytes and bottom of the littoral zone is utilized. This was estimated by measuring biological oxygen demand, but the share of bacteria and littoral fauna, notably Protozoa, is not known. The decline of the submerged macrophytes, on which the organic matter was found to accumulate in dense stands of the plants, will also influence the output of the littoral zone.

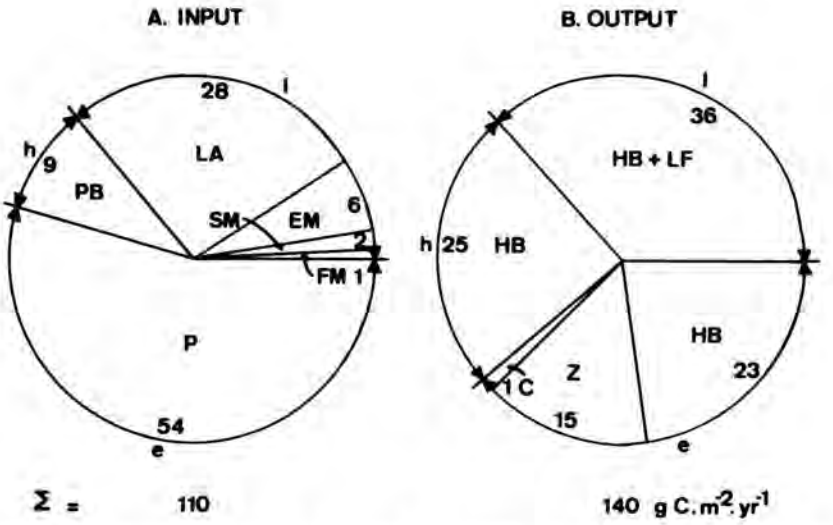

Fig. 11. Annual carbon budget for 1979. A. Input: net organic carbon production by autochthonous producers. B. Output: net carbon dioxide and methane production by consumers and decomposers.
Explanation: numbers in the sectors represent percentages; the letters e, h and l on the periphery of the circle indicate the sectors epilimnion, hypolimnion and anaerobic sediments, and littoral; C, carnivores; HB and PB, heterotrophic and phototrophic bacteria; LA and LF, littoral algae and fauna; EM, FM and SM: emergent, floating-leaved and submerged macrophytes; P, phytoplankton; and Z, zooplankton. Σ denotes the integrated values of input and output.

Below the 7 m contour the sediments are permanently anaerobic. The organic matter reaching here may have previously been broken down aerobically for short or long periods. It enters a complicated series of microbial degradations, with carbon dioxide and methane as end-products in a ratio of about 1:3. It is not known to what extent the methane either is utilized by methane oxidizing bacteria or escapes to the atmosphere. In the budget the methane was included in the produced carbon dioxide. But should all the methane be utilized, thus part of the carbon be incorporated in the bacterial cells, the lake's total carbon output would decrease by about 6%, or 8 g $C.m^{-2}.yr^{-1}$.

Zooplankton is functionally a dominant compartment in Lake Vechten. The consumption of the herbivorous zooplankton, mainly cladoceran filter feeders, was 1.2 times higher than the net cell-carbon fixation of the phytoplankton. Of the carbon consumed by zooplankton 30% was respired.

The higher trophic levels are quantitatively of minor importance. The carnivorous zooplankton and fish were estimated to account for not more than 1% of the output.

Annual carbon balance

From the budget it appeared that in 1979 carbon dioxide produced by the heterotrophs considerably exceeded the organic carbon formed by the autotrophs. Of course, the difference may be realistic: the marked fluctuations in annual primary production may not necessarily be accompanied closely by those of the heterotrophic utilization. However, such differences may be due partly to methodological errors and to gaps in our knowledge of the lake.

Of the latter the following seems to be most important. Except the metalimnetic layers, little is known about transitional zones. First, the littoral down to about 1.5 m depth is littered with tree-branches and all sorts of debris and is extremely heterogeneous. Secondly, the sediments between 5 and 7 m depth are submitted to seasonal shifts from aerobic to anaerobic decomposition. These two zones comprise, respectively, 15 and 20% of the lake area. Also our inventory of the horizontal distribution of biomass in the various compartments is incomplete, especially regarding zooplankton and littoral communities.

Allochthonous inputs might explain the discrepancy between the carbon fixation and carbon dioxide production. The leaf-fall input was estimated to be maximal 7% of the phytoplankton production. Other external contributions probably will prove to be small, but carbon exchange associated with the annual replenishment of the lake water by seepage and precipitation, about 15%, could influence the budget substantially. However, the discrepancy widened after adjusting for the organic matter accumulated in the anaerobic sediments which was estimated to be equivalent to 15% of the phytoplankton production.

Finally, it must be mentioned that the information on a) inputs and transformations of dissolved organic carbon compounds (representing about 85% of the organic carbon in the limnetic region), b) potentially important processes such as grazing on littoral algae, c) chemosynthesis, and d) on anaerobic mineralization in the hypolimnetic water is only fragmentary. The latter could be estimated minimally, based on the reduced concentrations of electron acceptors after stratification. This, amounting to 3% of the total carbon output, is included in the budget.

2.5.3. Carbon flow on September 9, 1980

In order to establish the carbon concentrations and fluxes a 24 hours study was carried out on September 9, when both stratification (Table 1) and littoral vegetation were well developed. Vertical extinction was low, irradiance values being up to 5 W. m^{-2} at zero oxygen depth. This allowed considerable carbon fixation by the phototrophic bacteria (see below). That the conditions for these organisms were favourable was also evident from the abundance of chlorophyll in the lower strata.

The distribution of the macrophytes was again reduced compared with that in the previous year, i.e., the submerged vegetation was confined largely to a narrow belt between 1.5 and 3 m depth, and the emergent and floating-leaved species too had markedly reduced stand areas. Thus, the epipelic algae were the only producers in the major part of the littoral area, with approximately 20 mg chlorophyll-a m^{-2}.

The results of the measurements on the carbon flow are estimated for the whole

Table 12. Some characteristics of the limnetic region of Lake Vechten on September 9, 1980.

epilimnetic vertical extinction coefficient (ε, 400 - 700 nm)	0.56 m^{-1}
epilimnetic temperature (0 - 5 m)	18.7°C
hypolimnetic temperature (10 m)	8.5°C
thermocline depth	5 - 7.5 m
zero oxygen depth	6.5 m
chl a, 0 - 6.0 m	82.4 $mg.m^{-2}$
chl a, 0 - 9.6 m (incl. bacteriochlorophyll)	505 $mg.m^{-2}$

lake, and given as net fluxes in kg C. day^{-1} (Fig. 12). The carbon flow is subdivided into littoral, epilimnetic and hypolimnetic cycles, which are interlinked by processes such as diffusion, sedimentation and migration. Although autotrophic and heterotrophic compartments are distinguished, part of the estimates are based on measurements on communities containing both. As explained later, assumptions must be made in order to derive the contribution of these compartments; also these assumptions are decisive in the estimated net rate of change in the limnetic organic carbon. The daytime value of carbon fixation in the limnetic region was high, higher than any recorded during 1979. For the phytoplankton and phototrophic bacteria the estimates were, respectively, 12.1 and 6.2 kg C. On 24 hours basis,

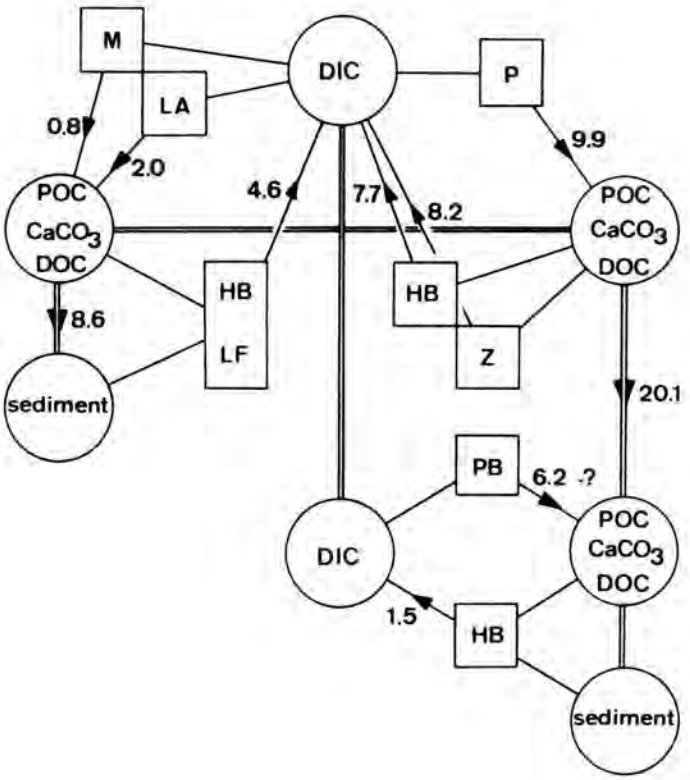

Fig. 12. Carbon flow on September 9, 1980. Circles represent pools of dissolved inorganic carbon (DIC), and of organic carbon in particulate (POC), dissolved (DOC) forms and the photosynthetic by-product ($CaCO_3$) which may absorb DOC. Transformations between the pools are effected by the functional compartments represented by squares. The double lines depict translocations of matter within the system. Symbols are as in Fig. 11. See text for further explanation.

the phytoplankton net production was considerably lower after corrections were made for the nocturnal respiration. A value of 9.9 kg was calculated assuming the respiration rate to be 10% of the maximum photosynthetic rate. With respect to the carbon fixation of the phototrophic bacteria, the knowledge about the *in situ* metabolism of these organisms did not warrant such a correction, hence the question mark in Fig. 12.

The aerobic mineralization in the limnetic region, derived from the oxygen exchange of seston concentrates, was equivalent to 12.4 kg C. Substracting the phytoplankton respiration from this gave 7.7 kg C as bacterial respiration. The tenuity of the estimates, and the importance of independent measurements on algal and bacterial respiration, may be illustrated as follows. Should the phytoplankton respiration rate double, the carbon fixation would decrease to 6.0 kg. In such a case, algae will contribute 100% to the seston respiration. Moreover, net change in the organic carbon due to phytoplankton and bacteria will increase almost threefold.

Also the zooplankton organisms contributed significantly to the carbon flow. Their respiration surpassed their assimilation, implying a decrease in biomass. The phytoplankton during this period was predominated by *Ceratium hirundinella*, a species considerably larger than the size fraction preferred as food by the zooplankton.

On integration it follows that the biological processes resulted in a decrease of the epilimnetic organic carbon, irrespective of the assumption for the phytoplankton and bacterial respiration. The conclusion drawn for the littoral region is similar. The macrophyte production, calculated on the basis of standing crop measurements, was distributed over the submerged, floating-leaved and emergent species in the ratio 1.8:1:2.2. The production of the littoral algae was for the greater part epipelic; epiphytic algae were estimated to contribute 15%. Also here, in order to establish separate fluxes for the algae and the bacteria and fauna, the algal respiration rate was assumed (Fig. 12) to be 10% of the maximum gross photosynthetic rate. In contrast with the epilimnetic subcycle, the assumption does not influence the net flow due to these compartments, since both respiration and photosynthesis were measured using the oxygen method.

At first sight the hypolimnion appears to be the only productive region. Ignoring the unknown quantity of nocturnal carbon dioxide release, the carbon fixation of the phototrophic bacteria was counter balanced only by loss from the mud in the form of methane (in Fig. 12, for sake of simplicity, this enters the DIC pool) and carbon dioxide, representing, respectively, 1.2 and 0.3 kg C.

The subcycles (Fig. 12) are interrelated by redistributions of the various carbon fractions.

Although no attempt was made to quantify exchanges of DIC, the measured concentrations give additional information about the biological processes. In the littoral zone diurnal fluctuations in DIC, like those in oxygen and pH, agreed with the results on the oxygen exchange rates, i.e., general predominance of heterotrophy during the 24 hours but positive net production in the parts exposed to sufficient light in day time. Neither the epilimnion nor the hypolimnion exhibited any change. The DIC concentration in the hypolimnion was approximately 1.5 times higher than that in the epilimnion. This difference indicates, of course, that heterotrophic carbon dioxide production exceeded the DIC lost due to diffusion to the upper strata and that fixed by phototrophic bacteria in the hypolimnion. This makes the results of September 9 very unexpected and stresses the need for more extensive research on the processes in the hypolimnion. (See also the discussion of the annual budget.)

In contrast with DIC, DOC concentrations were uniform both vertically and horizontally. This indicates an extremely low turn-over of most substances in this fraction.

POC was redistributed in at least three ways. First, the dinoflagellate *Ceratium hirundinella*, which predominated the phytoplankton on the basis of both biomass and numbers, exhibited a distinct diurnal vertical migration. Secondly, the zooplankton biomass varied markedly with depth, but the integrated areal values fluctuated considerably as well, suggesting a complex pattern of vertical and horizontal migration. These migrations, especially of the zooplankton, complicate the estimates of the relevant carbon fluxes, but supposedly did not involve exchanges with either littoral or hypolimnion. Thirdly, sedimentation is of outstanding importance in the distribution of organic matter for heterotrophic utilization

in the various parts of the lake. Using the sediment trap technique, the estimated sedimentation rates in the littoral and hypolimnion were, respectively, 8.4 and 20.1 kg C. day^{-1}. So far, it is unknown to what extent these high values represent downward net fluxes, but evidently the process needs to be investigated further, since our understanding of the system's carbon cycle will benefit greatly from clear balances for the three lake regions.

The data of September 9 have primarily descriptive value for the partitioning of the carbon cycle. The outcome, i.e., decrease in the epilimnetic organic carbon with sedimentation as the main cause, could not be compared with changes in the POC and DOC contents which were too massive: the epilimnetic POC amounted to approximately 200 kg, and DOC was almost six times as high. Therefore, the future studies will include measuring the changes on weekly intervals.

2.6. PROJECT 'THE RESTORATION OF THE LOOSDRECHT LAKES' (S. Parma)

As reported in paragraph 2.1 this research project will be a coordinated effort of five institutes and authorities. The Limnological Institute started with some preliminary observations concerning the exchangeable amounts of total phosphorus in the sediments (see 2.3.3) and the distribution of macrophytes (see 2.4.4). Studies are planned on the structure of phytoplankton, phosphate exchange at the mud-water interphase, production of autotrophs and heterotrophs and hydrology. The reports in future years will be more integrated.

At present chemico-physical data are collected and plankton is studied by the Municipal Waterworks, Amsterdam.

2.7. PROJECT 'POLDER RESEARCH' (R. Veeningen)

General

The project 'Dynamics of the concentration of dissolved oxygen in polder ditches' is financed by the Ministry of Public Health and the Environment and started in 1979. 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 field-work was started in the spring of 1980 in ditches in the vicinity of Nieuwersluis (polder Groot-Wilnis-Vinkeveen). A routine-programme including different environmental parameters was carried out in two ditches (four sample-points in each ditch) with the following dimensions: length c. 320 m, breadth 4-7 m and depth 0.3-0.7 m. These two ditches and another typical ditch were chosen to study the spatial and temporal variation of the dissolved oxygen concentration. Light and dark plexiglass enclosures were used to measure the production and consumption of oxygen in different compartments of the ditch. A distinction was made between the contribution of the sediment, the water-column, the macrophytes and the air-water interface in the oxygen balance.

The measurement of the oxygen-concentration in small water-samples during the time of enclosure was carried out with a simple flow-through-stirring-cell (see Fig. 13).

Environmental parameters

After the ditches had been cleaned mechanically in the autumn of 1979, the water column was greatly dominated by macrophytes and filamentous algae during the summer of 1980. Temporarily there were thick mats of filamentous algae either at the water surface or at the bottom. Dominant species of macrophytes were: *Sparganium erectum, Alisma plantago-aquatica, Potamogeton pectinatus, Ranunculus circinatus, Myriophyllum spicatum* and some *Chara* species. The spatial heterogenity in the ditch is largely caused by both the macrophytes and the filamentous algae.

In spring there was a bloom of phytoplankton giving rise to a chlorophyll-a concentration of up to 40 $\mu g.l^{-1}$. In summer and winter this concentration is mostly less than 5 $\mu g.l^{-1}$. Submerged macrophytes show a rich population of epiphyton, while chlorophyll-a could also be detected at the sediment. Thus, the amount of

photoautotrophs in the ditch is largely underestimated by quantifying chlorophyll-a in the free water only.

The water in the ditches is intensively coloured by soluble humic substances coming from the peaty soil. DOC varies seasonally from 10 to 20 mg $C.l^{-1}$. Because of the inlet of Rhine water in the polder system in early summer the chloride concentration increases from 80 to 240 mg $Cl.l^{-1}$. In autumn and winter the concentration decreases again to 80 mg $Cl.l^{-1}$. In spring and summer the two ditches were poor with respect to nitrogen and phosphorus. Ortho-phosphate and total-phosphate concentrations are always below 30 and 130 $\mu g\ P.l^{-1}$, respectively, and do not vary seasonally. The nitrate and on some locations the ammonia concentrations increase from 20 to 1000 $\mu g\ N.l^{-1}$ in the winter season.

Variations in dissolved oxygen concentration

The spatial and temporal variations in the dissolved oxygen concentration were measured every month at different moments during a few days. The oxygen, temperature and, occasionally, pH were measured *in situ* out on approximately 40 locations in the three ditches. The spatial and temporal variations of the dissolved oxygen concentration were large (see Fig. 14) and in some cases also a vertical gradient existed. Some preliminary conclusions are:

- the temporal variation strongly depends on the amount of sunshine and on the presence or absence of macrophytes and filamentous algae;
- locations without macrophytes or filamentous algae exhibit lower oxygen concentrations and smaller diurnal variations than those with a dense vegetation;
- the diurnal variation in a field of *Typha angustifolia* is as great as that on locations with submerged macrophytes but the minimum and maximum are much lower, the maximum mostly below 100% saturation;
- the locations with a thick mat of filamentous algae at the water surface exhibit a strong vertical gradient in oxygen concentration. Only the top layer shows a diurnal variation;
- the spatial and temporal variation of the pH and dissolved oxygen are closely related.

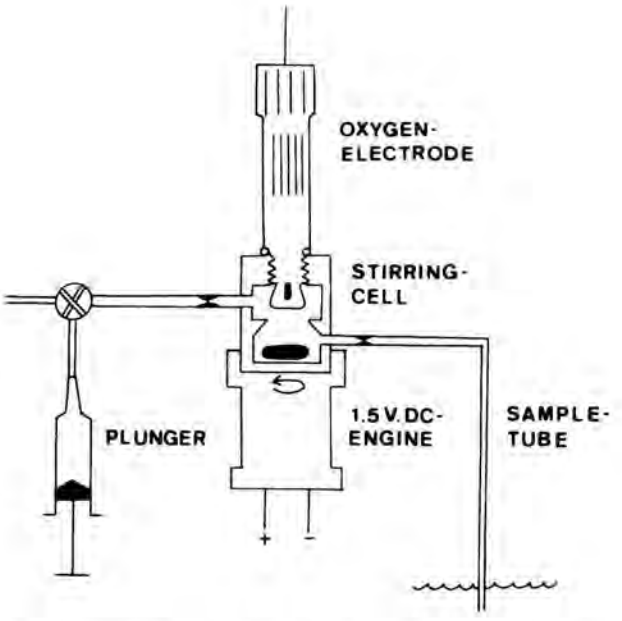

Fig. 13. A diagram of the flow-through-stirring-cell for measuring the oxygen concentration in small water samples. The sample is sucked in the cell (vol., 6 ml) with a plunger, while the magnetic bar agitates the water. After reading off the oxygen concentration the sample is pumped back into the enclosure via the sample tube.

Oxygen balance

The dimensions of a ditch make it suitable for a study of the oxygen balance with enclosures. However, in practice the efforts to enclose macrophytes *in situ* were not successful, mainly because of the physical structure of the stands. The sediment oxygen demand (SOD) ranged from 20-85 mg $O_2.m^{-2}.h^{-1}$. On sunny days there was also a net production of oxygen at the sediment ranging from 20 to 125 mg $O_2.m^{-2}.h^{-1}$. *In situ* incubation of small volumes of water (c. 100 ml) in light and dark columns revealed very small changes in oxygen content: net production and consumption rates ranged from 0.01-0.03 mg $O_2.l^{-1}.h^{-1}$. The sum of the activity of the sediment and of the free water was reflected in the changes in the oxygen content in the enclosures of the whole water column.

The sediment greatly influenced the oxygen balance in a ditch. Its relative contribution to the gross production and consumption ranged from 55 to 80%. These percentages refer to locations without macrophyte stands or filamentous algae. On locations with dense macrophyte stands or filamentous algae, however, a great deal of the variation in dissolved oxygen concentrations must also be ascribed to the activity of this vegetation, including epiphyton.

3. Tjeukemeer Laboratory

3.1. GENERAL INTRODUCTION (S. Parma)

The Tjeukemeer is a shallow freshwater lake (area c. 21 km^2, mean depth, c. 1.5 m). It forms a part of the Frisian reservoir system (Fig. 1). The uppermost sediment consists of a mosaic of peat (60%), sand (35%) and mud (5%). In winter the lake receives nutrient- 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^{2+} (range, 36-56 $mg.l^{-1}$) and HCO_3^- (range, 79-122 $mg.l^{-1}$) concentrations, the pH in this peaty lake is usually high, i.e., 7.5 and above. In the summer period, however, water from the IJsselmeer is allowed to enter the lake for agricultural purposes. The humic acid concentration of IJsselmeer water is much lower than that of the polder water but the chloride concentration is much higher. The increase in the chloride concentrations up to 200 $mg.l^{-1}$ gives the lake an oligohaline character.

A study of the food chain in the Tjeukemeer was started in 1966 within the framework of the International Biological Programme and finished in 1971. Subsequently, two workgroups were set up.

3.2. WORKGROUP 'ALGOLOGY'

3.2.1. Introduction (J.R. Moed)

Large populations of diatoms, blue-green and sometimes green algae are observed annually in the Tjeukemeer. The aim of the monitor programme is both to establish the magnitude and periodicity of these algal populations and to obtain information about the factors co-regulating their wax and wane (project A1). The regulating factors are being studied in the field and in the laboratory.

The varying physico-chemical regime of the lake may influence the availability of P, N, Fe and trace elements for algae. In other words, the hydrology may be co-regulating the algal periodicity. It would mean that its role is more than just that of transporting nutrients and algae. This is illustrated by the following data. During June the average Mn_{diss}-content in the Tjeukemeer amounted to 15 $\mu g.l^{-1}$. Thereafter rapid decrease to 1-2 $\mu g.l^{-1}$ coincided with a considerable inlet of polder water, in which the Mn_{diss}-concentration was 150-300 $\mu g.l^{-1}$. It follows that the created physico-chemical environment was not favourable to maintain manganese in 'dissolved' state (project A2.2).

In order to study the different physico-chemical forms of the nutrients, gel filtration, ultrafiltration and ion-exchange chromatography are being applied. The availability for algae will be tested in chemostats. Meanwhile the culture studies indicate that for evaluating the growth parameters μ_{max} and K_s the physico-chemical growth environment has to be taken into account (project A6.2).

Bio-assays are being performed to detect the factors co-regulating algal periodicity. The preliminary work in 1980 revealed that around June-July the positive effect on growth of the complete medium added to Tjeukemeer water was hampered in one way or the other (project A7.1). The possible role of Cu and Zn in inhibiting algal growth in the Tjeukemeer is being investigated (project A6.4). The field and laboratory data will be applied to an experimental model to simulate the algal periodicity in the Tjeukemeer.

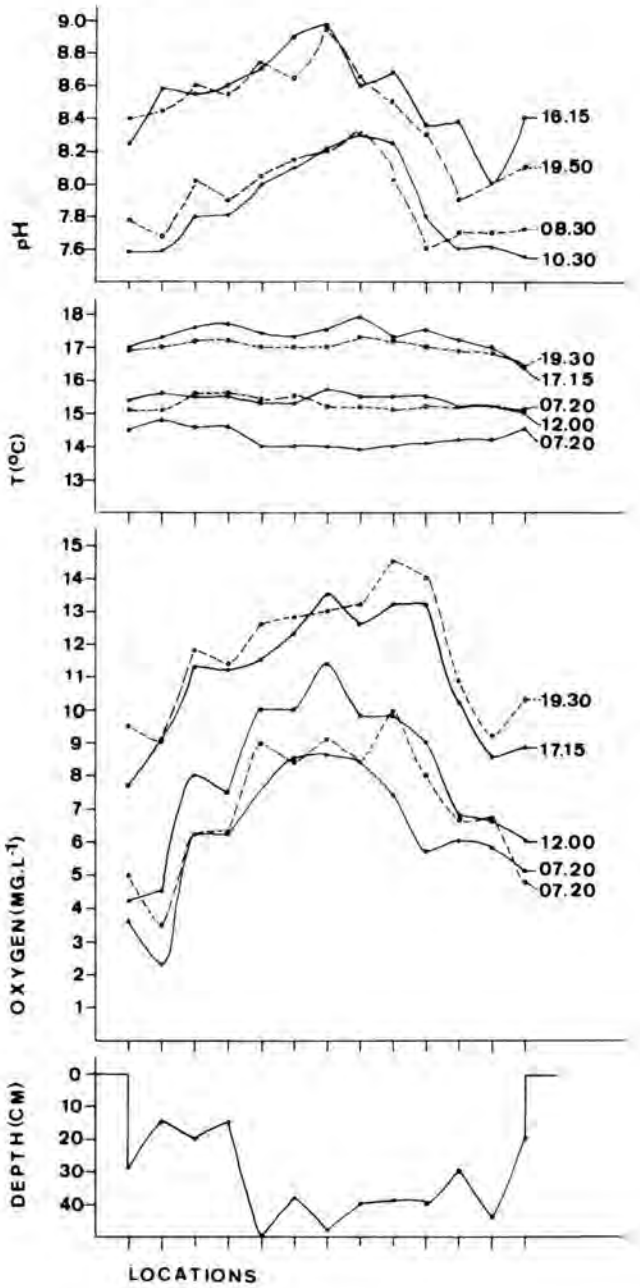

Fig. 14. The spatial and temporal variation of dissolved oxygen, water temperature and pH in a ditch (September 1980). The abscissae give the successive locations in the ditch. The values measured simultaneously at the successive locations are connected with each other. The depth at the different locations is given in the curve at the bottom.

The research programme for the period 1979-1983 is aimed at explaining the periodicity of abundant algal species in the Tjeukemeer as well as at quantifying the water flows into the lake. In this regard, the workgroup is cooperating with Ir. J.D. Leenen and Ir. H.G. Verhagen, both affiliated to the Laboratory for Hydraulics and Catchment Hydrology, Agricultural University, Wageningen. A chloride-model was developed in order to calculate the amount of water flowing into the Tjeukemeer (project A4).

The essential results this year can be summarized as follows:

1. Despite the fact that the summer of 1980 was a wet one, the algal periodicity in the Tjeukemeer generally showed the trends noted since 1972. In the summer period, however, centric diatoms occurred in relatively high densities.

2. Progress was made in the detection of possible regulating factors. Chemical monitoring and laboratory bio-assays revealed that especially N, Mn, Fe and Cu might affect algal growth in the lake. The presence of heterocysts in *Aphanizomenon flos-aquae* in the lake confirmed the supposed N-limitation.

3. The parameters to study the ability of the abundant algal species of the Tjeukemeer to compete were evaluated. Culture studies indicated that the growth parameters μ_{max} and K_S are a function of the physico-chemistry of the growth limiting substrate. Several *Oscillatoria* species were isolated.

4. The study of the hydrology of the lake was continued. Using weekly average chloride concentrations of 10 stations in the lake, and that of the inflow into the lake, the discretized mass-balance equation for the chloride gives a good estimate of the total inflow of water per week or 14 days into the lake.

More detailed information is reported in the next paragraphs.

3.2.2. Phytoplankton dynamics in the Tjeukemeer (project A1; H.L. Hoogveld and J. Voerman)

The aim of this study is to monitor the succession of species in the Tjeukemeer and to collect data on the inflow of algae from the IJsselmeer. Also, morphological and morphometric data on certain algal species are being gathered to calculate biovolumes (Fig. 15) and to identify factors regulating algal periodicity.

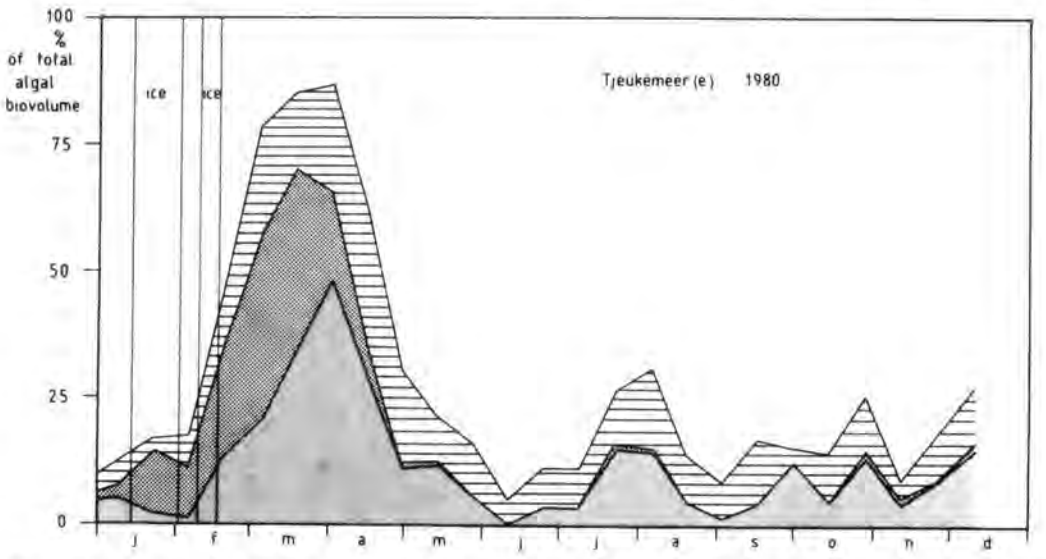

Fig. 15. Per cent distribution of total algal volume in Tjeukemeer-east:

Cyanophyceae; Chlorophyceae; Cryptophyceae; Bacillariophyceae

The overall pattern of the algal succession in 1980 agrees with the general trend observed since 1972. Nevertheless, it is striking that the percentage contribution of the blue-greens, namely, 70-90%, to the total algal biovolume in the summer of 1980 was relatively low, compared to 90-99% found usually. On the other hand, the contribution of centric diatoms, in particular, and the green algae *Scenedesmus* spp. and *Pediastrum* spp. relatively increased. In the wet summer of 1980, the Tjeukemeer received much polder water containing nutrients. Thus, conditions may have become more favourable for the diatoms and green algae. In spring the maximal density of unicellular centric diatoms (16,000 cells.ml^{-1}) is noteworthy. This density is much higher than that of *Asterionella formosa* (900 cells.ml^{-1}), *Melosira* spp. (4,300 cells.ml^{-1}) and *Diatoma elongatum* (3,100 cells.ml^{-1}). In summer, the numbers of *Melosira* spp., unicellular centric diatoms and *Scenedesmus* spp. were also high in the inflow water from IJsselmeer/Groote Brekken to the Tjeukemeer. This may have influenced the algal development in the Tjeukemeer as well, at least by way of inoculation.

The average length of the filaments of *Oscillatoria agardhii*, *O. redekei* and *Aphanizomenon flos-aquae* has been determined for some years. Certain patterns, probably reflecting the nutritional status of the Tjeukemeer for these species, may be recognized. During the period June-November the average length of *A. flos-aquae* did hardly change, in contrast to that of the other mentioned algae. In May-June *A. flos-aquae* showed increasing numbers of heterocysts per unit filament. In the period July-October the average length between two heterocysts was 150 µm. The presence of heterocysts points to limitation of inorganic nitrogen. The course of the numbers of heterocysts per unit filament was inversely related to the concentration of inorganic N_{diss} in the Tjeukemeer.

Also in 1980, the total algal volume per litre in the Tjeukemeer was significantly correlated with the chlorophyll-a concentration ($r = 0.83$; $P < 0.05$; $n = 23$).

3.2.3. *Nutrient dynamics in the Tjeukemeer* (project A2)

Determination of nitrogen compounds (project A2.1; H.A. Kramer and J.R. Moed)

In the previous years a batch method for the determination of nitrate was developed in which this compound is reduced to nitrite by zinc powder. This year it was found that NaCl, besides stimulating the nitrate reduction, also prevents nitrite from further reduction. It seems that the specificity of both the effects owing to NaCl is low.

To determine total nitrogen the alkaline persulphate method has been tested. In oxidizing ammonia in closed bottles, the ratio of the volumes of solution and air should not fall below 1.5:1. Comparing the persulphate and the Kjeldahl method ($n = 28$), the latter yielded 6% higher values for 0.2 µm filtered and 2% higher for non-filtered Tjeukemeer and polder water. Considering that these differences are small and Kjeldahl more laborious we plan to continue using the persulphate method.

Generally, the N-content in the filtrate, using 0.2 µm membrane filter, was almost similar to that using the glass fibre filter (Schleicher and Schüll) ($n = 14$) and it seems therefore that in Tjeukemeer water a N-fraction including particles with a diameter of a few micrometers is hardly present. On the average, the N-content in 0.2 µm filtrates was 5% lower in Tjeukemeer water and 2% lower in the polder water than in the respective glass fibre filtrates.

From April to November the $N_{org.diss}$-fraction in Tjeukemeer water varied from 700 to 3600 µg l^{-1} of N. Such variation is noteworthy, assuming that nitrogen limitation co-controls algal growth (project A7). The applicability of ion-exchange chromatography in characterizing the $N_{org.diss}$-fraction was tested. On the average almost 50% absorbed to a Dowex 2X-8 anion-exchange column, as against 30% to a Dowex 50 W-X8 cation-exchange column ($n = 15$). This method appears to be promising. Also, the application of the hydrophobic Amberlite XAD-2 resin is being tested.

Determination of Cu and Zn (project A2.1; J. Voerman, Th. de Boer and H. de Haan)

Preliminary work indicates that the concentrations of Tot-Cu and Tot-Zn in the Tjeukemeer are around and below the detection limit of our Flame Atomic Absorption Spectrophotometer technique (FAAS). Therefore concentrating these metals

by freeze-drying and binding on charcoal after complexation by Ammonium Pyrolidine Dithio Carbamate (APDC) was attempted. The concentrate was analysed using FAAS colorometry. Both techniques give comparable results for Cu. So far Zn has only been measured by FAAS. Intercalibration of our Cu analysis with that of the Frisian Water Authorities gave corresponding results. Though these results give confidence in our methods, these are laborious, time consuming and not direct. Direct and rapid analysis of Cu and Zn in Tjeukemeer water can be performed by applying Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Ecologically, Tot-Cu concentrations are of minor importance since only Cu ions seem to be available for phytoplankton. Methods to quantify dissolved and ionic Cu are in progress.

Monitoring nutrients (project A2.2; Th. de Boer, H.A. Kramer, G.J. Schrotenboer and J.R. Moed)

The main aim of this project is to identify the nutrients which possibly co-regulate algal periodicity in the Tjeukemeer. Like in the previous years, the temperature, turbidity, salinity, nutrients, chlorophyll-a and the algae in the Tjeukemeer were monitored.

From mid November to December 1979 polder water entered the Tjeukemeer, resulting in an increase of $E_{365}^{1.0}$ from 0.06 to 0.20, a value of 0.18 being normal for this period. In 1980, from March onwards the extinction value decreased gradually to its normal summer value of 0.06 (Fig. 16). The increase in July-August is due to the inlet of polder water. This is also true for the considerable increase, mainly during November, in 1980. In the periods January 12-31 and February 8-16 the lake was covered with an ice-layer. The inlet of IJsselmeer water started at the end of April but was very irregular and restricted (Fig. 16). Only during May-June and mid August-mid September did the chloride concentration increase due to inflowing IJsselmeer water. This is because of the abnormally wet summer.

In 1980 we started monitoring the Mn_{diss}-concentration (Fig. 16). During February-March the concentration dropped from 175 $\mu g.l^{-1}$ to almost zero. A slight increase was noticed: (i) during May-June, coinciding with the inlet of IJsselmeer water; and (ii) in December, due either to inlet of polder water or to a short ice-period or both. The decrease in July has already been considered (see 3.2.1). In addition, this decrease coincided with a decline of the pH from 10.2 to 9.0. Further, it is striking that during August-November the Mn_{diss}-concentration re-

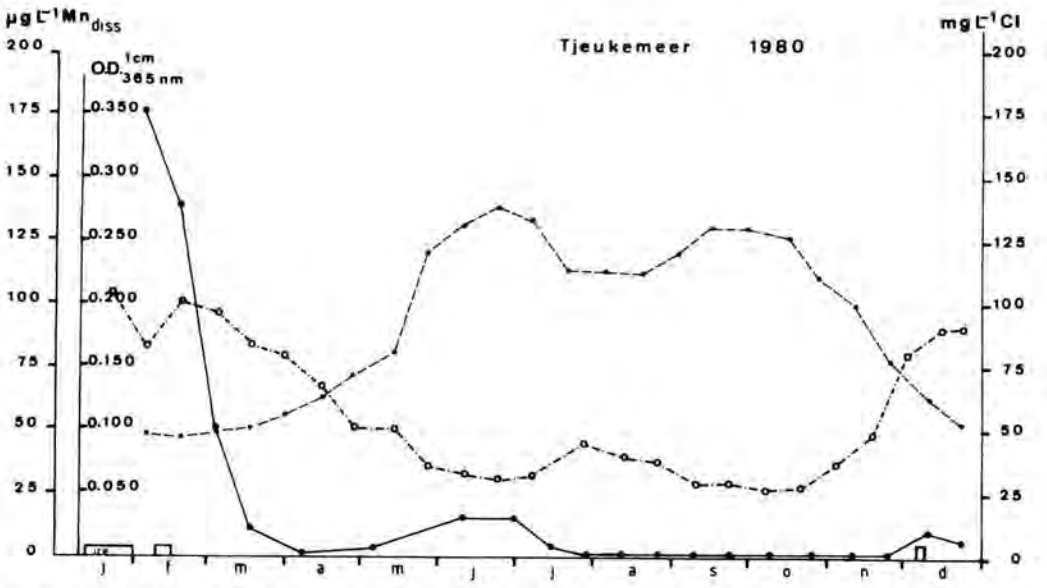

Fig. 16. Course of Mn_{diss} (.——.), chloride * --- * and optical density, O.D. $^{1cm}_{365nm}$ (O-.-O) in Tjeukemeer east in 1980. Manganese determined colorimetrically at pH 4.

mained almost at the zero. The role of precipitation by carbonate will be investigated. Also, in the bio-assay experiments such an effect will be considered.

The course of $Tot\text{-}P_{diss}$, $Tot\text{-}Si_{diss}$, $Tot\text{-}Fe_{diss}$, NH_4^+ and NO_3^- was also followed in 1980. The observed maximal values for P and Si, in February, did not exceed the postulated upper limits of 0.3 and 5 $mg.l^{-1}$ for the Tjeukemeer. The course of the (dissolved) nutrients is generally similar to that of Mn_{diss} (Fig. 16). The differences concern the rates of decrease in spring, the values of the summer level, the fluctuations around this level, and the rates of response to the incoming polder water in the last part of the year. Thus, in the period June-August the values of $Tot\text{-}P_{diss}$ and Si_{diss} increased considerably but only slightly in case of $Tot\text{-}Fe_{diss}$. Around mid October polder water entered the Tjeukemeer. The response in time was in the following sequence: $Tot\text{-}Si_{diss}$, E_{365}, $Tot\text{-}Fe_{diss}$, NH_4^+, $Tot\text{-}P_{diss}$, N_{diss}^{org}, NO_3^- and Mn_{diss}. These results again point to the unfavourable conditions for Mn to dissolve. In conclusion, it seems that during 1980 N, Mn and Fe were important in co-regulating algal periodicity. Si and P might have occasionally been involved.

3.2.4. Monitoring physical factors (project A3; H. Hoogveld)

The information on solar irradiation, lake turbidity and lake temperature can be used for a general description of the lake and for case studies. An example of the latter is mentioned under project A7.4, where data of temperature and solar irradiation are related to the differences in the initial growth rates of diatoms and blue-green algae. In 1980, like in previous years, solar irradiation was measured daily, using a Kipp solarimeter, and transparency (Secchi disc) and temperature at weekly intervals. No strikingly deviating values were recorded compared to those in 1979.

3.2.5. Hydraulics and hydrology of the Tjeukemeer (project A4; J. Voerman, R. Mevissen, J.D. Leenen (Agricultural University), N. van Benthem and J.R. Moed)

The main aim of this project, namely, the development of a model to calculate the inflow of water into the Tjeukemeer in a known period, was realized. Using the average weekly chloride concentrations of 10 stations in the lake, and that of the inflow into the lake, the discretized mass-balance equation for chloride gives a good estimate of the total amount of water flowing into the lake per week or 14 days.

With the wind acting as a driving force, a horizontal dispersion mechanism exists within the lake . Because of the small average depth (1.80 m) it is assumed that the mixing in the lake is complete.

A one-dimensional dispersion model based on a numerical solution of the convection-diffusion equation has been developed. The horizontal dispersion coefficient in this model was calibrated on the weekly chloride samples in the lake in 1971. An average dispersion coefficient of 1 $km^2.day^{-1}$ was found adequate to describe the variation in chloride concentration from one side of the lake to the other.

Furthermore, it was tried to relate the direction and the value of flow rates in the 'Follega sloot' to the inlet of IJsselmeer water at Lemmer. This will give us information to what degree the application of flow rate measurements can be used to follow the hydraulics in the Tjeukemeer. In the period August-November the flow rate in the 'Follega sloot' was measured almost daily. Data of four short periods of inlet of IJsselmeer water at Lemmer and the resulting increase of chloride-content in Tjeukemeer-west, were obtained. No correlation with the flow rate in the 'Follega sloot', however, was observed.

3.2.6. Bottom of the Tjeukemeer (project A5)

The execution of this project has been delayed. Due to financial reasons the regional Government agency was not able to assist us in preparing a new map of the Tjeukemeer showing the various depths. Therefore, apparatus needs to be purchased to fix sampling positions in the lake.

3.2.7. Availability of nutrients (project A6)

The aim of this project is to test the degree of availability of the various (phys-

ico-)chemical forms of nutrients for the abundant algal species. Fe, P, N and trace elements will be considered. This will provide information on the capacity of species to compete for the uptake of the various nutrients (K_S-values).

Cultivation of abundant algal species (project A6.1; H.L. Hoogveld, P.J. Timmer and J.R. Moed)

In 1980 the blue-green algae *Oscillatoria limnetica*, *O. redekei*, *O. agardhii* and the green alga *Planctonema lauterbornii* were isolated. *O. redekei* cells show a rose colour. This species will be further characterized (project A10).

Availability of iron (project A6.2; Th. de Boer and H. de Haan)

Our data on the size fractionation of some major and minor elements smaller than 2000 Å in the Tjeukemeer as obtained by ultra filtration are listed in Table 13. The main conclusions drawn from the data are:
1. Most of the Na, K and Si in Tjeukemeer water occur in dissolved forms, i.e., are smaller than 12.5 Å.
2. About 30% of the Ca and Mg occur in a size range between 15 and 18 Å. The latter is colloidal, most likely due to supersaturation with $CaCO_3$.
3. Remarkably, the size fractionation of neither Ca nor Mg follows that of org.C. Since most dissolved organic C in the Tjeukemeer is a constituent of fulvic acids, this finding raises doubt about the existence of humus-iron-phosphate complexes. However, the simultaneously occurring organic dissolved C, Fe, and P fractions between 140 and 2000 Å, point to humus-iron-phosphate colloids formed by peptization of colloidal $FePO_4$ and $Fe(OH)_3$, by fulvic acids. Whether the distribution of the elements over the size fractions is seasonally dependent is being examined.

Studies on the relationships between the physico-chemical form and the availability of Fe in Fe-limited chemostats of abundant algal species from the Tjeukemeer were continued. It was found that to achieve optimal Fe-limited growth of *Scenedesmus quadricauda*, a maximal amount of complexing ability is needed in the growth medium. Interpreting the effects of the EDTA concentration on the growth of *S. quadricauda* in terms of availability of Fe, these results are not unexpected.

The Fe-limited growth of *Oscillatoria limnetica* is also dependent on the EDTA concentration. Compared with *S. quadricauda*, less EDTA per atom of Fe is needed to achieve optimal Fe-limited growth of *O. limnetica*. Undoubtedly, this result reflects the differences in affinity for Fe of the two algae.

The most important conclusion from the studies on the effects of the EDTA concentration on the Fe-limited growth of the green and the blue-green algae is, that the growth parameters μ_{max} and X/S_R and, therefore, also K_S, are a function of the physical chemistry of the growth-limiting substrate.

Besides the complexing ability, the pH also affects the physical chemistry of Fe and, therefore, its availability. This is clearly demonstrated by the Fe-limited

Table 13. Size fractionation of some major and minor elements in 0.2 µm filtered Tjeukemeer water. Amounts are in %.

Element	< 12.5 Å	< 15 Å	< 18 Å	< 22 Å	< 140 Å	< 2000 Å
Ca	61	82	95	97	97	100
Mg	66	84	98	100	100	100
Na	89	96	96	99	99	100
K	89	95	96	99	99	100
P	13	14	22	45	45	100
N	82	87	89	97	99	100
Fe	1	2	9	9	12	100
Si	98	98	98	99	99	100
org. C	9	19	52	86	88	100

growth of *S. quadricauda* with (pH = 8.0) and without (pH = c. 10.5) a pH-stat. At pH 10.5, when Fe is expected to be less available than at pH 8.0, μ_{max} and X/S_R were found to be lower than at pH 8.0 (Table 14). Remarkably, however, a lower K_S at the highest pH was found. Possibly Fe-cell interactions and the formation of Fe colloids interfered with the determination of dissolved Fe (s) in the chemostat and led to erroneous μ-s curves. As may be expected the minimum cell quotum (q_0), i.e., the minimum Fe content of the algal cells for growth was found to be the same in both cases.

Table 14. Some growth parameters of *Scenedesmus quadricauda* in Fe-limited chemostats at pH 8.0 and pH c. 10.5.

pH	K_S ($\mu g\ Fe.l^{-1}$)	μ_{max} (day^{-1})	X/S_R ($mg.\mu g^{-1}$)	q_0 ($\mu g\ Fe.mg^{-1}$)
8.0	11	1.3	4 - 8	0.07
10.5	1 - 2	0.8	1 - 6	0.09

Availability of copper (project A6.4; Th. de Boer, J. Voerman and H. de Haan)

The main aim of this sub-project is to investigate to what extent Cu and Zn occur in phycologically toxic concentrations in the Tjeukemeer (see also A7.1).

The Tot-Cu concentration in the Tjeukemeer varies enormously, namely from 7 to 64 $\mu g.l^{-1}$. Remarkably, these variations do not appear to be related to the hydrological parameters of the lake. This questions the source of the high Cu concentration. Also the fluctuating Tot-Cu concentrations in the IJsselmeer are unexplainable. The high mean Tot-Cu concentration (30 $\mu g.l^{-1}$) of the Tjeukemeer is stressed, because since 1977 the level in the river Rhine did not exceed 20 $\mu g.l^{-1}$.

The value of the Tot-Zn concentration (9-24 $\mu g.l^{-1}$) in the Tjeukemeer, as determined by FAAS after APDC concentration, is preliminary.

Availability of nitrogen (project A6.5; L. Arkesteijn-Dijksman and J.R. Moed).

In 1979 laboratory bio-assay experiments indicated that omission of nitrogen in a mixture of Tjeukemeer water and a synthetic medium (M 26) strongly decreased algal growth. Prior to *in situ* bio-assays, parameters indicating N-limitation for algae in the lake itself are being tested. At present the ratio carotenoids: chlorophyll-a is being investigated.

3.2.8. Bio-assay experiments (project A7)

The aim of this project is to identify factors co-regulating the algal periodicity in the Tjeukemeer.

Detection of nutrient limitation (project A7.1; J.H. Coers, J. Schreurs, H. de Haan and J.R. Moed)

Bio-assays are being performed at the laboratory to identify nutritional factors. Following the multiple addition technique, in which to Tjeukemeer samples medium minus one nutrient is added, changes in growth are followed. The increase of growth in a complete medium is assumed as 100%. This is the so called high control (Fig. 17), compared to Tjeukemeer water called 'low control'. Optical density, chlorophyll and algal numbers are determined.

During 1979 the omission of trace-elements in the bio-assays enhanced phytoplankton growth. It is likely that the addition of trace-elements raised the concentration of one or more of these elements in the lake water to a growth inhibiting concentration. Since Tot-Cu concentration in the Tjeukemeer was relatively high, this element was considered to occur in phycologically toxic concentrations. Therefore, in 1980, the effect of the omission of the separate trace-elements on phytoplankton growth in the bio-assays was studied.

Although the analyses have not been finished, some tentative results can be reported:

1. It seems that from mid April till mid May the response, i.e., increase in the numbers of blue-green filaments, to the addition of complete medium is greatly positive, whereas such a response decreases gradually from mid May till July (Fig. 17). Around mid August a recovery is observed. Such a course points to an impaired bio-assay that needs further investigation in relation to the behaviour of Cu and Mn. The decrease of the numbers in the low controls and in the Tjeukemeer (Fig. 17) hints at the nutritional status of the lake water both in the laboratory - low control - and in the lake itself.

2. For the period mid April-mid May it appears that the omission of N, Mn-EDTA, P and Fe-EDTA - and not the EDTA alone -, reduced to a greater or lesser extent the growth of blue-green filaments in the flasks.

3. In 1980 the growth inhibiting effect of the addition of 6.7 $\mu g.l^{-1}$ of Cu, measured as optical density, is significantly correlated with the Tot-Cu concentration in the lake water ($n = 17$; $r = 0,456$; $P < 0.05$). However, it is not yet established that the actual Cu concentration inhibits phytoplankton growth in the Tjeukemeer.

Detection of regulating physical factors (project A7.4; J.R. Moed, J.H. Coers and H. Hoogveld)

The aim of this sub-project is to investigate if in January-February the initial growth rates of diatoms in the Tjeukemeer are higher than those of blue-greens and the physical factors which control this. In this lake, even during the early months, the diatom numbers are high and during the rest of the year the blue-greens dominate.

In 1980, diatom growth was observed at 2.5°C, and that of blue-green algae at 8°C. The corresponding values of daily irradiation (project A3) were 150 and 400 $cal.cm^{-2}.d^{-1}$. The role of the light and temperature is being investigated in laboratory bio-assays. To eliminate the influence of lacking organic growth factors, the lake sediment was added. Preliminary results show that addition of bot-

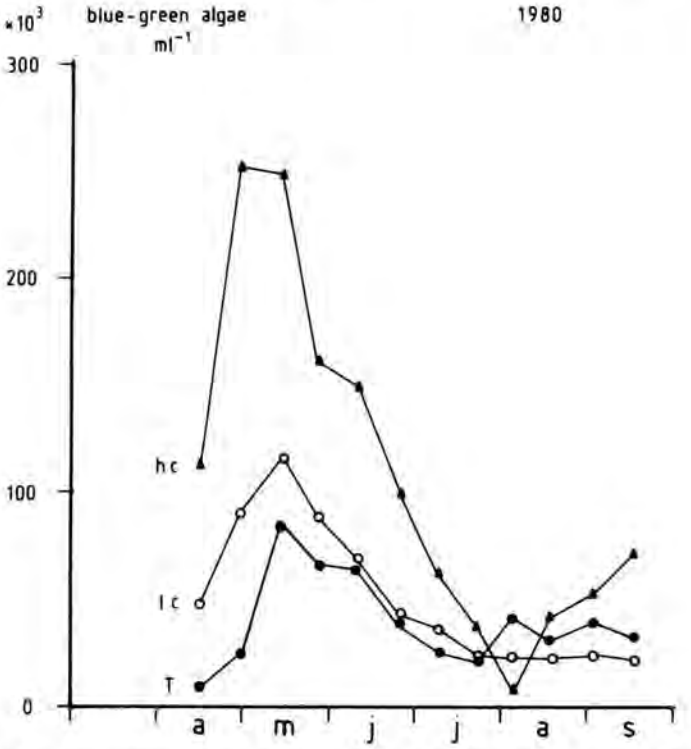

Fig. 17. Filaments of blue-green algae in the high control (h.c.) and low control (l.c.) laboratory bio-assays, and in Tjeukemeer-east (T) in 1980. The high control is a mixture of complete medium and Tjeukemeer water; in the low control medium is replaced by distilled water.

tom material retards rather than stimulates growth. This seems to be true for experiments performed at 4°C, but the decrease of numbers of algae counted at 8°C was marked. The tests for the lack of nutrients or for extreme bacterial activity were negative. Therefore it is tentatively concluded that bottom material of the Tjeukemeer may retard algal growth. For investigation of the mechanism involved, these experiments will be repeated.

3.2.9. Mineralization of algae (project A9)

The aim of this project is to investigate to what degree the catabolic functions may be linked to the algal periodicity. In this regard the applicability of dark incubation as 'tool' is being investigated. Experience was gained in studying the influence of dark incubation upon the viability of *Diatoma elongatum* (A9.1). This alga will be tentatively used in model studies.

The viability of Diatoma elongatum after dark incubation (project A9.1; J.R. Moed, H.L. Hoogveld and P.J. Timmer)

Initially, the relation between chlorophyll-a degradation products and dead cells was investigated. At present, the effect of various limiting growth conditions followed by dark incubation upon viability and morphological appearance is being investigated as well.

The problem of growth against the flask walls and of nitrite formation in the medium (up to 60 $\mu g.l^{-1}$ of N) delayed the progress of the investigation.

3.2.10. Taxonomy of blue green algae (project A10)

Oscillatoria redekei and O. limnetica (project A10.1; H.L. Hoogveld and J.R. Moed)

The taxonomical differences and similarities between *O. redekei* and *O. limnetica* are being investigated. Up to now these algae have been counted as two distinct species. Both are abundant in the period April-June almost every year. In a multi-species experiment a maximum of *O. redekei* filaments was followed by that of a species resembling *O. redekei* greatly, but showing reduced vacuolization (Progress Report 1979, Table 4). Therefore, it was assumed that *O. redekei* might be identical to *O. limnetica*, which has no vacuoles. Experiments are being performed to investigate this assumption. Attention was focussed at light and total content of ions as factors. The light intensity, light period and nature of the light were varied.

Starting with an *O. limnetica* clone, vacuolization could be induced to a degree half as much as observed in *O. redekei* in the lake. On the other hand, starting with the rose coloured *O. redekei* (project A6.1) vacuolization could be reduced by half. In both cases it is true for numbers as well as diameter of the vacuoles. In view of the results obtained so far the work is being continued.

3.3. WORKGROUP 'FOODCHAIN AND PRODUCTION STUDIES'

3.3.1. Introduction

The workgroup is engaged in a study of the relations between the fish populations and their food organisms (see Fig. 18). Population structure, population density, fecundity, mortality, growth, standing crop biomass and production are being estimated for both the fish species and the most important fish-food species. The latter include zooplankton (copepods and cladocerans) and macrofauna elements as chironomids, gammarids and the opossum shrimp, *Neomysis integer*. Much work is directed at 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 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 1^+ and older fish. A detailed study was started on the predation of pike-perch on 0^+ fish and on the competition between eel and bream for chironomids, and bream and 0^+ fish for zooplankton.

3.3.2. A discrete event-oriented simulation model of the subsystem 'fish and its food organisms' in the Tjeukemeer (project V1; J. Vijverberg and A.F. Richter)

As a first step to the modelling of the whole subsystem the population dynamics model 'INSTAR' was developed (Fig. 19). It is a discrete event-oriented simulation model for the analysis of population dynamics and for the estimation of production in cladocerans and copepods with continuous reproduction. It is based on the life cycle of an individual crustacean. Events can be birth, death or moulting. Mortality is computed by comparing simulated densities with observed field densities. Egg-mortality caused by the death of adult females with brood is taken into account. For input/output purposes, animals are lumped together into size classes.

Data used in the model are:

- Length, growth and moulting frequency as function of temperature. These data are obtained from laboratory experiments.
- Population density, fecundity and water temperature as function of time. These data are obtained from field observations.

The fecundity data connect the model system with the lower trophic level, the population density data with the higher trophic level.

3.3.3. Population dynamics and production of copepods and cladocerans in the Tjeukemeer (project V2; J. Vijverberg, C. Fortgens and Th.H. Frank)

The routine sampling programme employed since 1968 was continued. The density of most species was relatively low during 1980: *Eurytemora affinis, Bosmina coregoni, Diaphanosoma brachyurum, Daphnia cucullata, Ceriodaphnia pulchella* and *Chydorus sphaericus*. The densities of both *Daphnia hyalina* and cyclopoid copepods were very similar to those in other years, only those of *Bosmina longirostris* were unusually high.

The growth rates, development rates, and longevity of copepods and cladocerans were determined in the laboratory (Vijverberg 1980, see par. 4.1). Distribution patterns of zooplankton in the Tjeukemeer are described in de Nie, Bromley & Vijverberg, 1980 (see par. 4.1).

The simulation model 'INSTAR' was used to integrate the field data of *Daphnia* with the laboratory data on development rates, growth rates and length-weight

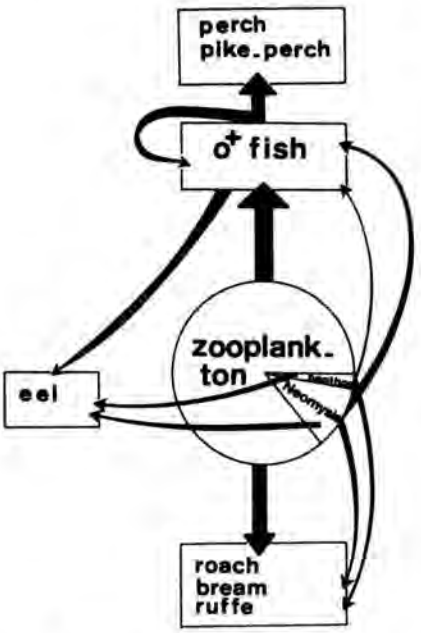

Fig. 18. Research field of the workgroup 'Foodchain and production studies' (1978-1983).

relationships. The population dynamics is mainly regulated by temperature and fish predation, the latter affects both the birth and mortality rates. Total annual production of *Daphnia* was 3.1-6.9 g organic matter.m^{-2}; the annual P/B ratio ranged from 25 to 40 for *Daphnia cucullata* and from 45 to 49 for *Daphnia hyalina*.

3.3.4. Population dynamics and production of chironomid larvae in the Tjeukemeer (project V3; J. Vijverberg, C. Fortgens and Th.H. Frank)

Samples were taken every 3 weeks from March to November at ten different stations in mud- and sand-substrates. Only some of the samples taken during 1980 have been analysed so far, but it seems that *Einfeldia* sp. was particularly abundant.

3.3.5. Autecology of Leptodora kindtii in the Tjeukemeer (project V4; J. Vijverberg and Th.H. Frank)

Samples for the estimation of population densities and population structure were taken fortnightly at two different depths. The samples have been stored, but not examined yet. Fecundity was assessed using live material.

3.3.6. Autecology of Neomysis integer (project V5a; J. Vijverberg and P.J. Mac Gillavry)

Since the population densities of *N. integer* were very low in the Tjeukemeer during 1980 (mean annual density c. 0.2 ind. m^{-2}), their diet and population biology were studied in the Slotermeer (see Student Report of P. Bremer, par. 4.4).

3.3.7. Abundance of macro-crustaceans in the reed-beds (project V5b; J. Vijverberg and H.W. de Nie)

The population densities of *Asellus* spp. and *Gammarus* spp. in the reed-beds were as low as in 1979.

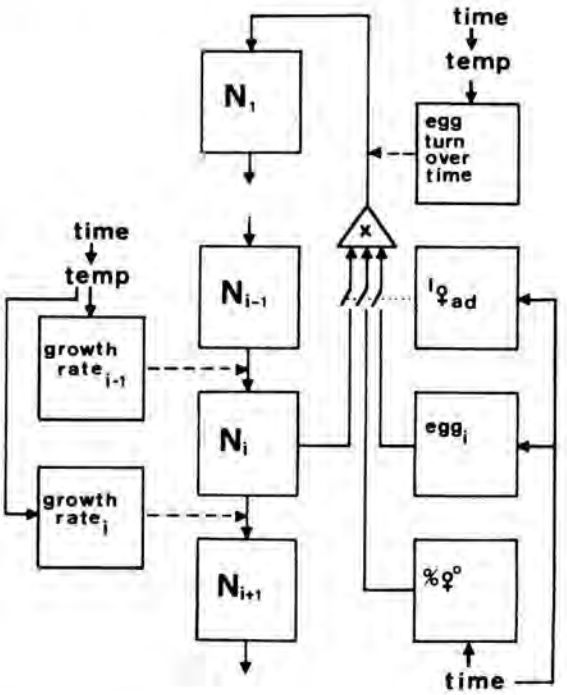

Fig. 19. Simplified flow diagram of the interdependence in the population dynamics simulation model 'INSTAR'. N_i = population density of ith size class, $l_{\varphi ad}$ = length of first adult female instar, egg_i = number of eggs of female in ith size class, % ♀° = percentage of adult females with eggs in relation to total number of females.

3.3.8. Ecology of 0⁺ fish in the Tjeukemeer (project V8; W.L.T. van Densen, E.G. de Boer, A.G. Frank-Landman, P.J. Mac Gillavry and A. Ypma)

Due to the low numbers at the end of 1979 the recruitment of the 0^+ smelt depended completely on the inlet of larvae from the IJsselmeer. During the dry spring of 1980 large volumes of water with smelt- and perch-larvae were let in. Because of the high abundance at the start of the season the survival rate was also high during the rest of the summer (depensatory mortality). Instantaneous daily mortality rates for 0^+ smelt and perch based on trawl samples were 0.014 and 0.018 respectively.

A remarkable situation existed for 0^+ pikeperch which had a very poor recruitment at the start of the season, but had optimal conditions to become a strong year class. At the end of June the density of small smelt was high enough for the young pikeperch to become piscivorous. The survival of 0^+ pikeperch was ensured by numerous 0^+ smelt and perch, forming a buffer against predation by older pikeperch.

0^+ smelt dominated the food of the 0^+ piscivorous pikeperch, but also roach, bream, perch and even pikeperch were consumed. During a 24 hour sampling period with 4 hour intervals in mid August, the stomach contents of piscivorous pikeperch appeared to be constant over the day but decreased between 21.00 hr and 05.00 hr in the morning to the minimum.

3.3.9. Ecology of smelt-, perch- and pikeperch-larvae (project V11; W.L.T. van Densen, E.G. de Boer, A.G. Frank-Landman, P.J. Mac Gillavry and A. Ypma)

The abundance of pikeperch larvae at the beginning of June 1980 was as low as in June 1979. This may have been due to starvation during the critical period (6-7 mm in length). The density of nauplii at the end of May was low (< 30 ind. l^{-1}), but it can be as high as 300 ind. l^{-1} in the Tjeukemeer. Further research will be directed at the feeding and survival of pikeperch larvae in relation to the occurrence of nauplii and young copepodites.

3.3.10. Population structure and feeding of I⁺ and older pikeperch and perch (project V12; W.L.T. van Densen, E.G. de Boer and A. Ypma)

The condition of pikeperch reached minimal values in June when forage-fish densities were low (Fig. 20). The considerable improvement during the summer was caused by the optimal feeding-conditions due to the presence of 0^+ smelt and perch. In contrast to the summer of 1979 the pikeperch grew in length and the productivity of the population based on dry weight at minimal mortality rates ($z = 0.2\ yr^{-1}$) was estimated at 0.67 for the growing season.

During spring the absolute fecundity and egg dry weight were related to the condition of individual females from Tjeukemeer and Grote Brekken (see Student Report by J.J.V. Meischke, par. 4.4). The relationship between the number of eggs (E) and the fork-length of the fish (L, mm) in the Tjeukemeer (1980) can be described by: $\ln E = 4.3807 \ln L - 14.789$ ($r = 0.748$, $n = 34$, $p < 0.0005$).

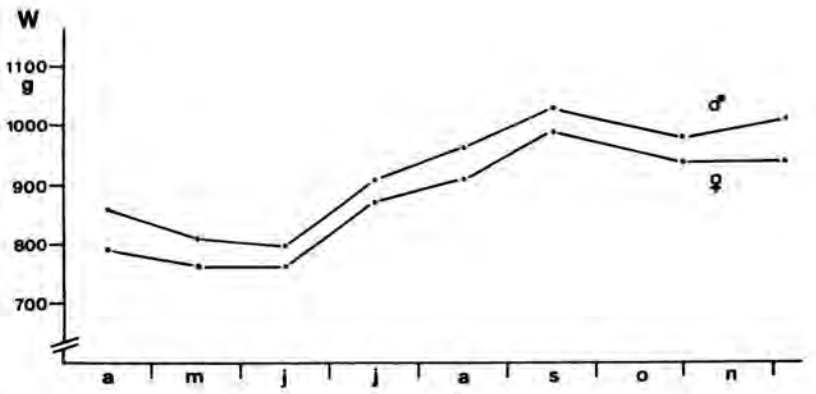

Fig. 20. Seasonal variations in the somatic weights (g fr wt) of 450 mm (fork-length) male and female pikeperch in the Tjeukemeer (1980).

3.3.11. Ecology of I^+ and older bream, white bream and roach in the Tjeukemeer (project V14; E.H.R.R. Lammens)

The cyprinid populations, viz. bream (*Abramis brama*), roach (*Rutilus rutilus*) and white bream (*Blicca björkna*) were sampled monthly by trawling at five stations in the Tjeukemeer. The length-frequency distribution, length-weight relation, sex, gonad weight, age and stomach contents were determined for each species.

Bream

In winter the feeding of smaller bream (< 30 cm in length) almost ceased and schooling increased. There was an inverse relationship between the magnitude of schooling and the length of the individual fish. This was reflected in the decreasing catches for the smaller fish. The distribution pattern of feeding bream was determined. The larger bream (> 30 cm in length) is located in places richer in chironomids. The mature bream (> 25.5 cm for males, > 27.5 cm for females) started feeding by late March and spawned during the third week of May. Growth was minimal in June and July and mature females, building up their gonads, increased in weight in August only.

Feeding conditions were very poor this year because of the very large numbers of young smelt and perch competing with the bream for zooplankton. For bream in the Tjeukemeer zooplankton is the most important food source. Only the year classes 1979 (5 cm) and 1975 (24.5 cm) grew, respectively, 4 and 2 cm and produced 25 and 15 kg fr wt.$ha^{-1}.yr^{-1}$. Of the mature year classes 1973 (29 cm) and 1970 (32.5 cm) only the females produced some gonad tissue, which accounted for only 2 kg fr wt.$ha^{-1}.yr^{-1}$. Mean annual bream biomass was c. 250 kg fr wt.$ha^{-1}.yr^{-1}$.

The food items based on the analysis of the stomach contents changed seasonally. The most important food items were: copepods, in April; *Daphnia*, in May and June; chironomids (mainly *Cladotanytarsus*, *Procladius*, *Cryptochironomus* and *Einfeldia*), in July and August; and copepods and benthic cladocerans (mainly *Alona* and *Leydigia*), in September and October.

Roach

This species was studied less intensively than the bream. Roach did not stop feeding during winter although it slowed down; this was also true for the gonad development. Spawning started a few days earlier than that of bream. Food consisted throughout the year of the snails *Valvata piscinalis* and *Bithynia tentaculata* and the mussel *Dreissena polymorpha*. Roach reached a length of 30 cm in 11 years, which is very good.

White bream

This species also was studied less intensively than bream. The diet is almost the same as that of the bream, but the feeding does not stop in winter. Spawning started a few days later than that of bream. White bream reached a length of 25 cm in 11 years, which is medium to good.

3.3.12. Ecology of the eel (Anguilla anguilla) in the Tjeukemeer (project V16; H.W. de Nie)

Eel was sampled regularly using different methods. Most fish was caught by electro-fishing during the day and by trawling at night. The length and weight were measured, stomach contents analysed and the otoliths were collected for age determinations.

To study the diurnal feeding periodicity, samples were taken at 4-hour intervals during a 24 hour period on August 20-21. The fishing was carried out at one station and the feeding of the bream was studied simultaneously by Lammens (project V14).

During the spring and early summer the length class 24 to 28 cm dominated the catch. In August the smaller classes (14 to 20 cm) became more abundant. The percentage of males in the group of eels larger than 28 cm varied between 74 and 97%. Eels sampled at the intake of the Bergumermeer Power Station during summer differed markedly from those caught in the Tjeukemeer. The length class 18 to 20 cm strongly dominated, more females were caught and the condition index was

24% lower than in the Tjeukemeer. In the Bergumermeer 6.0% had cauliflower disease while in the Tjeukemeer this was only 1.4%.

In June young perch and smelt became important food items for larger eel (> 27 cm). During the growth season the pupae of the chironomids *Einfeldia* and *Chironomus* and the macro-crustaceans *Gammarus* and *Asellus* were the main food items for all length classes.

A distinct diurnal variation in feeding intensity was observed (Fig. 21). The percentage of eels with completely filled stomachs varied from 7% in the afternoon to 70% in the early morning.

3.3.13. Ecology of 0⁺ fish in the Frisian Lakes (project V20; W.L.T. van Densen)

Most lakes received large amounts of smelt larvae via the incoming IJsselmeer water. Depensatory growth during the season depressed the size of smelt and perch at the end of the season.

Poor recruitment of pikeperch larvae was observed only in the Tjeukemeer, Grote Brekken and Langweerder Wielen. Only in Tjeukemeer and Bergumermeer did an appreciable proportion of 0⁺ pikeperch become piscivorous. In all the other lakes investigated the small planktivorous pikeperch were dominant at the end of the season. Their survival was ensured by large amounts of smelt and sometimes perch.

4. Publications

4.1. PAPERS PUBLISHED IN 1980

Best, E.P.H. - Effects of nitrogen on the growth and nitrogenous compounds of *Ceratophyllum demersum*. Aquat. Bot. *8*,197-206.

The effects of high concentration 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

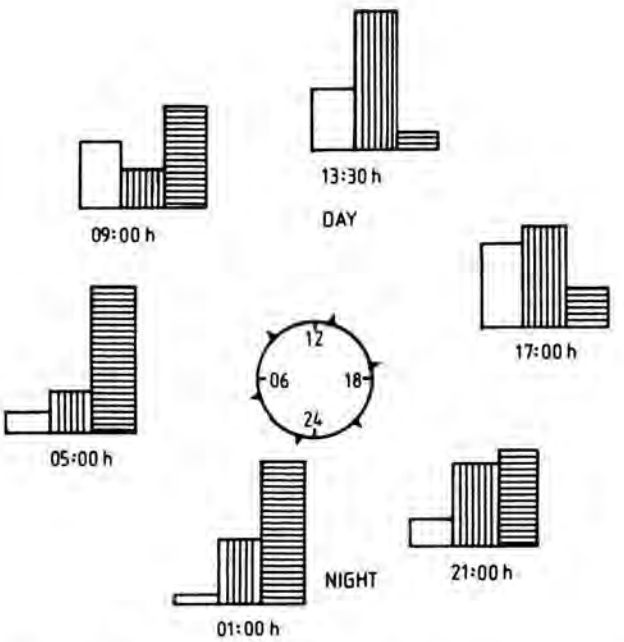

Fig. 21. Diurnal changes in the percentage stomach fullness of eel in the Tjeukemeer (August 20-21, 1980).
< 20% filled, 20-60% filled, > 60% filled.

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.

Best, P.H. - The submerged aquatic macrophytes in Lake Maarsseveen I: species composition, spatial distribution and productivity. In: Limnological research in the Maarsseveen Lakes, 1975-1980. Ed. J. Ringelberg; Dept. of Aquatic Ecology, Univ. of Amsterdam, p. 205-218.

See Progress Report 1979, p. 44.

Best, E.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 (Abstract). Hydrobiol. Bull. *13*, 112.

See Progress Report 1979, p. 34.

Cappenberg, Th.E. - Use of radio gas chromatography in studying break-down processes of organic matter in aquatic ecosystems. In: Agrochemical residue-biota interactions in soil and aquatic ecosystems. International Atomic Energy Agency, Vienna. 1980, p. 55-66.

See Progress Report 1979, p. 41-42.

Cappenberg, Th.E. - Carbon flow in the anaerobic ecosystem of a stratifying lake (Abstract). In: Abstracts of the Second International Symposium on Microbial Ecology, University of Warwick, Coventry, 1980, p. 93.

Anaerobic mineralization is an important but still little understood route of carbon flow in aquatic ecosystems, as in the anaerobic hypolimnion and sediments of Lake Vechten. Attention will be paid to the sedimentation rates and to the nature of the sedimented particulate organic matter to the break-down pathways and turnover rates of the important metabolic intermediates in methanogenesis, and to the distribution and activity of bacteria growing on these substrates. Emphasis will be laid on kinetic aspects, that is, the use of a few tracer (^{14}C) substrates is sufficient to quantify, by measuring their respective pool sizes and turnover rate constants, the major part of the carbon flow in the detritus food chain in sediments. Finally, the significance of the in- and output of C in respect to carbon cycling in the lake ecosystem will be discussed. (Complete text of abstract.)

Golterman, H.L., J. Voerman and H.W. de Nie - Phosphate loading of Tjeukemeer (in Dutch). H_2O *13*, 116-121.

Gons, H.J. - Periphyton in Lake Vechten, with emphasis on biomass and production of epiphytic algae (Abstract). Hydrobiol. Bull. *13*, 116.

See Progress Report 1979, p. 35.

Gons, H.J. and L.R. Mur - Energy requirements for growth and maintenance of *Scenedesmus protuberans* Fritsch in light-limited continuous cultures. Arch. Microbiol. *125*, 9-17.

Scenedesmus protuberans Fritsch was grown in light-limited continuous cultures with a light-dark cycle, at temperatures of 20° and 28°C. At 20° irradiances of 12 and 38 W.m^{-2} were used, at 28° 38 W.m^{-2}.

The relationships between growth rate and light uptake rate were of diphasic linear character. With the lower growth rates the relationships were defined with the parameters μ_e, i.e., the specific maintenance rate constant, and c, the 'true' efficiency of light energy conversion into biomass. The μ_e-value was dependent on temperature, the c-value on irradiance.

In cultures incubated in prolonged darkness decrease rates of biomass were comparable to the derived μ_e-values.

Both diphasic linear relationships between growth rate and light uptake rate and the same order of magnitude of μ_e-values could be derived from literature data on other green algae.

Gulati, R.D. and G. 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 *72*, 211-222.

See Progress Report 1979, p. 25.

Gulati, R.D. - A tribute (to Dr. S.V. Ganapati). Hydrobiologia *72*, 7.

Nie, H.W. de - Effects of thermal effluents from the Bergum power station on the zooplankton in Lake Bergum (Abstract). Hydrobiol. Bull. *13*, 100.
See for summary Internal Report 1980-2 by J.B.W. Wanders *et al.* (paragraph 4.3).

Nie, H.W. de, H.J. Bromley and J. Vijverberg - Distribution patterns of zooplankton in Tjeukemeer. The Netherlands. J. Plankton Res. *2*, 317-334.
See Progress Report 1979, p. 17.

Parma, S. - The history of the eutrophication concept and the eutrophication in the Netherlands. Hydrobiol. Bull. *14*, 5-11.
Eutrophication has been defined in various ways. Some authors stress the increase in nutrient concentration, especially nitrogen and phosphorus compounds, others the effect of the presence of these nutrients, viz. increase in biomass. A historical survey is given of the origin of the eutrophication concept as developed by Naumann and Thienemann. In the present paper eutrophication has been defined as the process in water by which the factors which stimulate autotrophic production become optimal.
Eutrophication in the Netherlands has been recognized only very recently as an alarming pollution problem.
Since about 1975 the problem got serious public and political interest. This resulted in 1979 in the so-called Fosfatennota ('Phosphate Report') of the Ministry of Health and Environmental Protection. This report recommends a reduction of phosphate loading in Dutch surface waters to values below 0.5-1.0 g. $P.m.^{-2}.y^{-1}$.
Restoration of eutrophic waters will be a laborious process.
A serious question is to what biological situation a water basin must be restored. The description of the 'original' situation is particularly difficult. Regarding this some methods are discussed.

Parma, S. (ed.) - Progress Report 1979 Limnological Institute. Verh. Kon. Ned. Akad. Wet. Afd. Natuurk. Reeks 2, *75*, 48 pag.

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. *89*, 509-532.
See Progress Report 1979, p. 42.

Vijverberg, J. - Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, The Netherlands. Freshw. Biol. *10*, 317-340.
See Progress Report 1978, p. 252.

Wanders, J.B.W. - Effects of thermal effluents from the Bergum power station on the phytoplankton in the Bergumermeer (Abstract). Hydrobiol. Bull. *13*, 98-99.
See for summary Internal Report 1980-2 by J.B.W. Wanders (paragraph 4.3).

4.2. PAPERS IN PRESS

Beattie, D.M. - Investigations into the occurrence of midge plagues in the vicinity of Harderwijk (The Netherlands). Hydrobiologia *80*, 147-159 (1981).
Between February and October 1977 a study was made of the total chironomid larval population in the Wolderwijd, a large, shallow Dutch lake. The main species distribution pattern, according to substrate and total larval population size, was of the same order of magnitude in both substrates. Eutrophication did not cause extraordinary large chironomid populations to produce excessive numbers of adult midges. The midge plagues experienced in the area are ascribed to the attraction of the midges to artificial light displayed by housing estates in the neighbourhood of the lake.

Best, E.P.H. - A preliminary model for growth of *Ceratophyllum demersum* L. Verh. Internat. Verein. Limnol. *21*.
A predictive model is presented to calculate growth and primary production of *Ceratophyllum demersum* during the year. Growth, i.e., production of structural plant material per stratified m^2 of water column, is considered to be regulated by the plants' metabolic pool and its photosynthetic carbon fixation. The main driving

variables are temperature and solar radiation. Light quantity in the water column is largely dependent on the depth distribution of plant biomass. Differences in photosynthetic activity due to senescence are considered. Nutrient relationships are not included in the model.

The simulated data are compared with measured production rates and standing crops for three depth classes of *Ceratophyllum demersum* in Lake Vechten, The Netherlands.

Best, E.P.H. - Hormonal interactions in *Ceratophyllum demersum*. Aquat. Bot.

Ceratophyllum demersum is a submerged aquatic macrophyte without roots. It occurs in winter in the dormant form and in summer in the vegetative form. In order to find a relationship between the plants' morphology and growth capacity, the endogenous contents of several phytohormones were determined during the year and their antagonism was tested.

In winter, when no growth occurs under natural conditions, the concentration of ABA was high and that of IAA low. In summer, however, when subsequently transitions from the resting period to strong growth and again to dormancy occur, the IAA concentration had its maximum and that of ABA was variable. The total GA activity was much lower in fast-growing plants than in dormant and quiescent ones. GA antagonized the action of ABA, but IAA did not show this effect.

The significance of the growth regulators investigated at present is discussed with respect to the plants' different physiological stages.

Best, E.P.H. - The submerged aquatic macrophytes in Lake Maarsseveen I: Species composition, spatial distribution and productivity. Hydrobiol. Bull.

See Progress Report 1979, p. 44.

Cappenberg, Th.E. - Microbial break-down processes of organic matter in anaerobic freshwater ecosystems. Advances in Microbial Ecology *4*.

See Progress Report 1979, p. 42.

Cappenberg, Th.E., L. van Breemen and J. Kaper - Microbial interactions in anaerobic mineralization: a case of interspecies hydrogen transfer. Microbiol. Ecol. *7*.

See Progress Report 1979, p. 42.

Duncan, A. and R.D. Gulati - The Parakrama Samudra Project, a study of a tropical lake ecosystem. III Preliminary results on the zooplankton. Verh. Internat. Verein. Limnol. *21*.

The paper summarizes the zooplankton results of a collaborative study of Parakrama Samudra, an ancient and one of the biggest man-made lakes in Sri Lanka. The study was carried out in Aug.-Sept. 1979 and March-April 1980.

The most striking result is the virtual absence of crustacean zooplankton. The rotifers were the main group present; their densities in the north basin (up to 3613 ind.l^{-1}) were higher than those in the south during Aug.-Sept. 1979. The densities (640 ind.l^{-1}) were much lower in March 1980. All rotifer concentrations at the inflow stations were much lower than those in the main body of the northern basin. Climatic factors, namely, rain fall and wind speed, and reservoir characteristics such as volume and rates of water inflow and outflow seemed to be important in affecting the abundance and growth of rotifer populations. The rotifers seem to exploit their potential for population increase in the absence of flushing and during calm periods. At present, little can be said about the effect of predation. However, the rotifer abundance and the near absence of crustacean zooplankton may indicate a classic situation of high predation in the latter and low pressure in the former. On completion of data analysis, modelling, particularly in view of the management pressure to which the lake is subject, may provide insight into the ecosystem dynamics.

Golterman, H.L. and A.G. Wisselo - Ceriometry, a combined method for chemical oxygen demand and dissolved oxygen (with a discussion on the precision of the Winkler technique). Hydrobiologia *77*, 37-42 (1981).

A ceriometric method is described in which Ce^{3+} salts are used for the determination of dissolved oxygen and Ce^{4+} salts for the determination of the chemical oxygen demand. The interference of COD in the O_2-determination, a common feature of most Winkler determinations, is corrected. The standard deviation is typically about 1% of the mean, and bias (inaccuracy) is very small. The method

is simple, quick and reliable. The precision and accuracy of the Winkler method is discussed and compared with that of the Cerium method.

Haan, H. de, T. de Boer and H.L. Hoogveld - Metal binding capacity in relation to hydrology and algal periodicity in Tjeukemeer, The Netherlands. Arch. Hydrobiol.

The Cu-binding capacity (Cu-BC) of Tjeukemeer water was measured between June 1977 and December 1979. The Cu-BC averaged 490 $\mu g.l^{-1}$ of Cu (range, 250-800 $\mu g.l^{-1}$); this high Cu-BC value is partly attributable to the humic nature of the lake.

Comparison of the Cu-BC and the colour of the lake (i.e., evaluating the influence of hydrology on the Cu-BC) strongly indicated that in 1979 the Cu-BC was controlled to a large extent by allochthonous organic matter (fulvic acids) from the surrounding polders. Though the polder water contained 2-3 times as much dissolved organic C as the lake water, the Cu-BC per unit of organic C of the lake water was consistently higher, reflecting the differences in algal biomass between the two types of water. Peak values of the Cu-BC between July and August in 1977 and 1978 were connected with the decline of dominant blue-green algal populations, in particular *Aphanizomenon* spp. and *Oscillatoria redekei*. A peak of Cu-BC in November 1977 was connected with a waning population of the green alga *Planctonema lauterbornii*. A high Cu-BC in October 1978 occurred simultaneously with a bloom of *Aphanizomenon* spp. and with very low concentrations of Fe, suggesting an active excretion of metal chelators by *Aphanizomenon* cells growing under Fe limitation.

Haan, H. de, G. Halma, T. de Boer and J. Haverkamp - Seasonal variations in the composition of fulvic acids in Tjeukemeer, The Netherlands, as studied by Curie-point pyrolysis-mass spectrometry. Hydrobiologia *78*, 87-95 (1981).

Three different molecular weight fractions of fulvic acid from Tjeukemeer, sampled monthly between January and September 1978, were studied by Curie-point pyrolysis-mass spectrometry. The winter samples apparently differed markedly in composition from the summer samples. These differences, which were particularly striking in the pyrograms of the high molecular weight fraction, can be explained in terms of fragment molecules attributable to polysaccharides, proteins and/or phenolic polymers.

Haan, H. de, J.B.W. Wanders and J.R. Moed - Multiple addition bioassay of Tjeukemeer water. Hydrobiologia.

Multiple addition bioassays of water from the eutrophic lake Tjeukemeer revealed that almost throughout 1979, when the lake was dominated by *Oscillatoria agardhii*, N and not P was the major algal growth limiting factor. At periods with very low concentrations of $Tot\text{-}Fe_{diss}$ in the lake, chelated Fe was a co-limiting factor. The results of this study further suggested that the concentrations of trace metals have almost reached levels toxic to phytoplankton. This was in particular the case when the lake received relatively humus-poor IJsselmeer water, e.g., water with a relatively low metal binding capacity.

Haan, H. de, D.J. Wijbenga and M.J.W Veldhuis - Aspects of the study of the availability of iron for phytoplankton in Tjeukemeer, The Netherlands (Abstract). Hydrobiol. Bull.

Gradually lowering the Fe concentration of medium MD26 (De Haan *et al.*, in prep.) it was found that the growth of *Oscillatoria limnetica* became Fe limited at a 4 times higher Fe concentration than that of *Scenedesmus quadricauda* when grown in chemostats at constant growth rate ($D = 0.3\ d^{-1}$). This means that the eucaryotic *Scenedesmus* has a more efficient Fe-uptake system than the procaryotic *Oscillatoria*.

The EDTA concentration, i.e., the metal complexing ability, of medium MD26 drastically affected the growth and pigment synthesis of *Scenedesmus* cells grown at Fe limitation. Apparently there is a maximal amount of complexing ability needed to reach an optimal pigment synthesis in Fe-limited growing *Scenedesmus*. This strongly suggests that not only the Fe concentration but also its physico-chemical form, i.e., its availability, effects the metabolism and Fe-limited growth of *Scenedesmus*. Compared with *S. quadricauda*, *O. limnetica* needs less EDTA per atom of Fe to reach optimal Fe-limited growth.

Besides the metal complexing ability the pH also affects the physico-chemical

speciation of Fe and therefore its availability. This was clearly demonstrated by the Fe-limited growth of *S. quadricauda* with (pH = 8.0) and without (9.9 ≤ pH ≤ 10.5) a pH-stat. At pH ± 10, when Fe is expected to be less available than at pH 8.0, K_S and μ_{max} were found to be quite smaller than at pH 8.0. Apparently the less available Fe becomes, the more affinity *S. quadricauda* must have to reach a certain μ_{max}.

The effect of the physico-chemical speciation of Fe on its availability was also demonstrated by comparing the Fe-limited growth of *S. quadricauda* at pH 8.0 with $NH_4Fe(SO_4)_2.12H_2O$ and the dissolved Fe fraction from Tjeukemeer water ($Tot\text{-}Fe_{diss}$) as Fe sources. It appeared from the K_S-values found that the natural Fe fraction is the least available.

The important conclusion from the results of these studies is that the growth parameters K_S and μ_{max} are a function of the physico-chemistry of the growth limiting substrate. Therefore, these growth parameters measured so far, without taking account of these physico-chemical effects, have only relative value.

Opstelten, W. - Diel patterns of phytoplankton productivity in Lake Vechten (Abstract). Hydrobiol. Bull. *14*, 219-220 (1980).

A review of the literature on phytoplankton primary production studies in lakes indicates that in most cases the daily primary production rates have been derived by multiplying the short-term (usually 3 or 4 hours) production measurement with a light factor. This factor is a ratio between the total irradiance during the day and during the period of incubation. The aim of the present study was to verify this method of calculation. Since the primary production rate is of great importance for the carbon cycle of lakes, this study can be regarded as a fundamental contribution to the integrated research of the ecosystem of Lake Vechten (Best *et al.*, 1978).

Photosynthetic carbon fixation was determined using the radiocarbon method of Steemann Nielsen (1952). The *in situ* measurements were carried out for 2 hours incubation periods starting at 15-30 minutes before sunrise and ending 15-30 minutes after sunset. Also measured were: water temperature, light penetration, oxygen and chlorophyll concentration. Total incident solar radiation data were obtained from the Royal Netherlands Meteorological Institute (K.N.M.I.) at de Bilt (situated at 5 km from Lake Vechten). The production during two consecutive 2-hourly periods, e.g., period 1 and period 2, were summed up and, using the irradiance data during the 4 hours, daily production was calculated. These calculations were repeated for period 2 and 3, and so on. The sum of all the short-term measurements during a day, i.e., the total daily primary production, was taken for comparison with the production calculated by using the sum of the production during two successive periods and the light factor.

Daily production rates obtained by extrapolating from short-term incubation periods both in the morning and in the late afternoon appeared to overestimate the 'real' daily production up to 100%. It is likely that in the morning the lack of nutrient limitation and relatively better physiological state of algae, and in the afternoon increase of phytoplankton biomass during the day can be considered as the factors causing the discrepancy between the calculated and 'real' production. On the other hand, the underestimation of the daily production rate based on midday time intervals on sunny days, was about 25%. It is attributed to photo-inhibition, especially in the present study where the measurements were limited to the upper water strata (0-4 m).

It is concluded that short-term *in situ* exposures between 10.00 and 14.00 hr appeared to be a good indicator of total primary production, although underestimates up to 25% on sunny days are to be taken into account.

4.3. INTERNAL REPORTS

Densen, W.L.T. van and C.J.J. Richter - Internal Report No. 06445205, Agricultural University Wageningen, Wageningen, The Netherlands.

This report contains the lectures given by both authors for students of the Agricultural University Wageningen.

Hoogveld, H.L. - Phytoplankton studies in the Tjeukemeer, 1979 (in Dutch). Internal Report 1980-5.

In terms of bio-volume, the Tjeukemeer is a blue-green alga lake. Diatoms play

a dominant part in spring only. In 1979 diatom development occurred later than in preceding years and evolved in a different way. Green and blue-green algae showed normal periodicities, but numbers of *Scenedesmus* spp. and *Oscillatoria agardhii* were higher than usual.

Algal development in the Tjeukemeer is largely autochthonous. The inflowing water shows the same periodicity as the lake; its algal concentrations are lower and are strongly diluted when flowing into the lake. Diatoms grew well at 4-6°C, whereas blue-green algae did not thrive below a temperature of 8-10°C (250 and 350 Cal. $cm^{-2}.24h^{-1}$).

Blue-green algae contain less chlorophyll per biovolume unit than diatoms and green algae, moreover their chlorophyll content differs from year to year. Therefore, chlorophyll seems to be less useful as a parameter for biomass.

Mean filament length of *Oscillatoria agardhii* and *O. redekei* varies during the year. The pattern of variations is roughly the same in different years. *Oscillatoria limnetica*, *O. redekei* and *Lyngbya* spp. are sometimes difficult to distinguish.

Kramer, H.A. - Reduction of nitrate into nitrite using a zinc-batch method (in Dutch). Internal Report 1980-1.

Quantitative reduction occurs when 5 ml 1.44 M NaOH, which contains 6.7 g neutralized Na_2-EDTA.2 H_2O and 240 g NaCl per liter is added to a 25 ml nitrate standard. Then 1 gram of zinc powder is added and the mixture vigorously stirred into suspension. The minimum time necessary for reduction is 6 min. and the maximum 11 min. Contrary to literature data no temperature influence in the range from 3°-30°C was found.

Interfering substances in the water sample can be removed by means of a 10 cm Al_2O_3-column at neutral pH. Without further dilution 50-1500 $\mu g.l^{-1}N$ as NO_3^-, including the nitrite content can be determined.

No significant differences between the zinc-batch and the reduction/distillation method were found during 1977-1979. Therefore the applied zinc-batch method is a good alternative for the time-consuming reduction/distillation method.

Parma, S. - History and morphometry of Lake Vechten (in Dutch). Internal Report 1980-3.

Lake Vechten, dredged in 1941, is situated in a riverine area characterized by slight depressions and broad river banks, forming an intensive network. The subsoil of the lake consists of deposits of an old river branch or of its banks.

The lake has two depressions more or less separated by a shallow ridge. For both depressions the areas and volumes are calculated separately. The lake has a surface area of 4,715 ha, a volume of 283 x $10^3 m^3$ and a mean depth of 6 m.

Wanders, J.B.W., H.W. de Nie, A.F. Richter, A.G. Frank-Landman and J.S. Swart - The effects of cooling water discharge on growth and development of plankton in Bergumermeer (in Dutch). Internal Report 1980-2.

Water movements

The Bergumermeer and its 4 connected canals are part of a complicated system of waterbodies which form the 'Friese Boezem' area in the north of The Netherlands. The water flow in this area is controlled by a system of locks and power mills. The Bergum Power Station (60 MW) of the Frisian Provincial Electricity Board is situated on the northern shore of the lake which has a mean depth of 1.3 m and a surface area of 4.64 km^2. Its northern half is separated by a breakwater into an inlet area in the north-west and an outlet area in the north-east. Over the whole year various quantities of water enter the Bergumermeer, mainly from the western canal (Prinses Margrietkanaal) and to a lesser extent from the southern canal (De Lits). The Bergum Power Station uses water from this system and releases it in a warmed condition. The degree of raised water temperature in the lake depends, on the one hand, on the amount and temperature of effluent released by the power station and the difference in temperature between in- and outflow water, and on the other hand, the general water-flow direction in the Friese Boezem system. During wet periods lake water flows out, generally, after passing the power station, via the northern canal (De Zwemmer) to the Lauwersmeer. In this case the heated water (22 $m^3.sec^{-1}$) does not enter the outlet area of the lake. When evapo-transpiration exceeds precipitation, lake water flows off

mainly to the eastern canal (Kolonelsdiep). In these relatively dry periods most of the heated water returns to the lake in the outlet area. Both these processes can, within certain limits, be manipulated. The most common situations are summarized as follows:

I. Closed sluice gates at the Lauwersmeer. In this case there is partial or total recirculation of heated water in the northern part of the lake. The percentage of surface water which is warmed is then the greatest.

II. Open sluice gates at the Lauwersmeer. 80 to 90% of the water pumped out there comes from the canal De Zwemmer, in which the thermal effluents from the power station are discharged.

a. When the volume of water discharged through the sluice is smaller than the quantity of thermal effluent discharged by the power station, not only does the water in the canal become warm but also a small part of the Bergumermeer. The water in the canal is slow moving so it is warmed up over a considerable period of time and the surface which is air-cooled is small.
b. When the volume of water discharged through the sluice is equal to or greater than the amount discharged by the power station, only water in the canal De Zwemmer is warmed. Over the whole length of the canal the rise in temperature can be considerable (5°C), however, the duration of the upwarming is minimal 8 hours.

Botanical Research

For 3½ years (1975-1978) water samples were taken regularly from 15 points in and around the lake in order to study the numbers and species of phytoplankton. In order to do this the chlorophyll content was determined. The results show that the chlorophyll concentrations of the inlet water are about 5% higher than those of water discharged by the power station. The southern half of the lake in which practically no raised water temperatures were found, has significantly higher chlorophyll concentrations (10-15%) than the inlet area. Water entering the lake from the west through the Prinses Margrietkanaal has considerably (10-15%) lower chlorophyll concentrations than the inlet area of the lake.

The phaeophytine percentage in the discharged water is significantly higher than that in the inlet water. Where the water stays warm phaeophytine production is increased. It can be concluded that in the power station effluents the break-down of chlorophyll is increased. But due to the raised temperature the production of chlorophyll is also stimulated. Because the production processes in plants, generally, depend on favourable light conditions, the net result of build-up and break-down of chlorophyll in the thermal effluent in this shallow lake is a positive one but in the deep canal De Zwemmer the result is negative.

The oxygen production and consumption in inlet and outlet water were examined monthly, at both the natural and raised temperatures. These quantities are good indicators for the physiological condition of phytoplankton in the water and are also of great importance to the primary production and the oxygen balance of the entire environment. It was demonstrated that an increase in temperature in the Bergumermeer water is a stimulant to both photosynthesis and respiration. The increase in oxygen production was, however, smaller than expected for the increase in water temperature. In contrast the oxygen consumption in the warmed outlet water was higher than expected. When the outlet water cools down quickly to normal temperature after discharge, the lower production and higher consumption in relation to inlet water can still be shown. The situation in the shallow and therefore quickly cooling lake is, as the data on chlorophyll content show, more favourable for oxygen production than in the deeper canal De Zwemmer.

Although higher water plants are outside the framework of these investigations, some attention was given to the Fringed Water Lilies near the inlet and outlet areas of the power station. The lilies at the outlet area begin to develop floating leaves earlier and they bloom longer (2-3 weeks). This advantage continues through the whole growing season. Due to the hydrological situation in and around the Bergumermeer it was not possible to determine if, due to long periods of warming-up, plankton organisms are noticeably affected.

Little is known of the zooplankton populations in the Bergumermeer before 1974. Because the zooplankton communities are similar it is feasible to compare the zooplankton population dynamics of the Bergumermeer with that of the Tjeukemeer. For this study samples were taken from 8 stations in the lake. The cladocerans and copepods were studied the most extensively. The population densities of cladocerans in the Bergumermeer differ little from those in the Tjeukemeer. Near the outlet the zooplankton densities in the surface layer decreased by 20-30%. The cladocerans *Bosmina longirostris*, *B. coregoni*, *Chydorus sphaericus* and *Daphnia* sp., and the cyclopoid copepodites (mainly of *Acanthocyclops robustus*) show higher densities in deeper water near the outlet. At 1.5 to 2 km from the outlet 'normal' densities are found in the surface layer and no higher densities in deeper water.

A vital staining technique was used to investigate the entrainment mortality of the cladocerans. The average increase in mortality during 3.5 hours of incubation at outlet temperature ranged between 2-3%. The entrainment mortality of *Leptodora kindtii* and the cyclopoid nauplii could not be investigated by this technique. For these organisms we compared densities at different depths. Average percentual mortalities ranged from 19 to 33% for *L. kindtii* to 27% for the nauplii. The effect of this mortality on the population dynamics of the zooplankton in the Bergumermeer could not be determined because of a constant water flow from the power station to other water bodies.

The Bergumermeer is an open system in which the water is continuously flowing. The long-term effect of the thermal discharge on growth and mortality of zooplankton populations could not be investigated in the field. By means of a mathematical model the influence of temperature increase on a theoretical *Daphnia* sp. population in a 'closed system' could be simulated. We used an 'event-oriented' computer model written in SIMULA. The number of eggs per animal, age distribution, and growth rate as a function of a given temperature were used as data. The temperature scenarios as realized in the Bergumermeer during 1975 and until 1977 were simulated. In this way the effects of temperature increases of 1, 3 and 5°C were tested. One of the interesting results is a definite effect on mortality rate in the spring.

Wisselo, A.G. - The cycle of phosphorus in Lake Vechten during 1979 (in Dutch). Internal Report 1980-4.

Sampling was performed at four stations in the open water of the eastern depression of Lake Vechten. PO_4-P, P_{diss} and P_{tot} were measured, from which P_{org} and P_{part} could be calculated.

No significant differences between the stations were found.

Seasonal fluctuations in the centre of the depression were larger than those near the shore.

At the same stations sedimentation rates were measured, using sedimentation traps. The rates near the shore were higher than those in the centre. This was partly due to a larger amount of inorganic material present in the traps near the shore.

Zippin, M. and P.H. Best - Studies on production and decomposition of *Phragmites australis*. Internal Report 1980-6.

Standing crop, C, N, and P contents were measured bimonthly in 1979 in a quadrat of 0.1 m^2, separately for shoots, roots and dead material. The growth curve was compared to a curve derived from plants tagged in April and harvested in the course of the year.

The biomass was maximal at the end of July when the plants had a shoot weight of 4.5 g ashfree dry weight.m^{-2} (dead material 15.2%, roots 15.9%, shoots 68.9%). The C, N, and P contents were maximal from April-July, 47, 2.8 and 0.36%, respectively, of ashfree dry weight. Most plant material ends up in the lake at the end of the vegetative period, thereby contributing the considerable amount of 536 kg ashfree dry weight to the lake.

Decomposition of *Phragmites* leaves was measured under experimental conditions (light-dark cycle: 12/12, temperature 15°C) in closed systems during 147 days. Compared were containers filled with a) lake water, b) lake water + sediment, c) lake water + sediment + plant material (in nylon litterbags, 1 mm mesh size). The plant material was collected in March from the lake bottom, so initial leakage of the nutrients had occurred already. It was freeze-dried and fragmented before the

start of the experiment. Ash-free dry weight, N and C content of the plant material decreased gradually to, respectively, 64, 54 and 66% of the initial values. The P content fluctuated due to accumulation of bacteria and their excretion products. Almost all C and N that had disappeared from the plant material during the first 100 days was recovered in the water phase. Subsequently, however, the nutrients accumulated in the sediments. Only about 10% of the total C and N content of the water phase was soluble (i.e. < 33 µm). The ortho-P content of the water phase increased substantially from 60 to 100 days incubation in the low-oxygen vessels b) and c), compared to that of the control probably given off by the sediment or originating from decaying algae and bacteria. Only a small part of the plant-P was recovered as ortho-P in the water phase.

The changes in numbers of bacteria and photosynthetic organisms were recorded (epifluorescence counting and pigment chromatography). In the water phase only obligate heterotrophic bacteria occurred, whereas in the sediment also phototrophs were found. Initially (0-60 days) the activity of both groups was greatly stimulated by the decaying plant material as demonstrated by, respectively, the production of considerable amounts of methane and the increase in pigments specific for phototrophic bacteria. After 60 days, algae, particularly diatoms, increased in numbers and photosynthetic bacteria decreased.

4.4. STUDENT AND TRAINEE REPORTS

Baks, A. - Effect of copper on primary production in Tjeukemeer water at different light intensities (in Dutch). Student Report 1980-1.

The effect of addition of $CuSO_4$ on photosynthesis at different light intensities was studied in Tjeukemeer water. At low light intensities (10-25 $\mu E.m^{-2}.S^{-1}$) photosynthesis was low and hardly affected by the addition of $CuSO_4$; at higher light intensities (50-220 $\mu E.m^{-2}.S^{-1}$) $CuSO_4$ lowered photosynthesis. The relationship found between Cu-concentration and photosynthesis at different light intensities suggests that Tjeukemeer water masks the toxicity of Cu by Cu-binding.

Bergsma, R. - Distribution of phytoplankton in Lake Vechten (in Dutch). Student Report 1980-23.

Eleven stations in the eastern basin of Lake Vechten were sampled fortnightly for chlorophyll concentration from April to August, 1979. At all sampling data no significant differences between stations were observed, except for one station in the littoral zone that showed a higher concentration during July and August. It is concluded that distribution of phytoplankton in the eastern basin is generally uniform.

Bloem, J. - The relationship between *Daphnia hyalina* and its food in the Tjeukemeer (in Dutch). Student Report 1980-14.

Gut contents of *Daphnia hyalina* were compared with excreted material, and changes were recorded 0, 10, 20, 30 and 40 min after capture. Most of the algal species ingested (filamentous blue-green algae and *Chlorococcales*) were only partially digested. This is probably due to the high concentration of algae in the Tjeukemeer.

Bremer, P. - Population dynamics and feeding ecology of *Neomysis integer* in the Slotermeer (in Dutch). Student Report 1980-20.

Neomysis was sampled in the Slotermeer and the Tjeukemeer during May-November 1980, with a trawl net at a speed of 1 $m.sec^{-1}$ for one minute.

Maximum average density in the Tjeukemeer was very low, 0.2 $ind.m^{-2}$; in the Slotermeer it amounted to 6 $ind.m^{-2}$. These differences are difficult to explain. Predation by fish and mortality caused by the cold winter of 1979 are probably important. Effects of migration and water turbulence could not be proven. Chloride concentration and food situation probably have no influence.

In the Slotermeer a significant preference was found for a sandbottom covered by a layer of mud.

Three generations were present, a spring generation (June-July), a summer generation (July-August) and a winter generation (from September onwards). The first two generations are described as cohorts. Fecundity equals $0.0522\ L^{2.34}$. In October reproduction stopped.

Production measured, using the growth of the spring generation, amounted to 91 $g.ha^{-1}$ dry weight in the Slotermeer and 0.29 $g.ha^{-1}$ in the Tjeukemeer; daily

specific production (P/B) was 1.1% and 1.3%, respectively. Production in the Slotermeer was 250-400 $g.ha^{-1}$.

Neomysis is an omnivore. Algae only accounted for less than 5% of the gut contents. A preference for *Planctonema* was found. *Oscillatoria* was rejected; filaments present in the gut were much shorter than those in the plankton samples. During the day, *Neomysis* lives on the bottom; during the night they swim through the whole water column. No males were found on the bottom during the night. In the night *Neomysis* hunts for zooplankton, the gut contained especially large quantities of *Bosmina*. There appears to be a diurnal periodicity in feeding; in the evening gut contents was highest.

Cevaal, G. - Comparison of grazing rates of *Daphnia magna* in four different places in the northern 'Vechtplassen' area (in Dutch). Student Report 1980-13.
See Progress Report 1979, par. 4.1.2.

Fokkema, D.S. - Diet of the pikeperch in the Tjeukemeer during the summer of 1979 (in Dutch). Student Report 1980-10.

The stomach contents of 1293 pikeperch, caught with trawl- and gill-nets, were investigated. On average 27% of the stomachs contained food. The highest percentage (85%) has been found in the 0-10 cm class which, in June, feeds mostly on plankton and benthos.

In July, August and September 80% of the prey was first-summer (0^+) fish, mainly ruffe and roach. Bream evidently is disliked as a prey. In most years the smelt is abundant in the Tjeukemeer and serves as main prey; in 1979 it was rare, causing the feeding conditions to be bad.

The percentage of stomachs containing food in relation to the hour at which the fishes were caught, revealed that pikeperch feeds mainly at twilight and to a lesser degree in the daytime.

Fokkema, D.S. - Selectivity of gill-nets for pikeperch in the Tjeukemeer (in Dutch). Student Report 1980-11.

Gill-net selectivity for pikeperch was calculated according to Holt's method from catches in nets of 48 and 51 mm half-mesh size. K, representing the relation between mesh size and length of optimum catchable fish was found to be 0.946. The standard deviation in the selectivity curve was 3.9 cm. After correction for net selectivity the size structure of the catch in gill-nets was compared with the one in a trawl-net. The catchability of larger individuals was lower with the trawl.

Comparison of efficiency in 51 mm nets of different twining, colour and thickness showed white 3-thread nets to be superior. In 35 mm nets no significant differences in efficiency were detected.

The percentage of pikeperch in the catch was independent of the saturation of the net.

Geraedts, J.M. - Compilation of data on the hydrology of the Tjeukemeer (in Dutch). Student Report 1980-2.

The direction of water flow in the Frisian waterways is determined by:
- water supply from polder districts and higher areas;
- intake of water in the south of the province, from the IJsselmeer;
- discharge of water through sluices in the north to the Wadden Sea, and by pumping in the south to the IJsselmeer;
- discharge of water to the adjacent provinces.

The wind too sometimes greatly influences the direction of flow. In the dry period water with a chloride content of about 200 $mg.l^{-1}$ flows to the Tjeukemeer from the IJsselmeer via the Follegasloot. In the wet period water with a chloride content of about 80 $mg.l^{-1}$ only comes in from the polders (c. 23,000 ha) and the higher areas (c. 35,000 ha); these two areas differ in regimes of draining and in water composition. Practically it is not possible to measure directly the quantities of water flowing through the waterways in and out of the Tjeukemeer. Seepage of water also greatly influences hydrology in some places, including the Tjeukemeer. As the area is hydrogeologically inhomogeneous, it is hardly possible to calculate the extent of seepage.

As the IJsselmeer water and the water coming from the polders and higher areas differ in chloride content, a rather accurate water balance can be drawn up by calculating the continuity equations of water and chloride. The following data are needed:

- chloride contents of Tjeukemeer and supplying ditches;
- precipitation on the lake, calculated as a weighted mean of the measurements performed by the Royal Dutch Meteorological Institute at Joure and at Lemmer;
- evaporation at the lake, measured at Leeuwarden and calculated with Penman's formula;
- changes in the storage of water in the lake measured by means of water gauges;
- volume of the lake.

By applying a simplified chloride model a relation could be found for the dry seasons of 1969-1971 between the calculated amount of water flowing into the Tjeukemeer via the Follegasloot and the measured amount of water taken into the Frisian waterways from the IJsselmeer. The relation is roughly: $Q_{Follegasloot} = 0.5 \times Q_{IJsselmeer} - 8$, in $10^6 m^3.(14\ days)^{-1}$. A comparable relation could not be calculated for the wet period as sufficient chloride data were lacking.

Hampsink, G.I.M. - Food consumption of bream during the summer of 1979 in the Tjeukemeer (in Dutch). Student Report 1980-7.

Monthly samples were taken at five stations during June-September 1979 and gut contents were examined. No great differences in food consumption were found between the five stations nor between bream of different lengths.

Bream was found to feed almost exclusively on zooplankton and chironomid larvae. The latter are only eaten when the larger zooplankton species are scarce. Zooplankton was consumed mainly in June and September, at a total amount of 225-550 kg fresh $wt.ha^{-1}$, about 75% of it was *Daphnia* and 20% cyclopoid copepods. In July and August chironomids were the main food items (200-500 kg fresh $wt.ha^{-1}$), particularly *Cladotanytarsus*, *Cryptochironomus*, *Procladius*, and *Glyptotendipes*.

Hermans, H. - Food composition and selection in the eel, *Anguilla anguilla*, in the Tjeukemeer (in Dutch). Student Report 1980-8.

Samples were taken fortnightly between May and October, 1979. By means of a narrow-meshed trawl-net 2039 eels were caught during the night, and 600 at daytime. Gut contents of 814 eels were studied. Pupae and larvae of chironomids, *Anodonta*, and *Unio (Mollusca)* were the most numerous food items.

Fulton's condition index (whole sample) varied between 0.140 (May) and 0.190 (August).

The length classes between 24 and 28 cm dominated the catches. Of the longer eels only 12% were females.

Jonkheer, G.J. - Anaerobic mineralization of *Chlorella* cell walls in the sediment of Lake Vechten (in Dutch). Student Report 1980-9.

Anaerobic mineralization plays an important part in the carbon cycle in Lake Vechten. Anaerobic mineralization of sedimented, complex organic matter was studied, using cell walls of the green alga *Chlorella* sp., a dominant species in Lake Vechten. Bacteria which could degrade the cell walls were isolated from Vechten mud. The break-down velocity of a good-growing culture was 1 to 2 $mg.day^{-1}$. Main products of the break-down were acetate, propionate, butyrate, and hydrogen.

The cell walls consist mainly of cellulose and hemicellulose. Experiments with different fractions extracted from the cell walls showed that the isolated bacteria were not very substrate specific; all fractions, except lignin, were more or less broken down.

In situ measurements with ^{14}C-labelled cell walls showed a break-down velocity of 40 to 90 $\mu g\ day^{-1}.cm^{-2}$ of sediment, which amounts to 800 to 2000 $kg\ C.year^{-1}$ in the whole lake. This is in the same order of magnitude, i.e., 1650 $kg\ C.year^{-1}$, as calculated earlier from sedimentation measurements.

Characterization of the sediments by CHN-analysis suggested that compounds in the upper layer of mud contain more N and are degraded faster than those in deeper layers.

Jooren, H. - Role of bacteria as food for zooplankton (in Dutch). Student Report 1980-4.

Experimental animals were allowed to graze for 10 min on ^{14}C-labelled food. After grazing they were killed and their radioactivity was measured.

From experiments with known bacteria and *Daphnia magna* it can be concluded that in small animals (length 1.0 mm) the filtration rate increases with decreasing food concentration. In larger animals (length 1.3-1.6 mm), however, it is constant

at different C concentrations. A possible explanation is, that the food particles are too small for the medium sized and large animals, so that they pass the filtering apparatus without being utilized. Therefore, despite changes in food concentration, food utilization is small, so that the animals are equally stimulated at all concentrations and the filtration rate remains constant. Experiments in which unknown bacteria and unknown algae were provided to *Eudiaptomus gracilis* had inconsistent results. A possible explanation for this could be the difference in size between the two food types.

Concluding one may say that both *D. magna* and *E. gracilis* consume bacteria. To what extent bacteria actually serve as food cannot be established with certainty, due to the preliminary nature of the experiments carried out.

Kromkamp, S. - Bio-assay experiments: addition of nutrients to detect limiting factors (in Dutch). Student Report 1980-19.

Linden, P.J.H. van der - Growth rate of *Daphnia magna* under laboratory conditions, and differences in food selection between juveniles and adults (in Dutch). Student Report 1980-18.

The growth rates of *Daphnia magna* at 18 ± 2°C were compared in two different culture media, one of which contained trace elements (A) and the other was Freeman medium (B). Laboratory-cultured *Chlorella* sp. was used as food.

The food concentration in the cultures was monitored by using simultaneously both the C.O.D. (Carbon in $mg.l^{-1}$) and photometric (extinction values at 772 nm) techniques. The correlation between the results obtained by using the two methods was significant ($r = 0.95$, $P < 0.05$, $n = 26$). Thus, the extinction measurements of the cultures may serve as a quick and suitable alternative for carbon estimates to regulate or monitor food concentrations in daphnid cultures.

The increase in length, dry weight and carbon content of the daphnids was followed from birth to the age of 40 days; biochemical composition, namely, protein and nitrogen content, and heat of combustion were measured at regular intervals as well. In both culture media, the animals grew up to 3 mm, attaining a weight of 40 $\mu g\ C.ind^{-1}$. The animals in Freeman medium were generally smaller with their birth rates two-fold higher, and nitrogen and protein contents somewhat lower than in the medium A.

The food uptake and selection were examined using the Coulter Counter technique. The food consumption of the newly-born and 28 days old animals was, respectively, $0.5 \pm 0.3 \cdot 10^6$ and $28.7 \pm 3.4 \cdot 10^6\ \mu m^3.hr^{-1}.ind^{-1}$. The latter age-group selected food particles larger than 3.24 μm positively, contrary to the erratic results for the newly-born animals. The apparent negative selection of particles smaller than 3.24 μm found for both age-groups was presumably because of generation of smaller particles due to egestion.

Meischke, J.J.V. - Numbers, growth and diet of 0^+ smelt, perch and pikeperch in the Frisian Lake District (in Dutch). Student Report 1980-16.

Decrease in numbers and increase in weight of 0^+-smelt, perch and pikeperch was compared in nine Frisian lakes; particular attention being paid to the comparison with the Tjeukemeer. The sampling was done in June and September 1979.

For smelt, daily mortality rates did not differ much between the lakes, but differences in abundance were considerable, with extremes of 0.8 $ind.m^{-2}$ in the Lauwersmeer and 72 $ind.m^{-2}$ in the Grote Brekken. This is caused by hydrological factors; smelt did not reproduce in the Frisian lakes, larvae coming from the IJsselmeer were carried along with the water currents. Growth rate was highest in the Lauwersmeer and lowest in the Grote Brekken. In June, 0^+-perch was rare, in September it was almost absent.

In June, the abundance of 0^+-pikeperch differed much between the lakes, the extremes were 0.5 $ind.m^{-2}$ in the Grote Brekken and 9 $ind.m^{-2}$ in the Bergumermeer. Causes for this are unknown; condition of the eggs, predation and food availability may be important factors.

Production and mortality of smelt and of pikeperch differed much between the lakes yet total mortality of smelt and pikeperch differed less, Tjeukemeer 4 $kg.ha^{-1}$, Grote Brekken 350 $kg.ha^{-1}$, the other lakes 80-150 $kg.ha^{-1}.yr^{-1}$.

Meischke, J.J.V. - Growth, condition, fecundity and egg quality of pikeperch in a good (Grote Brekken) and a bad (Tjeukemeer) food situation (in Dutch). Student Report 1980-17.

Foraging conditions for pikeperch in 1979 were much worse in the Tjeukemeer than in the Grote Brekken, resulting in significant differences in condition between the populations of these lakes.

Reproductive potential, determined as egg weight and number, was, however, the same for both lakes in 1980. Relative fecundity increased and dry weight of the eggs decreased with increasing condition of the fishes. These relations, which are not significant at $\alpha = 0,05$, agree with literature data for different fish species. Perhaps a significant difference might be found if a larger range of conditions could be studied.

Fat content and dry matter of the eggs did not correlate with condition.

Mennes, F. - Daily food intake of 0⁺perch (*Perca fluviatilis*), pikeperch (*Lucioperca lucioperca*) and smelt (*Osmerus eperlanus*) (in Dutch). Student Report 1980-5.

An important link in the ecosystem of the Tjeukemeer is the energy transfer from zooplankton to 0⁺fish. In order to determine this transfer it is necessary to know (among other things) how much zooplankton an 0⁺fish eats. This quantity can be estimated with the model $DR = 24 \times \bar{M} \times s$ (DR = daily ration, % (energy); $\bar{M}$ = mean daily stomach content, g; s = relative digestion speed, $hour^{-1}$).

During the growing-season of 1978 $\bar{M}$ was determined five times by catching fish in the lake at certain time intervals during a period of 24 hours. The mean stomach content, expressed as a percentage of body weight, decreased with increasing size of smelt and perch.

Laboratory experiments gave some data concerning s. 0⁺fishes first fed with unstained zooplankton were then allowed to eat red-stained zooplankton. The experimental groups were killed after different periods. The distribution of stained and unstained zooplankton in the stomachs in relation to the time that the fishes were allowed to eat red-stained zooplankton, produced s. All experiments were carried out at 20°C. For smelt and perch s decreased with increasing size of the fish, for pikeperch it increased.

By combination of $\bar{M}$ and s with the data on size and composition of the stock of 0⁺fish and the zooplankton during the season 1978, the predation pressure could be calculated. Smelt predated the *Daphnia* population up to a maximum of 21% per day. Winbergs' formula, in combination with observations on the oxygen consumption of 0⁺fish, and literature data produced a much higher predation pressure of smelt on *Daphnia*, namely 67% per day.

Schoon, A. - Distribution, growth and diet of larval smelt, perch and pikeperch (in Dutch). Student Report 1980-15.

From April up to the middle of June, 1979, weekly samples were taken from the Tjeukemeer and the waterways leading to the place where water from the IJsselmeer is pumped in. Larvae were caught by two hoop-nets (40 cm diameter) trailed for 30 sec just below the surface and at 1 m with a speed of about 2 $m.sec^{-1}$. A parent population of smelt in the Tjeukemeer was lacking, the larvae came from the IJsselmeer. Therefore, hydrological factors were responsible for the distribution of smelt larvae. Also perch fry, present in far lesser densities, was distributed by hydrological factors, despite the presence of an adult population in the Tjeukemeer.

Pikeperch fry also was present in small densities only, despite the enormous reproduction potential in the Tjeukemeer. These larvae did not enter from the IJsselmeer.

Up to the middle of June smelt fry was found mainly in the surface hauls. Perch fry did not show a preference for the surface or 1 meter depth. Until the 30th of May ($\bar{L}$ = 8 mm) pikeperch larvae were mainly found in the surface hauls. After this date ($\bar{L}$ = 13 mm) 1 m hauls contained more larvae, which were greater in average length than those in the surface hauls. Probably at a length of 8-13 mm pikeperch fry migrate to deeper water.

The stomach contents of pikeperch larvae at the transition from yolk to exogeneous food (6-7 mm length) consisted primarily of nauplii and copepodites. No correlation could be found between larval length and average length of the consumed organisms. It is, however, remarkable that on May, 16th, mainly nauplii were consumed, and a week later mainly copepodites by the same size-class.

Schreur, J. - Multiple addition bio-assay experiments (in Dutch). Student Report 1980-22.

Veldhuis, M.J.W. - Growth of *Scenedesmus quadricauda* in Fe-limited chemostats (in Dutch). Student Report 1980-21.

Growth of *Scenedesmus quadricauda* in Fe-limited chemostats at pH 8 was studied and compared with growth at pH 10. μ_{max}, K_S and q_o were calculated. K_S equalled 11 $\mu g.l^{-1}$ of Fe at pH 8 and 1 $\mu g.l^{-1}$ of Fe at pH 10. This difference is due to the decreasing availability of Fe which results from its decreasing solubility with increasing pH. K_S, therefore, depends on the physico-chemical form of the growth-limiting substrate. The maximum growth rate (μ_{max}) was higher (1.1 d^{-1}) at pH 8 than at pH 10 (0.8 d^{-1}) which was to be expected from the decreasing availability of Fe at increasing pH. As expected, the minimum quota of Fe per cell required for growth did not change much at both pH values.

Werf, B. van der - Determination of the concentration of Cu and its effect on photosynthesis in Tjeukemeer water (in Dutch). Student Report 1980-3.

The mean Cu concentration approximated 40 $\mu g.l^{-1}$, which is rather high. Probably the results of the analyses are influenced by the method used.

The effect of added Cu on the growth of algae was estimated by measuring photosynthetic activity. At concentrations lower than 100 $\mu g.l^{-1}$ of added Cu, no significant change of photosynthesis was found; at higher concentrations photosynthesis decreased considerably. Probably, Tjeukemeer water can bind c. 100 $\mu g.l^{-1}$ of Cu thus masking its inhibiting effect on photosynthesis. At higher concentrations free Cu-ions will become increasingly inhibitory to the photosynthetic system of algae.

Wittenboer, J.P. v.d. - Effect of paraquat on photosynthesis and growth of *Ceratophyllum demersum* L. and *Elodea canadensis* Michx. (in Dutch). Student Report 1980-12.

Under experimental conditions paraquat was added to *Ceratophyllum demersum* and *Elodea canadensis* in a concentration of 1 $mg.l^{-1}$. In both species growth was greatly inhibited by this herbicide within a short time. Sensitivity with respect to growth and photosynthesis differed between the species and with the developmental stages. Experimental evidence suggests that the different sensitivities for paraquat displayed by both species are caused by different uptake rates and morphology.

Wijbenga, D.J. - Effect of EDTA on Fe-limited growth of *Oscillatoria limnetica* (in Dutch). Student Report 1980-6.

Dry weight and chlorophyll-a content of *Oscillatoria limnetica* in Fe-limited chemostats were dependent on Na_2EDTA concentration. Optimum values of dry weight and chlorophyll-a were found using a $Na_2EDTA/NH_4Fe(SO_4)_2.12H_2O$ (weight/weight) ratio between 2 and 3. This result suggests that the half saturation constant, K_S, for the Fe-limited growth depends on the physico-chemical form of this element. Apparently, the addition of Na_2EDTA results in binding of Fe in a complex which can considerably affect its biological availability.

However, for optimum growth conditions in batch culture, a $Na_2EDTA/NH_4Fe(SO_4)_2.12H_2O$ ratio exceeding 4 is needed. This indicates that the growth rate might play a role in the biological availability of Fe.

5. Acknowledgements

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Delta Institute for Hydrobiological Research

Progress Report 1980

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CONTENTS

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 south-west Netherlands, with the aim of studying the environmental changes to be expected as result of the closing of the various river-mouths and sea-arms in this area.

When the Zuiderzee, in the centre of the Netherlands was closed by a dam and converted into the fresh-water Yssellake 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 an 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 1980 a permanent staff of personnel of 56 including 13 scientists. Additionally short-term contract scientists, assistants, guestworkers, students and trainees took part in the programme of the institute (Table 1).

Thanks are due to Mrs. E.S. Nieuwenhuize and Miss M.J. de Dreu, Mr. A.A. Bolsius, Mr. J. v.d. Ende and Mr. R.H.G. Kleingeld for the preparation of manuscript, 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 development of young marine species and for winter refuge of migrating European and Northwestasian birds.

The hydrobiological studies of the institute have been started in 1957 with 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 1980 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 1). The working-group themes are (i) the element cycling and food chains in the Grevelingen with extention in the Eastern Scheldt, (ii) structure, functioning and dynamics of ecosystems in brackish waters and (iii) ecosystems studies on salt marshes. A group on mineralization

Table 1. Scheme of personel (31 Dec. 1980)

WORKING GROUPS

Elements cycling and food changes

Botany:
Dr. P.H.Nienhuis (W.G. leader)
B.H.H. de Bree
J.M. Verschuure
G.C. Pellikaan (temporary)

Zoology:
Drs. R.H.D. Lambeck
E.G.J. Wessel
A.J.J. Sandee

Plankton:
Drs. C. Bakker
P. v. Rijswijk
J.C.M. Rijk

Primary production:
Drs. F. Vegter
P.R.M. de Visscher

Salt-marsh Ecosystems (S)

Communities:
Dr.Ir.W.G. Beeftink* (W.G. leader)
M.C. Daane
B.P. Koutstaal
W. de Munck

Populations:
Drs. A.W. Stienstra
Dr. A.H.L. Huiskes
M.M. Markusse
J. v. Soelen

Biomassa budget:
Drs. G.J.C. Buth (temporary)

Brackish waters (B)

Microbiology
Dr. A.B.J. Sepers (W.G. leader)
F.W. Melissen

Planktonbiology:
Ir. J.W. Rijstenbil
L. de Wolf

Entomology:
B.P.M. Krebs

Zoology:
Drs. C.H. Borghouts
R.H. Bogaards*
J.W. Francke
Drs. A.W. Fortuin (temporary)

Short-term projects (3 yr).

ZOWEC-R.W.S.
(Saline-Water Ecology)

Microbiological Research:
Drs. J.G.C.M. Goossens
Dr. H.J. Lindeboom
R.S. Minnaar
H.A.J. De Klerk

Fishery:
Drs. G. Doornbos
F. Twisk
R.H. Bogaards*

Sediment-water exchange:
Drs. P. Kelderman
A.M. van de Repe

Seston research:
Drs. E.T. van Ierland
A.P.A. de Booy

VEGIN-R.W.S.
(Effects storm-surge barrier salt marsh vegetation)
Drs. A.M. Groenendijk
M.A. Lievaart

ZACHTSUB-R.W.S.
(Ecological Research Zoobenthos)
Drs. J. Coosen
A. van den Dool

BALANS-R.W.S.
(Research Food-balance Eastern Scheldt)

Organic Matter Transport:
Drs. J.H.B.W. Elgershuizen
R.C. de Leeuw

Qualification and quantification of the microfytobenthos:
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Dr.Ir. W.G. Beeftink*
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Residence times:
DIHO - Shell
Drs. M. Smies

Litoral-water exchange:
Drs. L.A. van Geldermalsen

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(book, courses, excursions).
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J.C. Ruissen
E.F. v.d. Plasse (guestworker)

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Students and Trainees: (1980)
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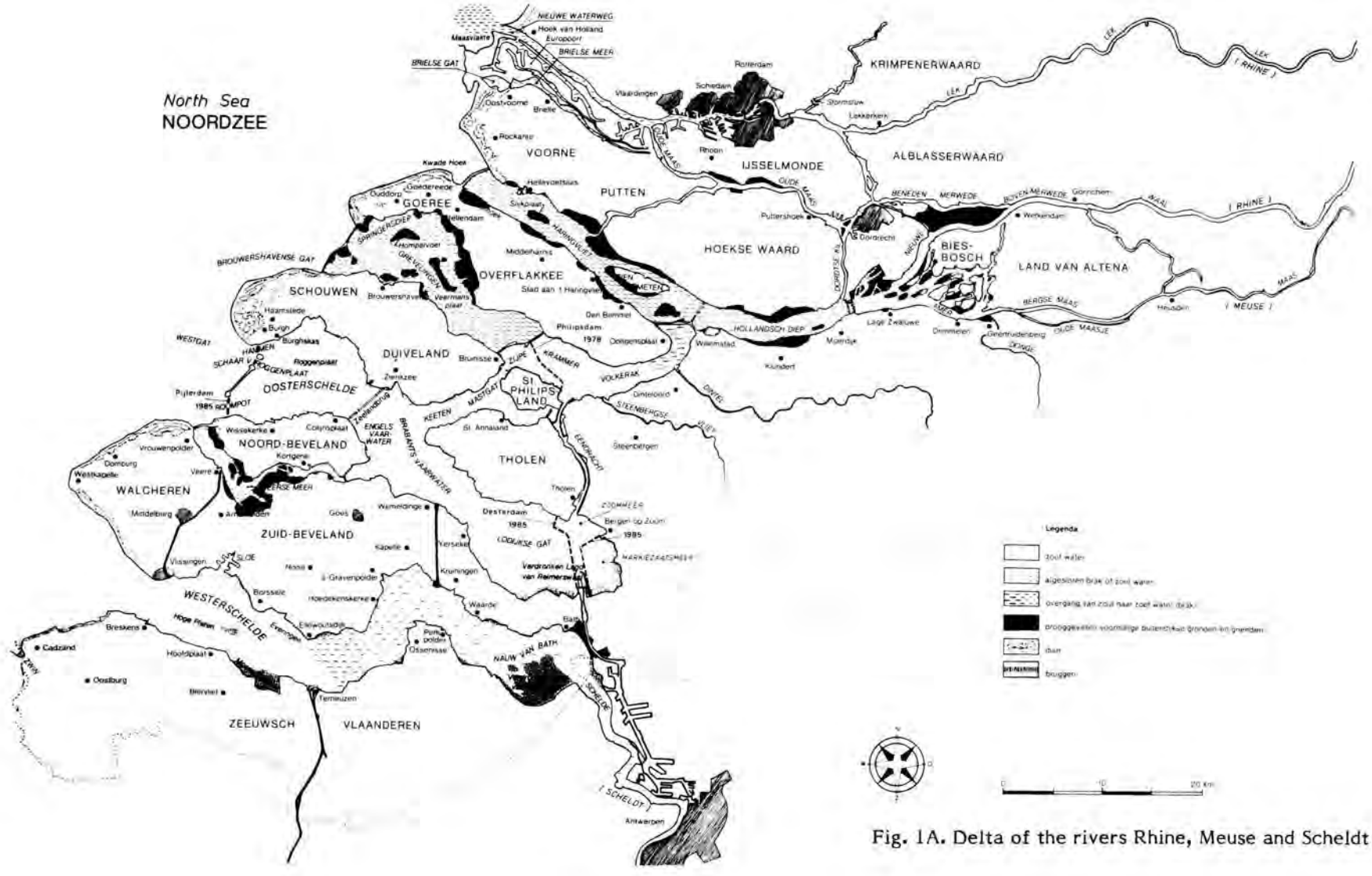

Fig. 1A. Delta of the rivers Rhine, Meuse and Scheldt

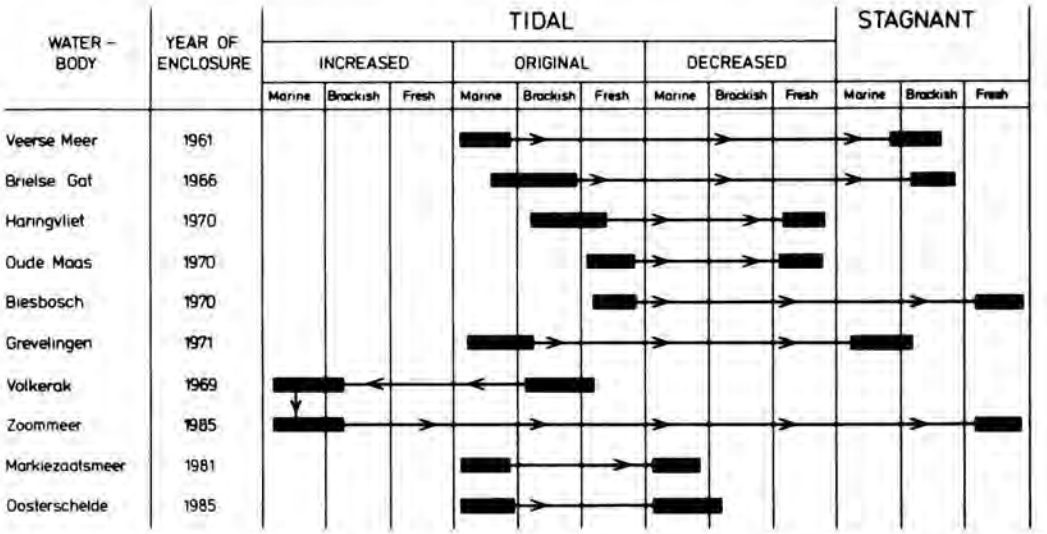

Fig. 1B. Hydrological changes due to the "Delta Plan"

studies is at present 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, B for the brackish-water investigations, S for the salt-marsh studies while A denotes miscellaneous subjects, among which are the pollution studies.

Since the studies of the Delta Institute for Hydrobiological Research are of a long-range character, the research planning is usually made for both the working-group projects and the more individually carried-out projects for a long period of years. Reference is made to the working plan 1980-1990 of the institute (Anonymous, 1980).

Reference

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

III. CONTRACT RESEARCH AND COOPERATION

The extensive pressure on the Delta Institute to produce data and results that can be applied for technical and environmental management purposes, has resulted in a number of short-term contracts between our institute and other organisations. The major part concerns 3-years research projects funded by the Delta Department of the Rijkswaterstaat for which the programmes were determined by mutual agreement between the Environmental Division (DDMI) of this Delta Department and our institute. These projects are: ZOWEC (salt-water ecology) in which 5 scientists and 5 technical assistants are engaged. This project will be reported in the Working group "Elements cycling and food chains"; ZACHTSUB (soft-bottom benthos), which involves 1 scientist and 1 technical assistant for the investigation of benthic organisms in the Volkerak; VEGIN (vegetation-inventory), being an experimental study on the prognosis of the effects from the construction of the storm-surge barrier in the Eastern Scheldt on the salt-marsh vegetation, involving 1 scientist and 1 technical assistent; BALANS (budget of organic matter in the Eastern Scheldt), which is a project starting in 1980, involving 5 scientists and 5 technical assistants. Cooperative studies with the Delta Department to be mentioned are WABASIM (water-basin modelling) with the Hydraulics Laboratory in Delft and the section DDMI of the Delta Department in Middelburg, as well as the project ZOUVER (salt-water refreshment, Grevelingen).

A smaller contract, financed for 30% by the Commission of European Communities, section Biology, concerns the analysis of plutonium in the Delta waters. The first contract of 1979-1980 is extended to 1984. The analyses are carried out by the ITAL at Wageningen and the Laboratoire Géologique at Paris (F). The Delta Institute carries out the sampling.

A joint cooperative investigation with closed budgets, and initiated through the Scientific Markets organized in Yerseke in 1977 and 1979, has been carried out with the Chemical Institute of the KFA at Jülich (FRG) on the heavy metal distribution in water, plants and organisms of the Delta area. Identically initiated but with funding of the CNRS-Paris, a joint project was carried out (1979-1980) with the Centre de Recherche Sédimentologique de Perpignan on the primary production and mineralization processes in Lake Grevelingen and Etang Salses Leucate in Southern France.

Since 1978 and ending in 1981 a joint research project on "Residence times of natural and anthropogenic substances in estuaries" is carried out with Shell Internationale Research Mij, The Hague, for which 1 scientist has been seconded to our institute.

IV. GENERAL ECOLOGICAL CONDITIONS IN 1980 (R. Peelen)

Temperature

January was a little colder, while February and March were warmer than normal. Till June there was little deviation from the average temperature; July was too cold while August and September were somewhat warmer than normal. October and November were colder than normal (Table 2).

Solar radiation

The representative station for the south-western Netherlands is at Flushing. The solar raditation quantities of 1980 were in May and half June till November higher than the normal values given for the Bilt (central Netherlands) (Fig. 2). Over the

Plate I. Lowering of the scuba operated beljars for the determination of the community metabolism. This metabolism is measured by recording the oxygen production and consumption rates of sediment and phyto and zoobenthos over a 30 hours period.

first 11 months Flushing recorded 356,400 Joules cm^{-2} and the Bilt 324,500 Joules cm^{-2}.

Rainfall
Dryer than normal were the months January, May (very), August, September (very) and November. Wetter were February, March, April, June, July (very much) and October (Table 2).

Windspeed
The winspeeds recorded in ½ m s^{-1} were close to the mean values for March, May, June, July, August and September. The weather was quiet in January, February and April. More wind was recorded in October and November.

Table 2. Climate and river-discharge conditions in 1980.

	Temperature °C			Rainfall mm $month^{-1}$		
	measur.	normal	deviation	measur.	normal	deviation
January	0.0	0.8	- 0.8	50.9	61.9	- 11.0
February	5.5	3.0	+ 2.5	53.0	46.4	+ 7.2
March	5.5	5.2	+ 0.3	59.0	39.1	+ 19.9
April	8.1	8.4	- 0.3	51.3	41.9	+ 9.4
May	12.1	12.1	0.0	6.0	44.1	- 38.1
June	14.8	15.3	- 0.5	85.5	48.9	+ 36.6
July	15.8	17.2	- 1.4	175.2	69.4	+ 105.8
August	17.5	17.4	+ 0.1	37.1	66.1	- 29.0
September	16.4	15.5	+ 0.9	18.3	72.8	- 54.5
October	11.0	11.5	- 0.5	84.2	70.2	+ 14.0
November	5.9	7.3	- 1.4	55.8	75.2	- 19.4

	Windspeed ½ m s^{-1}			River discharge x normal	
	measur.	normal	deviation	Rhine	Meuse
January	11	14	- 3	1 - 3/4	2 - 1
February	11	13	- 2	1 - 4	2 - 5
March	12	11	+ 1	1 - 2/3	1 - 3
April	10	12	- 2	1 - 1½	2 - ½
May	11	10	+ 1	1 - 3/4	½ - 1
June	12	11	+ 1	1	½ - 1/8
July	12	11	+ 1	1 - 3	1 - 4
August	12	11	+ 1	1½ - 1	1½ - ½
September	12	11	+ 1	1 - 2/3	1 - 1/3
October	14	11	+ 3	2/3- 1	½ - 1
November	17	12	+ 5	½ - 1	½ - 2
December				1 - 2	1 - 3

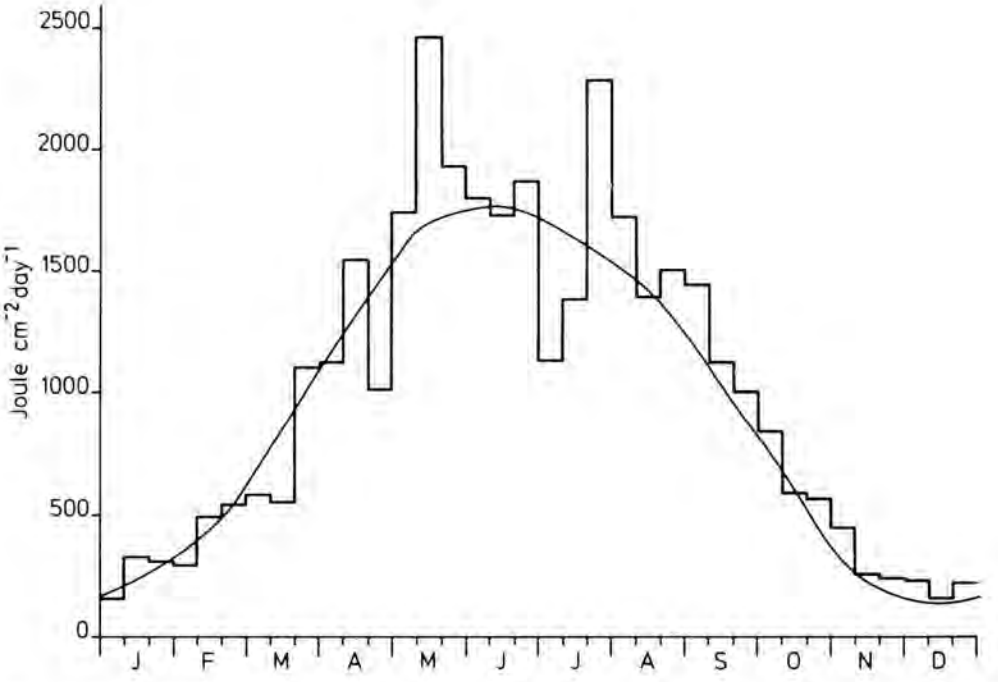

Fig. 2. Solar irradiation at Station Flushing (block diagramme) for 1980 as compared to normal irradiation at The Bilt (smooth curve)

River discharges and North Sea water exchanges

The average discharges of the three rivers Rhine, Meuse and Scheldt are respectively 2200, 330 and 95 $m^3 s^{-1}$. The relative dayly discharges are given in Table 2. The discharges of the river Rhine fluctuated from $\frac{1}{2}$ till 4 times the normal discharge. Extreme values were recorded on the 9th February (8586 $m^3 s^{-1}$) and on the 15th of November (1388 $m^3 s^{-1}$) at Lobith (German border). The fluctuations were this year much higher than in 1979. The river Meuse discharge fluctuated from 1/8 till 5 times the normal discharge. On the 9th of February the discharge was 1400 $m^3 s^{-1}$ and on the 15th of June it was 40 $m^3 s^{-1}$.

Technical works by Rijkswaterstaat in the Delta area

1. Construction of the piers for the Eastern Scheldt storm-surge barrier is in full progress. Nineteen piers are being built in building dock I in the mouth of the estuary and ten piers are completed. Another twenty-seven piers are under construction in building dock II, while the construction of the last series of the sixty-six piers needed is about to start.
2. The building of the lifting barge "Ostrea", that will be used to place the piers in position has started on 1st July. The vessel "Cardium" is partly being built in The Netherlands, partly in Western Germany. This ship is to lay the foundation mattresses for the surg-barrier piers. The laying of block mattresses to prevent bottom scour on both sides of the future barrier has been completed. Since 1973 4.5 km^2 of mattresses have been laid. The floating asphalt mastic factory "Jan Heymans" will be converted to a stone-dumping barge in 1981.

A sheet and a tube design for the gates have been tested. The tube design is more resistant to external stresses. Hydraulic model research of the half-open situation is still being carried out.

The dike connecting the barrier with the Isle of Schouwen is nearly ready.
3. The compartimentation works are running to schedule. The secondary dams will comprise a combination of sand- and stone closure, stone blocks will be tipped by lorry from a bridge. The diking of the Markiezaat of Bergen op Zoom is in full progress. This work must be completed by 1981.
4. The partial dike-reinforcement has been nearly completed. One-hundred-and-

ten km has been finished, and only 700 m at Yerseke and a small distance at Flakkee remain to be done before July 1981.
5. A siphon with a capacity of 100 $m^3 s^{-1}$ near Flakkee can transport water from the Grevelingen to the Eastern Scheldt to increase chlorinity. This syphon will become operational in 1982.
6. The sluice in the Brouwersdam was open until 29-2-1980 and closed from 01-03-1980 until 15-10-1980. Since then it has been open again.
7. The construction of the sluice for the drainage canal at Bath has been started.

V. WORKING GROUP: ELEMENTS CYCLING AND FOOD CHAINS (Code G + K)

V.1. Introduction (P.H. Nienhuis)

The working group "Carbon cycle in the Grevelingen" will terminate its integrated ecosystem studies 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 1985-1986, 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 an ecosystem study in the Eastern Scheldt.
A new research plan has been developed, based upon the results of the Grevelingen study, and dealing with the production and transformation of organic matter. Consequently, the working group has two main projects under study: a. Carbon cycle in Lake Grevelingen, and b: Food chains and production of organic matter in the Eastern Scheldt. The central question in 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.

In close cooperation with Rijkswaterstaat two 3-years projects have been launched in the course of 1979 and 1980, comprising 7 research-workers and 7 technical assistants (Code names: ZOWEC and BALANS).

The Eastern Scheldt study started in 1980 with a general approach of a number of significant compartments in the system: primary production and biomass estimates of phytoplankton and microphytobenthos, and biomass estimates of macrozoobenthos. Detailed information about the transport of organic and inorganic particles in the estuary has been gathered.

For the Lake Grevelingen much effort was put into a number of integrated, continuous 30-hours measurements, dealing with the metabolism - production and consumption - of the dominant components of the water column and the underlying bottom in an eelgrass bed.

A mathematical -ecological model of the aquatic system is under construction

Table 3. Range of environmental parameters measured bi-weekly in 1980 at station G 11, a 22 m deep central gully in Lake Grevelingen; DOC was measured incidentally

Grevelingen G11	0 - 2.5 m	17.5 - 20 m
Temp °C	1.7 -20.2	2.2 -18.2
°/oo Cl^-	15.3 -17.4	16.4 -17.5
Secchi cm	225 - 625	
O_2 mg l^{-1}	8.0 -12.8	4.5 -10.8
P-PO_4 mg l^{-1}	0.06 - 0.42	0.07 - 0.44
Si mg l^{-1}	<0.01 1.61	0.01 - 1.66
N-NH_3 mg l^{-1}	<0.01- 0.71	<0.01- 0.29
N-NO_3 mg l^{-1}	0.00 - 0.50	0.00 - 0.58
DOC mg l^{-1}	1.93 - 3.07	

in close cooperation with Rijkswaterstaat and the Delft Hydraulics Laboratory (Code name WABASIM).

The Brouwerssluice, between Lake Grevelingen and the North Sea, caused an open connection with the sea up to 29 February 1980. The sluice was closed from 1 March and reopened on 15 October. During the periods of open connection a total volume of 939 x 10 m^3 water from the North Sea was let in, which means that at an approximate constant water level 1.6 times the lake volume was exchanged in 1980 against seawater, stressing the estuarine character of the present lake.

Table 3 shows a number of environmental parameters, measured in Lake Grevelingen in 1980. The salinity of the water appeared to be high and relatively stable. The water was extremely clear. No significant stratification and consequent oxygen depletion could be discovered in the watermass shallower than 20 m. N-nutrients, especially $N\text{-}NO_3$, were estimated in extreme low concentrations during large periods of the year. DOC levels showed an estuarine character, comparable with the eastern part of the Eastern Scheldt.

V.2. Laboratory experiments on sediment/water exchange (G2) (P. Kelderman and A.M. van de Repe)

Mass budget calculations have shown the role of sediment-water exchange in Lake Grevelingen phosphate contents (Kelderman, 1980). Earlier studies of interstitial water of Lake Grevelingen sediment offered insufficient insight into the processes of sediment-water exchange (Kelderman, 1981). Alternatively, these processes have been studied by means of laboratory experiments under specified conditions.

Sediment cores were taken (30 cm depth, ϕ = 11 cm) at 4 sampling stations in the lake. Sediment types varying from medium- to muddy sand (Fig. 3) were representatively chosen according to bottom map of Lake Grevelingen (Nieuwenhuize et al., 1980). The sediment cores with overlying water (ca. 2 litres) were placed in the dark, at 5 °C in thermostatically controlled water baths. Overlying water was renewed regularly by (unfiltered) Eastern Scheldt water. After a week's recuperation time, temperature was slowly raised to the experimental temperatures. After three weeks 8 sediment cores (4 sediment types, duplicates) were at 5 °C, 8 at 10 °C, 8 at 15 °C and 8 at 20 °C. The same procedure was applied to control cores, containing Eastern Scheldt water.

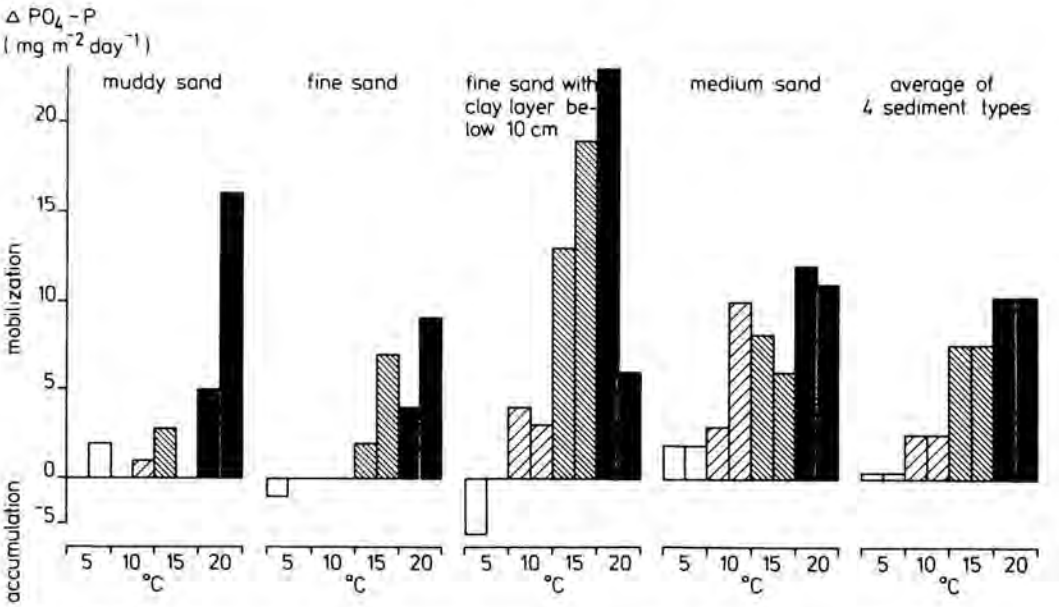

Fig. 3. PO_4^{3-}-P exchange between water and sediment at different temperatures and sediment types. Average results of 8 consecutive exchange data are given (duplicates). Overlying water contains ca. 300 µg P l^{-1}. Absence of bars denotes zero exchange

Next, sediment-water exchange was investigated by monitoring the overlying water for NH_4^+-N, (NO_2^- + NO_3^--N), PO_4^{---} -P, Si and, occasionally, P tot., N tot., pH and DOC. By addition of Eastern Scheldt water of known composition, a reasonable constant phosphate content in the overlying water was maintained. The sediment cores remained in good condition throughout the experimental period (ca. 4 weeks), as was apparent from the constant pH-values and the presence of actively pumping *Cerastoderma* spp., *Arenicola marina*, etc.

Results for the PO_4^{---} -P exchange are given in Fig. 3. With an adjusted overlying water content of ca. 300 ppb, P mobilisation from the sediment took place. Its magnitude was strongly dependent on temperature. Large differences existed between duplicate samples and between the different sediment types. The same picture holds for the silicate sediment-water exchange (Fig. 4). No specific trends could be discovered for the N and DOC exchange.

Follow-up experiments have shown that P accumulation sets in for higher P contents in the overlying water (> ca. 500 ppb, dependent on temperature and sediment type).

The phosphate exchange data are consistent with the P budget calculations (Kelderman, 1980), and with preliminary *in situ* experiments in Lake Grevelingen using rigid bell jars. Laboratory and field experiments on sediment-water exchange will be continued in 1981.

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Kelderman, P. - 1980. Phosphate budget and sediment-water exchange in Lake Grevelingen (SW Netherlands). Neth. J. Sea Res. 14 (3/4), 229236.

Kelderman, P. - 1981. Een interstitieel water onderzoek op vijf permanente meetplaatsen op de Grevelingenbodem in de eerste zes maanden van 1979. DIHO Rapp. en Versl. 1981-4, 57 pp.

Nieuwenhuize, J., J.M. van Liere and A.G. Vlasblom - 1980. Een bodemkaart van het Grevelingenmeer in 1979. DIHO Rapp. en Versl. 1980-6. 44 pp.

V.3. Statistical reliability of the bottom map of Lake Grevelingen (G2) (J. Nieuwenhuize, J.M. van Liere and C.H. Vos)

In order to check the reliability of bottom-sediment maps for the saline Lake Grevelingen in 1979 (J. Nieuwenhuize *et al.*, 1980, 1981), 36 bottom sediment cores were collected by SCUBA divers in 1980 in each of four areas in Lake Grevelingen, that differed

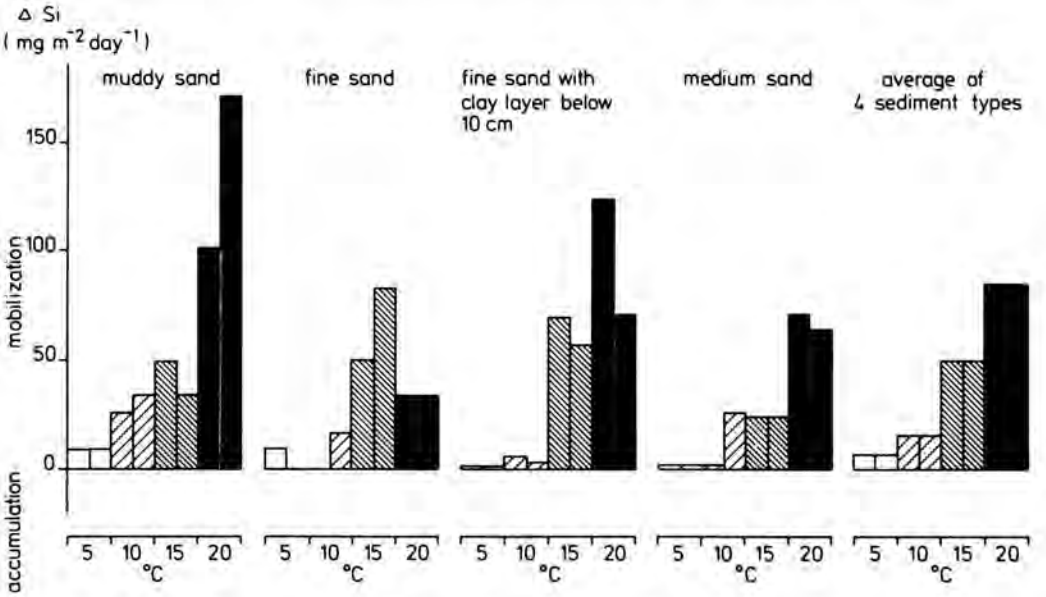

Fig. 4. Reactive-Si exchange at different temperatures and sediment types, see legend Fig. 3. Overlying water contains ca. 2500 µg Si l^{-1}

markedly in sediment type. In the samples the following parameters were determined: calcium carbonate, silt, particulate organic carbon, chlorophyll and the grain-size distribution.

The spatial and laboratory analytical variation between the samples was analyzed. The spatial coefficient of variation was four to twenty times larger than that of the laboratory analysis (Fig. 5). The variation in the value of the parameters within the area is fairly large, but it is concluded that the spatial values lay in general between the class limits of the bottom maps of 1979 (Fig. 6), where (----) is range of the class of the parameter in the maps of 1979 and (——) represents the mean and 95% confidence limits of the spatial values of 1980.

References

Nieuwenhuize J., J.M. van Liere and A.G. Vlasblom - 1980. Een bodemkaart van het Grevelingenmeer in 1979. DIHO Rapp. en Versl. 1980-6, 44 pp.
Nieuwenhuize J., J.M. van Liere and C.H. Vos - 1981. Statistische betrouwbaarheid van de bodemkaart van het Grevelingenmeer. DIHO Rapp. en Versl., 1981-1. 17 pp.

V.4. Particulate Organic Carbon (POC) in relation to plankton concentrations of Lake Grevelingen in 1980 (G1,3) (C. Bakker, P. van Rijswijk and J. Nieuwenhuize)

POC-concentrations were determined in 1 litre-samples of the water column at station G11, containing seston mainly composed of small phytoplankton and detritus.

Gradually increasing values were demonstrated in the course of the year. POC levels started with concentrations of 0.20-0.40 ppm in winter, rising during spring to 0.50-0.80 ppm and reaching a peak concentration of 1.05 mg l^{-1} in August. As a rule, highest concentrations in the water column were found in the surface layers (0-2.5 m depth), except for the month of August.

Weighted means of POC concentrations amounted to 0.46 ppm in spring (0.51 ppm in 1979) and to 0.66 ppm in summer (0.53 ppm in 1979). The annual mean was 0.52 ppm (0.47 ppm in 1979).

POC percentages of total seston dry weight varied from 5-20 in winter, from

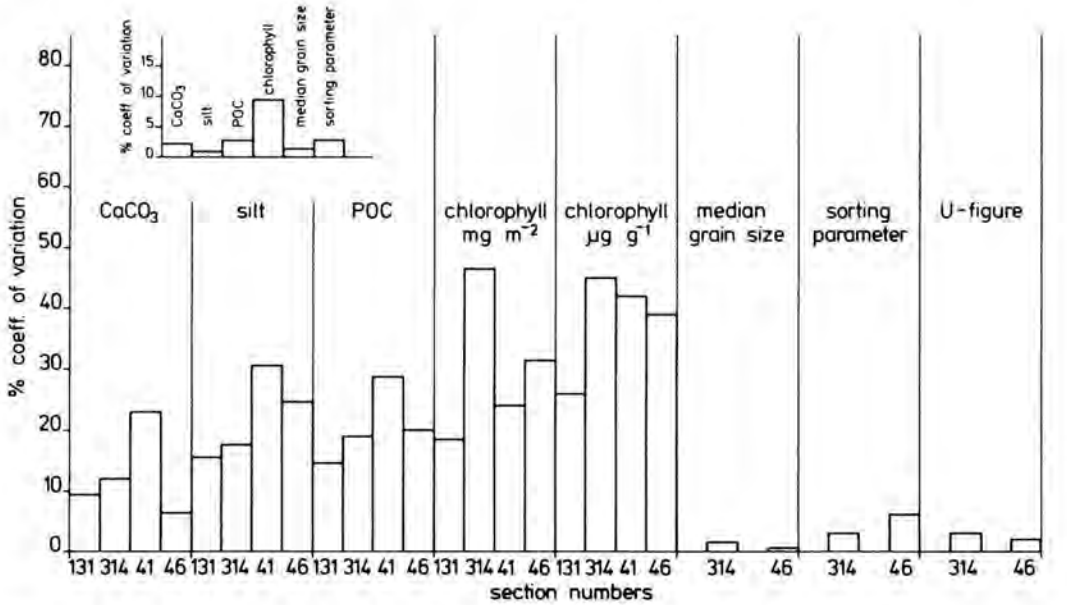

Fig. 5. The spatial coefficient of variation of some bottom sediment parameters. Insert: Coefficient of variation of some bottom sediment parameters; analyses were carried out in tenfold

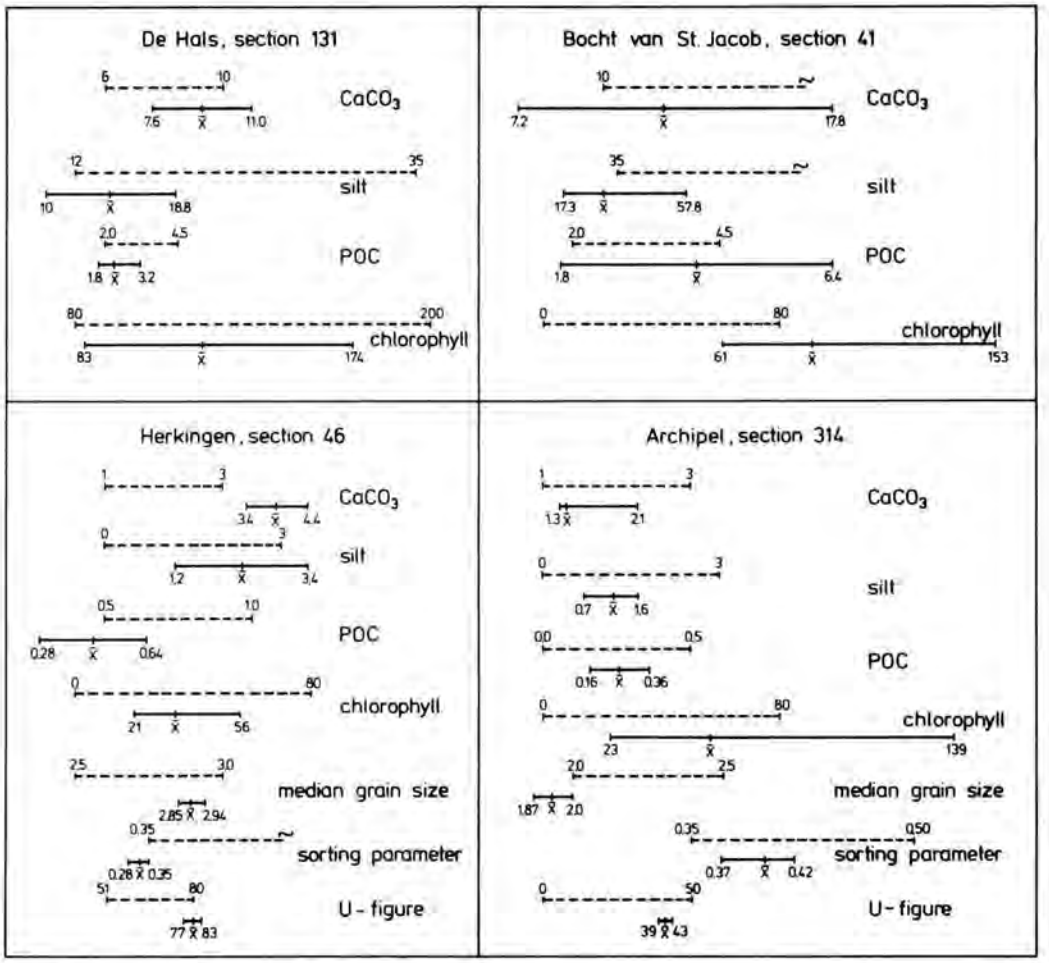

Fig. 6. Classification according to the bottom sediment maps 1979 (----) the 95% confidence limits (——) and the mean x of the sediment parameters of four areas

15-35 during spring and summer periods of algal blooms, with an incidental maximum of ca. 50% in summer.

POC content was also estimated in plankton samples of 100 l, filtered with nets of 63 µm mesh width, containing zooplankton, larger phytoplankton and detritus particles. Winter levels were low: less than 1 mg 100 l^{-1}; spring-and summer samples reached concentrations of 2-10 mg 100 l^{-1}. The differences between the POC contents of the (100 l)-zooplankton samples from winter and summer are therefore much larger than those of the (1 l)-phytoplankton samples during the same periods. In Fig. 7 the results of the analysis of the 1 l and 100 l samples are given. The main tendencies of the development of phyto- and zooplankton biomass in the course of the growing season are satisfactorily reflected. The 3 main phytoplankton- POC peaks (April, May, August) were followed by zooplankton-POC maxima, in all cases occurring 2 weeks later.

Seston dry weights of the zooplankton samples contained the highest POC percentages during spring with a mean value of 36% and peak values of ca. 45% when rotifers dominated or a mixed zooplankton was present, consisting of rotifers, barnacle larvae and copepods.

V.5. The effect of nitrogen enrichment and sediment on the eutrophication of Lake Grevelingen, simulated in four enclosures (J.A.C. Derks, Rijkswaterstaat)(G1, 2, 3, 4)

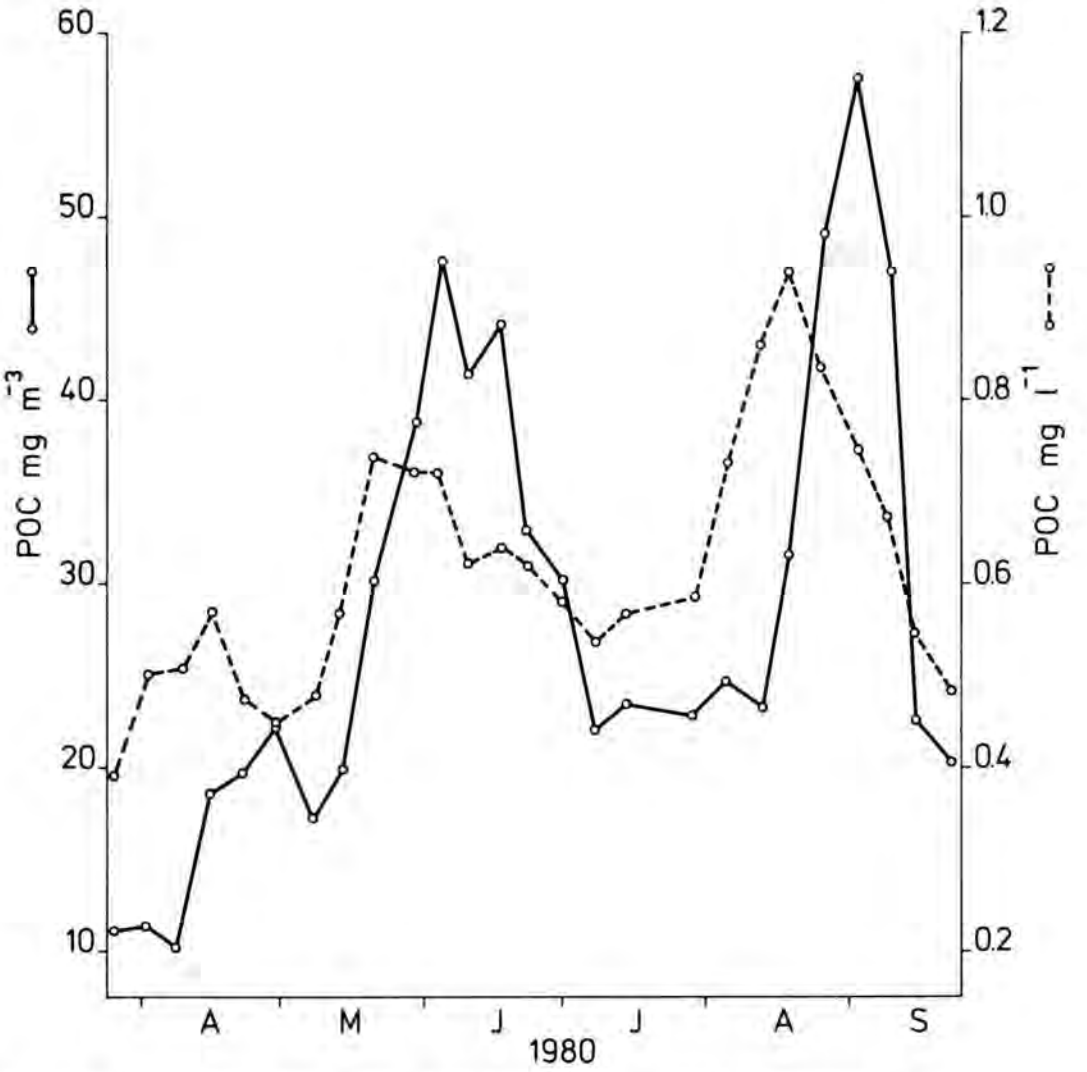

Fig. 7. POC concentration expressed as moving averages of 3 succesive weekly values (mg l^{-1}) and in 63 µm mesh netplankton (mg m^{-3}); samples are from April to September 1980 in Lake Grevelingen, station G 11.

Since the Brouwersluice has been used, netto import of POC from the North Sea and netto export of phosphate and silicium were calculated. In relation to nitrogen, a limiting factor for the development of phytoplankton, a netto export was calculated over the entire year, but calculations suggested a considerable import during the growing season (Stokman, 1980).

The effect of nitrogen enrichment and sediment on the eutrophication was measured in spring 1980 in four enclosures (ϕ 5.50 m; depth 1 m) (Derks, 1981).
The different combinations of environmental circumstances resulted in the following mean chlorophyll concentrations (Fig. 8) during the experiment,

no N-enrichment; no sediment contact; encl. 1: 5-4 µg l^{-1}
N-enrichment; no sediment contact; encl. 2: 20.6 µg l^{-1}
N-enrichment; sediment contact; encl. 3: 49.4 µg l^{-1}
no N-enrichment; sediment contact; encl. 4: 15.7 µg l^{-1}

surrounding Grevelingenwater 3.0 µg l^{-1}

By means of water and chloride mass-balance equations the enclosed water volumes were estimated. No exchange of water or nutriënts between the

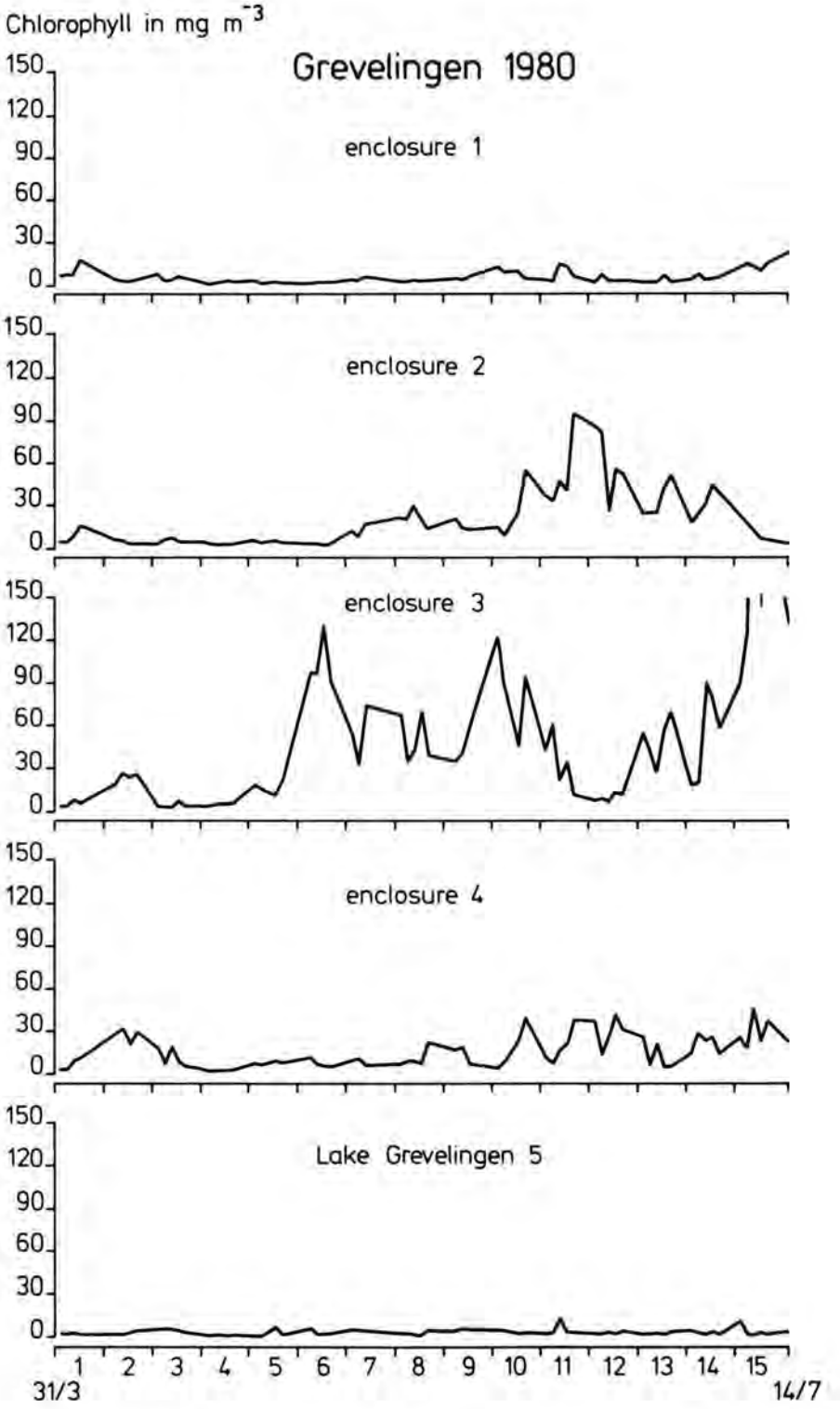

Fig. 8. Chlorophyll contents of enclosed waterbodies in Lake Grevelingen during a 15 weeks experiment (April-July 1980)

enclosures and the surrounding water could be calculated. Using mass-balance equations for the other measured nutrients (PO_4; P tot.; $N\text{-}NH_4$; $N\text{-}NO_3/NO_2$; N tot.; POC, PON and POP) denitrification and phosphate mobilisation from the sediment were recognised as important processes. The mean denitrification rate (in the N-added reservoirs) was about 100 mg N m^{-2} day^{-1} and the maximum velocity of phosphate regeneration was $\pm$ 25 mg $m^{-2}day^{-1}$ (in encl. 3). The estimated net sedimentation of nitrogen compounds was neglectable compared with the denitrification. The anaerobic sediment (encl. 3) and the sedimented organic material (encl. 2) were responsible for the denitrification process. The occurrence of denitrification under anaerobic circumstances was illustrated by the composition of the gas mixture, below the plastic bottoms, of the enclosures consisting of 88% nitrogen.

Both presence of nitrogen and sediment contact play a dominant role in the development of phytoplankton blooms. Since nitrogen (for diatoms also silicium) limits the biomass of phytoplankton in Lake Grevelingen, biomass and primary production were higher in the N-added reservoirs. N-limitation under natural conditions is probably caused by the enormous loss of nitrogen due to denitrification (Kessel, 1976).

Phosphate can be regenerated from the sediment also in the absence of eelgrass. This process is, either directly or indirectly, accelerated by the primary production process. Without sediment contact and after the addition of nitrogen phosphate becomes limiting for phytoplankton development. When contact with the sediment exists, phosphate is regenerated from the sediment.
This process continues until light becomes the limiting factor for the development of phytoplankton, due to selfshading. The situation occurring then is undesirable: biomass of phytoplankton > 200 µg l^{-1}; only a few decimetres secchi-disc transparency and strongly fluctuating pH and oxygen concentrations.

The role of the sediment in most nutrient cycles becomes clear if we consider the sediment primarily responsible for denitrification. Measurements of oxygen consumption (Goossens, 1981) indicate (with the exception of enclosure 1) that the sediment plays a dominant role in the mineralization process in the reservoirs. Mineralization in enclosure 2 occurred particularly on the plastic bottom, indicating that a new sediment was formed on the bottom of the enclosure. Finally the sediment has an enormous potential for phosphate regeneration.

Because the high sampling frequency (five times a week) in this experiment the dynamic character of the processes involved was clearly demonstrated (*e.g.* doubling of the phytoplanktonbiomass during one day). The variation of the chlorophyll content of phytoplankton as a function of light intensity became quite clear. The zooplankton concentrations were measured by Bakker and van Rijswijk. They were higher then in the surrounding Grevelingen and might play an additional role in limiting the phytoplankton biomass. Finally the role of macrozoobenthos in filtering the phytoplanktonbiomass was estimated and held responsible for the greater part of the difference between enclosure 4 and the surrounding Grevelingenwater.

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V.6. Phytoplankton of Lake Grevelingen during 1980 (G3) (C.Bakker, J.C.M. Rijk and P. van Rijswijk)

Phytoplankton was sampled at station G11 (depths of 2.5, 7.5, 12.5, 17.5 m) and at a nearby shallow area of ca. 3 m, using a 5 l hydrobios sampler. Sedimentation samples of 1 l were taken to study the composition and abundance of the smaller phytoplankton. So far only the 2.5 m depth samples have been studied.

In Table 4 mean cell numbers of ca. 10 species during the summer of 1979 and

Table 4. Average phytoplankton densities (2.5 m depth) during the growing season, average chlorophyll concentrations (2.5 m depth) and Secchi-disc visibilities in spring and summer of Lake Grevelingen, station G 11, in 1979 and 1980

	1979	1980
Algal species	cells ml^{-1}	cells ml^{-1}
Cryptomonas spp.	770	430
Eutreptiella spp.	700	200
Katodinumi rotundatum	390	450
Flagellates 4 µm diam	390	240
Coccoid µ-cells	64,000	42,000
Green rods	320	2,800
Nitzschia seriata	72	31
Rhizosolenia spp.	30	3
Chaetoceros spp.	3,450	1,540
Ditylum brightwellii	39	6
Chlorophyll a	mg m^{-3}	mg m^{-3}
spring (III-IV)	5.8	2.7
summer (V-VIII)	6.9	2.4
Annual mean	4.5	2.1
Secchi-disc visibility	m	m
spring (III-IV)	4.4	3.7
summer (V-VIII)	3.4	3.5

1980 have been listed, as well as chlorophyll and secchi-disc data. Chlorophyll-a concentrations were reduced with more than 50% in 1980 as compared with 1979. In agreement with these data is the smaller phytoplankton biomass in 1980, caused by lower abundance of the most dominant species. Especially the flagellates *Cryptomonas* spp. and *Eutreptiella* spp., the small diatom *Chaetoceros* spp. and the larger diatoms *Ditylum brightwellii* and *Rhizosolenia setigera/libetata* decreased strongly in number. Coccoid µ-cells diminished too, but small green rod-like cells (an unidentified alga, dimensions ca. 10 x 3 µm) increased nearly tenfold. Secchi-disc values in summer were more or less similar to those of 1979, but in spring the transparence of the water was less than in 1979.

The last 3 annual cycles occurred under 3 different hydrographical regimes. During 1978 Lake Grevelingen was still stagnant, without any influence from the North Sea. The Lake was characterized then by lower biomass figures throughout the year and an annual-primary production of ca. 150 g C m^{-2}. In 1979 the lake was continuously flushed with North Sea water (by means of a sluice in the Brouwersdam). Mean annual biomass of phytoplankton was doubled and primary production increased strongly to ca. 250 g C $m^{-2}yr^{-1}$. In 1980 flushing was limited to the first and last 2 months of the year; during the remainder of the year, *i.e.* the complete growing season, the lake was stagnant again, demonstrating a considerable decrease of biomass, accompanied however by still increased (ca. 228-326 g C $m^{-2}yr^{-1}$) primary production.

The agreement between chlorophyll- and phytoplankton data is satisfactory. POC contents on the other hand (see V.4), differed hardly from those of the preceding year, the summer values of 1980 being slightly higher. Primary production was

even significantly larger than the 1979 figure. These discrepancies cannot be explained yet and have to be studied further in detail; see also V.19.

Plankton sampling of Lake Grevelingen has been finished with 1980. Full attention will be given to a final evaluation of all measured parameters (and their mutual relations) relevant to phytoplankton: species composition and -abundance; biomass based on calculation of cell volume and expressed as chlorophyll a, particulate organic carbon and data of primary productivity.

V.7. The regulation of the phytoplankton primary production in Lake Grevelingen (G3) (A.B.J. Sepers, P.R.M. de Visscher and B.H.H. de Bree)

In this contribution the results of some preliminary experiments are described, set up in order to gain some insight into the main environmental parameters which regulate the phytoplankton primary production. For that purpose a multiple linear regression analysis was performed with the phytoplankton primary production as the dependent variable and a number of eight environmental parameters as independent variables.

The experiments were carried out during a one week period in June and September 1979 and in January and April 1980. In each experimental period the phytoplankton primary production was measured twice, applying the carbon-14 method. Samples were taken at station G 11-channel with a depth of 20 m and at station G 11-shallow with a depth of 2 m. Simultaneously the values of eight environmental parameters were determined: solar radiation, chlorophyll, silicate, phosphate, particulate organic carbon, ammonium, nitrate and temperature.

The multiple linear regression analysis was performed according to Nie *et al.* (1975). The basic regression equation is represented by

$$Y' = A + B_1X_1 + B_2X_2 + B_3X_3 + \ldots\ldots B_kX_k,$$

where Y' is the calculated value of the dependent variable Y (phytoplankton primary production), X_1, X_2, ... X_k are the independent variables (environmental parameters), A is the intercept on the ordinate and B_i are regression coefficients. The independent variables were entered into the regression equation one by one. The order of inclusion is determined by the respective contribution of each variable to the explained variance. The criteria for inclusion were significance of the partial regression coefficient at the 0.05 level and a tolerance value of 0.2 (proportion of the variance of the independent variable which is not explained by the independent variables already included in the regression equation).

For both sampling stations only two environmental parameters met the selection criteria for inclusion into the regression equation. At sampling station G 11-channel 65% of the variation in the phytoplankton primary production could be attributed to the combined variation of solar radiation and chlorophyll concentration (Table 5); at sampling station G 11-shallow 85% of the variation in the phytoplankton primary production was explained by the joint variation of temperature and nitrate concentration. At both sampling stations six other environmental parameters did not meet the criteria for inclusion into the regression equation.

Multiple regression analysis describes non-causal relations between variables. Therefore it is doubtful to draw definite conclusions about functional relationships

Table 5. Multiple linear regression of phytoplankton primary production in Lake Grevelingen on a number of environmental parameters

Sampling station	Variable entered	Multiple R	R^2	Significance (P)
G 11-channel	Solar radiation	0.712	0.507	0.001 (n = 56)
	Chlorophyll	0.804	0.647	0.001 (n = 56)
G 11-shallow	Temperature	0.880	0.744	0.001 (n = 22)
	Nitrate	0.920	0.846	0.001 (n = 22)

between the variables. The technique of multiple regression, however, permits the selection of the independent variables with the highest predictive value. A more detailed research into the relations between the dependent variable and the selected independent variables then results in a better knowledge of the processes which have a regulating power for the dependent variable.

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V.8. Zooplankton of Lake Grevelingen during 1980 (G4) (C. Bakker, P. van Rijswijk and J.C.M. Rijk)

Zooplankton samples were taken on 17 stations situated in deeper as well as in shallow places. For the procedure of proportional sampling and pooling of samples: see Progress Report 1979.

During the flushing period (November - February) species as the copepod *Calanus helgolandicus* and the Chaetognath *Sagitta setosa* entered the lake from the North Sea. Salinity was constantly high, also during the spring-summer period of stagnancy and varied between 16-17 $^{0}/_{00}$ Cl^{-}. During the preceding years the cladoceran *Evadue nordmanni* was incidentally observed in summer; in 1980 the species reached a maximum population density of 2-30,000 animals per m^3 in the beginning of June and replaced the maximum of *Podon polyphemoides*, normally occurring during that time. Towards July/August and in September and October several observations were made of ascidian larvae, presumably belonging to the newly introduced species *Styela clava*. This species has become very common in the Eastern Scheldt and is spreading in Lake Grevelingen.

Water temperature was abnormally low during 3 successive weeks at the end of June and the first half of July (ca. 16 ^{0}C) and consequently reduced numbers of larval stages of gastropods, cirripeds and polychaetes were found. After the rise of temperature to 19-20 ^{0}C in August several species demonstrated a more intense reproduction: *Podon*, *Acartia tonsa* and lamellibranchs.

The main biomass peaks of zooplankton developed in the beginning of June and the end of August, corresponding well to increasing phytoplankton densities two weeks earlier (see paragraph V.4 about POC). In agreement with the decrease of the average chlorophyll and phytoplankton biomass (see paragraph V.6), zooplankton biomass was lower in 1980, especially during summer. The distribution of biomass in the course of the summer 1980 was quite different from 1979, as the 1980 peaks increased successively in the course of summer (35 ppb C in June, 50 ppb C in July/August and 60 ppb C in September). The spring peaks of the rotifers (*Synchaeta*) were the lowest sofar measured and confirmed the impression of 1979 that the winter flushing regime, by enhancing water turbulence and decreasing Secchi-disc visibilities, suppressed the early development of the rotifers, which are well adapted to stagnant saline water.

During spring J.A.C. Derks (Rijkswaterstaat), started experiments with enclosures on a shallow place of the lake to study in detail the processes of wax and wane of the algal spring blooms (see V.5). Our institute analyzed the zooplankton samples in order to determine possible grazing pressure in the compartments. The preliminary data have to be analyzed in detail before conclusions can be drawn.
(Note: ppb = parts per 10^{9}).

V.9. *Gonionemus vertens* A. Agassiz (Hydrozoa, Limnomedusae) a new species in the eelgrass beds of Lake Grevelingen (G4) (C. Bakker)

The sheltered habitat of eelgrass beds is of significance for animals sensitive to strong wind-driven turbulence. From 1976 onwards the medusa of *Gonionemus vertens* is frequently found within the beds of Lake Grevelingen. The expansion of *Zostera* has created enlarged possibilities for the development of the medusa (Bakker, 1980, 1981).

For details about the habitus of the medusa: see Werner (1950). Adult specimens in Lake Grevelingen reach 3-4 cm. We did not yet discover the very small sessile solitary polyp stage.

The medusae are found in shallow places in tidal and non-tidal areas with a

littoral vegetation consisting of large brown algae, green algae or eelgrass species. The medusae are able to attach themselves to the thalli or leaves by means of adhesive organs at the distal ends of the tentacles. The medusa has the habit of turning over when the water surface is reached and then sinking downwards on its back with all tentacles extended. In that case the animal is fishing with an effective diameter of ca. 20 times the umbrella. Major food organisms in general are isopods (*Idotea chelipes*).

The occurrence of the medusa appears to be strictly limited to the summer months. Higher water temperatures are decisive for its development. This was clearly illustrated during 1980 when, after a cold period in early summer (June-July), the first medusae could be observed not earlier than late July at watertemperatures of > 18 ^{o}C.

The species occurs also in the harbour canal of Goes. In 1980 live specimens from Lake Grevelingen were transferred into aquaria of the institute and kept alive for months. Initially, we fed the animal isopods, but later small pieces of mussel meat were offered with good results.

The species shows a peculiar distribution. It is endemic in the coastal North Pacific Ocean. The hypotheses trying to explain the spread to Western Europe and the Atlantic coast of North America strongly suggest human influences *i.e.* distribution of the polyp via oyster transports (Edwards, 1976) and transport of the polyp as a member of the fouling community on ship's hulls.

Two articles have been prepared about the habits of the medusa, its behaviour as a littoral-bound species and its distribution (world-wide and in the S.W. Netherlands). The distribution is treated in more detail, especially its mechanism in relation to large-scale (global) and small-scale (local) range extension. It is the assumed occurrence of the polyps in shallow as well as in deeper tidal water that explains satisfactorily the findings of the medusa in the Netherlands.

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V.10. Phytobenthos in Lake Grevelingen (G5) (P.H. Nienhuis, B.H.H. de Bree and J.M. Verschuure)

A two years cycle of microphytobenthos production measurements was finished in 1980 (see also Progress Report, 1979). A preliminary estimate over 1979 and 1980 shows values of roughly 40-50 g C $m^{-2}yr^{-1}$ for the shallow areas of Lake Grevelingen. Production starts in February, shows a faint peak in spring, decreasing summer values and a slight second peak in early autumn. The picture is erratic and differs from locality to locality. Notwithstanding the high visibilities in Lake Grevelingen (Secchi disc values of 2.3 - 6.3 m in 1980) the overall C-14 production values are surprisingly low. The average estimates are about half as high as (*e.g.*) in the turbid Waddensea (cf. Cadée and Hegeman, 1974, 1977). A start was made in answering a number of methodological questions about the accuracy of the used methods, *viz.* diffusion of $^{14}CO_2$ into the sediment and concentration of non-labeled bicarbonate in the interstitial water of the sediment.

Chlorophyll data in the top-sediment vary between 50 and 200 mg m^{-2}, and show an erratic picture with relatively high winter values and no extreme peak values in summer. The full results of the biweekly production and biomass estimates will be published in 1981.

Further investigations were performed in the eelgrass (*Zostera marina*) community. The eelgrass system progressively developed during the period 1971-1978: in summer 1978 the plants covered an area of 4400 ha. The eelgrass production for entire Lake Grevelingen was estimated in 1978 at 91 g C $m^{-2}yr^{-1}$ (Progress Report 1979).

In 1980 this increase in production came to an end. A rough estimate of the distribution of *Zostera marina* in August 1980 revealed an area of approximately 2500-3000 ha. Up till now the causes of this decline are unknown. It should not be excluded that the changed hydrographical regime, owing to the management of the Brouwerssluice connecting the lake with the North Sea, influenced the eelgrass development in a negative way.

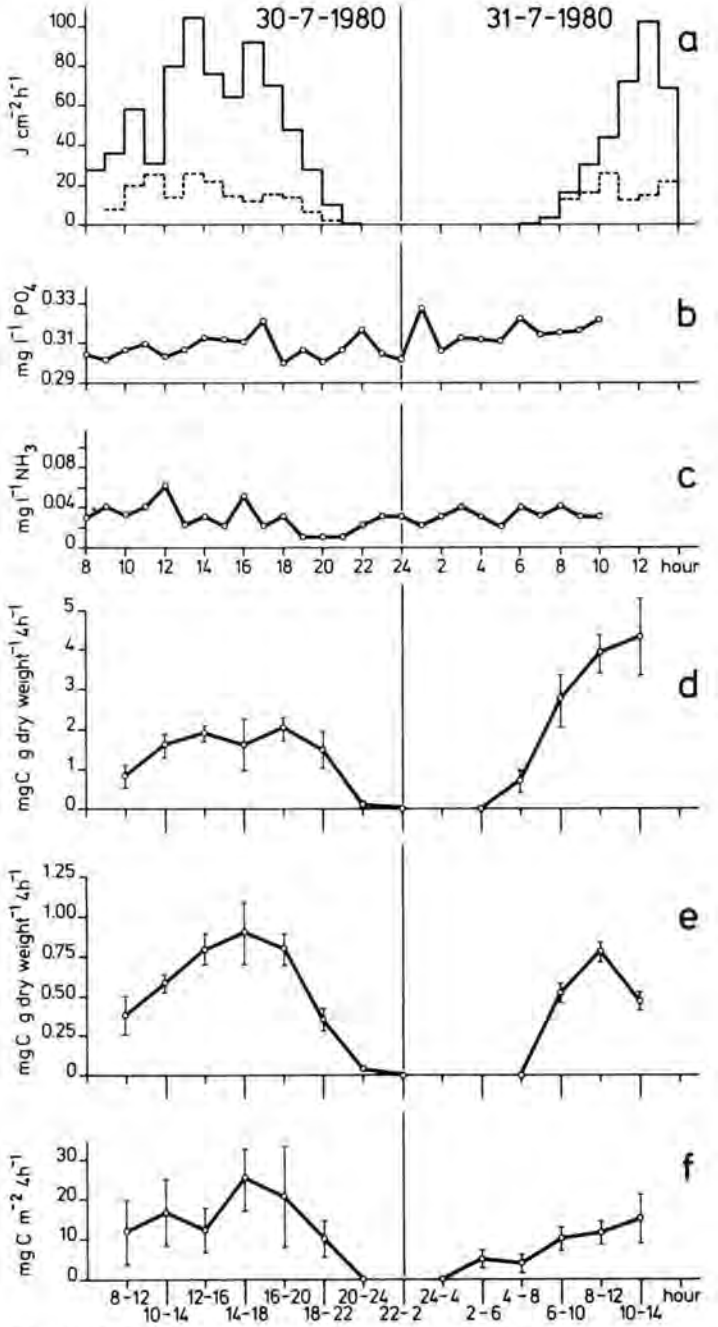

Fig. 9. A 30-hours measurement at a 1.5 deep eelgrass locality at Herkingen. a: uninterrupted line: insolation measured at Bommenede 8 m above water level; interrupted line: insolation measured in μE $m^{-2} s^{-1}$ at 1.5 m water depth and converted into J $cm^{-2} hr^{-1}$. b: $P\text{-}PO_4$ concentration in surface water. c: $N\text{-}NH_3$ concentration in surface water. d: production of *Zostera marina* shoots at 0.75 m water depth. e: production of *Chaetomorpha* at 1.5 m. f: production of micro (phyto) benthos at 1.5 m. Vertical lines: twice standard deviation of the mean

Work is in progress on a mathematical-ecological model of eelgrass dynamics in Lake Grevelingen, in cooperation with the Delft Hydraulics Laboratory. The model aims at a simulation of -temperature and light dependent - developments of the eelgrass vegetation in the lake.

In order to get a deeper insight into the eelgrass ecosystem metabolism, a number of 30-hours measurements were performed at a shallow eelgrass locality near Herkingen. Both production and consumption parameters were measured, either continually or interrupted, connected with a number of environmental parameters. Fig. 9 gives an example of a number of parameters measured at the end of July 1980 at an eelgrass site of 1.5 m depth. Roughly 30% of the solar radiation expressed in J $cm^{-2} hr^{-1}$ reached the bottom in a dense eelgrass stand. PO_4 and NH_3 concentrations in the surface water of the eelgrass bed did not show a marked day-night rhythm.

Primary production of *Zostera marina*, macroalgae and microalgae was estimated *in situ* with the C-14 technique. Fig. 9 shows that *Zostera marina*, with a standing stock of 97 g dry weight m^{-2}, was the most prominent primary producer, followed by the loose-lying alga *Chaetomorpha* and the microphytobenthos. Epibenthic algal production was ignored. An obvious relation exists between insolation and daily primary production. *Zostera* reached its maximum production later on the day than *Chaetomorpha* and the microphytes. The latter showed an extremely heterogeneous spatial production pattern (cf. standard deviations).

Full interpretation of these results together with a methodological elaboration will be given elsewhere.

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V.11. Decomposition experiments with *Zostera marina* (G6) (G.C. Pellikaan)

In 1977-1979 some aspects of the decomposition of eelgrass have been investigated in Lake Grevelingen (Pellikaan, 1979; Beunder, 1980; Groenendijk, 1981). Eelgrass detritus is transported from the meadows on the shore into the gullies, where it accumulates locally. In April 1980 the bulk of the bottom detritus in the gullies was derived from *Zostera*. It was fragmented to particles of 2-12 mm. The leaching rate of these leave fragments has been estimated. Mechanical fragmentation experiments with fresh leaves showed rapid fragmentation to small particles of leached cells and aggregated cell content.

In the field, decomposition of eelgrass is a complex of processes: 1. physical and biological fragmentation; 2. autolysis and leaching of DOM and nutrients; 3. colonization by bacteria and fungi; 4. microbial decay by the exoenzymes; 5. consumption by detritivores; 6. colonization of faecal pellets by microorganisms and subsequent decomposition.

Experiments were performed to unravel some aspects of this complex of processes. *Zostera* leaves were incubated in bottles with seawater and microorganisms under different conditions: aerobic - anaerobic; + and - antibioticum; fresh, green leaves and old, brown leaves; 6-11-16 °C. Frequently, samples were taken to determine pH, DOC and nutrients of the water. Dry weight, ash, POC, N_{tot}, PO_4-P, Na, K, Ca and Mg were analyzed in the detritus. Preliminary results of the series with green leaves under aerobic conditions are shown in Fig. 10. Note the sharp fall of DOC and the increase of pH and dry weight after 9 days. The C:N and C:P ratios in the detritus increased initially, indicating the leaching of N and P rich compounds; later on the ratios tend to decrease, possibly due to enrichment by microorganisms (Godshalk and Wetzel, 1978; Thayer *et al.*, 1977). Further statistical analysis and comparison with the other series are required to draw more definitive conclusions. Publication of these results is in preparation.

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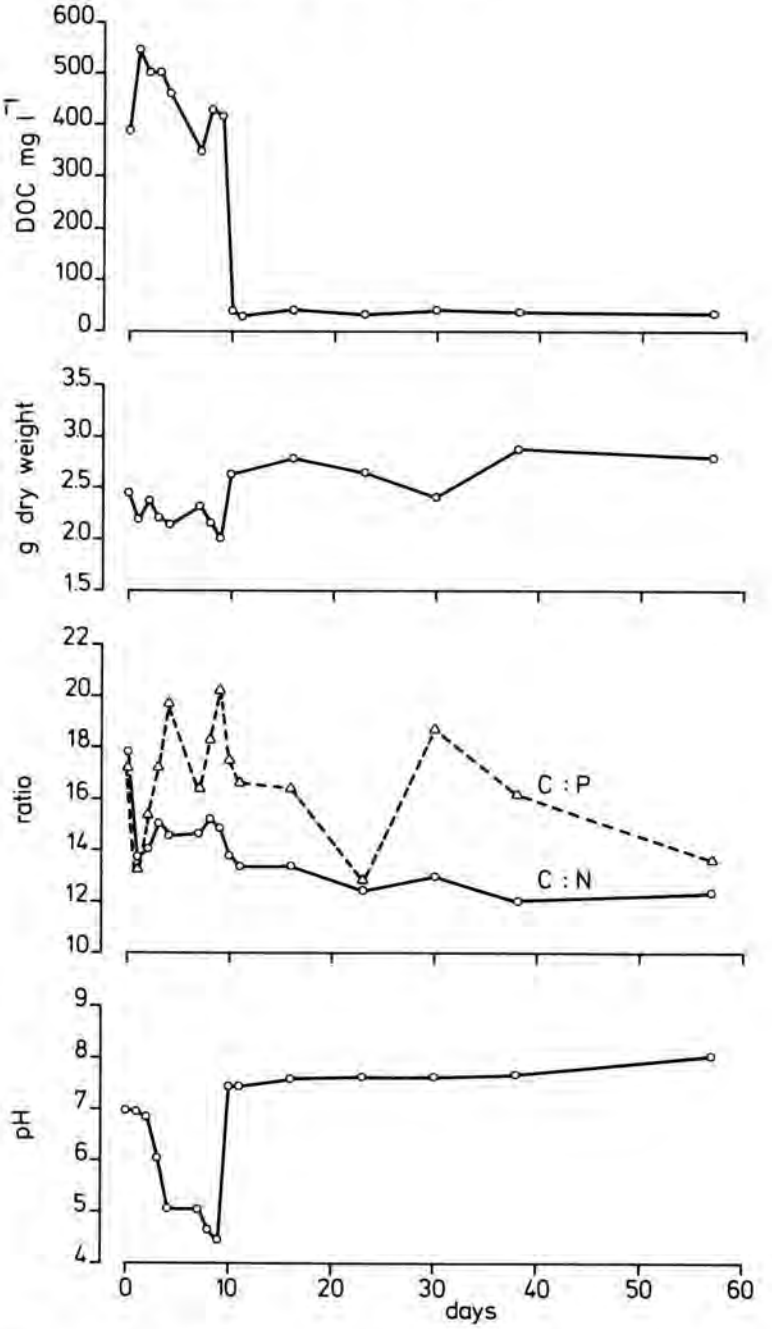

Fig. 10. Incubation experiments in 0.5 l bottles with decomposing *Zostera* leaves under aerobic conditions at 16 °C. Points in the figures are means of triplicates

V.12. Decomposition of plant matter (G6) (G.C. Pellikaan)

In order to get a better insight in the complex subject of decomposition in soil and in water, recent literature has been studied. The subject decomposition can be divided into seven sections:

1. Models of decomposition, detritus and herbivorous food chains, nutrient cycling, methods and terminology.
2. Chemical composition of plant matter and chemical changes during decomposition. Nutritive values, palatability and toxic compounds are included.
3. Decomposition rates, physical and biological fragmentation, particle size, leaching and particle formation.
4. Influence of physical and chemical factors on decomposition.
5. Role of bacteria: colonization and succession, biomass and production (free living or adhered), nutrient immobilization and detritus enrichment.
6. Role of fungi: colonization and succession, biomass and production, extracellular enzymatic activity, fungi- bacteria interactions, detritus enrichment and nutritive value.
7. Detritivores: micro, meio and macrofauna, feeding mechanisms, fragmentation and consumption of detritus. Food preference, attractiveness, particle size, ingestion and digestion of the food and the enzymes are involved. Faecal pellet production, chemical changes, recolonization and coprophagy. Uptake and release of DOM and nutrients.

In 1981 a report will be published, mainly containing a survey and a list of references.

V.13. Life history of *Praunus flexuosus* (Müller) (Crustacea, Mysidacea) in Lake Grevelingen (G7) (A.W. Fortuin)

As part of an investigation into the role of some epibenthic macro-crustaceans in Lake Grevelingen, *P. flexuosus* has been sampled at 5 stations in the period April 1978 - March 1979, using a bottom skimming planktonnet (Borghouts and Deelder, 1973). To illustrate the life history of *P. flexuosus* in Lake Grevelingen the population development at a shallow station (1.5 m), situated north of Veermansplaat in an eelgrass meadow, will be presented and described. With the exception of a station situated in a 10 m deep channel, harbouring comparatively few animals, the other stations showed a similar pattern.

The population compositions at the different sampling dates are presented in Fig. 11. At the start of the study, April 14, a few breeding females were already found, this number gradually increased to 70% of all females breeding at the end of May. At that time a considerable number of animals belonging to the new generation was found. On June 14 over 95% of the population consisted of animals belonging to this spring generation. The last representatives of the winter generation were found on July 26. The first breeding females of the spring generation were found on June 28. This number increased rapidly to more than 90% breeding on July 12. This led to a new wave of juveniles, appearing at the end of July. The breeding females of the spring generation were notably smaller than the females of the overwintering generation (mean length 16-17 mm to 21-22 mm), as was the case with males (spring generation 13-15 mm; overwintering generation 17-19 mm). These data also proof mature females to be longer than males of the same generation. On September 5 about 70% of the population belonged to the second cohort. Although the main part of this cohort will have originated from the spring generation, a minor part must have originated from females of the over-wintering generation, as breeding females of this generation were found as late as July 26. The last breeding females were found on September 20, all belonging to the spring generation. Consequently the over-wintering population of *P. flexuosus* in Lake Grevelingen is presumed to consist for the greater part of animals originating from the spring generation and for the smaller part of animals originating from the former over-wintering generation.

In general the same pattern as in Lake Grevelingen was found in Scotland by Mauchline (1971). However, only a small part of the females belonging to the spring generation was breeding during the summer, whereas in Lake Grevelingen almost all females did. Consequently the over-wintering population in Scotland consisted of a large number of animals belonging to the spring generation and

a small number of animals belonging to the summer generation. This may be explained by a different temperature in the two regions (Tattersall and Tattersall 1951). The localities sampled by Mauchline were situated at the west coast of Scotland and were in open connection with the sea.

In Lake Grevelingen, a closed waterbody, the summer temperature is in general higher than in the near by sea. According to Mauchline breeding continued in Scotland during the winter at a low rate. No indication for this has been found in Lake Grevelingen. The numbers of animals caught differ rather strongly (Fig. 11). Apart

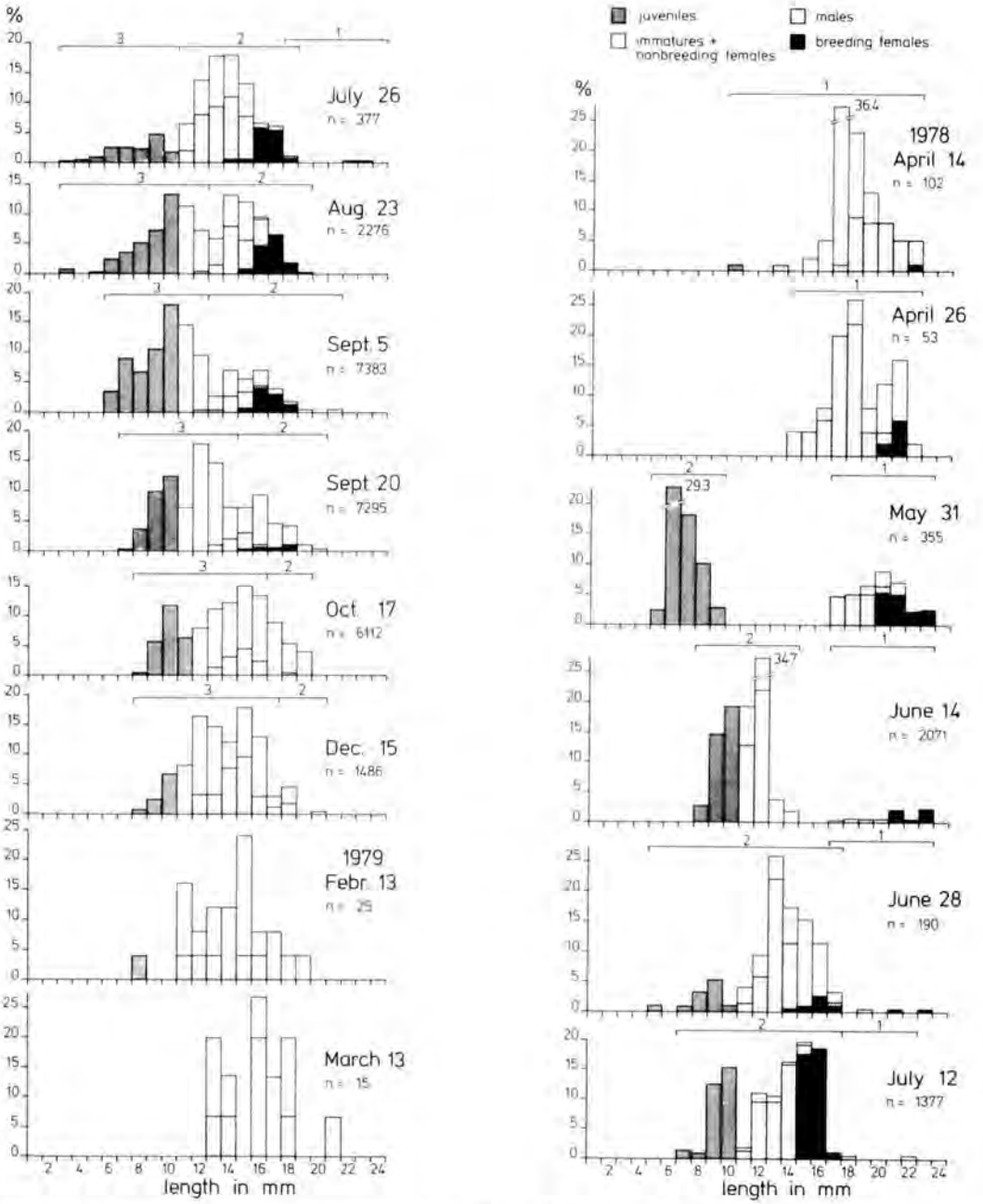

Fig. 11. Lenght-frequency distribution of *Praunus flexuosus*. Each bar represents the percentage of animals of one length related to the total number caught. The brackets above each distribution delimit separate cohorts. The boundary between cohort 1 and 2 is sharp, the boundary between cohort 2 and 3 is arbitrarily. No separate cohorts were indicated after December 1978

from expected seasonal differences, this was due to the experienced variation in the efficiency of the net used.

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V.14. The impact of eelgrass (*Zostera marina* L.) on the macrozoobenthos of Lake Grevelingen (G7) (E.G. Alkema and R.H.D. Lambeck)

An eelgrass vegetation influences its environment. In the water column the leaves will change light conditions, current velocities and perhaps even the amplitude of variations in oxygen concentration. In the bottom a dense root mat will be built up and the amount of detritus can increase, changing the sediment structure and the oxygen conditions.

These environmental changes will also have an impact on the local macrozoobenthos, living in as well as on or just above the sediment. The dense eelgrass meadows provide a shelter for several species and moreover food for some grazers. In the bottom detritivores may be favoured. These developments, however, may be at the cost of other groups.

In Lake Grevelingen the standing stock of *Zostera* increased considerably after the closure of the estuary in 1971: the area covered amounted to 1200 ha in 1968 and 4400 in 1978. A benthos survey in the shallow waters (0.70 - 2.10 m) of the lake in 1979 revealed a much lower biomass of the larger species (*i.e.* molluscs minus *Hydrobia ulvae* and some polychaetes) in the eastern part, mainly covered by *Zostera* compared with the western "bare" area, respectively 21 and 105 g ash-free dry weight m^{-2}.

These data provoked a study into the eelgrass - macrozoobenthos relation in Lake Grevelingen, that was started in spring 1980. In an experiment at two locations a pair of permanent quadrats has been set up at two water depths, i.e. 0.75 and 1.75 m. The quadrat size amounted to 7 x 7 m. In one quadrat of any pair the vegetation could develop undisturbedly, but in the other each fortnight the green vegetation was clipped away by SCUBA-divers. Unfortunately *Zostera* declined considerably in 1980 disappearing completely in one of the locations, hence halving the experiment. A possible response could be expected soonest from the small-sized species with a short generation-time, reason to direct the study to this category. Per six weeks in the inner 5 x 5 m segment (to rule out side-effects of the adjacent vegetation) 5 samples, each consisting of two cores of 28.3 cm^2 and 20-25 cm deep have been taken, which were flushed trough a 0.3 - 1.0 mm sieve combination and preserved in formalin. All molluscs, polychaetes and crustaceans have been sorted out, identified to species level, dried and ashed to obtain ash-free dry weights.

To have a more general picture, around August 1, at 20 stations of 0.4 to 2.0 m depth, vegetated as well as "bare" areas were sampled. At each spot 3 cores of 28.3 cm^2 were taken for the smaller organisms and 5 cores of 95 cm^2, sieved on a 1 mm screen, for the bigger ones.

With regard to the experiment, at both depths no significant difference could be demonstrated between both treatments in the period 28 March - 25 September. A considerable reduction in vegetation cover had obviously no short-term effect on the macrofauna. It has to be kept in mind that the in-bottom situation, *i.e.* the median grain-size, the detritus load and the dense root-mat, was the same in the paired quadrats.

The summer survey revealed a higher number of individuals and more biomass in the vegetated samples: 52 g ash-free dry weight m^{-2} compared with 21 g in the bare ones. A major part of this difference can be ascribed to epifaunal species, *e.g.* the snails *Littorina littorea* and *Nassarius reticulatus*, the bivalve *Mytilus edulis*, and several small crustaceans. Only the infaunal lugworm *Arenicola marina* appeared to be more common in the bare areas.

Results of this survey seem to confirm literature data, *e.g.*, Stoner (1980).

However, judging from the high amount of fragmented detritus and dead roots the "bare" stations were presumably covered with eelgrass in the preceding year. Because there is no difference in the bottom situation, the availability of a green vegetation with its own epifauna may be expected to raise the amount of animals. It is quite interesting to see, after just one season, a higher density and biomass of the lugworm in the bare areas. Possibly the lack of a strong living rootmat hampering its burrowing activities, may be responsible. This result indicates that parallel with a diminuation of the detritus load, a gradual shift towards another benthic community may occur. Only then a more realistic comparison between bare and *Zostera* covered bottoms is possible.

In 1981 the clipping experiment will be continued a complete year cycle. Moreover three transects at the extensive shallow Slikken van Flakkee, in 1977 sampled by Koniuszek (1979) will be resampled to study the long term effects of a *Zostera* cover.

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V.15. Net-efficiency of a small beam trawl for stickleback (*Gasterosteus aculeatus* L.) in shallow eelgrass beds (G9) (G. Doornbos and F. Twisk)

A small beam trawl is used in shallow water for sampling flatfishes and gobies. The net, simply called the 2 m beam trawl, has been described by Kuipers (1975). Although the beam trawl has not been designed for catching pelagic fishes, the three-spined stickleback (*Gasterosteus aculeatus*) is caught reasonably well, especially on bottoms overgrown with eelgrass (*Zostera marina*) and seaweed (*Chaetomorpha* spp.). In a comparative experiment between the 2 m beam trawl and a 3 m otter trawl the first caught an average of 2.9 times more stickleback per haul than the latter. At least over a short period of time, the rigid frame of the beam trawl prevents the plugging up of the mouth with plant material.

Sticklebacks are very abundant in eelgrass beds since these give them an excellent habitat and material for making their nests (de Graaf, 1979).

To quantify the fish it is necessary to know the selectivity of the sampling equipment used. To measure the selectivity of the 2 m beam trawl the following procedure has been applied. In an area with *Zostera* and *Chaetomorpha* a fine mesh seine was sailed out in the form of a rectangle. The lead line of the seine was looded down by a chain and rested on the bottom. The enclosed area amounted to about 400 m^2. At a distance of around 1000 m from this region, about 100 sticklebacks were caught and part of the first dorsal spine was clipped. Very few fishes died as a consequence of the handling. After one hour these marked sticklebacks were carefully released in the enclosure. They then had another period of about two hours to adapt to their new environment inside the enclosure. In the meantime 10 hauls with the beam trawl were made around the enclosure. Due to the abundant vegetation the fishing time per haul was restricted to $2\frac{1}{2}$ minutes, covering an area of 493 m^2. The enclosure was then fished out as well as possible by making successive hauls in it. After 15-20 hauls the surface was reduced by half and another series of hauls were made. This procedure was repeated three times. At the end the surface was reduced to about 2 m^2 and the remaining fishes were caught by a dip-net.

In three trials the mean percentage of recaptured marked sticklebacks was $76.2 \pm 3.8\%$ (Table 6). There was no difference between males and females, but young individuals seemed to be recaptured more easily than adult ones. With these data it is possible to correct for the escaped numbers of sticklebacks from inside the enclosure. By comparing these numbers and the numbers caught outside with the beam trawl, both related to an area of 500 m^2, an estimate of the net-efficiency of the beam trawl is obtained.

The mean efficiency for adult sticklebacks is $8.8 \pm 2.3\%$ (Table 6). There is a highly significant negative correlation ($P < 0.001$) between the length of the fish and the catch-efficiency (Fig. 12).

Only when the following conditions are assumed, our procedure of investigating

Table 6. Determination of the real number of sticklebacks (*Gasterosteus aculeatus*) per 500 m^2 and the net-efficiency of the 2 m beam trawl by marking and recapturing a known number of individuals in an enclosure placed in a shallow area (1 - 1.2 m deep) with *Zostera marina* and *Chaetomorpha* spp.

Date	Place	Inside enclosure				Outside enclosure	Eff. (%)	Remarks	
		Area (m^2)	Number released (marked)	% recaptured	Real N 500 m^{-2}	Mean N 500 m^{-2} ± s.e.	2 m trawl		
2-7-1980	Herkingen (east)	433.5	37	64.9	218	29.2 ± 3.7	13.4	♂♂ >	40 mm
			60	65.0	635	72.0 ± 16.3	11.3	♀♀ >	40 mm
3-7-1980	Herkingen (west)	405.7	74	79.7	479	16.2 ± 2.9	3.4	♂♂ >	40 mm
			27	81.5	458	14.2 ± 2.0	3.1	♀♀ >	40 mm
22-8-1980	Dijkwater	332.5	51	78.4	101.7	12.8 ± 0.9	12.6	♂ + ♀ >	40 mm
			41	87.8	18.8	7.7 ± 1.7	41.0	♂ + ♀ ≤	40 mm
				$\bar{x}$ =	76.2	$\bar{x}$ =	8.8	ind.>	40 mm
				s.e.=	3.8	s.e.=	2.3		

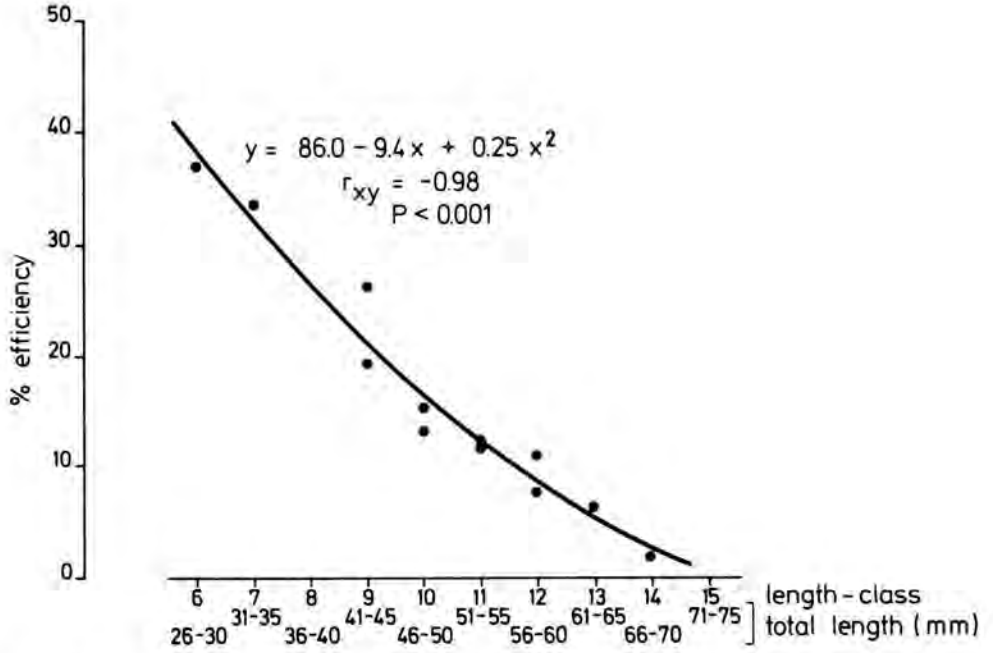

Fig. 12. Correlation between total body lenght and net-efficiency for the three spined stickleback (*Gasterosteus aculeatus*) for the 2 m beam trawl. Because of the low numbers, individuals smaller than 26 mm are excluded

the efficiency of the 2 m beam trawl will give correct data: 1). during the sailing out of the seine the same number of fish swim in as swim out of the enclosure. 2). the number of fish inside the enclosure is representative of the area. 3). the chance of escape out of the enclosure is the same for marked as for unmarked individuals.

In the Ythan estuary Healey (1971) determined the numbers of the sand goby (*Pomatoschistus minutus*) in an enclosure by making successive hauls in it. He found a negative correlation between the number of hauls made in the enclosure and the number of fish caught per haul. In this way he calculated the total number of sand goby that was originally present inside the enclosure. We did not find a similar correlation. The numbers remained more or less constant over a long series of hauls.

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V.16. Mineralization measurements in the water column (G10) (J.G.C.M. Goossens and R.S. Minnaar)

The mineralization of organic matter in the water is essentially an aerobic process. We chose to measure the oxygen consumption rate to quantify the carbon decomposition rate. In 1980 methods were developed and the field program was started.

During spring, oxygen consumption rates were measured twice a week in 4 enclosures in a shallow area (of 1 m depth). Simultaneously many chemical and physical parameters were monitored. In 2 of these enclosures NO_3^- concentration was kept above zero artificially. Gradually, rather different conditions developed in these enclosures. A preliminary analysis of the data set revealed no apparent relations between oxygen

consumption rate and other parameters as concentrations of NH_3, chlorophyll, phaeophytine and dissolved organic carbon. Detailed statistical analysis will be applied to reveal driving factors of the oxygen consumption rate.

Short term variations in oxygen consumption rate in the lake were investigated during 28 hour periods in spring, summer and autumn. Rather high variations of the oxygen consumption rate were demonstrated during the 28 hour periods in spring and summer. A dependence on time of the day was not obvious, due to the variation resulting from sampling of different watermasses. In autumn the variation was not very high.

The mean oxygen consumption rate (OCR) was much lower in autumn than in spring or summer even though water temperature differed hardly. The activity of the Electron Transport System (ETS) was measured too, following the procedure of Olańczuk - Neyman and Vosjan (1977). Altough the ETS activity showed a considerable variation, the ratio OCR/ETS was rather constant. ETS activity values should not be used as absolute values. OCR/ETS ratios larger than 1 occur frequently, showing lack of reliability of ETS activities as absolute values. Assuming that ETS values can be used for reasons of comparison the fact that OCR/ETS in autumn is much higher than in summer might mean that in autumn a greater part of the respiratory capacity is used.

In order to quantify the C mineralization in the water layer, the oxygen consumption rate was measured, from August on, every 3 weeks in samples taken at 34 stations all over the Grevelingen. A conversion factor of 0.29 is used to convert O_2 uptake to C mineralization (Sepers, 1981). The mean oxygen consumption rate shows a normal seasonal pattern. A close connection was observed between temperature and mean oxygen consumption rate in autumn. Assuming a temperature dependence of C mineralization and making an extrapolation to spring and summer we calculate a total C mineralization in the water of 250 g C $m^{-2} yr^{-1}$. Indications exist that total mineralization of both the water mass and the bottom amounts to more than twice the values calculated for the water column only. A considerable gap exists between total primary production and total consumption values which may partly be explained by differences in measuring methods (see further V.19).

In 1981 the field work will be continued and laboratory experiments will be initiated to get a better insight in substrate composition and substrate utilization processes.

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V.17. Production and mineralization in an eelgrass (*Zostera marina*) community during three 28-hour measurement periods in 1980 (G10) (H.J. Lindeboom and H.A.J. de Klerk)

Lake Grevelingen harbours a large eelgrass community which shows a vigorous primary production in the summer season. Recent studies yielded a wealth of information about the production of the eelgrass (Nienhuis and de Bree, 1980), but so far nothing was known about the consumption in this community. This study was carried out to investigate how production and consumption are related in the eelgrass fields.

During three 28 hour measurement periods in May, July and September 1980 the oxygen production and consumption in an eelgrass community were investigated using light and dark plexiglass enclosures equipped with a removable lid. Three enclosures were placed over the eelgrass community and two enclosures were placed on a patch where the overground parts of the eelgrass had been completely cut away. Two of the enclosures over the eelgrass and one of the enclosures over the barren sediment were darkened. With 15 minute intervals to allow fresh Grevelingen water to enter, the enclosures were closed for a 2 or 4 hour incubation period in which the oxygen evolution was recorded using YSI oxygen electrodes.

From the different oxygen uptake and release rates as measured over the 2 or 4 hour periods, the total oxygen production and/or consumption was estimated.

In order to obtain a 24 hour budget this was done for both the first and last 24 hours of the 28 hour measurement period (Note: there is a 20 hour overlap between the two 24-hour periods). Applying an oxygen to carbon conversion factor of 0.29 (Sepers, 1981) the amount of carbon fixed and the amount of carbon mineralized were estimated. For the different calculations it was assumed that the enclosures over the intact eelgrass gave the total mineralization or production of the eelgrass community, while the enclosure over the barren sediment gave only the production or mineralization of the sediment. Furthermore, it was assumed that respiration was the same in both the light and dark enclosures, and that primary production was the difference in oxygen evolution between a light and a dark enclosure. The results are shown in Fig. 13.

Both 24 hour periods in May and the last one in July showed a net production, while the first period in July and both periods in September/October showed a net consumption for the total eelgrass community. Both in July and September the overground eelgrass community showed a net production over a 24 hour period, while the sediment consistently showed a net consumption. This net consumption might have been underestimated since the shielding effect of the eelgrass was not present in the enclosures on barren sediment resulting in an increase of the bottom primary production. The results of all three measurements reveal that in the eelgrass field near Herkingen production and consumption are in the same order of magnitude. If production and consumption in the water column are included (unpublished data J.G.C.M. Goossens and P.R.M. de Visscher) similar conclusions can be drawn. Identical results were obtained in Vostok Bay (Cherbudgi and Tarasov, 1980). Monthly measurements performed throughout the year (unpublished data) indicate a slight overproduction of the eelgrass community followed by a physical removal of the eelgrass.

During the 28 hour measurement periods the primary production of both eelgrass and sediment were also estimated with the C-14 technique (De Bree, unpublished). Comparison of the results of both the O_2 and C-14 methods showed that the latter yielded considerably lower C-fixation values. It was concluded that it is impossible

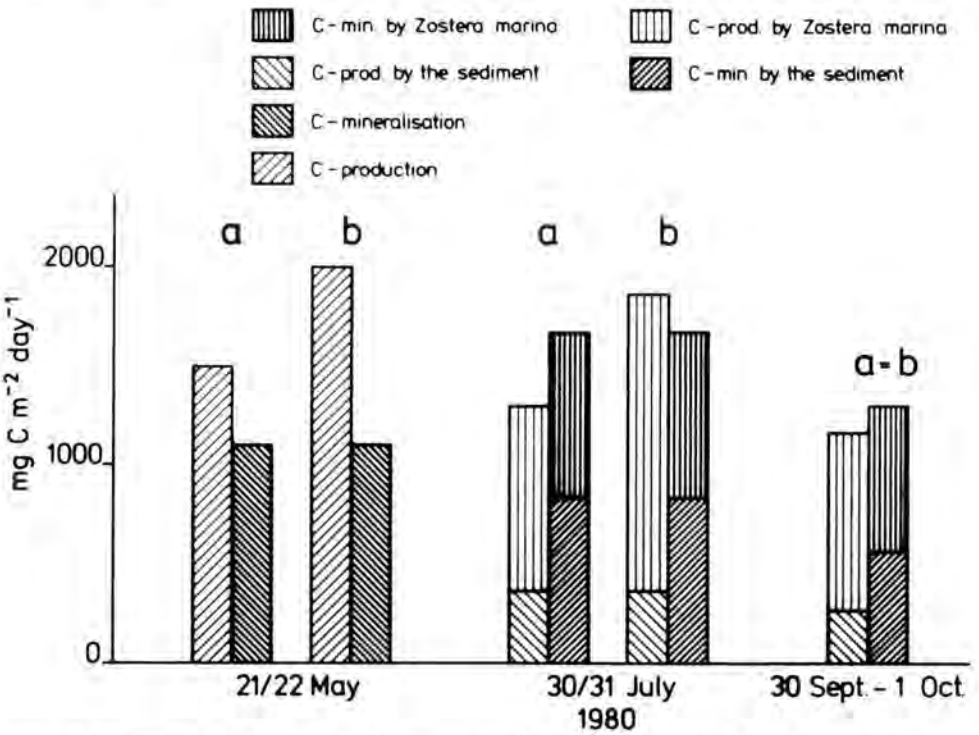

Fig. 13. Carbon production and mineralization of the sediment and the *Zostera marina* community during three 28 hour measurement periods. a: production and mineralization over the first 24 hours of the 28 hour period, b: production and mineralization over the last 24 hours. On May 21/22 no enclosures on barren sediment were employed. For further details see text

to draw C-mass balances based on both the C-14 and O_2 technique. In 1981 the research on this phenomenon will be continued (see V.19).

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V.18. Modelling the carbon cycle in the Grevelingen (G11) (J.H.C.Verhagen and I.de Vries, Delft Hydraulics Laboratory)

In 1980 a start was made with a mathematical ecosystem modelling approach for the Grevelingen in a joint project, called WABASIM-salt, between:
- the Delta Institute for Hydrobiological Research - the Environmental Hydraulics Branch of the Delft Hydraulics Laboratory - the Delta Department of Rijkswaterstaat.
The ecosystem model will contain primarily those components which have a major contribution to the carbon cycle. A C-budget scheme on a yearly basis is constructed for the situation before the opening of the Brouwerssluice (Fig. 14), based on biomass and production measurements of the Delta Institute and on P/B-ratios and growth efficienties from literature data. This scheme

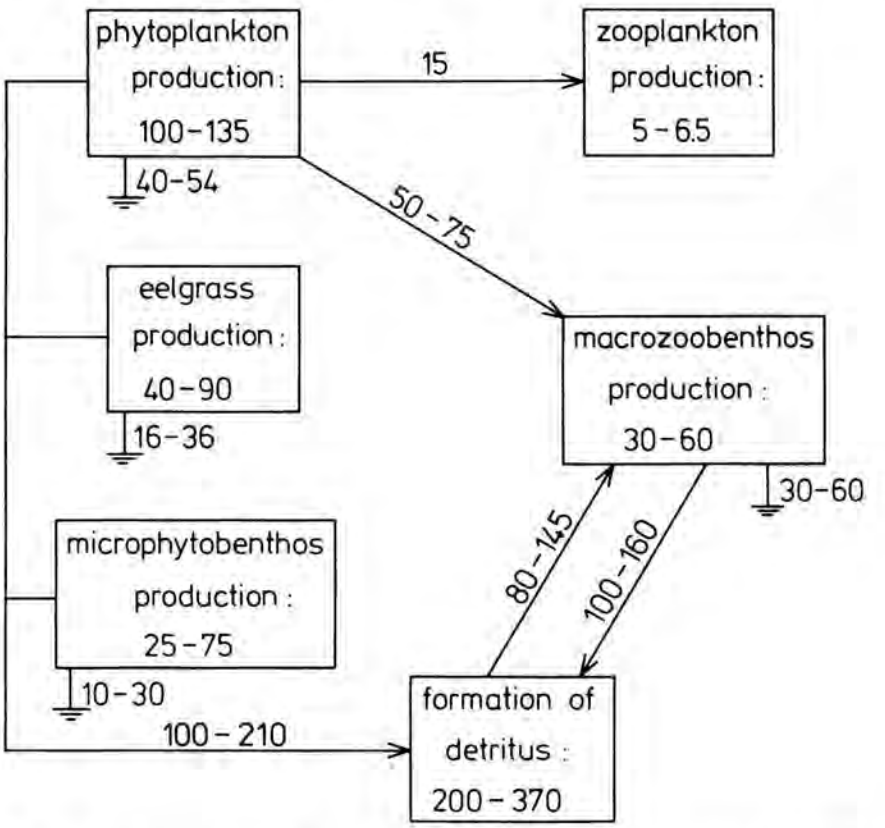

Fig. 14. Major components of the C-cycle in the Grevelingen before 1979. All values are in g C m^{-2} yr^{-1} averaged for the whole lake; arrows indicate consumption or detritus-production; ⊥ indicates respiration

will be differentiated in space (into three layers: 0-3, 3-5 and < 5 m depth and a bottom layer) and in time (into four seasons). In these schemes also the relaton between C-cycle and N-, P- and Si-cycles will be given.
In addition to this general budget-approach, dynamical models for the separate components will be constructed. These models finally will be coupled to a general salt water ecomodel.
For the eelgrass component a first model version is constructed, based on the extensive biomass surveys and production measurements by the Delta Institute. Growth and production of eelgrass are modelled as a function of radiation (and extinction) and temperature, and as a function of the amount and age of (the) eelgrass biomass that has been produced before. Decay of eelgrass (and as a consequence detritus production) is modelled as a function of age of the eelgrass plants and of wind-action (mechanical damage by currents). Basic assumption in the eelgrass model is the limitation of the shoot-density by space (space occupied by overground biomass). Using this assumption the typical bimodal shape of the shoot density curve on a yearly basis, caused by a generative and a subsequent second vegetative growth period, can be simulated.
Fig. 15 shows the observed and calculated shoot density year-curve and biomass year-curve in an eelgrass plot on 0.75 m depth.

V.19. Carbon budget 1980 for Lake Grevelingen (G1-11) (P.H.Nienhuis)

Primary production measurements and estimates for 1980 reveal rough maximum annual values per square metre of 70 g C for microphytobenthos, 40 g C for macroalgae, 60 g C for eelgrass and 150-210 g C for phytoplankton. Net POC import through the Brouwerssluice amounted to 10 g C which brings the total amount of particulate organic carbon available for consumers at a level of roughly 330-390 g C m^{-2} yr^{-1}. It should be stressed that the presented data are only rough estimates. Methodological discussions on the value of the C-14 method for microphytobenthos and macroalgae production measurements are continuing. The opening of the Brouwerssluice introduced unpredictable changes in the Grevelingen environment which hampered the interpretation of all primary productivity data drastically. One example will be given to illustrate this.

Phytoplankton primary production increased with a factor 1.5-2 after the opening of the Brouwerssluice at the end of 1978 (Table 7). Neither POC nor chlorophyll data did show such a conspicuous increase, comparing the 1978 and 1980 data. Chlorophyll biomass reveals even lower data in 1980 than in 1978 and 1979, which means that in 1980 a relative large production per biomass-unit should be assumed. An explanation for the increase in phytoplankton production might be found in the changed environmental conditions. The opening of the Brouwerssluice introduced N-rich coastal water by which the supposed N-limitation for phytoplankton during the period 1971-1978 was removed. Another point of interest is that the 1978 data have been based on a series of *in situ* measurements, and the 1980 observations on a large number of laboratory incubator measurements and only a few *in situ*

Table 7. Average particulate organic carbon, chlorophyll and phytoplankton for primary production at station G 11 in Lake Grevelingen 1978-1980 (columns 1-3). A rough estimate is given for the entire lake (column 4)

year	POC	chlorophyll	phytoplankton production	
	g m^{-3}	mg m^{-3}	g C $m^{-2}yr^{-1}$	
	G11	G11	G11	Grevelingen
1978	0.46	3.2	157	ca. 100
1979	0.47	4.5	249	ca. 160
1980	0.52	2.1	228-326	ca. 150-210

data. Incubator measurements may overestimate the proper *in situ* values, as was found in the Eastern Scheldt (see V.22).

Preliminary estimates of the annual C-mineralization in one square metre of the water column with the O_2 method amount to approximately 250 g C. Including a number of assumptions the C-mineralization of the bottom of the lake, an eelgrass bed near Herkingen, was calculated at approximately 340 g C. Assuming the same order of magnitude for the bottom sediments of entire Lake Grevelingen these data together with those from the water column lead to an overall mineralization rate of 590 g C $m^{-2}yr^{-1}$, which should be taken as a preliminary, very rough estimate.

The balance between primary production and mineralization (including O_2 consumption by animals) shows a negative difference of roughly 200-250 g C $m^{-2}yr^{-1}$ on the production

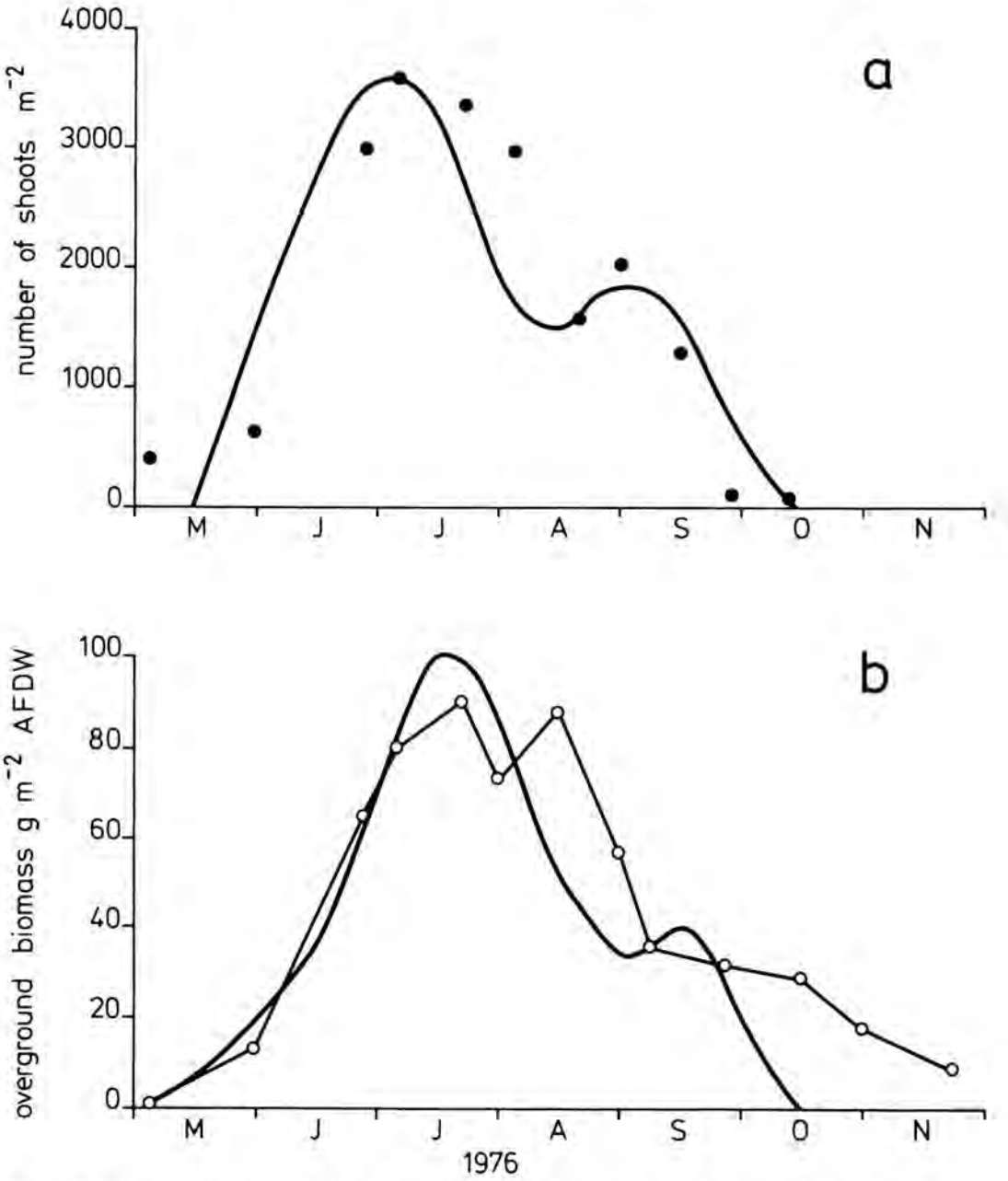

Fig. 15. Preliminary results of the eelgrass model. a: shoot density; dots: observed; in curve: calculated. b: overground biomass; connected dots: observed; smoothed curved: calculated

side. We are far from a reliable budget. The data contain many assumptions. Up to now it is impossible to draw a C-budget based on both the C-14 and the O_2-techniques. It is our aim to present a more precise budget over the year 1981, based on a number of integrated continuous 30-hours *in situ* measurements.

V.20. Transport of organic matter in the Eastern Scheldt estuary (K1) (J.H.B.W. Elgershuizen)

A flux measurement of organic matter in the Eastern Scheldt Estuary at Wemeldinge on 23 April 1979 indicated a transport of ca. 50 tons particulate organic carbon in eastern direction. This confirms calculations of Rijkswaterstaat from differences in average concentrations of seston along the axis of the estuary and from a tentative food balance of the Eastern Scheldt ecosystem. (Elgershuizen and Stortelder, in press).
Organic matter is transported by 1. dispersion; 2. a jump-like process (described earlier by Postma, 1954); 3. by advection to a lower extent. Phytoplankton may be transported eastwards mainly through flood channels and westwards through ebb channels.

Generally, particulate organic carbon is highly correlated with seston, but linear regression coefficients differ according to place and time.
Most of the organic matter (usually 60 to 80%) is detritus and falls slower than 1 m hr^{-1}.

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V.21. Seston analysis (K3) (J.H.B.W. Elgershuizen and E.T. van Ierland)

To fractionate seston in different parts (phytoplankton, zooplankton, detritus and anorganic particles), a choice was made to perform this on basis of the sedimentation rate and density of the particles. Therefore an osmotic acceptable gradient medium was developed and applicated in a sedimentation chamber and a zonal A rotor. First runs using phytoplankton and zooplankton cultures are promising.

Also attention was paid to the application of a FACS II Cell Sorter (courteousy TNO, Rijswijk, The Netherlands) that can sort particles be means of charging the particles with determined optical characteristics and dividing them according to their charge in an electric field. This apparatus succeeded in characterizing 8 phytoplankton species of 5 different phyla on basis of 3 optical parameters, and may therefore sort these species. (Trask *et al.*, in press).

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V.22. Comparison between *in situ* and incubator primary production measurements in the Eastern Scheldt (K3) (P.R.M. de Visscher)

The usefulness of the incubator technique has been tested for Lake Grevelingen during 1979 (see Progress Report 1979) with promising results: using an incubator an overestimate of 3% of in situ measured vertical production was observed.

Since the Eastern Scheldt estuary was included in the research area in 1980, the necessity of calibrating the incubator with *in situ* measurements was clearly understood because of the high variability of physical parameters (extinction, temperature, wave motion etc.) by tidal movement during the course of a day. The measurements were carried out monthly using the same water sample from nearby buoy 0-11 *in situ* and in an incubator. During the *in situ* incubation at 0-11 extinction was measured hourly. Solar irradiation was obtained from the Rijkswaterstaat stations Kreekrak (about 15 km east of 0-11) and Burghsluis at the mouth

of the Eastern Scheldt (about 40 km west of 0-11). A linear interpolation was made between the two stations in order to obtain the irradiation at 0-11. The hourly change of extinction was accounted for in the hourly irradiation at the surface resulting in rather realistic underwater light intensities for the construction of the photosynthesis-light curve in the calculation model (Osborne 1970; Gargas *et al.*, 1976; de Visscher, in press).

Simulated *in situ* productions obtained with the mentioned calculation model were compared with productions measured (Table 8). The comparison seems only convenient on April 14th, July 15th, October 16th and December 19th. The overall mean ratio is 1.46, generally leading to an overestimate of yearly production in the Eastern Scheldt of about 50% when purely based on incubator measurements. The relationship between *in situ* (x) and incubator (y) productions $y = 1.93 x - 6.71$ is very significant ($P < 0.001$ and $r = 0.91$). This linear relation intercepting the x-axis suggests that other factors, not yet simulated (*i.e.* light oscillations caused by wave motion), play a major role in this type of measurement (Fréchette and Legendre, 1978). It is the aim for 1981 to investigate the influences of tidal and meteorological phenomena in detail.

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Table 8. The comparison between *in situ* and incubator-measured primary productions at O 11 (Eastern Scheldt)

Date (1980)	*In situ* mg C $m^{-2}(4hr)^{-1}$	Incubator mg C $m^{-2}(4hr)^{-1}$	Incub. *In situ* ratio
March 17th	29.4	22.2	0.75
April 14th	183.6	201.6	1.20
May 12th	435.2	1401	3.22
June 12th	186.9	317.6	1.70
July 15th	125.5	136.0	1.08
August 14th	279.9	605.4	2.16
September 8th	a. 228.5	386	1.69
	b. 799.1	1278	1.60
October 16th	a. 13.4	13.8	1.03
	b. 15.3	9.6	0.63
November 18th	11.9	17.8	1.49
December 19th	10.2	9.9	0.96
			mean 1.46

a. before noon

b. after noon

V.23. Biomass and species composition of the microphytobenthos in the Eastern Scheldt (K5) (E.A.M.J. Daemen and M.T.T. Vereecken)

In May 1980 a study of the species composition and biomass of the microphytobenthos in the Eastern Scheldt was started as a part of BALANS, a joint project of Rijkswaterstaat and the Delta Institute. Microphytobenthos biomass is determined by measuring the chlorophyll a content, according to the method used for phytoplankton (Holm-Hansen *et al.*, 1965). The bottom-material is grinded in a bottle filled with acetone 90% as an organic extracting solvent for chlorophyll. The pigment content is measured spectrofotometrically or fluorometrically. Preliminary methodological investigations were carried out, demonstrating the necessity of calibrating the fluromometer with pure chlorophyll solutions. The first results showed that the chlorophyll content in the upper cm of the sediment may vary considerably in time and space (range: 10-500 mg Chl m^{-2}). Cell counts by means of epifluorescence microscopy will be used as an additional method for estimating the biomass of the microphytobenthos.
A statistical approach for the best sampling method was started. In collaboration with Rijkswaterstaat a bottom sediment map of the Eastern Scheldt is in preparation according to the method used by Nieuwenhuize *et al.* (1980) for Lake Grevelingen.

The species composition of the diatom flora has not yet been studied very intensively, nevertheless about 100 different species could be identified. In most samples, especially those taken during a diatom bloom, one or a few species were dominant, *e.g. Tropidoneis vitrea, Pinnularia ambigua* and *Navicula rostellata* in late summer (near Yerseke) or *Navicula viridula* and *Surirella gemma* in February. Several species occur more or less abundantly in almost every sample *e.g. Raphoneis amphiceros, Raphoneis surirella, Dimerogramma minor Cymatosira belgica.* By taking samples fortnightly at the same stations we will be able to reveal more details about the species composition and succession. Sediment composition (median grain size, silt-content etc.) may influence the species composition strongly as well as the biomass of the microphytobenthos. As soon as the bottom-map is available 10-15 permanent sampling stations will be selected for further investigation.

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VI. WORKING GROUP: BRACKISH WATERS (Code B)

VI.1. Introduction (A.B.J. Sepers)

In the Delta area of The Netherlands there is a great number of large and small waterbodies with very diverse characteristics. Beside the great differences in size there are also considerable differences regarding the influence of the bottom and the littoral zone on the metabolism of the aquatic system as a whole. The mean chlorinity varies from near zero to about 18 $^{0}/_{00}$ Cl^{-}. The variation in the value of the abiotic environmental parameters (stability in the meaning of constancy of the environmental parameters) shows great differences, resulting in a wide range of mean nutrient concentrations. The presence of this great variety of aquatic environments offers the opportunity to perform experiments in very different environments, so as to determine the most relevant parameters for the considered process. Moreover, it is possible to test hypotheses originating from laboratory experiments, in the natural environment.

In natural waters the abiotic environmental parameters vary continuously. The range of these fluctuations determines the stability. A comparison of a stable environment with an environment with a great variation in the abiotic parameters indicates that the community in the stable environment is characterized by a higher diversity. There occur more species and there exist more interrelations between the species than in less stable environments. Instable environments are generally characterized by communities with a relatively low complexity. Comparative research with organisms which are characteristic for stable and instable environments yields information about the fulfilment of a particular function within the aquatic system and indicates what the determinant parameters are.

In addition to this research dealing with the functioning of an ecosystem, it is the aim of the working group to set up investigations related to the structural aspects of an ecosystem. In this context the structure will be defined as the distribution of organisms in time and space, by which an instable environment will show a structure with a low level of complexity and a stable environment a more complex structure. It is the ultimate goal of this research to yield better definitions of conceptions which are in general use in order to characterize ecosystems, like for instance the diversity concept.

The development of communities in waters with strong fluctuations will be interrupted repeatedly with the resultant effect, that these communities remain in the pioneer stage of development. Investigations into the community structure during this phase of development and into the functioning of the organisms yield information about the phenomena which govern the development of ecosystems during the initial phase.

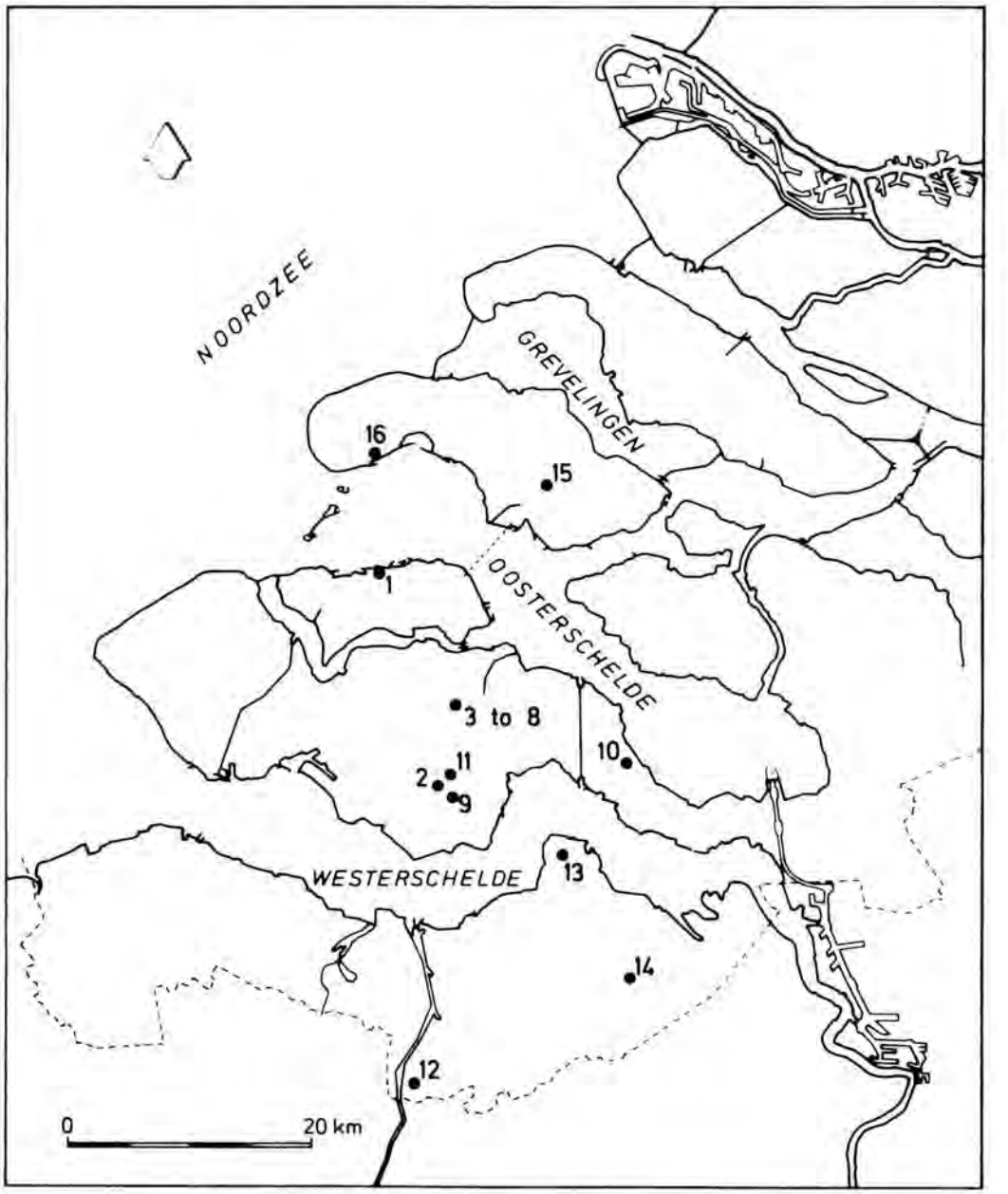

Fig. 16. Localities of distribution of *Dixella autumnalis*

VI.2. *Dixella autumnalis* Meigen (Diptera, Dixidae) in the Delta region of the Netherlands (B1) (B.P.M. Krebs)

Data about the distribution of Dixidae in The Netherlands are scarse. Altough the Dixidae were not a special topic of study, it is interesting to note that only the species *D. autumnalis* was found in this part of the country, while elsewhere more species of Dixidae occur. The larvae were found at 16 localities in the Delta region (Fig. 16) in various biotopes like pools, ponds and ditches. The chlorinity of these biotopes ranged between 0.01 and 3.4 $^{o}/_{oo}$ Cl^{-}. The chlorinity of 70% of the localities was lower than 0.5 $^{o}/_{oo}$ Cl^{-}. *D. autumnalis* appeared to prefer small, unpolluted waters (or the margins of larger lakes) with a dense (sub)emergent vegetation.

VI.3. Classification of brackish inland waters in the Delta region of the Netherlands (B2) (B.P.M. Krebs)

Chironomids are very common in brackish inland waters. During several years a study was made of the distribution of these midges. A number of different chironomid communities could be discerned with salinity as the imperative environmental parameter.

The collected distribution data could be covered by six groups, which were characterized by chironomid communities from limnetic to polyhaline waters. Within these groups a differentiation was made between dominant, abundant and accompanying species. Dominant species dominate the whole community; abundant species are common but not dominating; accompanying species are species of which the presence depends on other environmental parameters (*e.g.* vegetation, pollution level). In this way it is was possible to characterize the salinity of the water by a description of the chironomid community. The described method yields information about the salinity of the water in question over a certain period of time (in the order of months), whereas a chemical determination of the salinity reflects only the salinity at the time of sampling. Thus the community parameter offers more relevant information within the framework of ecosystem studies.

Briefly summarized and strongly simplified the following classification could be established. *Cricotopus sylvestris* and *Psectrotanypus varius* were taken as the dominant species for the limnetic group; the abundant species were *Chironomus plumosus, C. luridus, C. piger, C. annularius, C. thummi, Glyptotendipes barbipes, Procladius choreus* and *Cricotopus ornatus.* The accompanying species varied in relation to the value of other environmental parameters. Examples of accompanying species were *Xenopelopia nigricans, Ablabesmyia* sp., *Dicrotendipes lobiger* and *Pentapedilum uncinatum.*

The second group was dominated by *Chironomus annularius* and *C. piger* and was also characterized by the absence of *Cricotopus sylvestris* and *Psectrotanypus varius,* which were the dominant species of the first group; the brackish water species *Chironomus halophilus* was abundant, but not dominant. *Cricotopus ornatus* was also within this group an abundant species.

Chironomus halophilus was the dominant species of the third group. Abundant species were *Chironomus salinarius* and *Microchironomus deribae.* Accompanying species were *Procladius choreus, Chironomus piger, C. annularius* and *Cricotopus ornatus.*

In the fourth group *Chironomus salinarius* was the dominant species; *Chironomus halophilus* belonged to the abundant species of this group.

In the fifth group *Chironomus salinarius* was the dominant species, together with *Halocladius varians.* The accompanying species were *Chironomus halophilus* and *Glyptotendipes barbipes.*

The sixth group consisted of only two species: *Chironomus salinarius* and *Halocladius varians* without abundant or accompanying species. In many cases it was difficult to draw a clear distinction between the last two groups.

VI.4. Comparison between the carbon-14 and oxygen consumption method in relation to the determination of the activity of heterotrophic bacterial populations (B8) (A.B.J. Sepers and F.W. Melissen)

The activity of the microbial population in natural waters is often determined by measuring the oxygen consumption rate or the uptake of C-14 labelled organic

compounds. The oxygen consumption method involves the incubation of a water sample in the dark at *in situ* temperature. The oxygen consumption rate is determined from the difference between the initial oxygen concentration and the oxygen concentration at the end of the incubation period. By applying a suitable conversion factor, the uptake rate of organic carbon can be calculated.

Mineralization experiments with C-14 labelled substrates imply the addition of a known amount of a C-14 labelled organic compound to a water sample. Incubation occurs at the *in situ* temperature; it is stopped by the addition of acid. The carbon dioxide evolved from the oxidation of the substrate is fixed on filter paper that has been impregnated with a carbon dioxide fixing compound. After filtration of the sample, the carbon incorporated in structural cell material can be determined by measuring the radioactivity of the residue on the membrane filter. After measurement at several substrate concentrations, the uptake rate is plotted versus the added amount of substrate. A hyperbolic relationship indicates that the uptake of dissolved organic compounds fits Michaelis-Menten kinetics. From an appropriate linear transformation of the Michaelis-Menten plot the maximum uptake rate, the turnover time and the summation of the saturation constant and the natural substrate concentration, can be assessed. The maximum uptake rate is interpreted as a relative value for the population that is able to utilize the added substrate.

In the described experiments the mineralization activity of the microbial population was determined in the saline Lake Grevelingen in the Netherlands and in the Mediterranean Etang Salses Leucate in France by application of C-14 labelled pyruvate, glycollate and an amino acid mixture, and by measuring the oxygen consumption rate. Thereafter it was possible to compare both techniques.

The measurements in Lake Grevelingen were performed over an one year period. The experiments were carried out each quarter within a period of eight days, during which the oxygen consumption was determined three times and the uptake of the C-14 labelled substrates only once. The experiments in Etang Salses Leucate were carried out in June and September 1980 on two successive days. Each day the oxygen

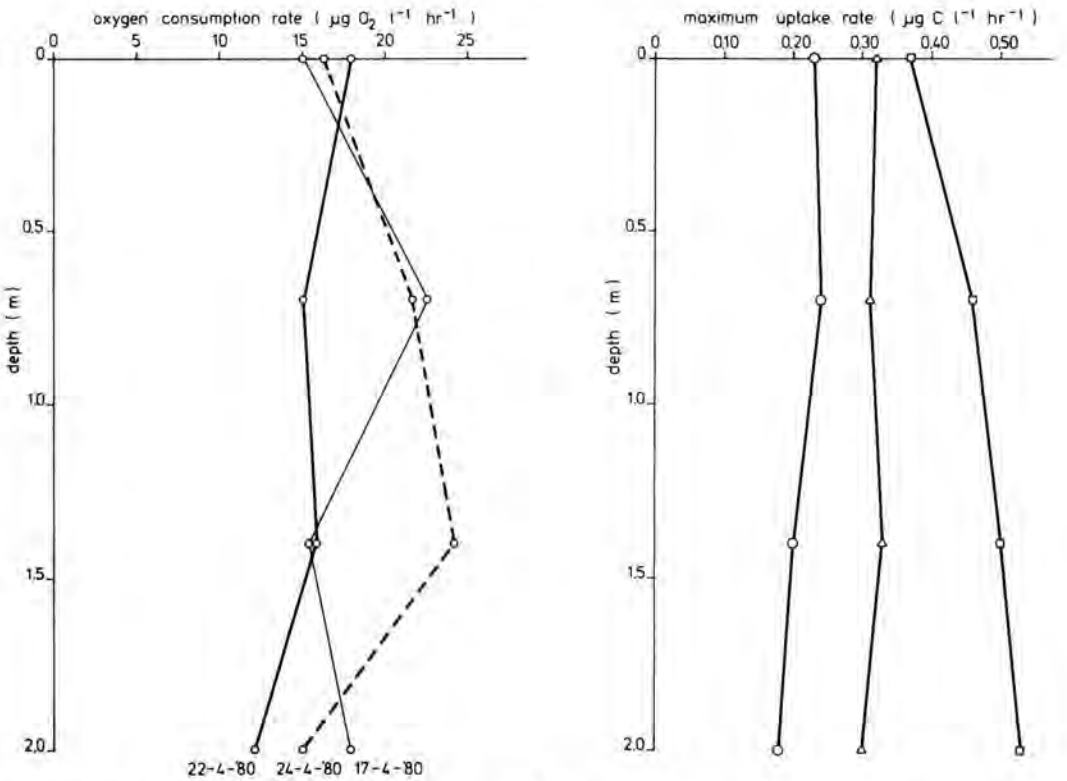

Fig. 17. The uptake of oxygen and of some ^{14}C-labelled organic compounds at sampling station G 11-shallow (depth 2 m) in Lake Grevelingen in April 1980, o = aminoacid mixture, □ pyruvate, Δ glycollate.

consumption was determined as well as the uptake of C-14 labelled pyruvate, glycollate and the amino acid mixture.

As a representative example of the experiments the results of the experiments carried out in April 1980 in Lake Grevelingen are shown. Fig. 17 shows the uptake of C-14 labelled pyruvate, glycollate and the amino acid mixture at a 2 metres deep location. The uptake was measured at four depths. There was no variation in maximum uptake rate with depth. A similar pattern was found for the oxygen consumption rate.

The experiments were also done at a sampling station with a total depth of 20 m. The uptake of oxygen and the organic compounds was measured at seven depths. Only for the amino acid mixture a decrease in the maximum uptake rate with increasing depth could be determined (Fig. 18). Considering the oxygen consumption rate no clear variation in relation to depth could be assessed.

From this type of plots (Fig. 17 and Fig. 18) the maximum uptake rate and oxygen consumption rate per square metre were calculated by integration. Table 9 shows the carbon mineralization rate (mg C $m^{-2}day^{-1}$) as calculated from the oxygen consumption rate (mg O_2 $m^{-2}day^{-1}$) applying a conversion factor of 0.29 for the conversion of the oxygen consumption rate to a carbon mineralization rate. The parentheses at the values of the maximum uptake rate of the labelled compounds indicate the maximum uptake rate as a percentage of the mineralization rate, calculated from the oxygen consumption experiments. These percentages are generally lower than 10%, except the data for the uptake of pyruvate and glycollate in January 1980, which amounts to 149% and 38% respectively. The same trend was found for the deep sampling station (Table 10). The maximum uptake rate of the added substrates was lower than 7% of the mineralization values from the oxygen consumption rate, except again the data for the uptake of pyruvate and glycollate in January 1980, which equals to about 30% of the mineralization rate calculated from the oxygen consumption rate.

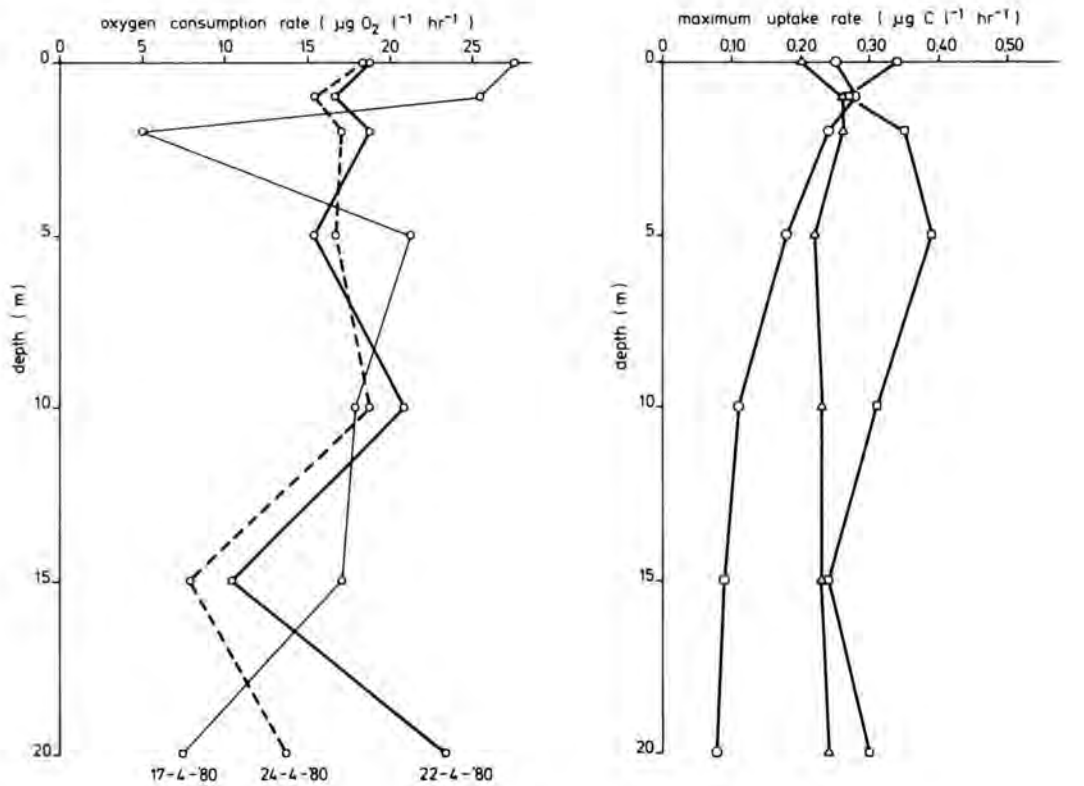

Fig. 18. The uptake of oxygen and of some ^{14}C-labelled organic compounds at sampling station G 11-channel (depth 20 m) in Lake Grevelingen in April 1980. For symbols see Fig. 17

Table 9. Comparison between the carbon mineralization rate as calculated from oxygen consumption experiments and the maximum uptake rate of C-14 labelled pyruvate, glycollate and an amino acid mixture. Sampling location: Lake Grevelingen (G 11-shallow (depth 2 m))

Month	Mineralization rate from oxygen consumption experiments (mg C m^{-2} day^{-1})	Maximum uptake rate (mg C m^{-2} day^{-1})		
		pyruvate[a]	glycollate[a]	amino acid mixture[a]
June 1979	557	21.3 (3.8)	6.8 (1.2)	10.2 (1.8)
September 1979	250	10.3 (4.1)	3.4 (1.4)	*[b]
January 1980	17	25.4 (149.4)	6.5 (38.2)	0.9 (5.3)
April 1980	251	22.5 (9.0)	15.2 (6.1)	10.5 (4.2)

[a] Parentheses indicate the maximum uptake rate as a percentage of the mineralization rate calculated from the oxygen consumption experiments.

[b] The uptake did not fit Michaelis-Menten kinetics.

Table 10. Comparison between the carbon mineralization rate as calculated from oxygen consumption experiments and the maximum uptake rate of C-14 labelled pyruvate, glycollate and an amino acid mixture. Sampling location: Lake Grevelingen G 11-channel (depth 20 m)

Month	Mineralization rate from oxygen consumption experiments (mg C $m^{-2}day^{-1}$)	Maximum uptake rate (mg C $m^{-2}day^{-1}$)		
		pyruvate[a]	glycollate[a]	amino acid mixture[a]
June 1979	4060	101.1 (2.5)	79.9 (2.0)	117.7 (2.9)
September 1979	2140	53.0 (2.5)	27.4 (1.3)	53.5 (2.5)
January 1980	219	68.8 (31.4)	71.6 (32.7)	9.6 (4.4)
April 1980	2230	149.2 (6.7)	111.5 (5.0)	67.2 (3.0)

[a] Parentheses indicate the maximum uptake rate as a percentage of the mineralization rate calculated from the oxygen consumption experiments.

In the Etang Salses Leucate the experiments were carried out only on surface samples. Here the maximum uptake rate of the amino acid mixture was 3.4 to 4.4% of the mineralization rate calculated from the oxygen consumption experiments (Table 11); the corresponding values for the glycollate and pyruvate uptake rate vary from 10 to 20% and from 8.5 to 35% respectively.

Considering the total data set for Lake Grevelingen it is clear that the maximum uptake rate of the applied organic substrates equals less than 10% of the carbon mineralization rate calculated from the oxygen consumption experiments. Only for pyruvate and glycollate higher values were found of about 30 to 40% with one exceptionally high value for pyruvate of 150%. However, these percentages were found in winter, just when the activity of the heterotrophic microbial population is very low.

In Salses Leucate higher maximum uptake rates of the ^{14}C-labelled compounds were found, relating this uptake to the oxygen consumption rate. But the maximum uptake rate is still always lower than 35% of the carbon mineralization rate calculated from the oxygen uptake rate.

Taking into account that maximum uptake rates were considered and that therefore the *in situ* uptake rate will always be remarkably lower than the maximum uptake rate, the results demonstrate that the uptake of C-14 labelled compounds represents a serious underestimation of the activity of the bacterial population *in situ*. The extent of the underestimation depends on the water type.

The results of heterotrophic uptake experiments in a chemostat, reported by Sepers and van Es (1979), are in agreement with the data of the reported field experiments. This research demonstrated that the maximum uptake rate of the growth limiting organic compound, as measured with C-14 labelled substrate, was lower than the actual uptake rate in the culture. Determination of the uptake of the growth limiting substrate through measurement of the oxygen uptake rate appeared to show a better reflection of the actual substrate uptake in the culture.

Thus the determination of the heterotrophic activity by measurement of oxygen consumption rates seems to offer a better insight into the carbon mineralization process in natural waters than the uptake experiments with C-14 labelled substrates.

Note: This work was part of a co-operative study with the Centre de Recherches de Sédimentologie Marine, Perpignan for which additional funding was obtained from the C.N.R.S. - Paris.

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VI.5. The impact of environmental fluctuations on phytoplankton communities in tidal and stagnant brackish waters (B10) (J.W. Rijstenbil)

The development of the phytoplankton community in two small brackish water lakes (Schelphoek and Westkapelle) and at two polyhaline sampling stations (Krammer-Volkerak and Eastern Scheldt) in the Eastern Scheldt estuary were compared with each other. It may be expected that fluctuations (in the sense of perturbations) in salinity and nutrient concentrations will have more pronounced effects in small lakes than in estuarine waters and greater well mixed lakes (Heerebout, 1970). It was hypothesized that the growth of certain phytoplankton species will be selectively stimulated by these environmental fluctuations.

In Lake Schelphoek (22 °/oo S), which is only supplied with rainwater and saline seepage, a springbloom of *Detonula confervacea* consumed most of the N-nutrients (Fig. 19). After the termination of this bloom the incorporated nitrogen sank into the anaerobic hypolimnion and the nutrient level of inorganic nitrogen in the epilimnion was not restored until winter.

In Lake Westkapelle (11 °/oo S) the nitrogen nutrients were never used up completely, which was due to the input of waste water. The algal succession started in spring with *Skeletonema costatum*, followed by a dense bloom of phytoflagellates in the summer (Fig. 19). In autumn the phytoplankton community was dominated by a small centric diatom.

At the estuarine stations Krammer-Volkerak (18 °/oo S) and Eastern Scheldt (26 °/oo S) only marine diatoms were involved in the phytoplankton succession

Table 11. Comparison between the carbon mineralization rate as calculated from oxygen consumption experiments and the maximum uptake rate of C-14 labelled pyruvate, glycollate and an amino acid mixture. Sampling location: Etang Salses Leucate (France)

Date	Mineralization rate from oxygen consumption experiments ($\mu g\ C\ l^{-1} hr^{-1}$)	Maximum uptake rate ($\mu g\ C\ l^{-1} hr^{-1}$)		
		pyruvate[a]	glycollate[a]	amino acid mixture[a]
250680	17.9	4.72 (26.4)	2.46 (13.7)	*[b]
260680	12.9	4.50 (34.9)	2.53 (19.6)	0.44 (3.4)
081080	13.3	1.13 (8.5)	1.29 (9.7)	0.58 (4.4)
091080	23.7	6.60 (27.8)	4.57 (19.3)	1.03 (4.3)

[a] Parentheses indicate the maximum uptake rate as a percentage of the mineralization rate, calculated from the oxygen consumption experiments.

[b] The incubation time was too long to permit application of the Michaelis-Menten kinetics.

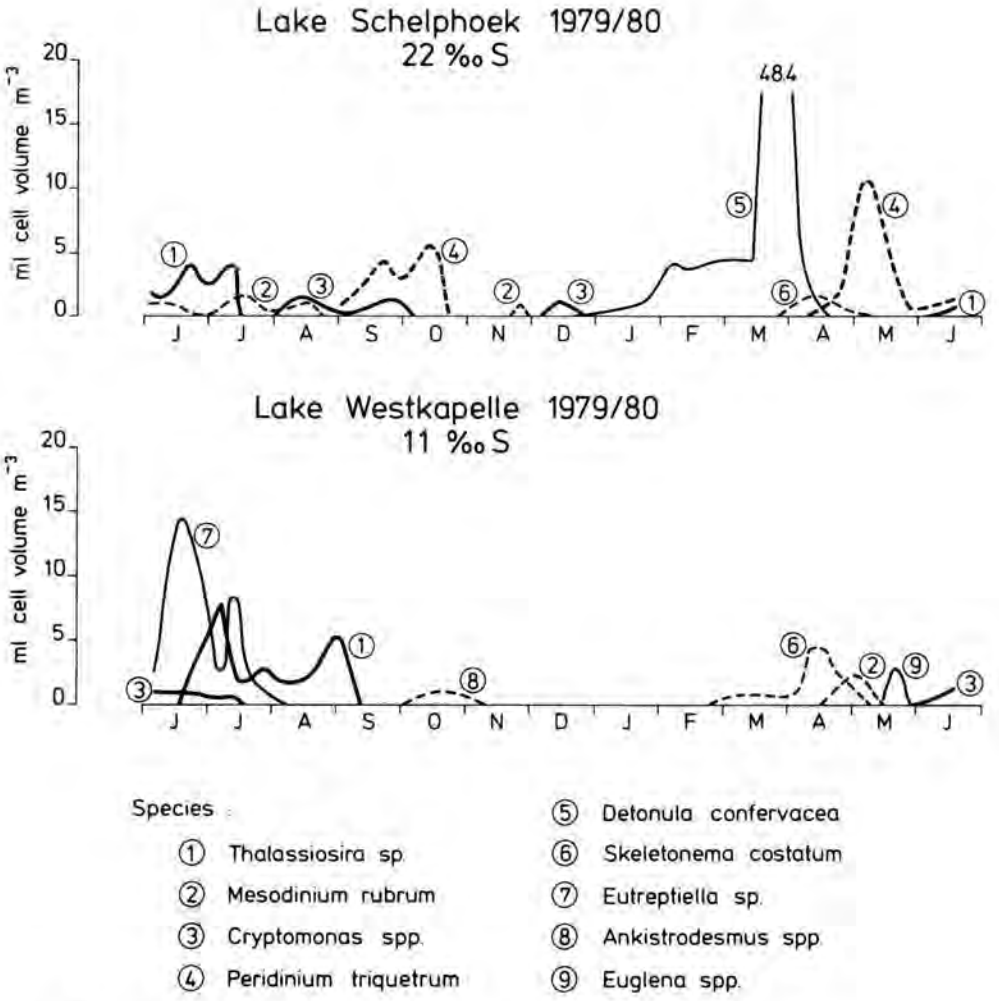

Fig. 19. Phytoplankton succession in small brackish lakes

(Fig. 20). Some of these species were characteristic for tidal waters and did not occur in the stagnant lakes. Other species, like *Thalassiosira nordenskioldii* and *Ditylum brightwelli*, may also occur in greater saline lakes (Bakker, 1978), whereas these diatoms do not survive in smaller brackish lakes.

The variations in the concentrations of the inorganic nitrogen nutrients in the sampled waters could be completely explained by the photosynthetic activity of the algal populations. Research is in progress into the effect of salinity fluctuations on the photosynthetic activity of some selected phytoplankton species, growing under a nitrogen limitation. The ultimate goal of this study is to gain an insight into the role of salinity fluctuations as a selection factor at the development of phytoplankton communities (Spektorov and Strogonov, 1980), of which the growth is limited by the nitrogen source.

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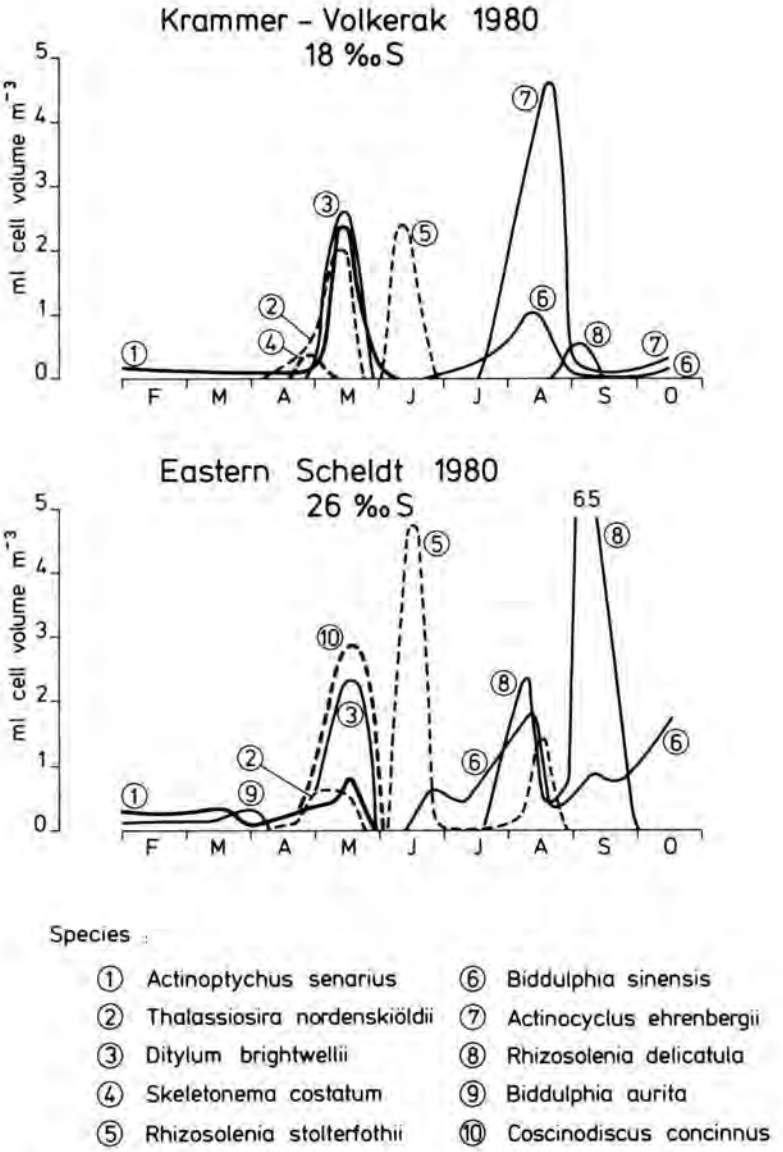

Species :

① Actinoptychus senarius	⑥ Biddulphia sinensis
② Thalassiosira nordenskiöldii	⑦ Actinocyclus ehrenbergii
③ Ditylum brightwellii	⑧ Rhizosolenia delicatula
④ Skeletonema costatum	⑨ Biddulphia aurita
⑤ Rhizosolenia stolterfothii	⑩ Coscinodiscus concinnus

Fig. 20. Phytoplankton succession in estuarine waters

VII. WORKING GROUP: SALT-MARSH ECOSYSTEMS (Code S)

VII.1. Introduction (A.H.L. Huiskes)

The understanding of the structure and functioning of the salt-marsh ecosystem in the south-west of the Netherlands is the objective of this working group. The group aims at this goal by describing the components of and processes in the ecosystem (*e.g.* communities, populations of plants and animals and cycling of materials and decomposition), by studying the interrelations between these components and processes, and by integration of the acquired results. The group also intends to contribute by these studies to the knowledge of ecosystems in general and to the generation of ecological theories.

The research activity of the group is divided over five projects, each of which subdivided in a number of topics. The components of the ecosystem, chosen for investigation, are on the community, the population, and - occasionally -the individual level. One project deals with production and decomposition processes in the salt-marsh ecosystem.

Although the research of the group is basically of pure scientific nature, some projects can be regarded as applied research: A joint project with the Institute for Applied Physical Chemistry of the Nuclear Research Centre (KFA), Jülich (GFR), the Institute for Atomic Sciences in Agriculture (ITAL), Wageningen (NL), the Laboratory for Geology, Paris (F), and the Netherlands Institute for Sea Research (NIOZ), Texel (NL), was devised to study the accumulation and pathways of anthropogenic pollutants in the salt-marsh ecosystem. A project, VEGIN, under contract of and in cooperation with the Public Works Department aims at the prognosis of effects of the construction and use of the storm-surge barrier in the mouth of the Eastern Scheldt on the salt-marsh vegetation.

Communities (S1)
W.G. Beeftink, B.P. Koutstaal and W. de Munck continued their work on the 500 permanent quadrats laid out on tidal and embanked salt marshes. Several data sets are being analyzed in cooperation with Dr. P. Hogeweg (State University, Utrecht) and her staff. Special attention was paid to the influence of the tidal movement as a factor for vegetational change and a certain amount of data were re-evaluated for this purpose (see VII.2). W. de Munck and W.G. Beeftink rearranged the relevés of dike, drift-line and quay communities. G.J.C. Buth terminated his survey of insect and arachnid fauna on the salt marsh near Oosterland. The results will be published after completion of the identifications (see VII. 3). K. Willems (State University, Ghent (B)) and A.H.L. Huiskes started a monthly survey of the meiofauna in the soil of the salt marsh South of Bergen op Zoom. B.P. Koutstaal, M.M. Markusse and W. de Munck developed a method for trapping seeds and seedlings transported by the tidal movement to and from the salt marsh south of Bergen op Zoom (see VII. 4). M.C. Daane continued work on water levels in salt-marsh soils and on salinity measurements. The integrated measurements of the salt-marsh environment by A.H.L. Huiskes, M.M. Markusse, J. van Soelen and B.P. Koutstaal were continued and extended.

Populations (S2).
The studies on the population dynamics of *Salicornia* spp. were continued with investigations by B.P. Koutstaal on seed germination under field conditions and on the release of seeds from dying and dead inflorescences. W. de Munck continued his study on the predation of *Salicornia* spp. by insects and molluscs. A.H.L. Huiskes, M.M. Markusse and J. van Soelen continued and extended the study of the population dynamics and autecology of *Aster tripolium* ecotypes. The demographic work on this species of the students M. Ham and E. Miedema was continued by J. van Soelen and M.M. Markusse; this work will be extended to other ecotypes and other sites. J. van Soelen investigated the influence of predation by insects on *A. tripolium* in various sites (see VII.5).

Experimental research on the autecology of *A. tripolium* ecotypes was carried out by M.M. Markusse and A.H.L. Huiskes and by A.W. Stienstra (on secondment to the State University, Utrecht) (see VII. 6). B.P. Koutstaal started work on the demography of the drift-line species *Atriplex hastata* and *A. littoralis* as part of a combined project on the ecology of drift-line species in cooperation with Dr. J. Rozema (Free University, Amsterdam) (see VII.7).

Production, decomposition and accumulation of organic matter (S3).
Since 1979 pilot studies on production, decomposition, transport and storage of organic matter on salt marshes have been carried out by A.M. Groenendijk. In March 1980 a start was made to study a complete decomposition cycle of plant materials of some dominant salt-marsh plants. P.F.M. Verdonschot and L. de Wolf started the investigations (see VII. 8). In August 1980 P.F.M. Verdonschot was succeeded by G.J.C. Buth, both deputized for A.W. Stienstra (see VII. 8 and VII. 9). K. van Koppen completed a student project on the role of *Orchestia gammarella* Pallas on the decomposition of plant debris washed ashore (see VII. 10).

Ecotoxic effects of pollutants (S4).
The joint sub-project with Dr. M. Stoeppler of KFA, Jülich (G.F.R.) on the trace-metal contents of soils and plants of some marshes in the Eastern and Western Scheldt was continued with new analyses of samples (see VII. 11).

Ecological effects of tidal management (S5).
A.M. Groenendijk and M.A. Lievaart commenced work on the influence of prolonged inundation on the growth and seed production of salt-marsh species. The research was carried out in the field and under controlled conditions in a former oyster storage basin and in the greenhouse. F. de Loos completed a student project on the effects of inundation on three salt-marsh grasses viz. *Puccinellia maritima*, *Festuca rubra* f. *littoralis* and *Elytrigia pungens* (see VII. 12).

VII.2. The tidal factor as an agent for vegetational change in the salt marsh (S1) (W.G.Beeftink)

It was hypothesized that patterns in the salt-marsh vegetation are in equilibrium with mean tidal submergences. Deviations in these sub-mergences, subnormal as well as supernormal, could then only exert changes in the vegetation when exceeding a threshold value. Those deviations in submergence can easily be effected by tidal changes caused by building embankments and barriers in estuaries or bays.

From earlier work (Beeftink 1979) it appeared that these threshold values largely vary with the height at which the plant communities occur with respect to the tides: Plant communities from low marsh areas Spartinetum endure an increase of flood level of at least 30 cm, while at the upper of the tidal influence communities with Agropyro-Rumicion crispi elements are vulnerable for an increase of 1 or 2 cm only.

The question is now whether a natural increase in tidal submergence would exert vegetational changes. Because this increase is always temporarily the vegetational changes will have the character of fluctuations in floristic composition and structure. From experiences with human-induced tidal changes these fluctuations can only be expected in the upper marsh areas. Indeed, the shifting character of Agropyro-Rumicion elements in Armerion communities is a well-known phenomenon in permanent plot studies (unpublished data). From communities growing in lower marsh areas, however, only poor results could be gained up to now. In the 20 years permanent plot programme of the research group die-back in *Halimione portulacoides* has been found as a consequence of a period of more than two years in which the monthly averages of high tides nearly constantly exceeded the overall average high tide level with 1-18 cm. In this case however, the marsh area concerned was influenced by withdrawal of groundwater towards the adjacent polder by drainage, hence by a human-induced factor as well.

The question remains now how much a tidal decrease will affect the vegetation. A permanent decrease in flood level will also have much more influence in the upper marsh area than in the lower ones. The effect of temporary tidal decrease depends highly on the magnitude and period of the decrease, and the season in which it happens. In both cases species of higher marsh areas will invade lower ones according to their specific ecological characteristics.

Reference
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. Blackwell, Oxford, 77-93.

VII.3. Insects and arachnids of salt marshes (S1) (G.J.C. Buth)

Most terrestrial communities consist of at least three interacting trophic levels: plants, herbivores and natural enemies of herbivores. So far the research group has mainly investigated the first trophic level, plants. To initiate zoological research on salt marshes, a pilot study on salt-marsh insects and arachnids had been started in May 1979; in June 1980 this study was stopped.

The aim was to assess how many species can be found in salt marshes, where those species can be found and what their function is in the salt-marsh ecosystem. Only those species were considered that have a habitual requirement for some part of the salt-marsh environment at some stage of their life cycle (Foster and Treherne, 1976). Because of the short time available, attention was given mostly to herbivores. In particular data of Collembola, Hemiptera, Lepidoptera, Coleoptera

and Acarina were collected, in cooperation with some entomological departments of universities and institutes. Data were collected by sampling insects on salt marshes, but were also retrieved from literature and collections.

On the salt marshes in the s.w.-Netherlands about 320 species of marine insects and arachnids can be found. The Diptera, Coleoptera and Hemiptera seem to be the most abundant orders on the salt marshes. The data of the different orders have been summarized in tables and will be published soon.

Some data are collected about herbivory of insects on salt marsh plants, especially on *Aster tripolium*. Aphids, cicadas and larvae of butterflies are mainly herbivores of vegetative plant parts, while beetles and larvae of butterflies and flies can cause a great loss - more than 90% - of seeds (Miedema and Ham, 1980). Effects of herbivory and the numbers of herbivore insects seem to differ strongly from year to year. In 1980 the endophagous Microlepidoptera *Bucculatrix maritima* was very numerous on the salt marshes. In May about 70% of the *Aster* plants on the salt-marsh near Stroodorpepolder showed one or more leaves with mines of the larvae. In the summer *Bucculatrix* was seen on every salt marsh where *Aster* was present. At the end of July was observed on the salt marsh near Ellewoutsdijk, that in vegetations dominated by *Aster*, this species was completely defoliated by the larvae at several places over more than 30 m^2. Also many aphid-colonies of *Aphis tripolii* and *Macrosiphoniella asteris* were found on *Aster* at that time. The heavily attacked plants showed some re-growth at the end of August. They flowered poorly, more than a month after the normal flowering time.

References

Foster, W.A. and J.E. Treherne, - 1976. Insects of marine salt marshes: problems and adaptions. In: L. Cheng (ed.) Marine insects. North-Holland Publishing Co., Amsterdam, 5-42.

Miedema, E. and M. Ham - 1980. Demografie van *Aster tripolium* L. DIHO Stud. Rep. D12 - 80 pp.

VII.4. Transport of seeds on the salt marsh of Bergen op Zoom (S1) (B.P. Koutstaal, M.M. Markusse and W. de Munck)

In autumn of 1979 a field study was started on the Bergen op Zoom salt marsh on the transport of seeds of salt-marsh species by the tides. This study has been continued and extended in 1980. This investigation is a continuation of a study on the seed release of *Salicornia* plants, growing on different heights of the marsh. Sets of nets with a diameter of 20 cm and a mesh-size of 60 µm were placed, from the low-water line up to the high marsh (dependent on the water level) with intervals of 1-4 weeks. Half of the nets were facing the ebb stream the other half the flood. In October and in the first weeks of November only a few seeds and seedlings were collected. Later on many seeds and seedlings of *Salicornia* spp., *Aster tripolium*, *Spartina anglica*, *Atriplex* spp., *Suaeda maritima* and *Triglochin maritima* have been trapped in the nets. This corresponds with the data obtained from the seed-release study on *Salicornia*. From the middle of January on the number of harvested *Salicornia* seeds decreased, while the amount of seedlings increased. In the middle of February hardly any seed was found, but on the other hand the number of seedlings became numerous. Probably many of these seedlings have been uprooted by the tides. Most times the "flood nets" contained considerably more seeds and seedlings than the "ebb nets".

VII.5. Survey of insects overwintering in inflorescences of *Aster tripolium* L. (S2) (J. van Soelen and M.M. Markusse)

Aster tripolium is a perennial growing in saline environments inside the dike as well as outside the dike. During its whole life cycle, different insects attack the plants, which has a big influence on the development of the plant population, especially in the later life stages during seed ripening.

In 1979 research on the population biology of *A. tripolium* was started. Anticipating on further investigations it was decided to start in the autumn of 1980 with a survey of insects found in *A. tripolium* stems which could possibly attack the plants in

the coming growing season. Because an influence of the salinity on the dispersal of the insects was also envisaged, there was sampled at four stations along the Western Scheldt and on one station along the Eastern Scheldt.

On each station, on a high and a low lying site, 30 inflorescences were sampled, cut open in the laboratory and the insects counted and sent for further identification to the Plant Protection Service (PD) in Wageningen. One sampling (December 1980) was dropped, because the marrow of the stems was too wet to count the insects properly.

In the course of the winter there was a decrease in numbers of insects. It is not clear yet if this was caused by mortality or by a migration of the insects to the rosette.

The beetle *Agapanthia villosoviridescens* de Geer was found in the eastern parts of the Western Scheldt and also in the Eastern Scheldt. This could be an influence of salinity, or maybe a geographical influence.Fig. 21a shows the numbers of fly pupae per station. These pupae are not yet identified.Figure 21b shows the number of caterpillars of *Phalonidia affinitana* Douglas, which are able to mine the tissues of leaves and stems, but consume also the seeds.

VII.6. The influence of salinity on two ecotypes of *Aster tripolium* L. (S2) (A.H.L. Huiskes and M.M. Markusse)

A pilot experiment was set up to study the influence of salt on the growth of two ecotypes of *Aster tripolium*. One ecotype occurs in the tidal marshes along the Eastern and Western Scheldt, the other in inland salt marshes.

Seedlings of the two ecotypes were placed on 5.5 l buckets filled with nutrient solution (Steiner, 1961). After two weeks NaCl was added to the nutrient solution in portions of 50 mmol each three or four days until one third of the plants had 400 mmol NaCl in their nutrient solution, one third had 200 mmol NaCl and one third 0 mmol NaCl. Each treatment had 5 replicates per ecotype. The solutions were changed every fortnight. After 21 weeks the plants were harvested. Roots, leaves, dead leaves and fresh leaves were separated and dried for 36 hours at 70 ^{o}C. The air-dry weights were measured thereafter. Compared with the plants grown on 0 mmol NaCl, the plants grown on 200 mmol NaCl had more dry weight, which was especially due to an increase in root weight (Fig. 22). At the higher NaCl-concentration both root- and shoot growth were decreased. Montfort and Brandrup (1927) describe the same phenomenon for *A. tripolium* but at much lower concentrations ($\pm$ 80 and $\pm$ 160 mmol NaCl respectively). The "tidal marsh" plants are more robust in general, with broader and longer leaves but with fewer shoots per plant (Fig. 23). NaCl in the nutrient solution appeared to have an adverse effect on shoot production of the "inland marsh"-ecotype; 200 mmol NaCl seems to stimulate the shoot "production" of the "tidal marsh"-type but this difference with the shoot number at 0 mmol NaCl was not significant.

References

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VII.7. Population ecology of *Atriplex littoralis* and *Atriplex hastata* (S2) (B.P.M. Koutstaal)

In April 1980 investigations started to study the differences in population dynamics and population ecology of two Chenopodiaceae, *viz. Atriplex littoralis* (Shore Orache) and *Atriplex hastata* (Hastate Orache). The field work has been carried out on two salt marshes: one on a marine marsh north of Krabbendijke (Stroodorpepolder) (Eastern Scheldt), the other on a brackish marsh near Ossendrecht (Hinkelenoord) (Western Scheldt). Of both species natality, mortality, length and fenology have been recorded along a transect from the upper part of the marsh up to the dike (Stroodorpepolder) or up to a closed *Elytrigia pungens* vegetation (Hinkelenoord). The Stroodorpepolder-

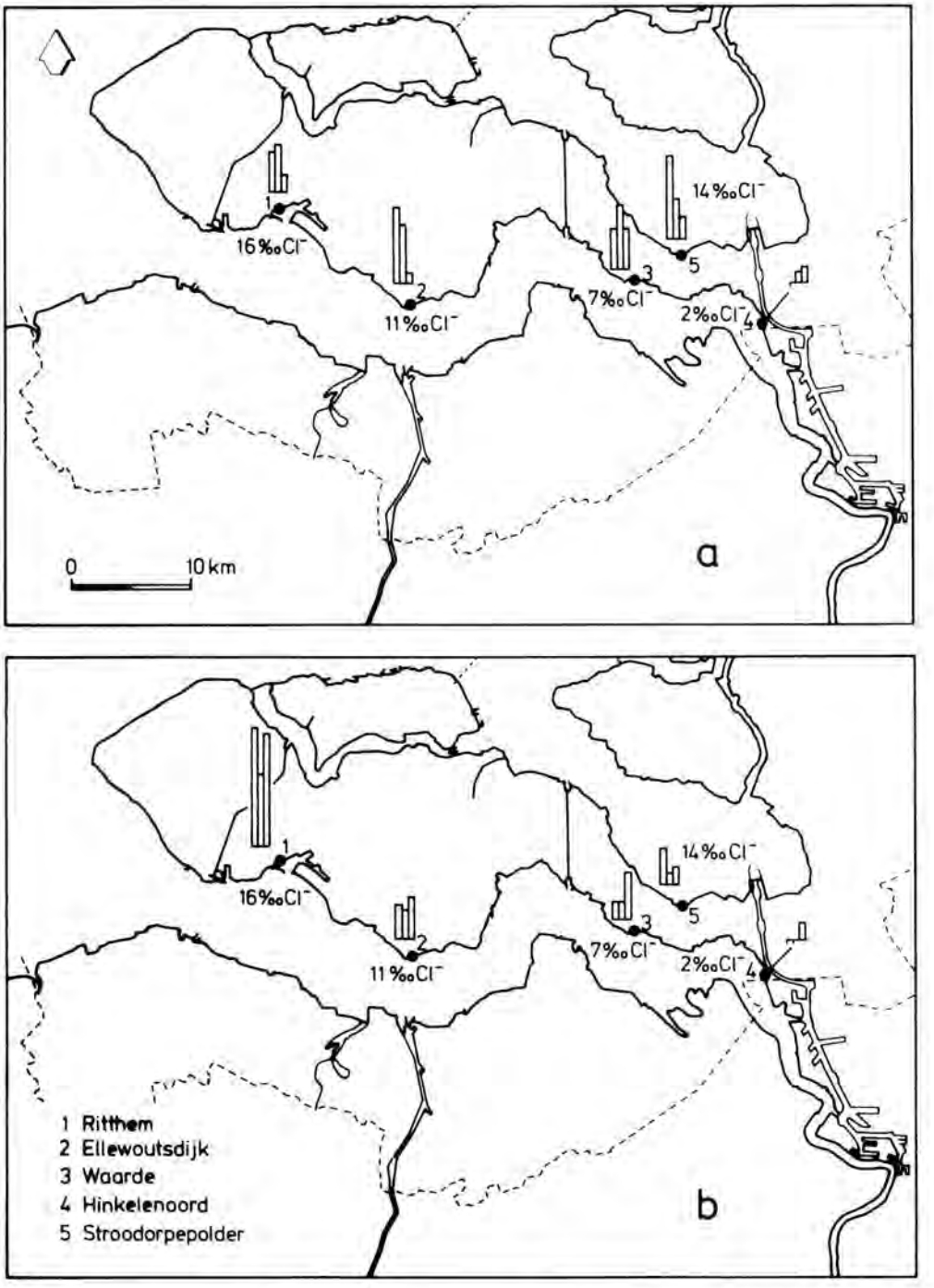

Fig. 21. a: Number of fly pupae of four stations along the Western Scheldt and one station at the Eastern Scheldt, sampled in November 1980 and January and February 1981. b: number of caterpillars of *Phalonidia affinitana* Douglas, sampled on four stations along the Western Scheldt and one station at the Eastern Scheldt in November 1980 and January and February 1981. The numbers of the high-lying sites are shown

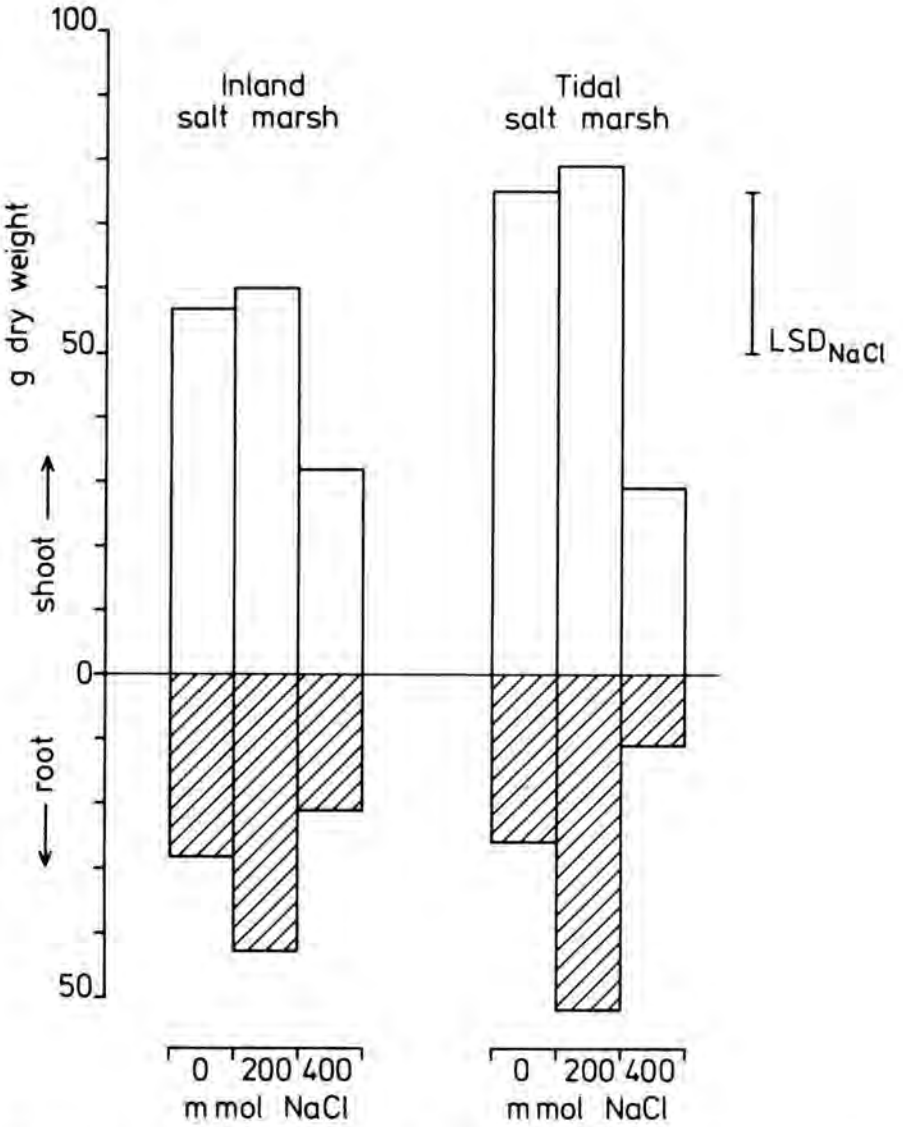

Fig. 22. Shoot- and root dry weights of two *Aster tripolium* ecotypes grown on nutrient solutions of different salinity. The values are the averages of the weights of five plants. LSD_{NaCl} = Least Significant Difference of salinity

transect had a length of 16 metres, the Hinkelenoord-transect was 10 metres long, both divided into plots of one square metre. Plant weights were recorded in plots parallel to the transect. *A. littoralis* occurred only in the upper part of the Stroodorpe-polder-transect, where plant debris have been thrown up against the dike face. At the Hinkelenoord-transect *A. littoralis* was absent, except at the higher located places against the dike face, where hardly any other salt-marsh species grew. On both *A. littoralis* habitats on plant debris, water stress during neap tide periods may be the factor of absence of salt marsh species and high mortality of the present *A. littoralis* plants.

It was tried to find a relationship between the composition and structure of the vegetation and the growth and biomass production of those *Atriplex* species. During the growing period, in all plots of the Hinkelenoord transect a considerable mortality took place, due to different causes, *e.g.* shading out by *E. pungens* (plot no. 10). There was a relatively small amount of seedlings that participated eventually in seed production (September). In the lowest part of the transect (plots no. 1 and 2) *Agrostis stolonifera* occurred (up to 40%) and in the highest part *E. pungens*

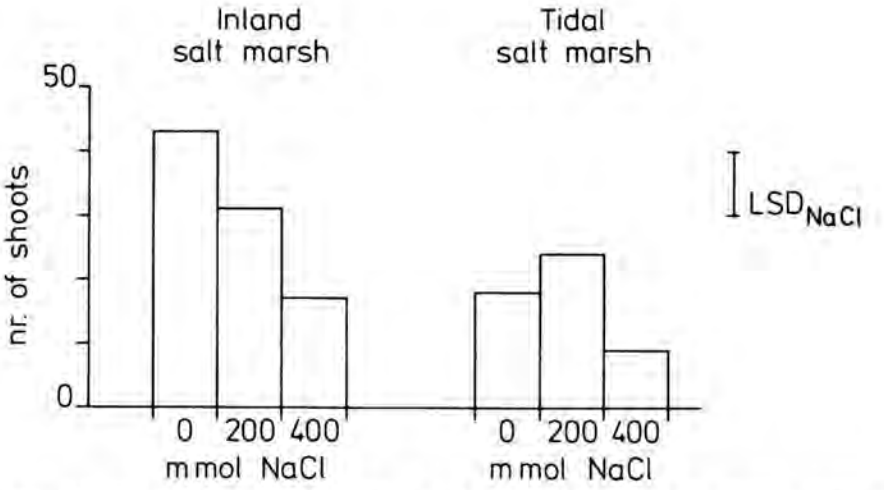

Fig. 23. The number of shoots of two *Aster tripolium* ecotypes grown on nutrient solutions of different salinity. The values shown are the averages of the number of shoots of five plants

(up to 45%) (see Table 12). Both species seem to have a negative influence on the development of *A. hastata* (see Fig. 24), and so did the vegetation in plot no. 6 (*Puccinellia maritima* 40%). These preliminary results give the impression that, with a few exception (plots no. 8 and 9), the decrease of *A. hastata* may be due to the high abundance of (tall) grasses. Differences in biomass of *A. hastata* existing in the juvenile phase, increase in the course of the growing period (Fig. 24).

VII.8. Abiotic and biotic factors in the decomposition process of some halophytes (S3) (G.J.C.Buth, L.de Wolf and P.F.M.Verdonschot)

Research on the decomposition processes of salt-marsh plants gives an insight in the budget of organic materials and nutrients of salt marshes and the surrounding estuarine waters (Kruczynski et al., 1978). Mainly in North America such research has been done the last years. However, American salt marshes differ strongly from European salt marshes.

Since April 1980 the loss of dry weight and of mineral components during decomposition of shoot litter of *Halimione portulacoides*, *Spartina anglica* and *Elytrigia pungens* in relation with the population dynamics of the litter fauna are measured, both in a salt marsh in the Eastern Scheldt and in controlled experiments.

At three sites of the salt marsh: submerged in a creek; on the lower marsh; and in plant-debris washed ashore, 1215 nylon bags of 20 x 25 cm, filled with dried litter, were placed. Different mesh sizes (300 µm, 1 mm, 1 mm with some 0.5 cm perforations) were used to partition the effects of microbial, meiofaunal plus microbial, macro and meiofaunal plus microbial activities on the decomposition processes. Each month 3 x 27 bags were retrieved from the marsh, the contents were dried, weighed and analyzed for chemical composition. Each two months all animals from 3 x 9 bags were collected, identified on order or family level and counted.

In the laboratory physical decomposition processes were studied (van Geldermalsen *et al.* unpublished). All organisms in 300 µm mesh bags were destroyed by using chemicals. Microbial activity was studied by monthly respiration measurements of decomposing litter. At the end of December 1980 the remaining litter was about 50-20% of the initial dry weight. The litter of the three plant species differed strongly in decomposition rate (Fig. 25). The place on the salt marsh had pronounced effects on the decomposition processes. On the marsh factors influencing decomposition seem to fluctuate less (Fig. 25).

References

Kruczynski, W.L., C.B. Subrahmanyam and S.H. Drake - 1978. Studies of the plant community of a North Florida salt marsh. Part II. Nutritive value and decomposition. Bull. Mar. Sci. 28, 707-715.

Table 12. Vegetation records of the different plots along the transect marked out on the salt marsh of Hinkelenoord. The figures in the table are cover percentages. The relevés were made on 13 June 1980.

Quadrat No.	1	2	3	4	5	6	7	8	9	10
% cover	75/95*	70/85	80/85	80/80	85/85	85/85	85/85	80/85	75/80	50/75
Elytrigia pungens				7	1/2		1/2	1	10	45
Scirpus maritimus	4	5	5	4	5	4	4	4	4	2
Agrostis stolonifera	40	20	1/2	1/2	1/2	2			2	
Cochlearea officinalis					2	1	2	2		
Puccinellia maritima	3	2	1	1	5	40	60	50	15	1
Atriplex hastata (number of examples)	20(368)**	40(229)	60(523)	50(640)	65(987)	35(469)	30(523)	40(587)	35(629)	2(16)
Aster tripolium	15	4	15	20	7	4	3	4	4	1

* The first number is the cover % of live plants; the second includes also the dead plant material.

** The figures in brackets are the number of plants.

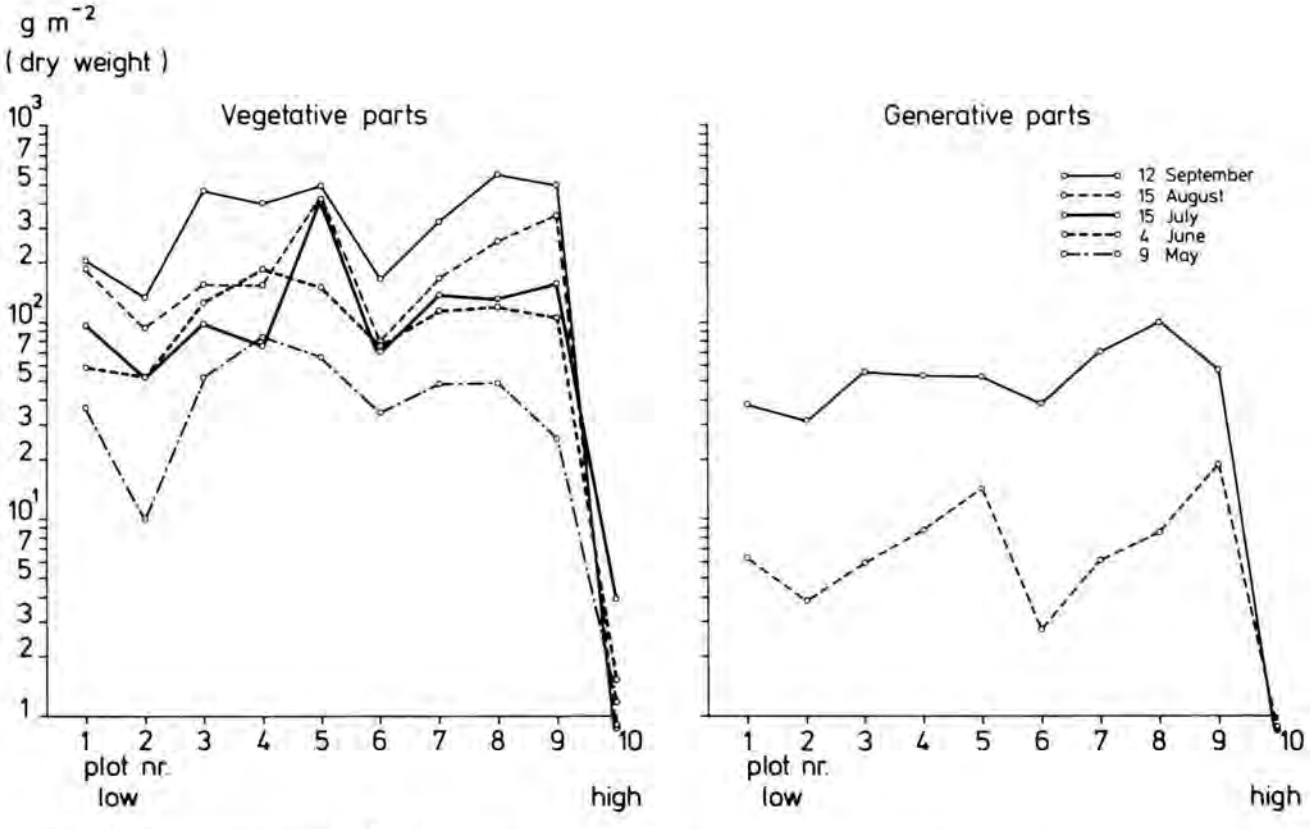

Fig. 24. Dry weight in g m^{-2} of *Atriplex hastata* growing along a transect on the salt marsh of Hinkelenoord

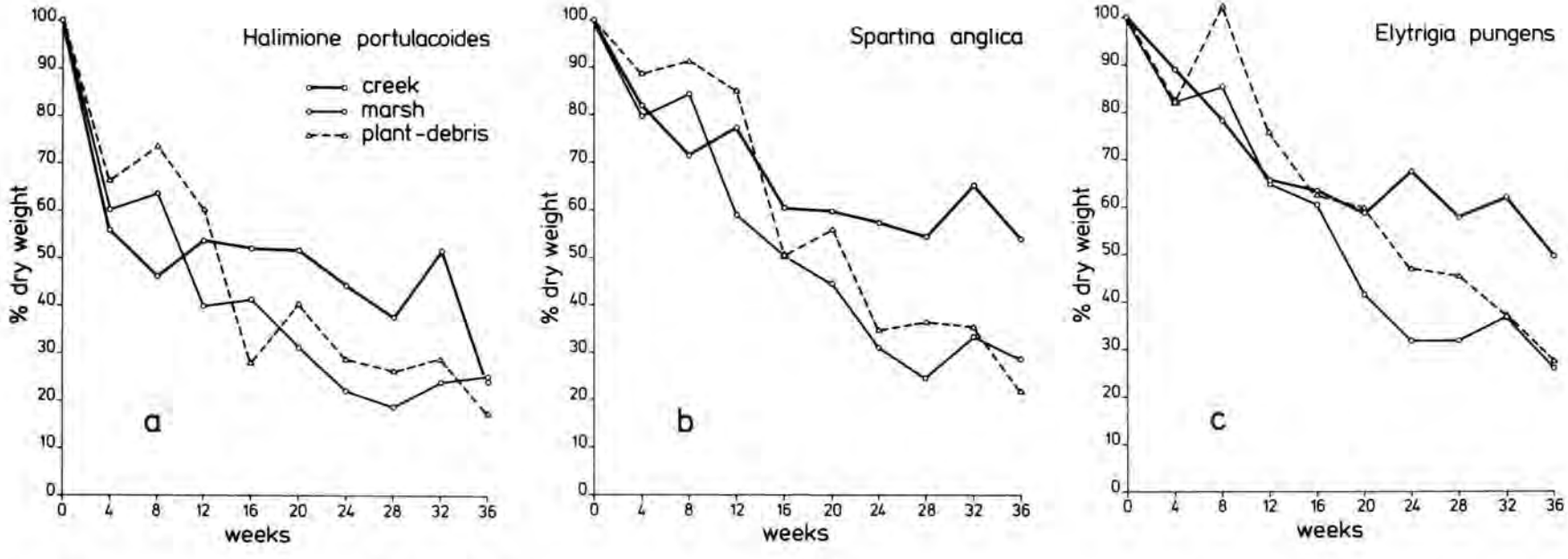

Fig. 25. Percentage of remaining dry weight, in litterbags with 1 mm mesh size, of shoot litter of a: *Halimione portulacoides*, b: *Spartina anglica* and c: *Elytrigia pungens* from April to December 1980

VII.9. A simple method for separating micro, meio and macrofauna from plant litter (S3) (L. de Wolf and G.J.C. Buth)

While studying the dynamics of fauna in decomposing litter of salt-marsh plants, problems occurred with the sorting and counting of the micro, meio and macrofauna. Decomposition was studied by using nylon bags, filled with dried litter. Several methods had been tried out to separate the animals from the litter, but none of these methods was satisfying for separating the total litter fauna.

Hand picking resulted in a fairly good separation but this was very time consuming. To make the sorting by hand easier a new, relatively fast method was devised for dividing the plant debris plus fauna into three fractions by washing it through a washing apparatus, formed by four 'three-dimensional' sieves (Plate II).

The sieves fit in each other, so that the coarsest sieve is the inner one and the finest sieve the outer. The frame of the sieves was made from brass strips, 3 cm wide. The sieving surface of the two smaller sieves was constructed from brass plates with pores of 1 mm and 3 mm respectively. Inside the frame of the two bigger sieves, nylon gauze of respectively 64 µm and 300 µm mesh size was fixed. A litter bag is placed in the inner sieve, the bag itself is removed and the sample is thoroughly washed with a strong jet of tapwater. Within three minutes a sample is washed through and the three fractions can be collected from the sieves.

The method was tested several times. About 95% of the fauna was extracted only some Nematoda or Oligochaeta remained in stems of *Spartina anglica*.

VII.10. The role at *Orchestia gammarella* Pallas on the decomposition rate of dead salt-marsh plants (S3) (K. van Koppen)

Every year after the growing season considerable amounts of dead plant parts are deposited along the dikes of the Eastern Scheldt. This fresh deposit is quickly colonized by several invertebrates of which *Orchestia gammarella* is one of the most abundant and certainly the largest. The role of macro-invertebrates on decomposition of dead plant material has been emphasized by several authors (Danell and Sjöber, 1979, Mason and Bryant, 1972, and Teal, 1962). On a dike along the Stroodorpepolder salt marsh a litterbag experiment has been carried out to determine the influence of *Orchestia* on the decomposition rate. The litterbags were filled with leaves and stems of *Spartina anglica*, the main component of the wrack layer. Half of the 50 litterbags (mesh size 0.3 mm) were made accessible for *Orchestia* by means of a few larger holes (0.7 cm). During a period of 70 days for the accessible litterbags a fractional loss rate of 0.0052 d^{-1} was found, while the fractional loss rate (k) at the inaccessible litterbags amounted to 0.0036 d^{-1} It is not clear, however, to what extent this difference in loss rate has been caused by *Orchestia*; other invertebrates especially Oligochaetes may play an important role as well.

Examination of the macro fauna in the litterbags, showed that macro-invertebrates were more abundant in the accessible bags than in the inaccessible ones. The number of Coleoptera found was greater than that of *Orchestia*. Both types of litterbags contained large numbers of Enchytraeïdae (Oligochaeta).

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Mason, C.F. and R.J. Bryant - 1975. Production, nutrient content and decomposition of *Phragmites communis* Trin. and *Typha angustifolia* L. J. Ecol., 63 - 71-95.

Teal, J.M. - 1962. Energy flow in the salt-marsh ecosystem of Georgia. Ecology 43, 614-624.

VII.11. Storage of trace metals in salt marshes of the Eastern and Western Scheldt (S4) (W.G.Beeftink)

On the basis of mutual assistance between Dr. M. Stoeppler (Institute for Applied Physical Chemistry, Nuclear Research Centre, Jülich, G.F.R.) and our working group the investigation on trace-metal contents in the salt marsh has been continued

with further analyses of soil and plant samples. For Pb, Cu, Cd, Ni and Hg-analyses the Institute in Jülich applied mostly electrothermal and cold-vapour AAS, after digesting with HNO_3 (plants), and HNO_3 and HCl (soils). Solutions containing high amounts of Cd (plants) or Pb and Cd (soils) were analyzed by flame AAS.

Four marshes (Stroodorpepolder marsh in the Eastern Scheldt; Ellewoutsdijk, Waarde and Bath marsh from Terneuzen to the Belgian frontier in the Western Scheldt) were selected. In each marsh three sample plots (low, middle and high marsh area) were located.

Clay-metal correlations were highly significant for Pb, Cu and Ni, but only weakly for Cd. The marshes in the Western Scheldt close to the port of Antwerp (Bath and Waarde) showed significantly higher levels of Cd and Cu in the soil than

Plate II. The three - dimensional washing sieve apparatus

the other marshes (Table 13). Low and middle marsh areas had higher contents of these metals than the higher marsh areas. In plants Pb-levels were significantly lower in the Ellewoutsdijk marsh, compared with the other marshes, and significantly higher in the lower marsh area, compared with the higher areas. Cd in plants was lower in the Eastern Scheldt, and higher in the Waarde marsh, Hg-contents in the plants showed significantly higher levels in the Bath marsh.

According to these methods of analysis mean values of trace metals in plants were for Pb: 3-8 ppm, for Cd: 120-1975 ppb, for Cu: 4-18 ppm, for Ni: 0.6-2.6 ppm, and for Hg: 24-84 ppb.

VII.12. The influence of tidal management on salt-marsh angiosperms (S5) (A.M. Groenendijk and M.A. Lievaart)

One of the tasks of the State Public Works Department is an integral assessment of the hydraulic, ecological and social-economic consequences of the construction and use of the storm-surge barrier in the Eastern Scheldt. The staff of the VEGIN-project who started their research in 1980, are engaged in a case-study on the effects of reduction of the tidal amplitude and the effects of active tidal management on salt-marsh vegetation.
The accent of the investigations carried out lies on research of the consequences of an elongated inundation of salt-marsh plants during their various life stages. In the greenhouse, and in a former oyster storage basin as well as on the salt marsh itself elongated inundations are simulated using tide simulators, *i.e.* a basin in which a tidal regime can be imitated and portable pump systems for the experiments on the salt marshes.

Preliminary results of the inundation experiments in the greenhouse indicate an influence of the water temperature on the effect of inundation on seedlings. At 25 ^{o}C seedlings of *Aster tripolium*, *Triglochin maritima* and *Plantago maritima* showed a clear die-back (Fig. 26). *Salicornia stricta* was less affected and showed even a slight increase in growth.

Most of the species, however, manifested only a decrease in growth rate after inundation for periods between 2 and 8 days (at 15 ^{o}C). In general a period of 4-8 days inundation resulted in a more severe reduction of growth than a two days inundation when only a slight growth reduction occurred.

In the oysterbasin inundation experiments have been carried out with *Halimione portulacoides*. Elongated inundation up to 8 days caused leaf fall but the plants recovered quickly after the end of the experiment. Between 2 and 4 days inundation flower buds started to decay. The relatively fast decay of flowers and flower buds has also been noticed during experiments with *Limonium vulgare* and *Plantago maritima* on the salt marsh.

During September, October and November a short-term student project performed by F. de Loos on the effects of inundation on three salt-marsh grasses *viz. Puccinellia maritima*, *Festuca rubra*, f. *littoralis* and *Elytrigia pungens* after their flowering.

Table 13. Significancies in trace - metal contents of soil and plant samples from salt marshes in the Eastern (S) and Western (E, W, B) Scheldt. L, M, H = low, middle and high marsh area. S = Stroodorpepolder, E = Ellewoutsdijk, W = Waarde, B = Bath marsh. Underlinings: non -significancies according to the method of the least significant difference. Sequence of symbols from low to high values

	soil samples		plant samples	
	salt marshes	marsh areas	salt marshes	marsh areas
Pb	SBEW >.05	HLM >.05	ESWB <.001	HML <.05
Cu	SEWB <.05	HLM <.05	SWEB >.05	MHL >.05
Cd	SEWB <.001	HML <.01	SEBW <.01	LMH >.05
Ni	SBEW >.05	HLM <.05	EWBS >.05	HML =.05
Hg			ESWB <.01	MHL >.05

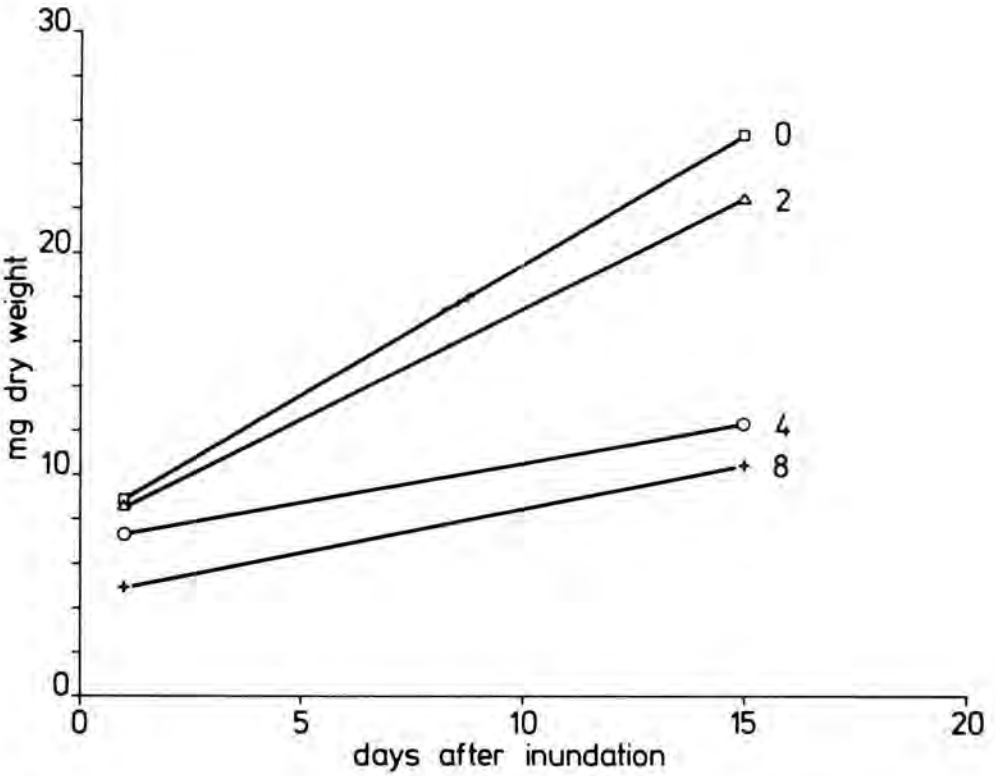

Fig. 26. Inundation experiments with *Aster tripolium* seedlings in the greenhouse. The regression lines of the 2 days, 4 days and 8 days inundation treatment and the control, depicting the increase in dry weight, show a significant non - convergence ($P < 0.001$)

Experiments have been carried out both in the oyster basin and on the salt marsh. On the salt marsh inundation was simulated using aluminium cylinders (diameter 50 cm; height 60 cm) which were driven into the sediment. With a pump system the cylinders were kept filled with seawater from a nearby creek to compensate for the seepage through the sediment. In the experiments in the oyster basin and on the salt marsh periods of inundation were resp. 4 days and 8 days. In the oyster basin a number of pots was 8 days inundated till ground level, this in order to keep the soil completely waterlogged. The results of the experiments on the two locations were in good agreement with each other (Fig. 27). *Festuca* showed little resilience against inundation. In the oyster basin as well as on the salt marsh there was a distinct die-back of plant material. *Puccinellia* and *Elytrigia* demonstrated a far better resilience against inundation. The field experiments also suggest a temperature influence on the resistance of plants against inundation.

VIII. A-SUBJECTS (MISCELLANEOUS)

A number of research projects is not covered by the programmes of the working groups, being either some investigations to be completed from previous projects, or pilot investigations for future programmes. Among the subjects are those on bird countings together with other organizations, a project on benthic fauna in the Volkerak (ZACHTSUB), flora and fauna records of littoral communities, intercalibration of chemical methods, pollution studies on organochlorine compounds, heavy metals and radionuclides, bottom-water exchange processes and model studies on residence time of natural and anthropogenic substances in estuaries.

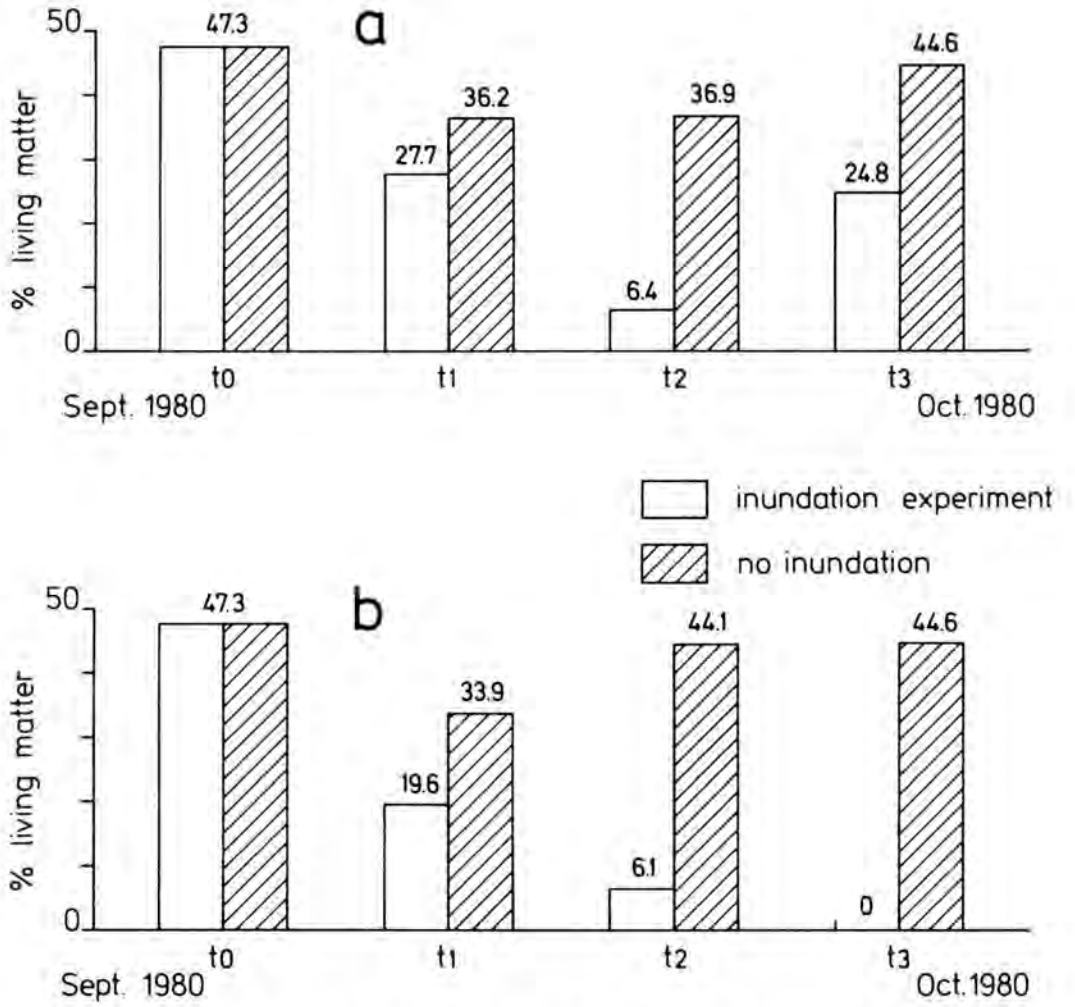

Fig. 27. The proportion of live plant material in experimental plots with a *Festuca rubra* sward before and after inundation. t_0 = just before inundation; t_1 = just after inundation; t_2 = ten days after inundation; t_3 = twenty days after inundation. a = inundation for four days; b = inundation for eight days

VIII.1. The implications of the closure of the Grevelingen on the number of waders foraging at the Roggenplaat (Eastern Scheldt) (A1) (R.H.D. Lambeck, A.J.J. Sandee and L. de Wolf)

The Delta area is one of the major wader areas in western Europe. The intertidal mud flats are visited by birds en route as winterers. However, this particular feeding habitat suffers much from the big hydraulic engineering projects within the Delta-plan framework. Since closing an estuary implies a more or less fixed water level and therefore the inaccessibility of nearly all the mud flats, it forces the great majority of the waders to leave from the area.

It can be asked what happens with these locally expelled birds. Do they have to leave the Delta area or is there sufficient foraging capacity on the mud flats in the remaining estuaries? In the 1960's it was not possible to organize a big monthly census, in which tens of people participate, as is carried out presently. However, observations demonstrated that near all of the waders foraging on the Roggenplaat, a large intertidal "island" in the western Eastern Scheldt (Fig. 28), had their hightide-roosts along the south coast of the island of Schouwen. Accessibility of these roosts and observation conditions were very good and it was possible to count all wader concentrations during one hightide period. This provided the possibility to monitor the wader numbers of one particular feeding area during the year and hence to

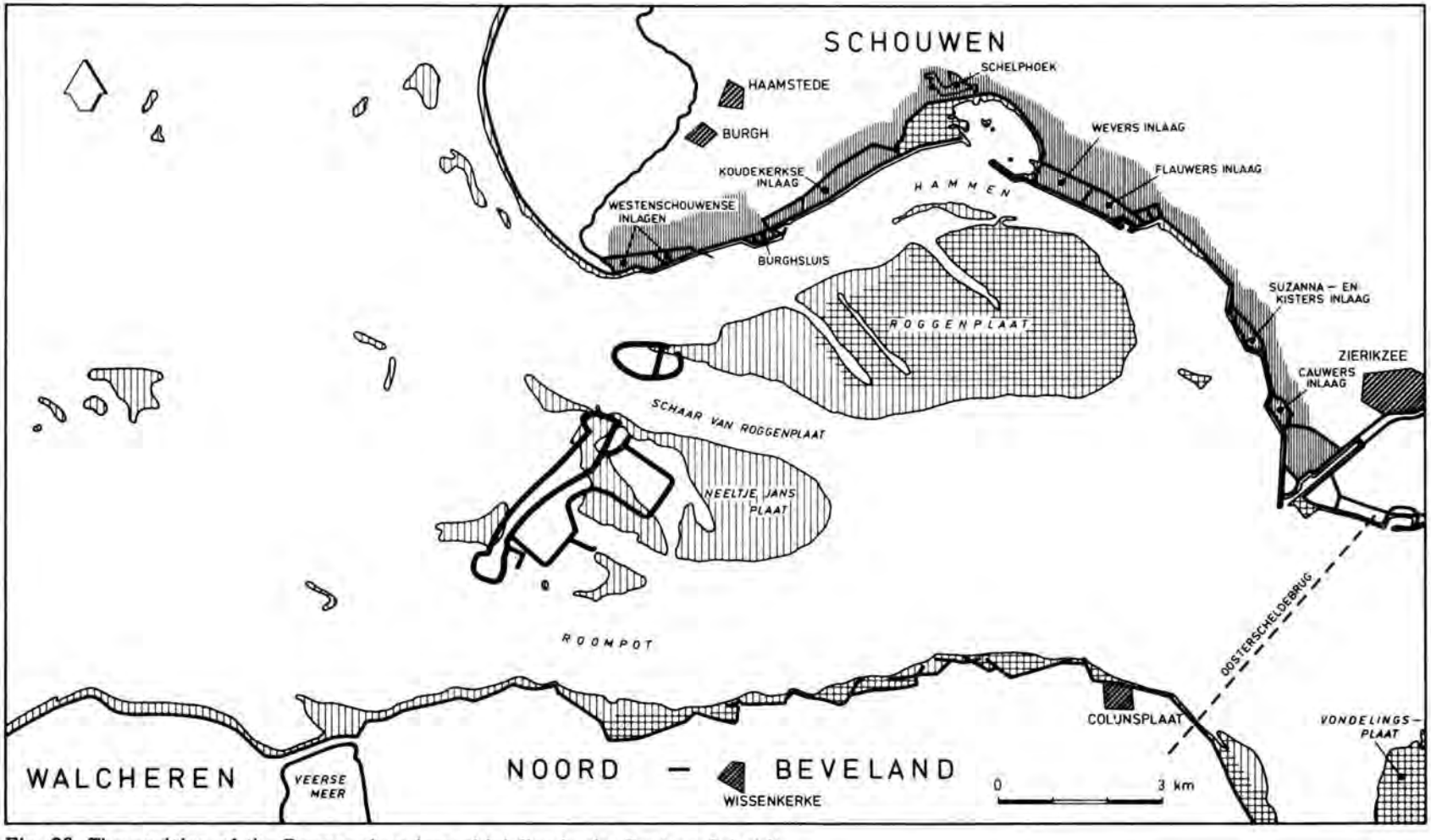

Fig. 28. The position of the Roggenplaat intertidal flat in the Eastern Scheldt.
The hatched zone along the coast of Schouwen marks the area surveyed for highwater roosts of waders. Over the 1964 situation the so far completed parts of the future storm-surge barrier is drawn.

detect the possible implications of closure operations elsewhere in the Delta region.

After some introductory work, a monthly census was started in the winter season 1964/65, that is still continuing. Because in the first three years not the whole coastline was censused, a later starting season has to be accepted for some species. Conditions have been standardized as much as possible: the time of hightide is about the same at all counting dates, the route is a fixed one and even the two main observers are still the same.

During this study two estuaries in the northern part of the Delta area have been closed: the Haringvliet in November 1970 and the Grevelingen in May 1971. Apart from some specialist species (*e.g.* the avocet *Recurvirostra avosetta*), waders in the brackish and silty Haringvliet were not very abundant. In the Grevelingen estuary, however, about 50,000 waders, among which 25-30,000 oystercatchers (*Haematopus ostralegus*), lost most of their feeding grounds. Therefore, possible changes at the Roggenplaat were expected from the winter 1971/72 onward.

The best index of the use of a feeding area is the number of bird-days spent per species. Based on twelve counts per year estimates per season (running from 1 July until 30 June) were made for nine common species (see later Fig. 30). By calculating 3- or 5-year moving averages, to suppress part of the variations due to *e.g.* counting errors and population changes, trends could be made much more easily visible. Also developments in the seasonal maximum number counted and occurrence patterns throughout the seasons were analyzed.

For the oystercatcher and the bar-tailed godwit (*Limosa lapponica*) a significant increase in the number of bird-days as well in the maximum number counted per season could be demonstrated after the closure of the Grevelingen. For the latter species this is illustrated in Fig. 29. A further increase in the number of bar-tailed godwits after 1975, a phenomenon occurring in the whole Eastern Scheldt, showed that also other unknown internal (local food supply) and external factors (changes in habitats elsewhere, a population increase) may play a decisive role in number developments.

As regards the other common species, the extend of changes possibly occurring was too small to override the "natural" variations.

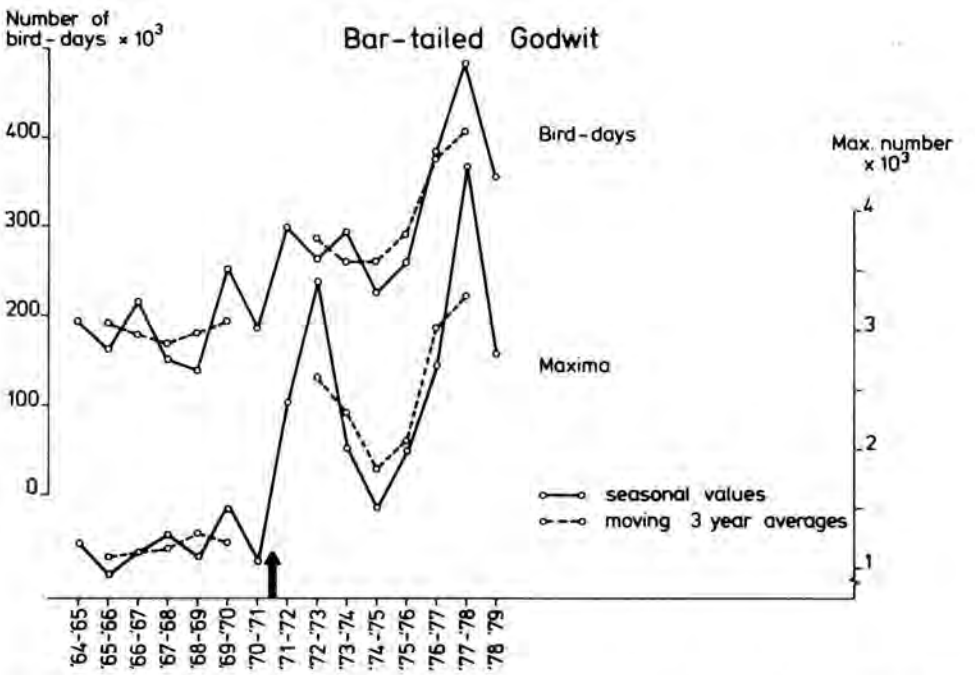

Fig. 29. Patterns in seasonal (1 July - 30 June) maximally counted numbers and bird-days calculations for the Bar-tailed Godwit (*Limosa lapponica*), foraging at the Roggenplaat (Eastern Scheldt), in the period 1964/65-1978/79. The arrow indicates the closure of the Grevelingen estuary

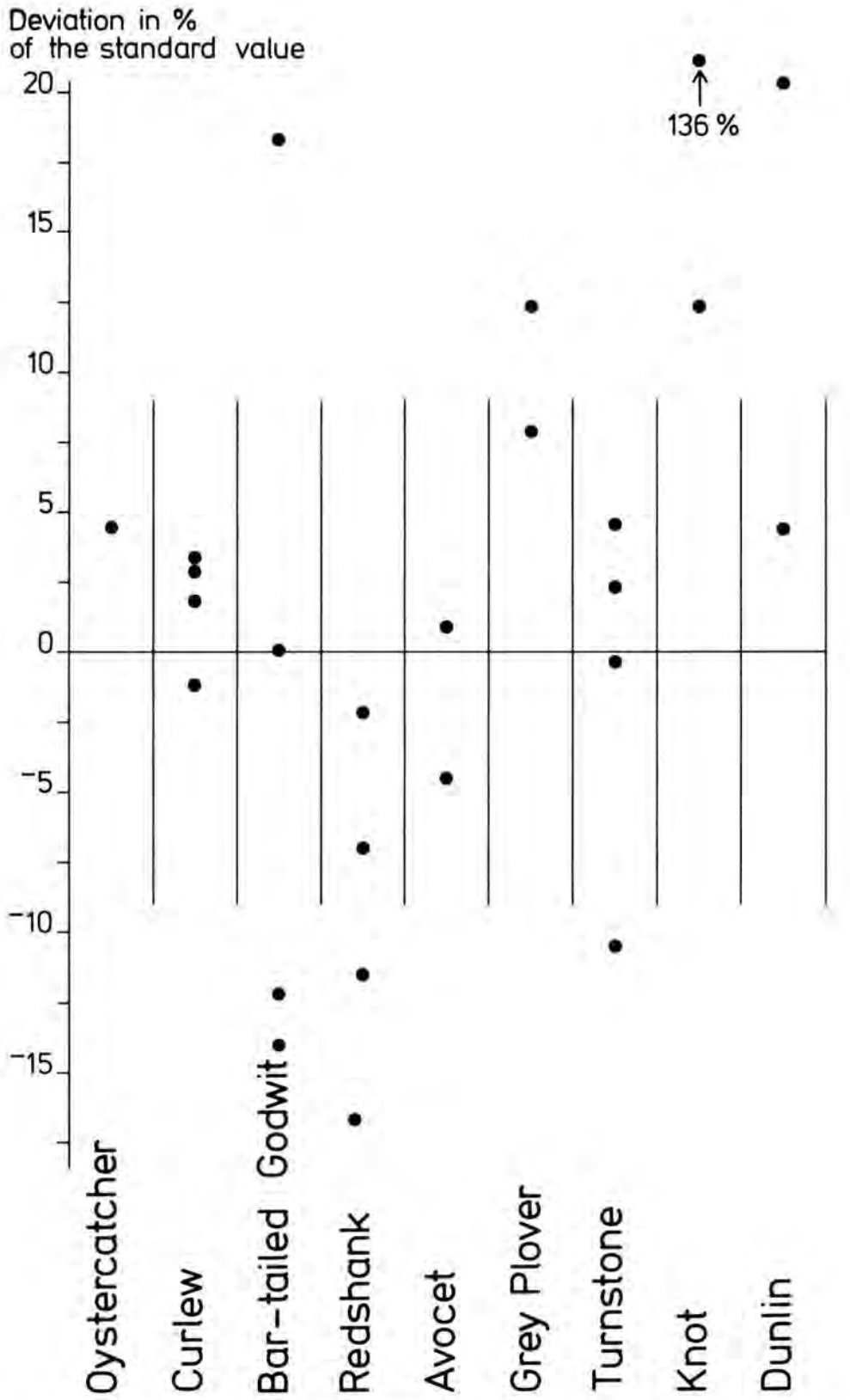

Fig. 30. The influence of extra counts on bird-days estimates for nine species of waders. The deviation of estimates of all counts available (N_c = 16 - 23) is expressed in percentages of the standard (N_c = 12, hence without 4 - 11 extra counts) estimates

One may ask whether one count per month is sufficient for a reliable picture of the use of the Roggenplaat. In the first four seasons a variable number of extra counts was carried out. Seasonal bird-days totals were also calculated on the basis of all counts available. In Fig. 30 the deviation of the standard number of 12 is expressed in percentages of standard bird-days values. With the exception of one very aberrant figure for the knot (*Calidris canutus*) all deviations were within 20%, even for the small species as dunlin (*Calidris alpina*) and turnstone (*Arenaria interpres*) that are hard to count accurately. This indicates that, although a higher census frequency certainly would improve the results, the present counting schedule provides a fairly reliable index. This is also shown by the rather regular patterns of increase and decrease in total bird-days and maximum number, not only for the bar-tailed godwit (Fig. 29) but for all other species. Which implies a certain correlation between succeeding seasonal figures.

VIII.2. Possible changes in intertidal benthos-communities after a freshwater run-off (A2) (J. Coosen and A. v.d. Dool)

Since the construction of a dam and sluice between the Krammer-Volkerak estuary and the Haringvliet in 1969, there has been an increase in chlorinity in the former part of these waters resulting in a chlorinity-gradient of 8 to 16 $^{0}/_{oo}$ Cl^- along the transect Volkerak-Krammer-Keeten-Eastern Scheldt. The brackish-water fauna survived this environmental change, whereas marine fauna immediately started to colonize the area. This resulted in an increase in the diversity of organism (Wolff, 1973a, 1974; Wolff and de Wolf, 1976).

From 1977 on 50 $m^3 s^{-1}$ freshwater is running off through the Volkerak sluices, to maintain an undisturbed fresh water basin north of the dam and to investigate the effects of fresh-water runoff on the Krammer-Volkerak estuary. An experiment was performed in which the amount of fresh water was temporary doubled to 100 $m^3 s^{-1}$ in the period November 1st, 1979 to March 26, 1980. A considerable change in the chlorinity gradient was observed during this period. To study the possible changes in the benthic fauna, the project "ZACHTSUB" was started in December 1979.

The benthic fauna in the area was studied by means of samples taken at 6 transects across the tidal flat from high to low water mark (Fig. 31). These transects were sampled in October 1978, 1979 and 1980 and in March 1979, 1980. The results of 36 stations sampled in March 1979 and 1980 are presented. The samples were taken with a corer of 0.005 m^2 to a depth of about 30 cm and with a total surface area of 0.045 m^2 . The samples were washed in the field through a 1 mm sieve and the residue was sorted by hand and with use of a stereo-microscope in the laboratory. All animals were identified to species levels, except for the oligochaeta. Ash-free dry-weight (AFDW) for 4 groups of organisms (1: *Arenicola marina*; 2: other Polychaeta; 3: Mollusca; and 4: Crustacea) was determined according to Wolff and de Wolf (1977).

Sediment analysis was carried out in November 1979 and April 1980. The median grain-size data varied from 2.47 to 3.84 phi-units (methods see Wolff, 1973b). The height of the sample stations in relation to N.A.P. varied from + 1.10 m to - 1.50 m; most stations were situated above N.A.P.

To measure the changes in the composition of the benthic fauna before and after the 100 $m^3 s^{-1}$ freshwater runoff experiment we compared, next to the occurrence of the species, the species diversity of the March-1979 and 1980-samples, using two indices:
(1) the number of species per 100 individuals; obtained by Sanders rarefaction-method, and (2) the Shannon-Weaver information function.
We also compared the biomass-values for 1979 and 1980 of the 4 groups mentioned before. Table 14 enumerates the species occurring in the 6 transects in 1979 and 1980. Most frequently found were the following species: (in frequency-order) *Hydrobia ulvae*, *Capitella capitata*, *Scoloplos armiger*, *Eteone longa*, *Arenicola marina*, *Heteromastus filiformis*, *Corophium arenarium*, *Macoma balthica*, *Nereis diversicolor*, *Cerastoderma edule* and *Pygospio elegans*. They occurred in more than 50% of the stations, often in more than 75%. No significant change was observed in the abundance of these species, from 1979 to 1980, except for *Heteromastus filiformis* which was not found in transect IV in 1980.

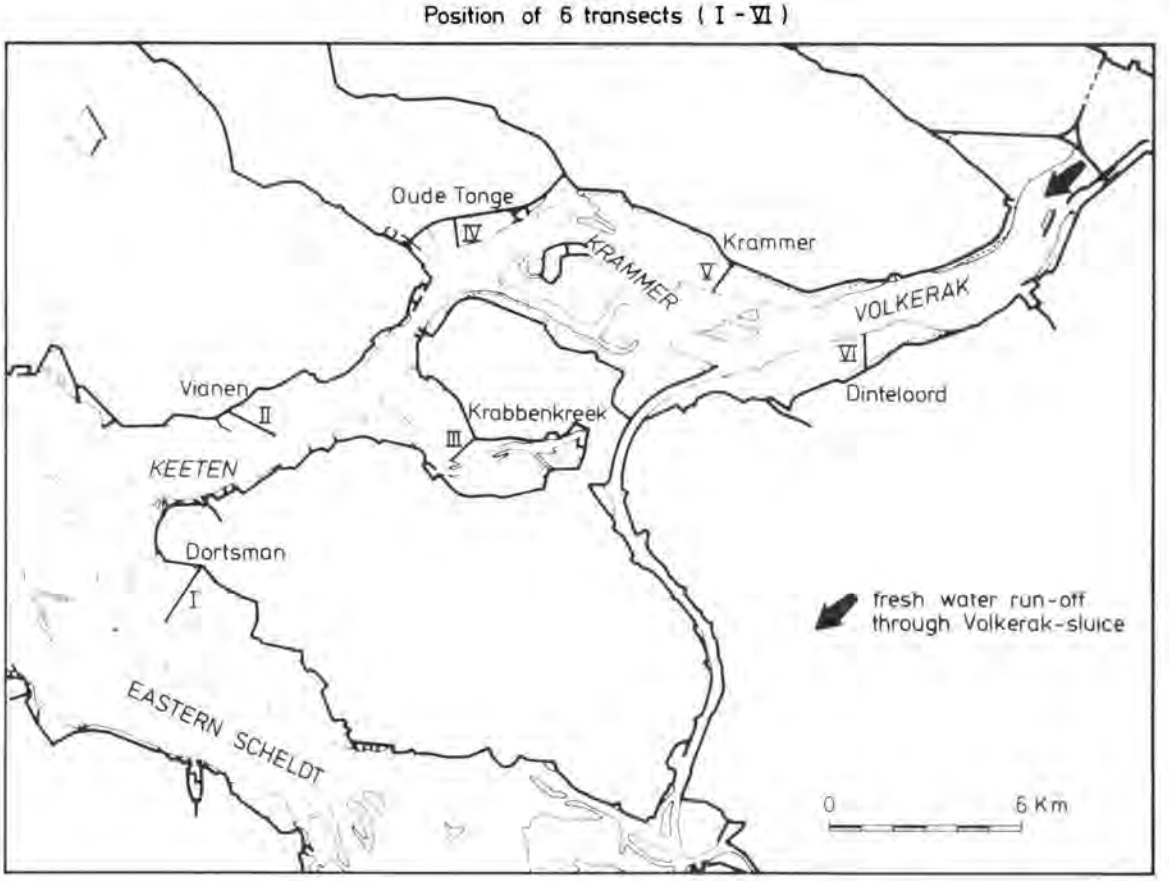

Fig. 31. Map of the Krammer-Volkerak area. The positions of the transects (I-VI) are indicated by solid lines

Most of the remaining 24 species were found in one or two stations only: 5 only in 1979; 9 only in 1980 and 10 in both years. Six species occurring in the transects III, IV and V in 1979, were absent in the 1980-samples. Table 15 gives these species, their occurrence and their lower chlorinity limit according to literature. It is possible that their absence in the 1980-samples is due to the lowering of the chlorinity at the transects III, IV and V. However, *Nepthys hombergii* was found in transect VI at a chlorinity of $\pm$ 9 °/oo. Furthermore, the number of samples in which these species were found in 1979 is very low.

Sanders rarefaction method allows us to estimate the number of species per 100 individuals. It is independant of sample-size. The calculated values show that for all transects the medium values do not vary very much from 1979 to 1980. Individual stations may vary however. No significant correlation was found with the lowering of chlorinity in the transects. The same conclusion can be drawn from the comparison of the Shannon-Weaver index values of the 1979 and 1980-samples. Although some stations showed a higher diversity-index value in 1980 (especially stations in transects I and III) and others the contrary (esp. in transects II and IV), the overall picture for most transects remained the same.

In 22 of the 36 stations biomass increased from 1979 to 1980. The values for each station in 1979 and 1980 are given in Fig. 32 and 33. Average values for each transect are relatively low: from 5 to 9 g m^{-2} AFDW in 1979 and from 6.7 to 12.2 g m^{-2} AFDW in 1980 (compared with the Waddensea, Beukema, 1974), except for transect II in which a musselbed station was included (1979: 20.3, 1980: 63.9 g m^{-2} AFDW). No significant correlation could be found between the change in chlorinity and biomass figures.

Summarizing we may conclude that six species, present in some of the 1979-

Table 14. Species occurring in 36 stations (position given in m from mean high water mark) along the transects I-VI in March 1979 (open squares) and 1980 (black squares)

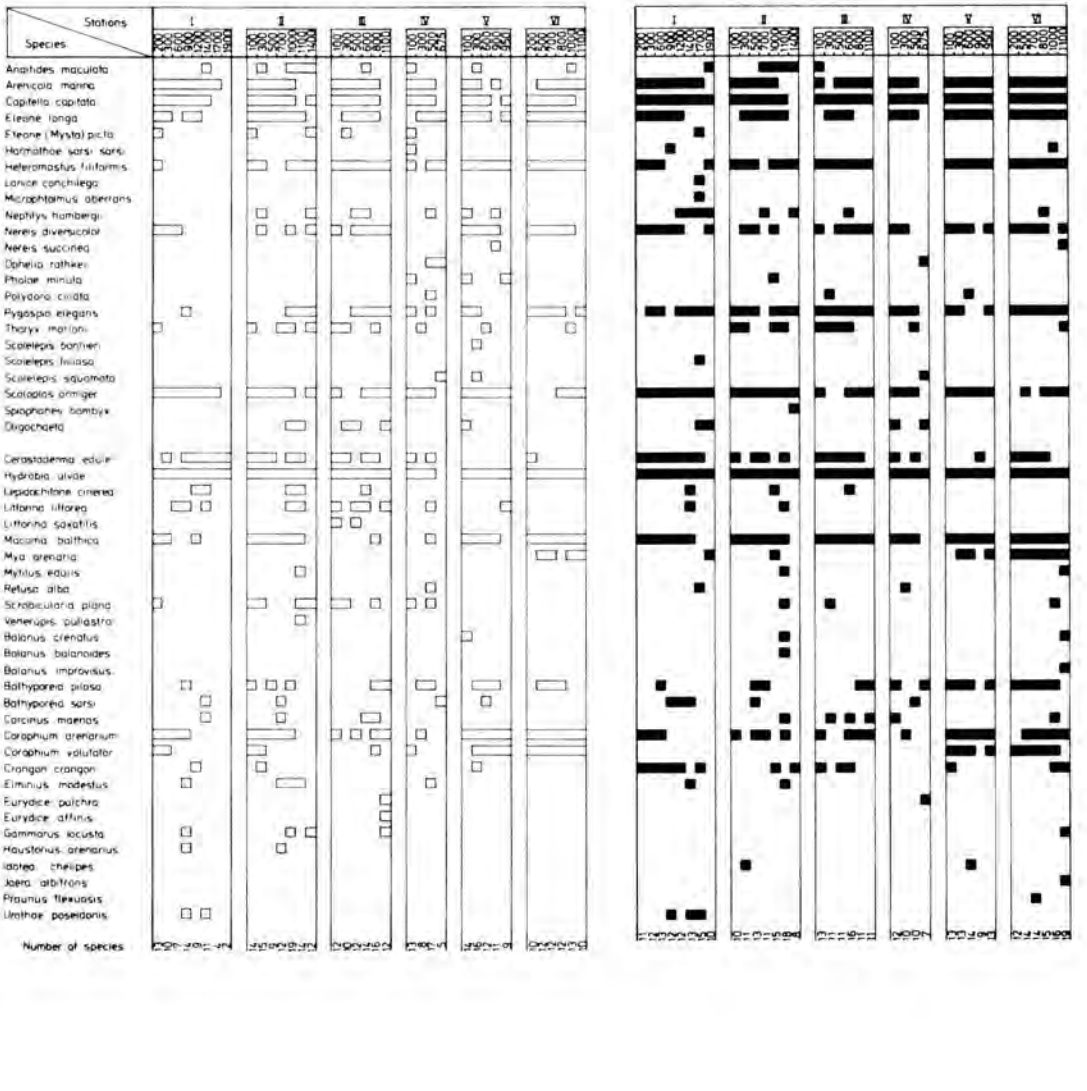

Table 15. The average interstitital chlorinity and median grain size for each transect in Marsh 1979 and 1980.

transect	average chlorinity of interstitial water in $^{o}/_{oo}$ Cl^{-}		average median grainsize with extremes in u-units	
	March 1979	March 1980	March 1979	March 1980
I	17.33	15.44	2.84(2.62-3.12)	2.79(2.49-3.03)
II	16.44	14.28	3.04(2.94-3.20)	2.99(2.90-3.15)
III	15.24	13.13	2.99(2.88-3.13)	2.95(2.65-3.14)
IV	15.41	12.01	2.52(2.47-2.58)	2.55(2.51-2.6)
V	12.74	8.81	3.13(2.75-3.39)	3.19(3.02-3.39)
VI	12.04	9.22	3.24(3.12-3.39)	3.26(3.04-3.84)

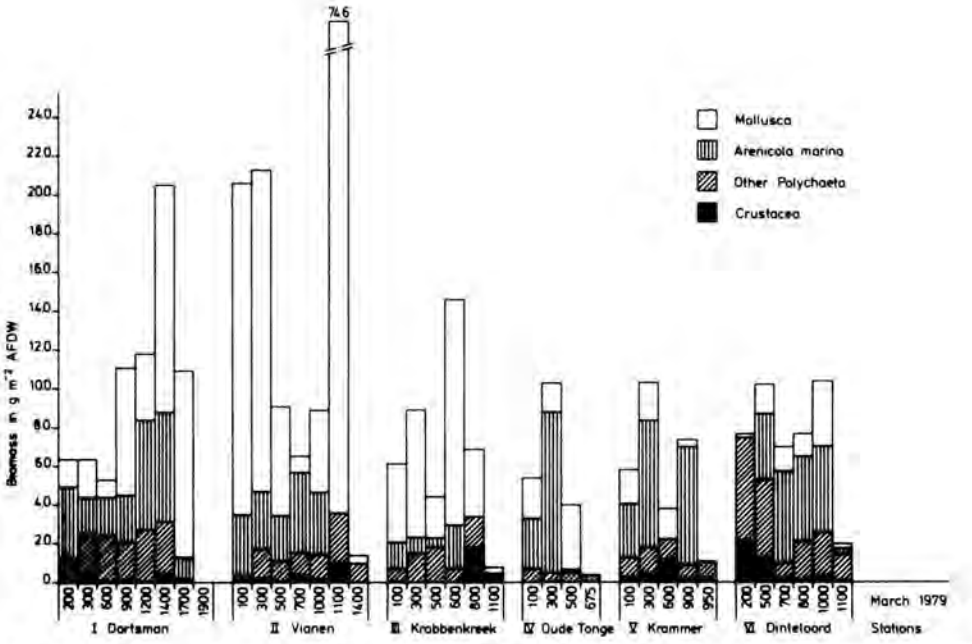

Fig. 32. Biomass for four groups of organisms from stations samped in March 1979

samples of transects III, IV and V, were not found there during the 1980-survey. Their absence might be an indication for their temporal disappearance from these transects. The observed changes in diversity and biomass did not show a clear correlation with the change in chlorinity. More macrobenthic samples taken before and after the experiment are in preparation. On the basis of the present data, one may conclude that the temporal lowering of chlorinity has had no considerable effect on the intertidal benthos-community.

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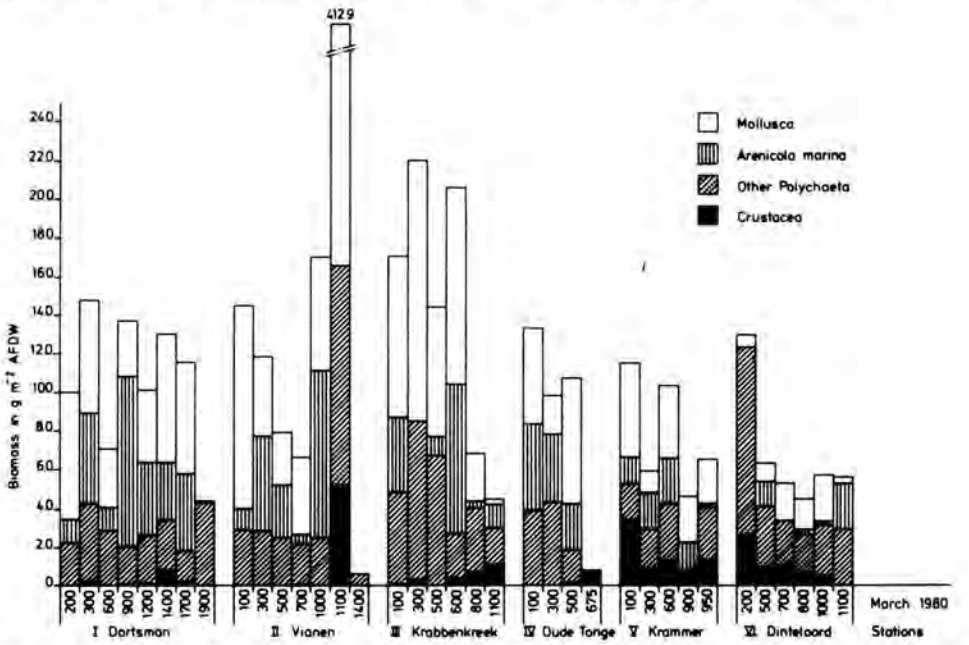

Fig. 33. Biomass for four groups of organisms from stations sampled in March 1980

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VIII.3. Organochlorines in mussels (A9) (J. Nieuwenhuize, J.M. van Liere and E.K. Duursma)

The general distribution of organochlorines in the Delta area was investigated by analysis of mussels sampled from different locations. The species sampled were *Mytilis edulis* for the marine and brackish waters and *Dreissena polymorpha* for the fresh-water region. Musselflesh was freeze-dried and grinded while the extraction and clean-up procedure occured according to an adapted method of Holden and Marsden (1969). The results are presented in Fig. 34 for PCB's on fat basis and reflect the influence of contaminated Rhine and Meuse water on the northern Delta area and the influence of the contaminated Scheldt on the Western Scheldt estuary. Values in the Eastern Scheldt and Grevelingen are relatively low.

Identical features were found for hexa and pentachlorobenzene, α HCH and p,p'-DDD. Dieldrin is detectable in the Volkerak, Grevelingen, Eastern and Western Scheldt at 0.3-0.5 ppm, but was masked in the chromatogramme of the GLC for the Haringvliet-Hollands Diep by a high peak of undefinable origin (probably an industrial product).

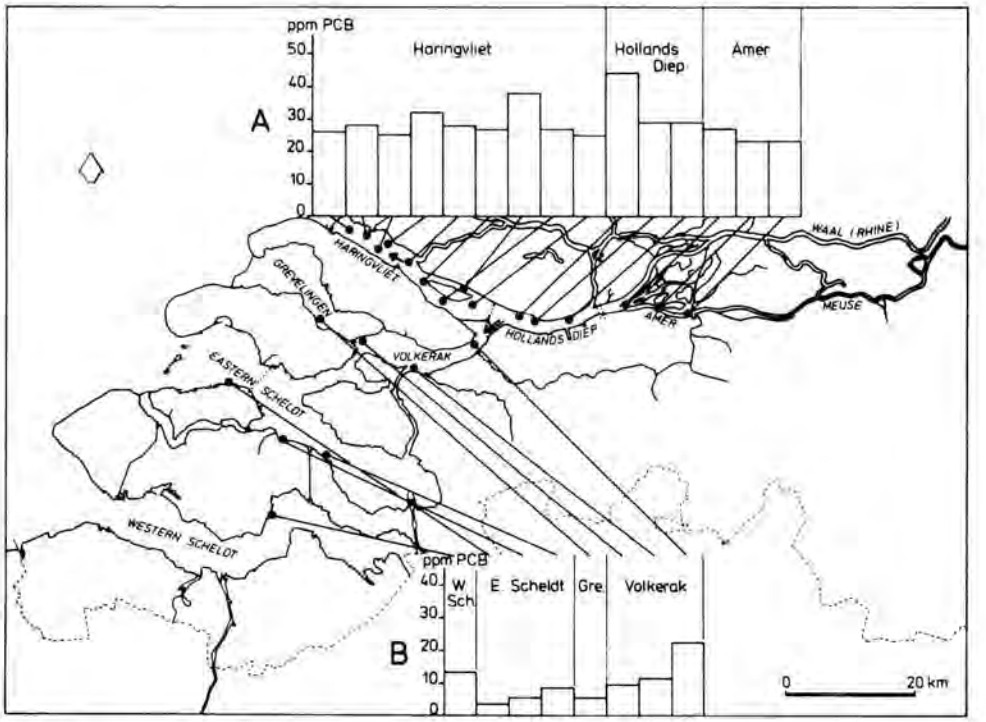

Fig. 34. PCB's in *Dreissena polymorpha* and in *Mytilus edulis* (B) in ppm per fat basis. Samples are taken in November 1980

Reference
Holden, A.V. and K. Marsden - 1969. Single-stage clean-up of animal tissue extracts for organochlorine residue analysis. J. Chromatog. 44, 481-492.

VIII.4. Biological half-life of some organochlorines in the mussel *Mytilus edulis* (A9) (J. Nieuwenhuize, J.M. van Liere and E.K. Duursma)

In order to determine the loss of organochlorines from contaminated mussels an experiment was set up for 148 days where mussels, sampled from a region (Grevelingendam) with relatively high amounts of organochlorines, were transferred to a 140 l basin of the aquarium building in Yerseke. About 29 kg adult mussels were kept in running sea water (from Eastern Scheldt) with a continues refreshment of 12 l min^{-1}. At regular time intervals mussels were sampled and analyzed on their fat content and the organochlorine spectrum. In the 148 days period the fat content of the mussels dropped regularly from 5.73% to 3.80% on dry weight basis. Except for PCB's all other organochlorines showed a drop in their content both on dry weight and on fat basis. The PCB's increased from 12.9 to 25.8 ppm per fat basis and from 740 to about 1000 ppb on dry weight basis, showing that probably some additional accumulation took place.

The apparent half-lives of the pesticides are calculated from the amounts per fat with the formula

$$C_t = C_o e^{bt}, \text{ where } t_{\frac{1}{2}} = \frac{\ln 0.5}{b}$$

applying a regression technique with $\ln C_t = bt + a$, in which then $a = \ln Co$.
The $t_{\frac{1}{2}}$ average values range between 7 and 322 days (Table 16).

The results show that in the organochlorines other than PCB's the loss is time consuming at least too long a process to have mussels decontaminated for consumptive purposes.

VIII.5. Release of chlorobenzenes from estuarine sediments in the absence and presence of benthic organisms (A10) (D. Monnikendam and M. Smies)

In order to study the release of four chlorobenzenes from estuarine sediments in the absence and presence of benthic organisms, we collected sediment from a tidal flat in the Eastern Scheldt. The sediment was wet-sieved to remove macrofauna and debris and portions were placed in glass beakers. Mixtures of 1,2,3-trichlorobenzene (concentration 14 ppb dry sediment), 1,2,4,5-tetrachlorobenzene (6 ppb), pentachlorobenzene (5 ng g^{-1}) and hexachlorobenzene (2 ppb) were added to the beakers. After addition of two *Nereis* sp. or two *Crangon crangon* to treatment beakers, these were placed in containers with aerated Eastern Scheldt water at a constant 15 ± 2 °C in the laboratory. At regular intervals beakers were removed for analysis of the chlorobenzene concentration in the sediment. The total duration of the experiment was 78 days, during which period the beakers were checked every two days and dead animals replaced.

Except for tetrachlorobenzene it was not found that the presence of the benthic organisms promoted the release of chlorobenzenes from the sediment. However, for all compounds, except hexachlorobenzene, it was found that there was a significant removal from the sediment over time, apparently through desorption. Losses varied between 10 and 60% over 78 days for tri, tetra and pentachlorobenzene without any indication of compound-specific differences. For hexachlorobenzene no significant loss occurred.

VIII.6. Extraction of estuarine sediments for chlorobenzene analysis (A10) (J.A. van Zetten and M. Smies)

Recoveries of chlorobenzenes from estuarine sediment samples, analyzed according to our institute's standard procedure, were poor. Typical recovery percentages were in the order of 12% (1,2,4,5-tetrachlorobenzene), 20% (pentachlorobenzene) and 40% (hexachlorobenzene). Since the clean-up stage, elution from an Al_2O_3-

Table 16. Parameters of loss experiment of organochlorines in mussels and biological half life with 95% confidence limits with 95% confidence limits a and b regression coefficients of ln $C_t = bt + a$; St_b = Student's statistic for regression coefficient b

Compounds analysed	Initial conc. ppm fat basis	Time of experiment days	Number of data	a	b	St_b	Significance of regression	Half-life days
PCB (50%) chlorinated	12.9	148	23	13.55	0.103	3.75	< 0.01	-----
pentachloro-benzene	0.13	70	13	0.130	-0.0011	2.29	< 0.05	15 ± 19
hexachloro-benzene	0.07	70	13	0.072	-0.0010	3.16	< 0.01	7 ± 12
α HCH	0.16	148	24	0.218	-0.0009	4.24	< 0.001	127 ± 258
γ HCH	0.65	148	24	0.330	-0.0006	1.54	> 0.05	322 ± 236
dieldrin	0.35	148	24	0.347	-0.0011	5.20	< 0.001	159 ± 329
p,p'-DDD	0.11	148	24	0.152	-0.0002	0.78	> 0.05	

Table 17. Recovery (%) of chlorobenzenes from duplicate spiked sediment samples, extracted with different solvents for two hours

Spikes	Extractant		
	pentane	hexane	hexane-acetone
1,2,4,5-tetra-	17.4	49.2	5.4
chlorobenzene	8.8	49.2	14.0
pentachloro-	24.1	52.4	18.0
benzene	24.5	51.0	20.8
hexachloro-	49.7	66.6	46.9
benzene	55.5	76.9	63.1

column, caused only 20% loss on recovery, we compared different organic solvents for sample extraction. Besides the standard extractant pentane we tried hexane and a 1:1 hexane-acetone mixture. Extraction was carried out in a soxhlet-apparatus for two hours. Table 17 shows the results for duplicate samples, indicating that hexane was most effective. We also compared extraction periods of 2, 4 and 6 hours and found that extraction for longer than four hours did not increase the yield of the recovery.

VIII.7. Seasonal variation in the nutrient composition of the anoxic interstitial water in a tidal area (A11) (L.A. van Geldermalsen)

The nutrient composition of interstitial water in a tidal flat was evaluated throughout one season by monthly sampling of a transection in the intertidal area situated near the Stroodorpepolder. The transection consists of 5 points ranging from the low water mark to the marsh border. At each point 8 hollow

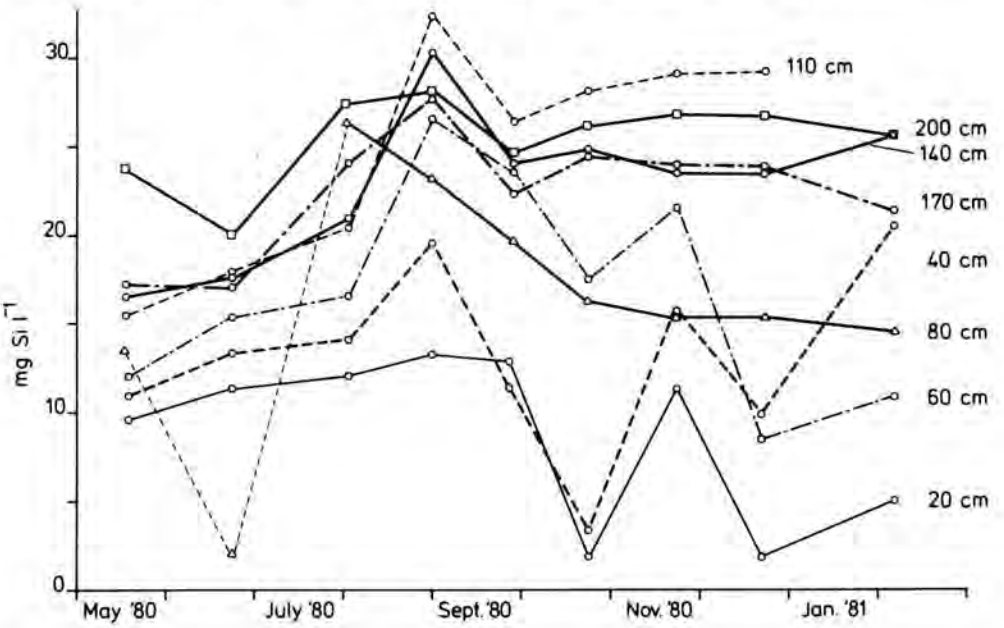

Fig. 35. Silicate content at eight different depths in the intertidal area near the salt marsh Stroodorpepolder

pipes of lenghts varying from 20 to 200 cm were drilled into the sediment. Holes in the pipewalls allowed interstitial water to percolate into the pipes. These holes were covered with a plankton net with a mesh size of 40 µm. Standard procedures were used to analyse these samples for orthophosphate, sulphate, silicate, ammonia and chloride content. Fig. 35 shows the silicate concentrations at point 1, nearest to the marsh border, at eight different depths and for a period of 7 months. Silicate shows a slight decrease in concentration in the summer months whereas in the fall this decrease is rapid for the upper parts of the sediment, followed by a slower decrease in concentration in the deeper layers.

Ammonia (Fig. 36) shows a decrease during the summer at all depths, but it returns to its approximately original level in the fall.

More data on the transection are gathered to evaluate the dynamics of the interstitial water in the anoxic region of an intertidal mudflat. Knowledge of these dynamics will give us a better understanding of the sediment-water exchange of mud flats.

VIII.8. Construction of a wave-maker system in a flume (A11) (L.A. van Geldermalsen)

In order to study the impact of waves on tidal flats a flume was constructed near the aquarium building. The flume measures 25 x 1 x 1.75 m^3 (lenght x width x depth). At one end of the flume a bottom-hinged paddle generates waves; at the other end waves are absorbed on a talus of perforated stones.

The paddle is powered by an electromotor equipped with an crank wheel setting. Waves of different lenght and height can be obtained by adjusting the frequency and the stroke of the paddle and regulating the water level height.

After installation, the equipment was tested to find out if it met the requirements. Fig. 37 shows wave heights (top-crest) and wave lenghts for two stroke lenghts

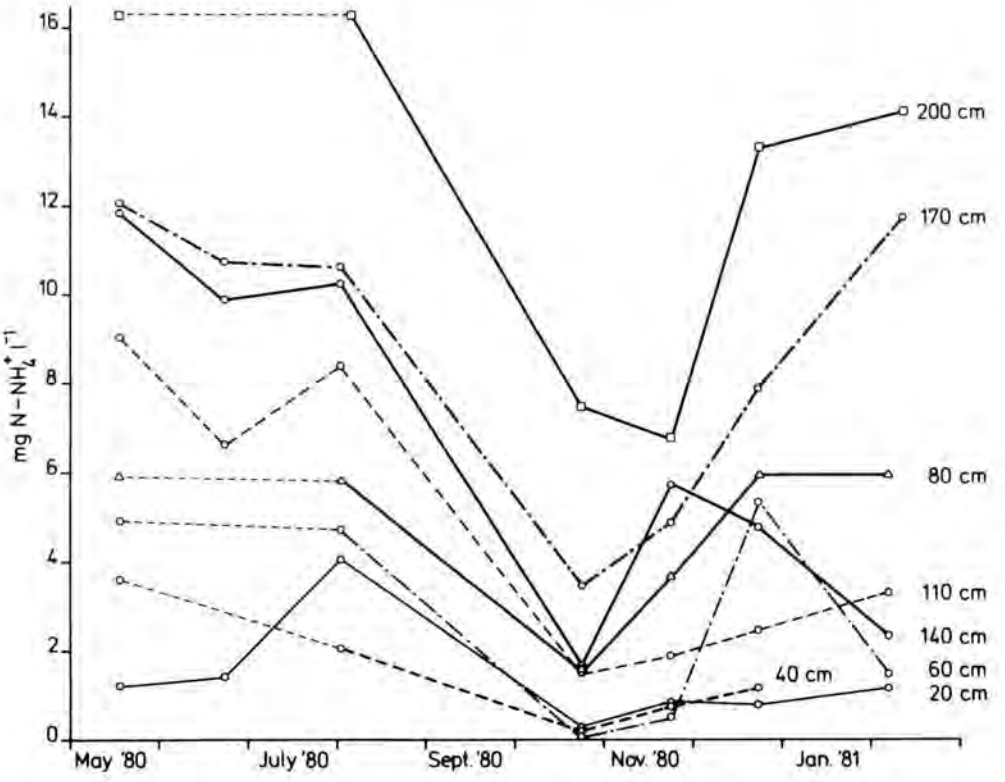

Fig. 36. Ammonia content of eight different depths in the intertidal area near the salt marsh Stroodorpepolder

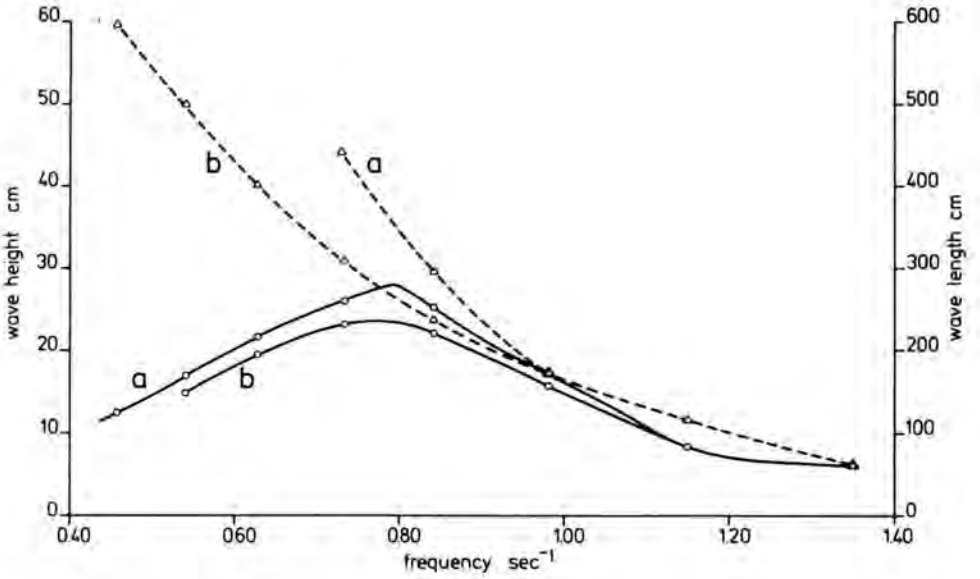

Fig. 37. Frequency dependent wave height (top-crest: ——) and wave length (-----) at 2 different stroke lenghts, a=32.5 and b=24 cm, in the flume at a waterdepth of 150 cm

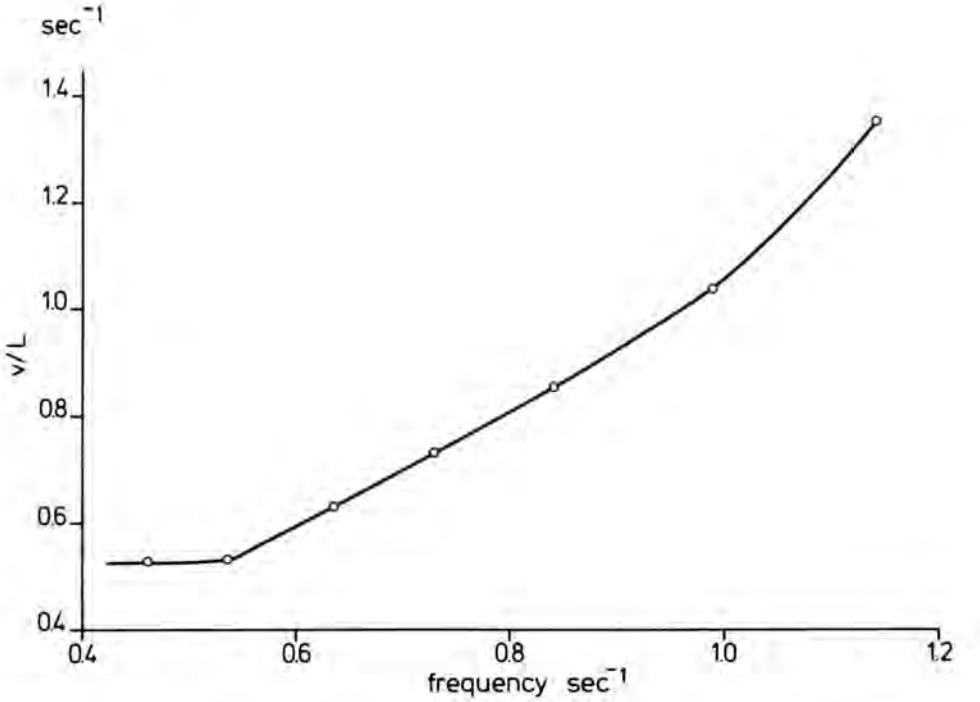

Fig. 38. Wave frequency dependence of wave velocity (v) over wavelength (L) in the flume at a waterdepth of 150cm and at a stroke length of 32.5 cm

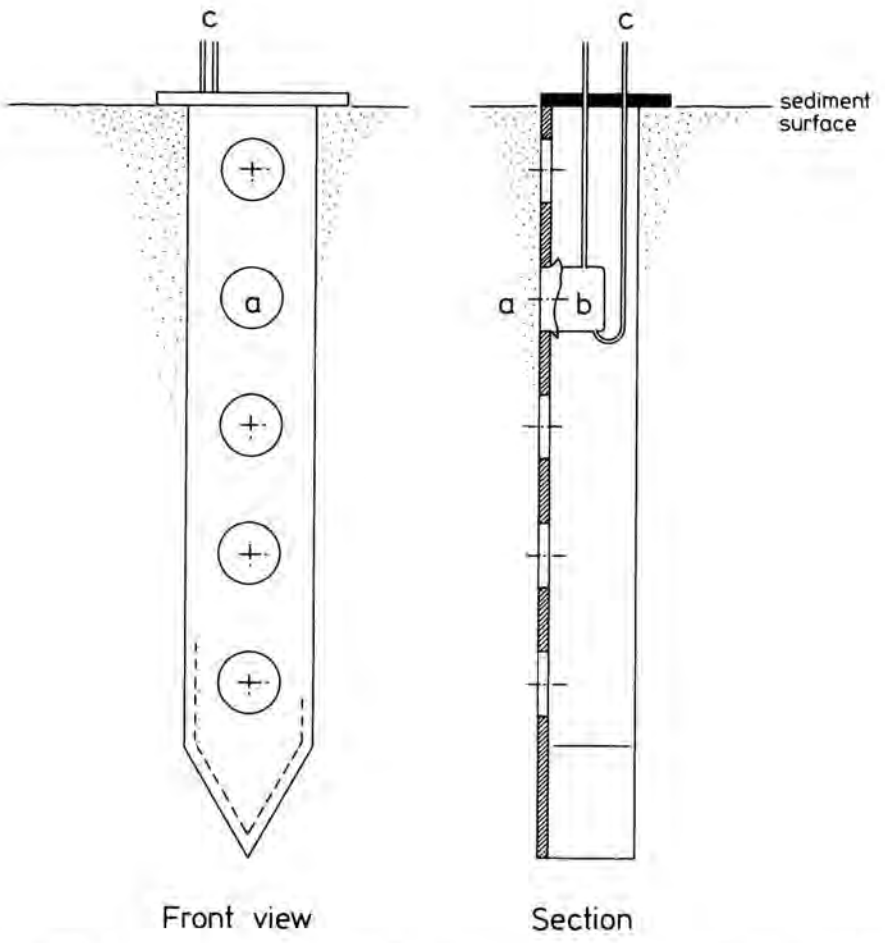

Fig. 39. Apparatus for interstitial water sampling. One sampling point (a) in the apparatus is shown. The sampling cup is covered by a dialysis membrane which allows nutrients to enter the sample cup. (b) The sample can be withdrawn via the tube (c) after which the cup is refilled with fresh seawater. One apparatus has 20 sampling points and reaches 30 cm in the sediment

at different frequencies of the paddle. The figure shows that with increasing frequency the wave height increases, but it decreases at higher frequencies. This is due to the steepness criterium which states: $\frac{H}{L} \leq 10$. If waveheight exceeds one tenth of the wave length, the wave will fall over and break. Two empirical equations are valid for waves in shallow seas: wave velocity (v) is inversely related to the frequency(ν), and wave length (L) is inversely related to the square of the frequency.

$v = 1.56 \frac{1}{\nu}$ This yields $\nu = \frac{v}{L}$

$L = 1.56 \frac{1}{\nu^2}$

Fig. 38 shows that this equation holds true in the system for the frequency range $0.60 > \nu > 0.95$ sec^{-1}. This frequency range will be used in experiments on sediment-water exchange to simulate natural conditions.

VIII.9. An interstitial water sampler for *in situ* sampling in intertidal areas (A11) (L. van Geldermalsen)

Much information can be obtained from a time series of nutrient concentrations in interstitial water at particular points in tidal flats. However, the values found when using the 'core-method' are unreliable. This is due both to the spatial differentiation

of nutrient concentrations and to changes in nutrient concentrations during the handling of the sample in the laboratory. Spatial differentiation of the so called 'patchiness' can be evaluated when using an interstitial water sampler at a fixed point in the sediment. The water is occasionally suctioned through a hollow pipe and analyzed. The disadvantage of this method is the induction of a ground water flow and therefore the disturbance of the natural equilibria of the interstitial water composition. A method often used is the semi permeable bag luried in the sediment. This does not disturb the ground water flow and is easily handled. The disadvantage is that it can only be used once.

Fig. 39 shows an apparatus which was designed to incorporate the good qualities of both methods. After testing the apparatus, the results were compared to those using the 'core' method, see Fig. 40.

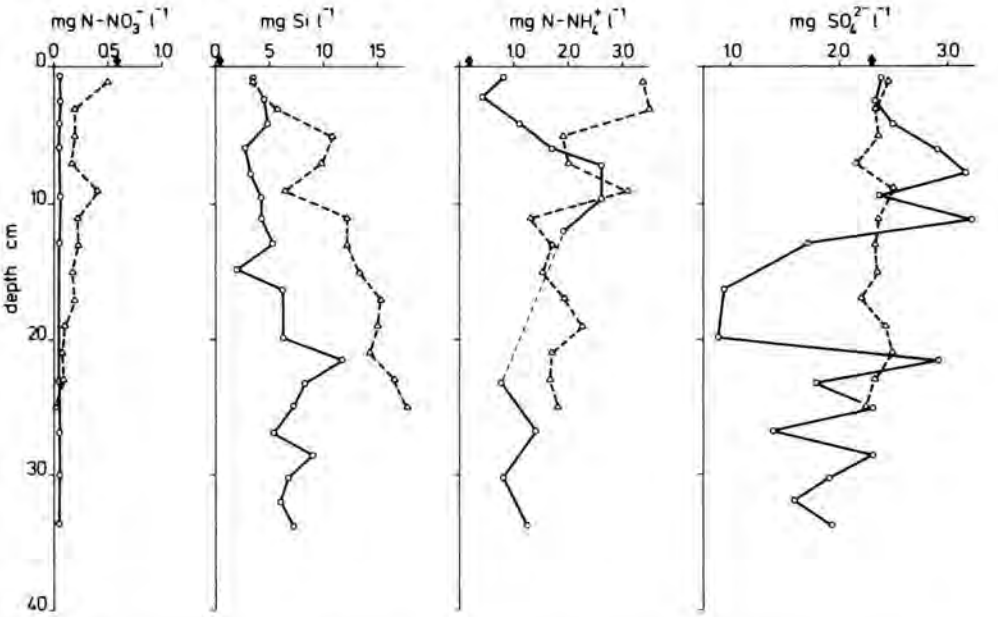

Fig. 40. Concentrations of four nutrients in the interstitial water of a tidal flat near the 'Hoek van Nieuwlande' in the Eastern Scheldt in November 1980. Two methods were used to sample interstitial water: the first method (o——o) employs the apparatus described in Fig. 39. In the second method cores were taken to the laboratory, frozen, and sawed in slices of 1 cm; the sediment was then defrosted and interstitial water was squeezed out through a 0.45 µm filter (Δ-----Δ). The samples were analyzed according to standard procedures. The arrows indicate the concentrations in the overflowing seawater

VIII.10. The book 'Changing Delta Waters' (R. Peelen)

The preparation of the 450 pages Deltaboek is progressing. From chapter 1 the lay-out is completed. Other chapters (until 6) are under various stages of editing, correction and illustration research.

IX. ARTICLES SUBMITTED FOR PUBLICATION OR IN PRESS

(Δ - 195 = publication number of the Delta Institute)

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Centraalbureau voor Schimmelcultures

Progress Report 1980

Edited by G.S. de Hoog

Oosterstraat 1, 3742 SK Baarn,
The Netherlands

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

CONTENTS

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 Dutch 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 1979 the total number of strains maintained was about 26.000, 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 and each year courses are given, e.g. on general and on human and animal mycology.

Scientific staff

(as from December 1st, 1980)

Dr. J.A. von Arx, director (general mycology, Ascomycetes, Melanconiales)
Dr. G.A. de Vries (human and animal mycology, Actinomycetes)
Dr. M.A.A. Schipper (Mucorales)
Drs. E.J. Hermanides-Nijhof (Fusarium, Aureobasidium)
Dr. H.A. van der Aa (Sphaeropsidales)
Dr. G.W. van Eijk (biochemistry)
Dr. W. Gams (Acremonium, Verticillium, Mortierella and other soil fungi)
Dr. R.A. Samson (Penicillium and related genera, entomogenous fungi)
Dr. G.S. de Hoog (Dematiaceae, yeast-like Hyphomycetes)
Dr. J.A. Stalpers (Basidiomycetes)
Dr. A.C.M. Weijman (chemotaxonomy)
Dr. C.A.N. van Oorschot (thallic Hyphomycetes)
Drs. W. Windig (pyrolysis-mass spectrometry)
Drs. R.P.W.M. Jacobs (Oomycetes)

Yeast division

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

Introduction

After a period of almost 20 years at the CBS, Mrs. A.J. van der Plaats-Niterink retired on May 1st, 1980. Her interest was directed toward the Oomycetes and she concentrated her research on the taxonomy and ecology of *Pythium* species, which are known to include important plant parasites. She finished a first draft of a *Pythium* monograph in the first half of the year and a key to the species a few months later. A list of 1130 references and citations was worked out by R.P.W.M. Jacobs; the text was edited by W. Gams and the illustrations were jointly prepared for publication.

The preparation of the 'Compendium of soil fungi' by K.H. Domsch, W. Gams and T.H. Anderson reached a final stage. The book was due to be published at the end of the year.

The book 'The resupinate non-poroid Aphyllophorales of the temperate northern hemisphere', by J.A. Stalpers and W. Jülich (Rijksherbarium, Leiden) appeared in December in the Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen.

Though still recovering from a serious illness, J.A. von Arx was able to complete the manuscript for the third edition of his 'Genera of Fungi sporulating in pure culture' by the end of June; the book is scheduled to appear early in 1981. The chapters on yeasts, most parts on Hyphomycetes and a new key to the Mucorales were rewritten during 1980. A number of new illustrations were added. W. Gams cooperated in the compilation of the list of more than 1100 references and helped with the preparation of the final draft.
The new edition includes the following taxonomic conclusions. In the yeasts two orders are distinguished, viz. the Endomycetales for the ascigerous yeasts and the Sporobolomycetales for the basidiomycetous yeasts.
The anamorphic taxa of the Endomycetales are brought together in the family Candidaceae. The family Endomycetaceae is accepted and delimited in accordance with Redhead & Malloch (Can. J. Bot. 55, 1701-1711. 1977), though with some restrictions, and now contains only taxa with galeate (hat- or helmet-shaped) ascospores.
The taxonomy of the 'black yeasts' is not yet settled and the specialists disagree about the delimitation of genera and species e.g. de Hoog - Stud. Mycol. 15. 1977; Ajello, Cole, McGinnis - PAHO, Sci. Publ. 356. 1978; McGinnis - Sabouraudia 17, 145-154. 1979; Carmichael *et al.* - Genera of Hyphomycetes. 1980; Gams and Samson in Domsch *et al.* - Comp. Soil Fungi. 1980. A number of CBS strains were reinvestigated and the genus *Fonsecaea* was reintroduced for two human-pathogenic species characterized by the formation of dark, catenate blastoconidia. The delimitation of *Ramichloridium* and *Rhinocladiella* is not yet clear. The genus *Exophiala* was accepted in the sense of McGinnis (1979, *l.c.*).

For the new fully revised edition of the Dutch handbook on cecidiology ('Het Gallenboek'), H.A. van der Aa wrote an introductory chapter on mycocecidia and enlarged on several descriptions and illustrations.

Contributions towards the theory of taxonomy were proposed in a paper by G.S. de Hoog. Three different, partly conflicting parts were distinguished, viz. (1) nominal representation of data, which may include numerical procedures; (2) the distinction of groups and their ordering, which implies logical reasoning on the basis of preestablished criteria, and (3) nomenclature, which has a more or less practical intention. In anticipation of any classification the structure of relationships has to be studied in more detail, particularly in little differentiated groups of microorganisms. Within this scope, the existence of the phenetic species was questioned.

Mycological taxonomy

OOMYCETES

R.P.W.M. Jacobs has been appointed to succeed Mrs. A.J. van der Plaats-Niterink and will also concentrate his research on the Oomycetes. In order to define the scope of future investigations and to familiarize himself with the Oomycetes, he initiated an inventory of the aquatic fungi of one of the Maarsseveen Lakes near Baarn.
Several genera were represented in the first isolates. For most of these isolates, particularly the *Pythium* and *Phytophthora* species, identification was impossible because no sexual structures were produced. The different sporangial characters, growth rates and colonies indicate that these isolates include several species.

At present Oomycete slant cultures are transferred every two months. In elaboration of the work done by Boesewinkel (Trans. Br. mycol. Soc. 66, 183-185. 1976), a number of trial cultures were stored in water in an attempt to minimize transfer. For short-term storage each fungus was allowed to colonize the entire surface of the agar slant and then the tube was filled with distilled water. First results indicate that this method allows transfer at 6 month intervals without loss of viability or change in morphology. For long-term storage blocks of agar cultures were transferred to small screwcap bottles filled with distilled water and left at room temperature. This technique may offer a promising alternative to the preservation of Oomycetes under paraffin oil.

ZYGOMYCETES

The study of the genus *Rhizopus* by M.A.A. Schipper was continued. The

investigation will include the strains present in the CBS collection, supplemented with cultures sent for identification and fresh isolates.

Most revisions of the genus attribute much significance to the occurrence of striations on the sporangiospores. However, doubt has arisen about the value of this character for species delimitation. *Rhizopus microsporus* has rhomboidal, striate sporangiospores, while *Rh. oligosporus* and *Rh. rhizopodiformis* are described as having subglose, smooth spores.

The type strains of *Rh. bovinus* and *Rh. pusillus*, both regarded as synonymous with *Rh. rhizopodiformis* by Scholer (Habilitationsschrift, 1970), as well as the CBS strains 289.71 (identified as *Rh. oligosporus*) and 258.79 (identified as *Rh. rhizopodiformis*), which appear to be characteristic representatives of their species, all produced zygospores on mating with *Rh. microsporus*. Mating experiments are being extended for further evidence of conspecificity. J.A. Stalpers has offered his assistance in reevaluating the significance of the above features with SEM.

The practical interest in the genus *Rhizopus* is increasing. Species of *Rhizopus* have been known for their fermenting activities. Today some are known to be a common cause of mucormycosis. Recent observations indicate that isolates from sorghum malt used for brewing beer in South Africa produce toxins (C.J. Rabie, Tygerberg, South Africa pers. comm.). The Mucorales strains received from Dr. Rabie for identification in view of suspected toxin production, also included some species of the genus *Mucor*.

A contribution to a chapter on potentially pathogenic Mucorales for the book 'The fungi pathogenic for humans and animals' (ed. D.H. Howard, Los Angeles) was completed by H. Scholer (Basel), E. Müller (Zürich), and M.A.A. Schipper.

An extensive study of mating reactions in *Mucor* species was carried out by M.A.A. Schipper and J.A. Stalpers. Several inexplicable 'aberrations' were encountered, which could be explained by the hypothesis of gene translocation ('jumping genes') developed for *Saccharomyces cerevisiae* (Hicks *et al.* - Nature, Lond. 282, 478-483. 1979) which allows switching of opposite mating types. Both mating types are supposed to be present on the same chromosome, although only one is expressed. The HO-gene (for homothallism) permits frequent switching while the ho-counterpart normally prevents this. The hypothesis offers an explanation for the fact that homothallism and heterothallism are not absolute conditions, and also explains observed heterothallic tendencies in homothallic strains and *vice versa*.

R.A. Samson and J.A. Stalpers presented a poster on SEM studies of asexual sporogenous structures in Zygomycetes at the seventh European Congress on Electron Microscopy in the Hague. Examples were given of the existing types of asexual sporogenesis in this group.

ASCOMYCETES AND THEIR ANAMORPHS

The study of species which have been described in *Phyllosticta* was continued by H.A. van der Aa. The greater part of the year was spent on the preparation of a manuscript on the excluded species, consisting of a check-list, augmented by commentary. About 2800 epithets will be treated in alphabetic order, each with the relevant bibliography, condensed data on typification, host range and material examined; poorly described taxa are briefly redescribed. About 50% of the species were studied from type and/or secondary collections, sometimes from pure cultures. Many of the remaining species can only be reidentified up to generic level, or are excluded from *Phyllosticta* on the basis of the description. Unexpected difficulties arose in obtaining the original diagnoses of certain species, especially of those described between 1920 and 1960.

Collection, isolation, lyophilized preservation and provisional description of new strains of *Coniothyrium sensu lato* was also continued. Special attention was paid to several strains of an undescribed species which was isolated from soil and plant debris collected by J.A. von Arx on Gran Canaria. The species is characterized by abundant production of greenish crystals, at present being investigated by G.W. van Eijk.

In further cooperation with the CBS chemical division, H.A. van der Aa also studied a strain of *Herpotrichia rhodosticta* which produces a characteristic yellowish or reddish pigment. This fungus was collected by W. Gams in Colombia;

in pure culture it only produces a *Pyrenochaeta* anamorph.

The formerly unknown *Aposphaeria* anamorph of *Melanomma sanguinarium* was obtained from ascospore isolations from material collected by R. Weiler (Ravensburg, F.R.G.) on dead apple branches.

W. Gams prepared a manuscript on a new genus, *Chaunopycnis*, which contains soil fungi with loosely knit conidial fructifications, intermediate between those of Coelomycetes and Hyphomycetes.

In 1979 the idea of publishing a multi-authored 'Compendium genericum Hyphomycetum' was launched by S.J. Hughes (Ottawa, Canada) and W. Gams will be one of the contributors. This work will include critical evaluations of the types of all known genera of Hyphomycetes, with accurate statements on the validity and circumscription of each genus. Full information on all taxonomic and ultrastructural studies on these genera will also be given. The authors hope to provide a numerical code for synoptic generic identification. W. Gams has revised his documentation for this purpose.

The monographic study of *Verticillium* and *Gliocladium* being conducted by W. Gams was strongly stimulated on receiving a number of isolates from dark spots on *Agaricus bitorquis* sent in by A. van Zaaijen (Proefstation voor de Champignoncultuur, Horst). These isolates are similar to *V. fungicola*, the common cause of the dry bubble disease of mushroom, but differ in their temperature maxima. Infection experiments were carried out with a number of isolates, including some from the CBS collection, which all produce white colonies with erect conidiophores and which were isolated from a variety of host fungi. Many of the strains were found to exhibit sharp temperature responses with maxima around 25°C, while species of the section *Prostata* have much higher temperature maxima. A revision of the white species with erect conidiophores will probably be completed within a few months.

A new genus of Hyphomycetes, *Pleurodesmospora*, parasitic on insects and rust fungi and formerly known as *Gonatorrhodiella coccorum*, was introduced in a joint paper by R.A. Samson, W. Gams and H.C. Evans (Kew, U.K.).
Further W. Gams prepared corrections of his paper on *Chloridium* and some other dematiaceous Hyphomycetes (Stud. Mycol. 13. 1976), and reported the finding of a new *Cladobotryum* species with penicillate conidiophores and retrogressive conidiogenesis in a polder forest in Eastern Flevoland.

Miss A.C. Stolk (retired) and R.A. Samson continued the revision of the ascomycetous genus *Eupenicillium*. Much attention was paid to the morphological structure of anamorphs and to the ascospores. Detailed comparisons of *Penicillium* anamorphs revealed that several *Eupenicillium* anamorphs are identical or similar to some monoverticillate Penicillia, *P. raistrickii*, and related species and species of the *P. restrictum* group. Ascospore ornamentation was examined by SEM to clarify the delimitation of related *Eupenicillium* spp.

In cooperation with D. Malloch (Toronto, Canada), R.A. Samson continued the revision of the cleistothecial Ascomycetes. The research in 1980 was focussed on the species of the *Aphanoascus* complex, and isolates described as *Aphanoascus* sensu Udagawa.

J.A. von Arx started a study of some Ascomycetes with arthroconidia e.g. those described in the genera *Monilia, Botryomonilia, Arthrographis, Oidiodendron* and *Geomyces*. Typical *Monilia* species represent anamorphs of Sclerotiniaceae and form blastoconidia with a broad base in acropetal chains.
The mature conidia are separated from each other at random by characteristic conjunctive structures. In *Monilia sitophila* (the red bread mould) and in other anamorphs of *Neurospora* species, the conidia are arthric and separate in usually basipetal succession. The anamorphs of *Neurospora* will therefore have to be classified in a separate genus, which is close to the *Arthrographis* anamorphs of *Pithoascus* and *Petriellidium* and to the *Oidiodendron* and *Geomyces* anamorphs of *Byssoascus* and *Pseudogymnoascus*.
Another characteristic of this group of fungi is the ascospores, which are 1-celled, fusiform, pigmented, striate or punctulate and often provided with two apical germ pores. The genera *Diplogelasinospora* with 2-celled, pitted ascospores and *Arthrographis*-like conidia, and *Faurelina* with striate ascospores and 2-celled arthroconidia are also related.

The revision of *Chrysosporium* and allied genera by C.A.N. van Oorschot appeared as Studies in Mycology no. 20. A new revision of the hyphomycetous

genera *Arthrobotrys*, *Dactylella*, *Dactylaria*, *Genicularia*, *Monacrosporium* and related genera was initiated. Herbarium and live material is being collected to augment the CBS collection. The fungi in this group mostly produce hyaline, 2- or more-celled conidia sympodially at one or more loci of a macronematous conidiophore. An overall monograph is required to erase the present apparent disagreement concerning the delimitation and conservation of the numerous genera which have been erected. The taxonomic value of determinate or proliferating conidiophores, non-differentiated, swollen or extended conidiogenous heads, presence or absence of denticles, enlarged apical or medial conidial cells, and conidial appendages will have to be evaluated. In addition, the vast number of species now attributed to this group require comparison and possible reclassification. Many of the species concerned attack nematodes and part of the study will involve the use of live bait to induce the development of trapping devices such as adhesive knobs, hyphal networks, and constricting rings.

Continuing the study of entomogenous fungi from South America, R.A. Samson submitted a paper in co-authorship with H.C. Evans (Kew, U.K.), on a new species of *Beauveria* and *B. amorpha* comb. nov.

A dematiaceous fungus isolated by K.J. Kwon Chung (Bethesda, U.S.A.), from a skin lesion on a cat was identified by G.A. de Vries as *Cladosporium bantianum*. This isolate was compared with four CBS cultures of *C. trichoides* and two of *C. bantianum*. It was concluded, that both species should be maintained separately. The observations made were conveyed to Dr. Kwon Chung in contribution to a paper on the rediscovery of *C. bantianum*.

In the anamorphic Mycosphaerellaceae, J.A. von Arx reintroduced *Heterosporium* (its synonymy with *Cladosporium* has never been accepted by plant pathologists) and *Fulvia* was again reduced to synonymy with *Cladosporium*. Additional superfluous names were found for *C. fulvum*, a parasite of tomato and other Solanaceae. The *Cercospora-Cercosporella* complex, which comprises more than 1200 fungi causing leaf-spots, has been attributed several additional generic names, some of which were recently described, e.g. *Ramulispora* Miura (1920), *Cercoseptoria* Petrak (1925), *Pseudocercosporella* Deighton (1973), *Thedgonia* Sutton (1973) and *Paracercospora* Deighton (1979). These genera are morphologically similar and can hardly be distinguished from *Cercosporella* and *Pseudocercospora*. *Isariopsis* and *Cercosporidium* should be reduced to synonyms of *Cercospora*.

Two strains of a new species of *Sporothrix*, both isolated from calf skin in Rumania, became available through O. Constantinescu (Bucharest). They are described in a joint article with G.S. de Hoog as *S. catenata*, a species belonging to the *fungorum* group of *Sporothrix*. The species is characterized by long conidial chains, and liberated conidia may produce budding cells.

Budding in yeast-like Hyphomycetes shows wide variations; the various processes were studied by G.S. de Hoog. Most *Sporothrix* species reproduce abundantly with almost no budding cells; apparently the yeast state is not an essential part of the anamorphic life cycle of these fungi. The budding cells are often variable and of irregular shape. In other species, such as several *Exophiala* species, most vigorous propagation is found in strains with budding cells. Here liberated propagules usually do not germinate, but repeatedly produce a succession of discrete daughter cells. Cultures in which this initial-stage budding is lost tend to show a decrease in conidiation. In a third group of yeast-like Hyphomycetes suspension plating results in two types of colonies; one which is mainly hyphal and another which shows mostly budding cells. The colonies become similar in a later stage, and single-spore isolations of each again give rise to the original two types of colonies. The occurrence of two phenotypes under identical environmental conditions was particularly clear in the newly described genus *Hyphozyma* de Hoog & M.Th. Smith.

All seven *Hyphozyma* strains were non-fermentative, utilized KNO_3 and ethylamine HCl as their sole source of nitrogen, grew at 37°C, but not in a vitamin-free medium. The diazonium blue B test was negative for all seven strains indicating the ascomycetous nature of the cultures. With respect to the growth capacity on 31 standard carbon compounds, the examined strains may be divided into three approximate groups. One strain was able to grow with 28 compounds as a sole carbon source. A second group (three strains) used ten compounds as carbon source and showed variable results with five additional carbohydrates.

The third group (including very mycelial cultures) utilized eleven compounds as carbon source, whereas growth results were variable with one additional carbohydrate.

Carbohydrate analyses, carried out by A.C.M. Weijman, support the erection of *Hyphozyma* in that the rare compound rhamnose was present in intact cell hydrolyzates of all strains. Rhamnose was absent from the morphologically similar *Phialophora hoffmannii*.

The taxonomic significance of the above elucidation of life cycles of anamorphic fungi was also demonstrated in *Trichosporiella sporotrichoides* by C.A.N. van Oorschot. The strains present in the CBS collection are indistinguishable in their hyphal states, but on suspension plating one appeared to have initial-stage, another late-stage budding, and a third no budding at all. In addition, pseudomycelial structures were found in shaken liquid cultures. The differences found were supported by the results of carbohydrate pattern analyses conducted by A.C.M. Weijman. The presence of xylose in some representatives clearly indicates a basidiomycetous relationship. Other strains show a more ascomycetous yeast-like pattern.

An isolate of a black yeast-like fungus from a case of human chromomycosis in Spain appeared to represent a hitherto undescribed genus. The isolate grows restrictedly in culture, meristematically forming clumps of obliquely septate cells. These clumps gradually fall apart into smaller entities which pass through a similar process of meristematic growth and disarticulation. G.S. de Hoog proposed the new term 'sarcinic' for this process and a new genus, *Botryomyces*, is being described to accommodate the fungus.

ENDOMYCETES AND BASIDIOMYCETOUS YEASTS

The revision of the genus *Dekkera* and its anamorph *Brettanomyces* was continued by M.Th. Smith. The guanine + cytosine percentages of *Brettanomyces abstinens*, *B. custersii* and the invalidly described species, *B. nanus* (Scheffers - Nature, Lond. 210, 533-534. 1966), were determined. The data on the range and the average plus standard deviation of the G+C percentages, as well as on the salient physiological characteristics of these species, are listed in Table 1.

A detailed morphological study was made to evaluate *Brettanomyces nanus*. Light-microscopic observations complemented with TEM studies demonstrated that in the cultures examined vegetative reproduction is bipolar and percurrent. In this respect they differ from the type strains of other *Brettanomyces* species, which show multilateral vegetative reproduction confined to apical (polar) zones. Since the mode of reproduction is considered an important generic feature, the three examined cultures could not be placed in the genus *Brettanomyces*. A manuscript giving a more adequate taxonomic classification of these cultures in a new genus has been completed.

L. Rodrigues de Miranda obtained ascospores in mating experiments with the CBS strains of *Candida ingens*. Comparison of morphology and assimilation and fermentation patterns of *C. ingens* with those of *Dipodascus ovetensis* showed that they are conspecific. *Dipodascus ovetensis* is homothallic whereas *Candida ingens* is heterothallic.

Mating experiments with other *Candida* strains gave the following results: Matings between compatible isolates of *Candida freyschussii* produced oval spores, 1-2 per ascus. *Candida iberica* strains resulted in independent cell conjugations on Difco malt agar, but no ascospores could be found. *Candida macedoniensis*, CBS 600, 2080 and 2081 gave conjugations and ascospores after mixing with *Kluyveromyces lactis*, CBS 2360; strains CBS 2079 did not show any sexual activity. *Candida kefyr*, CBS 834 and 1970 gave conjugations and ascospores after mixing with *Kluyveromyces lactis*, CBS 2360; strain 609 did not show any sexual activity.

The genus *Saccharomycopsis* was revised by J.A. von Arx in cooperation with J.P. van der Walt (Pretoria, South Africa). Only the type species *S. capsularis* is maintained in the genus; it is a soil-borne mycelial yeast with oblate, bi-valvate ascospores. *Saccharomycopsis fibuligera*, a common yeast on food, has galeate ascospores and the earlier name, *Endomyces fibuliger* is reintroduced. Two species isolated from fruit and fruit juices, with saturn-shaped, spiny ascospores

Table 1

Species	nr. of strains	% GC range	% GC averages±s.d.	Fermentation: glucose	galactose	sucrose	maltose	lactose	Growth on: glucose	galactose	sucrose	maltose	cellobiose	trehalose	lactose	raffinose	melezitose	soluble starch	D-xylose	L-rhamnose	glycerol	adonitol	mannitol	sorbitol	methyl-alpha-glucoside	NO_3
B. abstinens	1		38.85±0.27	+	+	+	-	-	+	+	+	+	+	v	-	-	-	-	-	-	+	-	-	-	+	+
B. custersii	1		39.46±0.27	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	+	+
B. nanus	3	40.2-41.4	40.86±0.43	+	+	-	-	-	+	+	-	-	+	+	v	-	-	-	-	-	+	-	+	+	+	-	-

and clavate conidia, were transferred to the genus *Endomycopsella*. For the teleomorph of the economically important, lipolytic yeast species *Candida lipolytica*, a new generic name, *Yarrowia* van der Walt & von Arx, is proposed. This genus is not directly related to any of the other genera of the Endomycetales.

R.A. Samson, J.A. Stalpers and A.C.M. Weijman elucidated the taxonomic position of *Filobasidiella arachnophila*. The species proved to be identical to *Aspergillus depauperatus* and closely resembles *Filobasidiella neoformans* and other basidiomycetous yeasts.

An evaluation of glucuronic acid and inositol assimilation capacities as criteria used to separate *Cryptococcus* from other anascosporogenous yeasts was continued by L. Rodrigues de Miranda.

The authentic material of the genus type species, *Cryptococcus mollis*, from the Leiden Herbarium, was investigated by light-microscopy as well as by TEM. The structure of the walls of most of the cells in this material is that of a basidiomycetous yeast, which makes it quite probable that *C. mollis* can be regarded as typical for the group of species now aggregrated in *Cryptococcus*.

A short publication was prepared by W. Gams and J.A. von Arx on the validation of the genus *Symbiotaphrina*. The comparison of Sporobolomycetales (red yeasts) with genera such as *Exobasidium*, *Microstroma*, *Protomyces*, *Kabatiella*, *Taphrina* and *Ustilago* was continued by J.A. von Arx and G.W. van Eijk in cooperation with J.P. van der Walt.

The present taxonomic delimitations of the genera *Sporobolomyces* and *Bullera* are regarded as questionable. A.C.M. Weijman and L. Rodrigues de Miranda examined all type strains of species of both genera by GLC analysis. The presence of xylose indicates a clear basidiomycete affinity among these yeasts. On the basis of xylose distribution and other criteria some taxonomic rearrangements need to be proposed.

In a project by G.W. van Eijk, G.S. de Hoog, M.Th. Smith, A.C.M. Weijman and W. Windig, a sample of red yeasts was studied from morphological, physiological and biochemical points of view. These fungi are known to be morphologically variable, largely due to flexible responses to changes in environmental conditions. G.S. de Hoog found several types of conidiation in most strains. In *Rhodosporidium* comparable states of propagation were usually remarkably similar. In most cases the upper part of the colony was mucous and consisted of discrete budding cells, the cells propagating by basipetal growth; sympodial complexes of cells were formed in the substratum. One of the mating types of *Rh. toruloides*, however, deviated fundamentally by not showing any percurrent growth. In several strains disarticulating hyphae and chlamydospores were observed. The difference between chlamydospores and teliospores in these fungi is not yet fully understood and is being studied in more detail. In most CBS cultures of species of *Sporobolomyces* sympodial conidiogenesis was predominant, although basipetal conidiogenesis usually also occurred. The two types were less markedly distinct than in *Rhodosporidium*. Often daughter cells were produced from such a broad base that their liberation could be referred to as arthric. The occurrence of ballistospores was also studied. Ballistospores and basipetal budding cells may disappear relatively easily in *Sporobolomyces*; they are most abundant in fresh isolates.

To examine the intra-strain reproducibility as well as inter-strain variability of physiological characteristics, growth experiments on 33 carbon sources were

performed by M.Th. Smith in three independent parallels of the red yeasts mentioned above. In intra-strain reproducibility, the percentage of compounds giving reproducible growth results varied from 81.8% to 100%. In inter-strain variability, after having taken into account the intra-strain variability, the percentage variable characteristics in the genus *Rhodosporidium* was 24.2% in one species and 45.4% in the second. In the genus *Sporobolomyces* these values were 39.3% and 48.4%.

G.W. van Eijk, A.C.M. Weijman and H.J. Roeymans investigated chemical characters of red yeasts, focussing on the analysis of volatiles, carbohydrates, fatty acids and carotenoid pigments. A distinct qualitative difference between *Rh. infirmo-miniatum* and *Rh. toruloides* was reflected in the volatile and carbohydrate profiles. In *Rh. infirmo-miniatum* mannan is insignificant, xylose and distinct volatiles being present, whereas *Rh. toruloides* is characterized by the dominance of mannan, volatiles being inconspicuous. Among the common compounds in the neutral fractions of both species, one substance was apparently specific for *Rh. infirmo-miniatum*, and can be used to discriminate between the two species. The data obtained support the view that *Rh. infirmo-miniatum* is related to *Filobasidium*.

Differences in chemotaxonomic characters were not detected between the two species of *Sporobolomyces*. Production of volatiles was not observed. Similar gas-chromatographic profiles were obtained when analyzing the sterol fractions. Ergosterol was the major sterol for all strains. The gas-chromatograms of the fatty acid methyl ester fractions also showed no qualitative differences. C18:1, C18:2 and C16:0 acids were the principal fatty acids. The UV-visible spectra of the yellow-orange extracts were similar to each other and resembled that of a mixture of β-carotene and torulene.

For taxonomic applications the methodology of pyrolysis-mass spectrometry (Py-MS) required improvement. Therefore W. Windig spent much time experimenting with factor analysis, such that pyrograms can be interpreted in terms of basic chemical components. The principle is outlined below.

When a component shows a different occurrence in various compared samples, this is reflected by differences in pyrolytic fragments and, consequently, by changes in a set of peaks in the respective series of pyrograms; these peaks are thus correlated.
This provides a means to determine which masses or parts thereof indicate a particular chemical component. Subspectra representing chemical components are then abstracted and quantified by means of factor analysis. The method was adapted to Py-MS of fungi and tested by means of a set of spectra of *Rhodosporidium toruloides*, *Saccharomyces cerevisiae* and *Filobasidium capsuligenum*. The chemical data obtained with this factor analysis technique agreed with published results.

An analysis of some strains of the genus *Rhodosporidium* showed that the cluster of *Rh. infirmo-miniatum* was homogeneous, but that of *Rh. toruloides* was heterogeneous, being divisible into two subclusters. The deviations were mainly based on differences in protein, pentose and deoxyhexose contents.

Strains of the genera *Sporobolomyces* and *Filobasidium* were also studied, with some strains of the genus *Rhodosporidium* being included as a reference. A close relationship was found between *Sporobolomyces* and *Rhodosporidium*. *S. roseus* was found to be homogeneous, but *S. pararoseus* was heterogeneous, mainly due to different protein and hexose contents.

In order to test the reproducibility of Py-MS data of yeasts, duplicate strains of *Rhodosporidium* were grown with two-week intervals and compared. Calculations of dissimilarity yielded comparable results, indicating that some of these chemical fingerprints are of taxonomic significance. Carbohydrate analysis for the taxonomy of fungi by A.C.M. Weijman was extended to about 610 strains for different projects. Together with G.W. van Eijk, he presented a poster entitled 'chemotaxonomic aspects of yeasts and yeast-like fungi' during the 12th international IUPAC Symposium on the chemistry of natural products.

R.A. Samson and J.A. Stalpers continued their scanning electron microscopy (SEM) studies on several groups of fungi. They displayed a poster on conidiogenesis of yeasts at the third international fungal spore symposium in Gwatt, Switzerland. In this study representative species of most genera were arranged according to the type of conidiogenesis.

BASIDIOMYCETES AND THEIR ANAMORPHS

J.A. Stalpers completed a study on diseases of grasses caused by pink Basidiomycetes in collaboration with W. Loerakker (Plantenziektenkundige Dienst, Wageningen).

Three species are involved: the causal agent of the Red Thread Disease, *Laetisaria fuciformis*, a clampless species with multinucleate cells, and two species with clamps and binucleate cells for which a new genus, *Viretomyces*, has been erected. The genus comprises the new species, *V. rhodopellis*, and a species known as *Galzinia rhodopellis*.

A fungus was found which overgrew and consequently killed fern prothallia in Finn-peat. Actual pathogenicity could not be established. The fungus, which has a conspicuous sclerotial state, was identified as *Athelia coprophila*. The identification of the sclerotial anamorph, which belongs in *Minimedusa*, necessitates a revision of the basidiomycetous *Sclerotium* species with regular sclerotia.

J.A. Stalpers and R.A. Samson started a revision of the genera *Antromycopsis*, *Sclerostilbum* and *Tilachlidiopsis*. The type species of these genera are anamorphs of Basidiomycetes and are congeneric, but most of the remaining species are not basidiomycetous. The revision of *Sporotrichum* will be finished in 1981. Most of the available type specimens have been studied. It has proved necessary to extend the study to include the genera *Ptychogaster* and *Ceriomyces* Corda, which both contain anamorphs of Basidiomycetes.

G.W. van Eijk and D.M.X. Donnelly (Dublin, Ireland) examined the crystalline material produced by several strains of *Heterobasidion annosum*. The compound ($C_{15}H_{16}O_3$) is found to be a new isocoumarin derivative. A closely related substance with a carbonyl instead of a methyl group was isolated by Dr. Donnelly from the carpophore. The structures of both compounds could be determined by synthesis of the isocoumarin carboxylic acid derivative. Isocoumarins of fungal origin isolated to date are biosynthesized via cyclization of a polyketide. However, the isocoumarins from *H. annosum* may arise via the farnesyl pathway as in fomannosin, the phytotoxic sesquiterpene of this organism.

The isolation of a sesquiterpene alcohol ($C_{15}H_{26}O$) from cultures of a *Stereum* species was described by G.W. van Eijk in CBS Progress Report 1976. A complete structure of this metabolite from *Xylobolus frustulatus* (= *Stereum frustulosum*) was not given. A comparison of the mass spectrum with those of other known $C_{15}H_{26}O$ sesquiterpenes in cooperation with A.P. Bruins (State University, Groningen) revealed that the compound was torreyol, a sesquiterpene with a marolane ring structure. The identification as torreyol agreed with the other physiochemical data obtained (NMR, IR and UV). A specific rotation $[a]_{289}^{25} = +99.99°$ (c = 0.6) was measured. To date (+) -torreyol of fungal origin has only been isolated from *Clitocybe illudens* (Nair and Anchel - Lloydia 36 : 106. 1973).

Mycology at the service of other sciences

ECOLOGY

An invitation to deliver some lectures on mycology and soil microbiology during a course at the 'Universidad Nacional' in Bogotá, gave W. Gams the opportunity to visit various areas in Cundinamarca, Colombia. Fungal collections were made from Páramo vegetation (above 3000m alt.), well-known and frequently visited by Colombian botanists, the Andes forests (between 2000 and 3000m alt.), which are particularly rich and still little explored and a tropical area (450-500 m alt.) with savannah vegetation. The latter is generally dry and less interesting for mycologists, but these areas interspersed with swamps of palms and other trees provide an El Dorado of microfungi. The 250 specimens were partly distributed to other specialists, and the remainder were studied by W. Gams who isolated a variety of microfungi, particularly *Acremonium* species.

G.A. de Vries collected 60 soil samples from Eastern and Southern Flevoland, and these were analyzed for the presence of keratinophilic fungi. The following species were isolated: *Arthroderma cuniculi* (anamorph: *Chrysosporium* sp.), *A. curreyi*, *A. tuberculatum* (anamorph: *Myceliophthora* sp.), *A. uncinatum* (anamorph: *Trichophyton ajelloi*), *Anixiopsis* sp. (anamorph: *Chrysosporium*

tropicum), *Nannizzia cajetani* (anamorph: *Microsporum cookei*), *Myceliophthora vellerea* and several as yet unidentified strains. The above-mentioned *Anixiopsis* species was already known from Southern Flevoland, a total number of three strains of this species now being known from the Flevoland polder. It will be described as a new species in the near future.

On invitation from the British Mycological Society, R.A. Samson presented a taxonomic review of the thermophilous fungi at a symposium on fungi in extreme environments at Birmingham (U.K.). The concept of a book on the thermophilous fungi, was discussed with M.T. Tansey (Bloomington, U.S.A.) during his visit in July. The study of an unknown thermophilous *Coprinus* species was continued. Extensive experiments showed that fruit-bodies only appear on dung extract agar with lupine stems at 45°C. Several *Chaetomium* species whose taxonomic position is doubtful were examined in partial cooperation with A. Carter (Toronto, Canada).

In a study with C.W. McCoy (Lake Alfred, Florida, U.S.A.), R.A. Samson continued the morphological examination of the mite parasite *Hirsutella thompsonii*. Several new strains received from R. Canbrera (Cuba) were included in these studies and the best sporulating strains were chosen for further experiments on mass production. A.J. van Winkelhoff (student, State University, Utrecht) investigated the sporulation and cultural characters of several mutants of a wild strain of *H. thompsonii*, obtained by UV irradiation. Another student of the same university, M. Rombach, studied the taxonomy of the genus *Hirsutella* and prepared new keys to the many taxa with R.A. Samson.

The ecology and taxonomy of various *Cordyceps* spp. on *Cephalotus atratus* and related ant species were investigated by R.A. Samson and H.C. Evans (Kew, U.K.). These *Cordyceps* species are characterized by the presence of *Hirsutella* and *Synnematium* anamorphs and have not yet been described. Extensive collections and observations of the ant populations revealed some interesting ecological aspects concerning these ant pathogens and their role in the natural control of the populations.

NUTRITION AND MYCOTOXINS

Spores of both healthy and virus-diseased fruit-bodies of the edible mushroom, *Agaricus bisporus*, were studied with the SEM by J.A. Stalpers and A. van Zaaijen (Proefstation voor de Champignoncultuur, Horst), in order to detect the morphological differences reported by Nair (J. Aust. Mushroom Grow. Assoc. 2, 22-24. 1976). No differences could be found.

Taxonomic descriptions and illustrations of about 100 common mould species were prepared by E.S. Hoekstra (guest worker) and R.A. Samson for a guide to food-borne fungi. The forthcoming book also includes contributions from various authors on isolation techniques, fungal growth, mycotoxins, fermentation by moulds preservatives and heat resistance. Much time was spent reviewing and editing the various manuscripts.

MEDICAL AND VETERINARY MYCOLOGY

The medical mycological division took part in a new research project on the significance of moulds in aspecific respiratory allergy. In October and November 37 petri plates exposed in different houses were qualitatively and quantitatively analyzed for moulds and Actinomycetes.

A technical assistant from the State University at Groningen was trained in the mycological technique and diagnosis required for this project. The project, which is subsidized by the 'Netherlands Astma Fonds' is carried out under supervision of H.J. Sluiter and K. de Vries (both of the State University, Groningen).

Together with G.T. Cole (Austin, U.S.A.), R.A. Samson prepared a chapter for 'The fungi pathogenic for humans and animals' (ed. D.H. Howard, Los Angeles), reviewing the various modes of conidiogenesis and sporangiospore formation in pathogenic microfungi. Various other CBS members have also contributed to this book (CBS Progress Report 1979).

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