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Netherlands Institute for Brain Research

Progress Report 1977

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The Netherlands

On the 1st of January 1977 the Netherlands Institute for Brain Research commenced operations as an institute of the Royal Academy of Arts and Sciences (KNAW). Although some aspects of the research undertaken are new to the Institute, its history goes back more than 70 years.

Historical background

At a meeting of the International Association of Academies held in Paris in 1901, the anatomist Wilhelm His proposed that research into the nervous system be placed on an international footing. This resulted in the formation in 1904 of the International Academies Committee for Brain Research, which set itself the task of "organizing a network of institutions throughout the civilised world, dedicated to the study of the structure and function of the central organ ...". The committee 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 also wished "... to introduce order into the chaos brought about by the gradually increasing confusion because of differences in nomenclature ...". They hoped to be able to persuade governments to establish Academy institutions for brain research. The first country in which their efforts were successful was the Netherlands.

In 1905, on the basis of a report drawn up by C. Winkler and L. Bolk, the KNAW approached the government with the request that a national Central Institute for Brain Research be set up in Amsterdam. Following the government consent, the municipality of Amsterdam provided a building and research material, and the government provided the Dfl. 10,000 required to equip a laboratory and an annual Dfl. 14,300 for salaries and other running costs. The Netherlands Central Institute for Brain Research opened on the 8th of June 1909; it was housed in a wing of the newly erected Department of Anatomy and Embryology of the Municipal University of Amsterdam, and was the first institute for pure research in the Netherlands.

The first director was Dr. C.U. Ariëns Kappers, who later combined this post with an "extraordinary" professorship at the University of Amsterdam. He gained international fame with his work in the comparative anatomy of the nervous system. A considerable number of the human and animal brains used in his research are essential for the understanding of the normal and pathological structure and function of the nervous system. That

his contention is still valid, is evident by the central place this approach occupies in the research program of the institute to-day. In 1923, to make the staff eligible for state pension, the Institute formally became a State Institution by Royal Decree; the supervision, however, was left in the hands of the KNAW.

Dr. Ariëns Kappers died in July 1946 and was succeeded by Dr. B. Brouwer, whose principal field of study was pathological anatomy. He had previously been an assistant director of the Institute, and was professor of neurology at the University of Amsterdam at the time of his appointment. A member of his staff, Dr. J. Drooglever-Fortuyn, who was later appointed professor of neurology at the State University of Groningen, introduced electrophysiology to the Institute's field of research. Professor Brouwer died in 1949. In response to a proposal put forward by the supervisory committee, the government agreed to a reorganization and expansion of the Institute designed to allow for the gradual setting up of a number of departments and thus for a multi-disciplinary approach to brain research. It was felt that in this way the original aims could be more readily achieved.

In 1952, Dr. S.T. Bok, professor of histology at the State University of Leiden, was appointed director. Shortly afterwards he was also appointed "extraordinary" professor of neurobiology at the University of Amsterdam. One of the pioneers of quantitative analysis of the cortex, Professor Bok, actively encouraged multi-disciplinary research at the Institute. He retired in 1962 and was succeeded by Dr. J. Ariëns Kappers, previously professor of anatomy and embryology at the State University of Groningen. The University of Amsterdam appointed him "extraordinary" professor of neuroanatomy. His 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. He was due to retire on the 1st of August 1975.

Recent history

When professor Ariëns Kappers tendered his resignation a year or so before his official date of retirement, a procedure was set in motion to appoint his successor. On the 26th of November 1974, the section of sciences of the Royal Academy of Arts and Sciences wrote the Ministry of the recommendation of the supervisory committee and the representatives of the Institute, proposing Dr. H.G.J.M. Kuijpers, professor of anatomy at the Erasmus University in Rotterdam, as the next director. However, on the day on which professor Ariëns Kappers was due to retire, his successor had still not been appointed because of discussions about building plans, guarantees for the budget and special research facilities for professor Kuijpers not being finished. The retiring director was asked by the Ministry to remain at his post for a further two months or for such shorter period as may be sufficient to appoint a successor.

On the 15th of August 1975, the director was unexpectedly and verbally informed by the secretary of the supervisory committee that the government had decided to close the Institute as from the 1st of January 1977. On the 27th of August 1975, the Director-General of Higher Education and Research, together with the head of the personnel department of the Ministry of Education and Science and members of the supervisory committee, conveyed to the staff of the Institute the government's decision (which apparently was taken without prior discussions with any of the many scientific bodies available for consultations) and a letter was read aloud to them from Dr. G. Klein, State Secretary for Education and Science, stating that financial problems forced the government to cease the activities of a number of medium-sized research-institutions. The reasons given for the closure of their own Institute were that it no longer fulfilled its original "central" function, that the staff turnover was too high, and that the Institute could be transformed into an effective one only if new premises were built, staff structure were changed and substantial additions were made to the equipment.

On that same day, the staff set up a committee which set out to do everything possible to have the decision reversed: a memorandum and a subsequent detailed report were submitted in which the validity of the reasons underlying the decision was contested. They were both sent to the parliamentary standing committee for education, science research and development, and a delegation from the Institute attended a hearing held by the committee on the 15th of December 1975. The Institute received a large number

of letters of support and declarations of solidarity with its effort to continue its research from universities, scientific institutions and individual researchers from all over the world. Dr. D.F. Swaab, a member of the staff, was appointed acting director by Dr. G. Klein on the 1st of November 1975. Representatives of the Institute and governing board of the Academy had several discussions with Dr. Klein and, after much effort, parliamentary support for the Institute was pledged in the form of an amendment deleting the term "liquidation" from the budget thereby enabling the Institute to carry on its activities in 1976, and in the form of a motion formulated to prevent a similar situation arising in the future. The form in which, and the extent to which the Institute could continue its research, was made dependent upon the findings of an evaluation committee consisting of professor H.B.G. Casimir (chairman), professor D. de Wied and professor J. Joosse.

The Casimir Committee's report was presented to the State Secretary in February 1976. It stated that various elements of the research conducted by the Institute was unquestionably of a very high standard, and recommended that the various lines of investigations be grouped under one central research-theme entitled "maturation and adaptation of the nervous system". This would not only be unique as far as the Netherlands was concerned, but would also be of considerable social relevance. The work was to be planned around five research-groups, composed of scientists with highly divergent backgrounds such as biologists, physicians, biochemists, physicists, engineers and psychologists. A committee called "Recon" was put in charge of carrying out the Casimir's Committee's recommendations and to study the possibilities of transfer of those researchers who could not be placed in the new formation. For the next five years, the formal status of the Institute, now named the "Netherlands Institute for Brain Research" will be that of an Academy Institute, as it was fifty years ago; the continuation of the research program itself has been guaranteed indefinitely beyond the five years mentioned above.

The members of Recon, appointed by Dr. Klein (viz. the two representatives of the Ministry Drs. J.A.M. Goemans and F.G.E. van Haersma Buma, the acting director of the Institute Dr. D.F. Swaab, the observer representing the professional bodies Drs. W.L. Bakhuis and the two scientific advisors Prof. Dr. D. de Wied and Prof. Dr. J. Joosse) were relieved of their duties upon completion of Recon's tasks on the 21st of June 1977.

A committee consisting of Prof. Dr. H.B.G. Casimir (chairman), Prof. Dr. R.A. Crone (representing the medical faculty of the University of Amsterdam), Prof. Dr. J. Joosse, Prof. Dr. A.H.M. Lohman, Dr. F.H. Lopes da Silva and Prof. Dr. D. de Wied convened several times in 1977 in the presence of the director of the KNAW (Mr. J.Th.A. Klarenbeek), the acting director of the Institute (Dr. D.F. Swaab) and his manager (K.E. de Roos) and initiated a selection procedure laid down for the appointment of a director of the Institute, who would also occupy the chair of neurobiology at the University of Amsterdam.

In accordance with the Casimir Committee's recommendations the central research theme is being investigated by five multidisciplinary research groups: I. Adaptability of the nervous system of adult organisms; II. Interaction of nerve cells and behavior during maturation; III. Interaction of the nervous system and hormones during maturation and adaptation; IV. Development and correctability of behavior patterns; V. Neurophysiological aspects of the maturation and adaptation of the nervous system.

The reorganization of the Institute had important consequences regarding the personnel situation and the research projects. Several projects could largely continue their original research aims within the framework of the present five groups, others had to initiate a new program within the central theme, while those who could not continue their work at the Institute, could be transferred, at least for the next five years, to various University Departments. The present progress report will thus reflect not only the results of the first year of reorientation around the central theme, but also the completion of various projects that will be discontinued. Apart from the research data obtained by the Institute's research groups, and their Servicing departments, assistants, guestworkers, students and apprentices, several other aspects of the Institute-members' research and teachings are mentioned in this report, such as the papers that were read, the seminars and lectures that were given, the working visits and conferences that were attended, the visitors, mainly from abroad, their seminars and the postgraduate activities (*e.g.* the Xth International Summerschool of Brain Research and a postgraduate course in neuroendocrinology). Concerning the equipment it is worth mentioning that the Institute's computer has been reconditioned in 1977, and approval was obtained from the Ministry to replace the old electronmicroscope in the years 1978-1979.

D.F. Swaab

GROUPS AND PARTICIPANTS

Director: vacancy (acting director: Dr. D.F. Swaab)

Manager: K.E. de Roos

I. Adaptability of the nervous system of adult organisms
(including histological technicum) (p. 9)

Dr. H.B.M. Uylings (leader)

Drs. K.C. Hodde

vacancy

Drs. W.A.M. Veltman

I. Mataw

B.M. Przybylski-Zweesaardt

A. de Ruyter

M.A.P. Vrielink-Van Dam (until September 1977)

II. Interaction of nerve cells and behavior during maturation
(including department of electron microscopy) (p. 15)

Dr. M.A. Corner (leader)

Dr. R.E. Baker

Dr. W.L. Bakhuis (until September 1977)

Drs. H. Bour

Dr. H.J. Romijn (head, dept. of electron microscopy)

M.T. Mud

A.Ph.J. Richters

P. Wolters

III. Interaction of the nervous system and hormones during
maturation and adaptation (p. 22)

Dr. D.F. Swaab (leader)

Dr. G.J. Boer

Dr. K. Boer

Drs. R.M. Buijs

Dr. J. Dogterom

Drs. F.W. van Leeuwen

J.W.L. van Buuren-Nolten

B. Fisser

C. de Raay

C.M.F. van Rheenen-Verberg

F.G.M. Snijdwint

IV. Development and correctibility of behavior (p. 38)

Dr. N.E. van de Poll (leader)

Dr. J.P.C. de Bruin

Drs. J.G. van Oyen

vacancy

T. Dijkstra

V.D.J. Noltén

V. Neurophysiological aspects of the maturation and adaptation of the nervous system (including computer department) (p. 49)

vacancy (leader)

Ir. K. Kuypers (acting leader, head computer department)

Drs. C.V. de Blécourt

vacancy

R.M. Nooy

vacancy

P. Bonnie (H.T.S.-volunteer)

Eschophysiology (p. 52)

Drs. B. Bermond

Drs. A.P. van der Meché

Monaminergic mechanisms in rat brain (p. 56)

Drs. M. van Wijk

Secretaries

S.W. Lust-Bosboom (until December 1977)

J. Sels

M.M. Smidt

J. van der Velden

Animal care

F. Harkema

R. Hofer

Library

Drs. C. Winkler

Electronical workshop (p. 58)

J. Overdijk

Mechanical workshop (p. 60)

A.W. Kamstra

E.W. Moes

Administration

J.H. Oudshoorn (head)

P.A.M. van der Poel

H. Sijsma

Drawing department

H. Stoffels

Photography department

A.T. Potjer (head)

T.C. Sypkens-Potjer

General technical service

J.C. de Jong (head)

L. Tibbertsma

Canteen

C. de Groot

Household service

H.H. Barbé-Scheermeyer

C. de Haas-Joele

J.N. Pals-Cappon

C. Salgado (until March 1977)

M.A. Scheermeyer-Beuker

M. de Vos-Harthoorn

I. ADAPTABILITY OF THE CENTRAL NERVOUS SYSTEM OF ADULT ORGANISMS

Participants

Dr. H.B.M. Uylings (biologist, 100%)

Drs. K.C. Hodde (M.D., 100%)

Drs. W.A.M. Veltman (physicist, 100%)

vacancy (neuroanatomist)

I. Mataw (technician, 100%; from September 1977)

B. Przybylski-Zweesaardt (technician, 66%)

M. Vrieling-Van Dam (technician, 100%; until September 1977)

A. de Ruyter (technician, 100%; from November 1977)

The research is directed towards the potency of "adult" brains for plasticity or recovery after retardation due to stunting factors (e.g. hormonal, nutritional or environmental) acting during development. "Normal" development, retardation in this development, and recovery potencies are investigated in several components of the central nervous system such as neurons, dendrites, synapses and glia.

This project is partly represented in the FUNGO workgroup "Development of Brain and Behavior".

Theme 1: Adaptability of pyramidal cell dendrites in the neo-cortex of adult rats

In rabbits indications were found that dendrites of pyramidal cortical neurons in adult mammals are still capable to form new branches. With regard to the current opinions on neuronal growth this finding was found of such a value that it has been examined more extensively in rats in collaboration with Prof. M.C. Diamond (University of California, Berkely, U.S.A.). This year the statistical analysis of these experiments has been completed.

An increase in the number of branches and in the length of terminal segments was found in the basal dendrites of visual cortex pyramidal cells of adult rats after exposure to standard, and even more after exposure to "enriched" (i.e. in a large cage, containing more rats and more toys) conditions. The analysis showed that the mode of this outgrowth in the adult rat is similar to that observed in the cortex of normal immature rats. Besides the results for the basal dendrites, oblique dendrites changed also in the same manner. It can be concluded that the entire dendritic tree of pyramidal neurons in adult rats has extended its surface, providing a new area for the establish-

ment of synaptic connections with other nerve cells. These results point to the possibility that, under certain conditions, a retarded outgrowth of dendrites of pyramidal neurons in development may be (partially) recovered in later life.

For the quantitative study of the branching patterns of neuronal processes in rats under different conditions, many measurements are necessary. In collaboration with J. Overdijk (electronic workshop), A. Kamstra (mechanical workshop), and K. Kuypers (computer department), a semi-automatic "3 Dimensional Dendrite (3 DD)" tracking system was developed and constructed, since such a system is not commercially available and the few existing digital systems were found to be not only too expensive but also unnecessarily complicated. The programming (K. Kuypers) for the 3 DD system has been completed this year. After extensive trial measurements and gauging of the system, the 3 DD system is now operational for the measurements of nerve cell processes in thick Golgi preparations (see also below, Theme 3). A paper describing this system (Overdijk *et al.*), is in preparation.

Theme 2: The influence of hormones on the development of the cortex

Influences of peptide hormones (*e.g.* vasopressin) on behavior have been reported frequently in the literature. Nothing is known, however, about the influence of vasopressin on the development of cortical neuronal organization. In collaboration with group III (Interaction between the nervous system and hormones during maturation and adaptation), the investigation of the development of the brain of Brattleboro rats was started. The first studies concern the development of body weight, wet and dry brain weight, and cortical and subcortical weight of Brattleboro rats heterozygous and homozygous for the recessive factor for diabetes insipidus. Furthermore, determinations are executed on the DNA, RNA, protein and lipid content of brain halves. The other brain halves are processed for Nissl and Golgi-Cox staining to visualize the neurons and dendrite patterns, respectively. Postnatal stadia selected for biochemical and anatomical analyses were day 12, 16, 24 and day 180. Before the quantitative analysis of number of neurons, dendritic patterns and synapses is started, the above-mentioned examinations and cortical thickness measurements will indicate the cortex areas to be examined in more detail.

In order to make counting of number of neurons possible, it is necessary to discriminate between neurons and (the different

types of) glial cells. This is performed in semi-thin and Nissl sections. For synapse counting, the E-PTA (ethanolic phosphotungstic acid) contrasting method will be used. In order to facilitate precise localization of the area under consideration, a method was devised for concurrent application of different tissue preparations. With a tissue chopper 200 μ m sections were made of fixed brains. From 3 adjacent sections, one was treated for routine EM, the second with E-PTA, and the third was Nissl stained for LM examination serving as a reference for the first and second section, enabling precise marking of the desired area. Semi-thin serial sections, both perpendicular and tangential to the pia have been made for orientation on cellular level, and the EM work is now in progress.

Before buying the Kontron MOP AM-02 system, also the various other systems for quantitative EM and 2-dimensional LM studies were tested. The MOP AM-02 will be connected on line with the Interdata computer.

Theme 3: The structural and functional development of the cortex

The postnatal development of non-pyramidal neurons in rat's visual cortex was measured by Dr. G.J. Parnavelas (University College, London) in Golgi Cox sections of day 4, 6, 8, 10, 12, 14, 16, 20, 24 and 90, made by himself. These measurements (+ 700 neurons) were obtained with the newly developed semi-automated "3 DD" system, and are stored on magnetic tape. The analysis of the data is now in progress.

Theme 4: Comparative anatomy of the brain stem (K.C. Hodde)

During a stay with Prof. S.O.E. Ebbeson (San Juan, Puerto Rico), the work on ascending spinal systems in the nurse shark, *Ginglymostoma cirratum* was completed. The ascending spinal systems were studied with the Nauta and Fink-Heimer techniques after hemi-sections of the spinal cord were made.

The distribution of the debris of degenerated ascending spinal fibers in the spinal cord was found to be restricted to the dorsal and lateral funiculi. Terminal degeneration was also observed in the gray substance. The dorsal funicular fibers are not segregated into a fasciculus gracilis and a fasciculus cuneatus, but form a single bundle issuing fibers to the gray substance of the spinal cord, the dorsal funicular nucleus (NFD), and the vestibular complex. Some dorsal funicular fibers also appear to contribute to the spinocerebellar tract. It is not possible to distinguish subdivisions in the

NFD as was already noted in amphibians, which suggests a common origin of the recipients of ascending dorsal column fibers.

The degenerated lateral funicular fibers are segregated into three fasciculi issuing fibers medially as they ascend through the brainstem. The largest target of these fibers, by far, is the reticular formation, but diffusely organized axons also reach 1) the gray matter of the spinal cord, 2) the dorsal motor nucleus of the vagus, 3) nucleus "A" of the medulla oblongata, 4) the central gray substance of the brainstem, 5) the granular layer of the cerebellar cortex, 6) the cerebellar nucleus, 7) the nucleus intercollicularis, 8) stratum profundum of the optic tectum, and 9) the dorsal thalamus.

The zone of spinal input in the thalamus does not overlap with the visual area, a finding comparable to those in other vertebrates, which provides strong evidence against Herrick's view that modality-specific thalamic nuclei evolved from a multimodal "nucleus sensitivus".

The results of this study, compared with the striking similar findings in other classes of vertebrates so far described, indicate that all vertebrate groups appear to have the same basic components of ascending spinal projections.

Theme 5: Angio-architecture and scanning EM

The method on Scanning Electron Microscopy of bloodvessel casts was completed.

It was confirmed unequivocally that simple morphological criteria can be applied to distinguish arteries from veins beyond any doubt. This observation also applied to light microscopy of sectioned material, stained with various methods, especially in differential interference microscopy of thick sections.

As an assay of the validity of these criteria the rat cochlea was revisited. Here capillary beds are well-known, and the effective filling of the vascular system with the casting medium was illustrated with the complete filling of all capillary beds. The existence of arterio-venous anastomoses, suggested in the literature, was established beyond any doubt.

One important conclusion from inspecting the vascular system in the rat brain was that the cortical blood supply in the cerebrum is very different from the cerebellar cortex supply, where the capillary network is constructed as closed loops with the main in- and outflow in the subcortical area. The cerebral supply has "open" loops with main in- and outflow to the menin-

ges.

In coöperation with A.Ph.J. Richter, a pilot study was done to apply combined techniques to tissue culture: phase-contrast microscopy, scanning and transmission EM. It turned out to be possible to consecutively locate a given cell while growing in the culture chamber, inspect the surface features in scanning EM, and have transmission EM images of that particular cell after re-embedding the critical-point dried specimen.

Papers read

- K.C. Hodde, A. Miodonski, C. Bakker and W.A.M. Veltman. Scanning Electron Microscopy of microcorrosion casts with special attention on arterio-venous differences and application to the rat's cochlea. Xth Symposium on SEM-IITRI, Chicago, March 1977.
- K.C. Hodde. Microcorrosion casts of rat brain vascular system in SEM. Hershey Medical School, Penn. State University, Hershey, May 1977.
- K.C. Hodde. Neurobiological application of SEM, New Jersey Medical School, New Jersey, June 1977.
- H.B.M. Uylings, K. Kuypers, W.A.M. Veltman and M.C. Diamond. Dendritic outgrowth in the visual cortex of adult rats induced by environmental conditions. 18e Federatieve Vergadering van Medisch-Biologische Verenigingen, Leiden, April 1977.
- H.B.M. Uylings, K. Kuypers and W.A.M. Veltman. Environmental influences on the neocortex in later life. 10th International Summer School of Brain Research, Amsterdam, July 1977.

Working visits, training meetings and attended conferences

- K.C. Hodde. Working visit to Dr. S.O.E. Ebbeson, Laboratoria de Neurologia, San Juan, Puerto Rico, March-June 1977; attended: Kongress Deutsche E.M. Gesellschaft, Münster, 5-9 September 1977; Conference and course on advanced SEM, Cambridge, 26-30 September 1977; Course on Transmission EM, University of Amsterdam, 17-27 October 1977.
- H.B.M. Uylings attended the 4th Intensive Study Program of Neurosciences Research Program (M.I.T.), Boulder, Colorado, June 20-July 1st. Working visits to Dr. G. Vrensen, I.O.I., Amsterdam.
- W.A.M. Veltman and K.C. Hodde. Working visit to Dr. H.J. Houthoff, University of Groningen, Dept. of Pathology, 25 November 1977.

Visitors from abroad

Prof. Dr. J.R. Wolff (Max-Planck-Institut, Göttingen, D.B.R.), March 1977; Drs. G.P. Wilkin and P.L. Woodhams (Medical Research Council, London, England), July 12, 1977; Dr. M. Berry (University of Birmingham, England), July 14, 1977; Dr. R.V. Stirling (National Institute of Medical Research, Dept. of Developmental Neurobiology, London, England), July 15, 1977; Dr. J. Zimmer (University of Aarhus, Denmark), July 15, 1977; Prof. Dr. M.R. Issidorides (University of Athens, Greece), September 22-23, 1977; P. McConnell (University of Birmingham, England), November 23 - 24, 1977; R. Buskirk (graduate fellow Rockefeller University, N.Y., U.S.A.), December 20, 1977.

Guest workers

Dr. J.G. Parnavelas (University College, London, England). 3-dimensional measurements of postnatal development of non-pyramidal neurons in rat's visual cortex. March and April, July 16-20, 1977.

Dr. A. Miodonski (Jagellonian University and Medical Academy, Krakow, Poland). Blood vessel injection and casting techniques and S.E.M. techniques. July 17 - August 8, 1977.

Miscellaneous

K.C. Hodde. Referee for Xth Symposium on SEM, IITRI, Chicago.
H.B.M. Uylings. Member organizing committee 10th International Summer School of Brain Research; received a fellowship of Neurosciences Research Program (M.I.T.) for the Fourth Intensive Study Program in Boulder, Colorado, U.S.A.; referee (1 x) Journal Neurocytology; co-editor of "Maturation of the Nervous System", Progress in Brain Research volume; leader of a FUNGO project.

II. INTERACTION OF THE NERVOUS SYSTEM AND BEHAVIOR IN DEVELOPMENT

Participants

M.A. Corner (physiologist, 100%)
R.E. Baker (embryologist, 100%)
W.L. Bakhuis (biologist, 100%, until October, 1977)
H.L.M.G. Bour (biologist, 100%, per September, 1977)
M. Mirmiran (M.D., 100%, guestworker, from September, 1977)
H.J. Romijn (biologist, 100%)
vacancy (per October, 1977)
M. Mud (technician, 80%)
A.Ph.J. Richter (technician, 100%)
P. Wolters (technician, 100%)

The work of the group this year has continued along two lines which have been reported in past progress reports. On the one hand, the problem of synaptogenesis has been pursued, especially with respect to factors which may determine the specificity of interconnections in the nervous system. On the other hand, further work has been done on spontaneous behavioral rhythms in early life, and the question of their physiological interpretation. Neither the circuitry underlying the rhythmicity of nervous activity, nor the role which it may play in the developing organism have as yet been elucidated.

A. The curious phenomenon of the misdirected wiping reflex, which develops in frogs subjected to the grafting of skin in an abnormal location prior to a certain larval stage, holds promise of shedding light on the selective formation of synapses during development. Such selectivity, of course, lies at the basis of the adaptive behavior patterns and potentials genetically programmed into each organism. Specifically, there is a possibility that sensory nerve fibers obtain information, needed to selectively make functional central connections, from the peripheral target tissues. Such a process would contrast with the selective outgrowth shown by various other classes of neurons in establishing contacts with their proper target cells. If one were able to locate those cutaneous neurons, within the sensory ganglia, which normally innervate the back, respectively the belly, it would become simply a matter of establishing whether or not their receptive fields have changed position under the influence of the altered peripheral tissue. This could be done in principle either by a morphological technique (uptake of a labelled substance by the peripheral endings and transport to the cell

soma) or by a physiological one (electrical recording of the impulses generated in neuronal somata following mechanical stimulation of the skin).

B. Hatching in birds marks a transitional period between pre- and postnatal life. Moreover, it bears a certain resemblance both behaviorally and electroencephalographically to the late embryonic period: large amplitude slow waves dominate the EEG, while variable trains of stereotyped generalized movement bursts occur against a background of low muscle tonus and intermittent twitching. This same EEG pattern is characteristic of the normal state of sleep after hatching, and the motor bursting (together with the EEG sleep pattern) can be caused to reappear by means of certain types of movement restraint. The physiological study of the hatch process can be expected, therefore, to shed light on the neurological mechanisms underlying sleep activity rhythms, and their relationship to the system responsible for pre- and postnatal behavior patterns.

(This project is represented in the FUNGO workgroup "Development of Brain and Behavior")

Theme 1: Cutaneous afferent projections of spinal ganglia in normal and skin-grafted frogs

The electrophysiological mapping experiments of thoracic sensory ganglia in *Discoglossus pictus* were completed this year. Control frogs showed essentially the same ganglion topography as had been found earlier in *Rana esculenta* (the European green frog): the ventral surface shows a predominance of belly over back units, especially in the caudal half, whereas the dorsal surface (especially in the rostral half) projects primarily to the animal's back. In addition, a statistically significant difference was found in both species studied between belly and flank cell distributions, with the former being concentrated in the lateral part of the ganglion, and the latter clustered more medially. This differential topography is inconsistent with the hypothesis that sensory neurons acquire their full specificity only after making contact with peripheral target tissues (the "end-organ modulation" hypothesis of Weiss and Sperry). We conclude, therefore, that spinal ganglion cells are already programmed prior to axonal outgrowth for innervation of specific regions of the frog's body.

Several plausible mechanisms for explaining, on the basis of a switch in the peripheral projections of specified populations

of afferent neurons, the misdirected behavioral responses which often develop in skin-rotated frogs were then put to the test. Peripheral searching of axons for the properly matching skin type could be discounted, since the receptive field of a given nerve trunk generally failed to invade areas from which the misdirected reflexes were elicited. Selective stabilization of (initially multiple) afferent collaterals was dismissed after we found that no multiple receptive fields could be recorded from sensory units in frogs having belly or back skin all around the body. Finally, the mechanism of degeneration-regeneration of the misconnected nerve fibers was disproved by our failure to find any difference between control and skin-rotated frogs with respect to the topography of belly and back cutaneous neurons in the spinal ganglia. This was established first by using electrophysiological recording techniques, and was subsequently confirmed by labelling (with cobalt) the cells projecting to the back via dorsal nerve trunks. Our general conclusion is that the original population of sensory neuroblasts may in fact not be functional in skin-grafted frogs, but rather has been replaced by a population which in normal development would have degenerated (as a result of improper matching with the available receptor organs). We are now trying to obtain evidence for such a possibility by means of autoradiographic identification of spinal ganglion cells "born" at different stages of development.

A series of experiments was also done employing electrical stimulation of the skin. Well-directed wiping reflexes could be consistently evoked from all parts of the body even by single shocks. Unit recordings established that only one impulse is elicited per shock in any given fiber, and that the arrival time of a nerve impulse in the ganglion varies greatly with the stimulus location within the receptive field. These facts make it inconceivable that positional information is being transmitted via the sensory nerves in the form of impulse patterns (as has been suggested by Székely and others). There is therefore no reason at present to doubt the adequacy of the conventional model of sensory encoding, based upon anatomically distinct input channels rather than upon coded messages, to explain positional information signalling in frog cutaneous reflexes.

The planned continuation of our work on the origins of afferent innervation patterns will shift its emphasis to the central nervous system (spinal cord and lower brainstem) and exploit the possibilities for experimental manipulations afforded by the cultivations of living tissues in vitro. Routine culture condi-

tions have been worked out this year for ganglion, spinal cord and medulla neurons, and attempts are currently under way to trace the sites of central afferent terminations and the overall process of synaptogenesis, using light and electronmicroscopic techniques. This is to be supplemented by electrophysiological mapping experiments once the setup now under construction becomes operational.

Theme 2: Spontaneous motility patterns during sleep in neonatal chicks and rats

The research in the control of hatching behavior was rounded off this year, and written up in the form of a doctoral dissertation (W.L. Bakhuis) and a 'hoofdvak werkstuk' (H. Bour). A number of unexpected new aspects could be added to our knowledge of the physiology of hatching in birds.

Two aspects of climax behavior can be distinguished: the stereotyped motility pattern of the behavioral unit, and the rhythmical occurrence of the behavior (the behavioral unit is repeated every 10 to 30 seconds). The stereotyped pattern only occurs when the freedom of movement of the animal is very restricted, whereas the rhythm occurs if the animal is prevented from leaving its place while, in addition, its possibilities for postural adjustment are limited. After the onset of climax, maintained external conditions (unbreakable egg and constant ambient temperature) will lead to response waning, possibly as a result of habituation, which is a function of time and of the degree of departure from the optimal incubation temperature. On the basis of behavioral and polygraphic criteria, and with the aid of certain drugs, it was shown that climax behavior occurs while the animal is either asleep or drowsy but, in contrast to what was expected, not during the phase of paradoxical sleep. Paradoxical sleep only occurs when the tendency for climax behavior has fallen to a low level.

Further work was continued on the infant rat as model for the ontogeny of sleep mechanisms in mammals. This year the emphasis was shifted to free-moving rather than restrained rat pups.

During early developmental stages after birth, rats show a high level of vigorous body-motility during sleep, which gradually declines during the first two weeks of neonatal life. The relationship between the occurrence of this spontaneous motility and different sleep-stages is now being investigated, using sleep-stage indicators such as eye-movements, muscle tonus

and the cortical electroencephalogram. Polygraphic recordings (EEG, EOG, EMG) of sleep-waking cycles have been made in free-moving rat pups of 2 to 26 days old, in the company of litter-mates. A very similar picture of periods with atonia, muscle twitching and REMs can be observed in young pups (of 2 to 12 days) as well as in older animals, where such periods are always characteristic of paradoxical sleep phases as defined according to EEG criteria. The consistency of this pattern in different ages is being investigated in order to establish comparable behavioral stages for the analysis of qualitative and quantitative aspects of spontaneous sleep-motility.

These baseline studies will provide the necessary basis for evaluating the effectiveness of our attempts to suppress paradoxical sleep (and paradoxical sleep-like periods) in neonatal rats during the first weeks of life. This line of investigation has been started with a pilot study of the usefulness of several different kinds of drugs (tri-cyclic antidepressants, catecholamine antagonists, MAO-inhibitors) for PS-suppression in young rats. So far, *o*-methyl Dopa and chlorimipramine have been proven to be effective in PS-inhibition in animals from 3 days of age onwards. The effect of PS-suppression during development on the adult animal will be examined anatomically and behaviorally, as well as physiologically.

Students

- H. Bour (biology, Gem. Universiteit, Amsterdam) "Relationship between hatching behavior and stage of vigilance in chicks".
S. de Boer (biology, Landbouw Hogeschool, Wageningen) "Polygraphic and behavioral studies of sleep in neonatal rats".

Doctoral dissertation

- W.L. Bakhuis (University of Groningen) "The Causal Organization of Climax (Hatching) Behaviour in the Domestic Fowl (*Gallus domesticus*)".

Papers read

- Baker, R.E. "Development of afferent innervation patterns".
10th Internat. Summer School of Brain Research, Amsterdam, July 1977.
Baker, R.E. "Studies on the mechanism of misdirected tactile reflexes in skin-grafted frogs". Symposium "Form-shaping movements in Neurogenesis", Uppsala, September 1977.
Baker, R.E. "Cutaneous afferent connections in normal and skin-rotated frogs", Anatomy Dept., Debrecen Medical University

- (Hungary), November 1977.
- Bour, H.L. "Hatching and vigilance in the neonatal chicken".
Animal Physiology Lab., Gem. Univ. Amsterdam, June 1977.
- Bour, H.L. "States of vigilance during hatching in chicks".
Ned. Ver. voor Fysiologie, December 1977: Neurophysiology
section meeting, Rotterdam.
- Corner, M.A. "Comparative studies of early behavior development".
Dept. of Biological Psychiatry, Univ. of Groningen, January
1977.
- Corner, M.A. "Experimental studies of afferent reflex develop-
ment in frogs". 5th Internat. Neurobiology Symp., Magdeburg
(DDR), May 1977.
- Corner, M.A. "Development of cutaneous afferent connections in
frogs". 27th Internat. Congr., Internat. Union Physiol. Sci.,
Paris, July 1977.
- Corner, M.A. "What mechanisms may underlie early spontaneous
motility in vertebrates?". 27th Internat. Congr., Internat.
Union Physiol. Sci., Paris, July 1977.
- Corner, M.A. "Motility rhythms in early life: comparative and
physiological considerations". 10th Internat. Summer School
of Brain Research, Amsterdam, July 1977.
- Romijn, H.J. "Electron microscopy of endocrine processes in the
pineal gland of the rabbit". Joint Dutch-Belgian Anatomy
Meeting, Hasselt, October 1977.

Foreign visitors (without seminars; for those who gave seminars
see list)

Dr. R.V. Stirling, Natl. Inst. for Medical Research (London).

Visits abroad

R.E. Baker to: Univ. of Uppsala (Prof. C.-O. Jacobson) September
1977; Debrecen Medical Univ. (Prof. G. Székely) October-Novem-
ber 1977; Oxford Univ. (Dr. Horder); and
Natl. Inst. for Medical Research, London (Dr. Gaze) November
1977.

M.A. Corner to: Academy of Medicine, Magdeburg, DDR (Prof.
Matthies) May 1977; and
Univ. of Uppsala (Prof. C.-O. Jacobson) September 1977.

Miscellaneous

R.E. Baker, organizing and editorial committee, 10th Internat.
Summer School of Brain Research, Amsterdam, July 1977; Fellow
of the Neth. Pure Research Organization (ZWO), at Debrecen
Medical Univ. (Hungary) October-November 1977.

M.A. Corner, advisory board of "Developmental Psychobiology" and of "Developmental Neuroscience"; invited discussant, Réunion de l'Assoc. des Neurobiologistes Ontogénétiques, Paris, May 1977; co-chairman of session on "Circadian Rhythms" at the 9th Internatl. Congress of EEG and Clinical Neurophysiology, Amsterdam, June 1977; organizing and editorial committee, 10th Internat. Summer School of Brain Research, Amsterdam, July 1977; coördinator guest-speakers program, Neth. Inst. for Brain Research; workgroup leader FUNGO Werkgemeenschap "Brain and Behavior Development".

III. INTERACTION BETWEEN THE NERVOUS SYSTEM AND HORMONES
DURING MATURATION AND ADAPTATION

Participants

Dr. D.F. Swaab (M.D., 100%)

Dr. G.J. Boer (biochemist, 100%)

Dr. K. Boer (M.D., 100%)

Drs. R.M. Buijs (biologist, 100%)

Dr. J. Dogterom (biochemist, 100%)

Drs. F.W. van Leeuwen (biologist, 100%)

Dr. P. Pevet (biologist, 100%; University of Amsterdam)

Drs. M. Visser (gynaecologist, guestworker since October 1977, 66%)

J.W.L. van Buuren-Nolten (technician, 50%)

B. Fisser (student-technician, 100%)

C. de Raay (technician, FUNGO, 100%; since July 1977)

C.M.F. van Rheenen-Verberg (technician, 80%)

F.G.M. Snijdwint (technician, FUNGO, 100%)

In this project the production and secretion of hormones by nerve cells (neurosecretion) and their action on the various target organs, including the brain itself are studied. Neuroendocrine mechanisms are involved, *e.g.* in diuresis, reproduction, brain development, behavior and diseases like diabetes insipidus, toxemia of pregnancy, disturbances of lactation, parturition and mental disturbances. Emphasis is laid (1) on fundamental aspects of neurosecretion (*e.g.* on the sites of hormone production and the mechanisms of hormone release), (2) on neuroendocrine systems in maturation (also of the brain itself) and parturition, and (3) the role of neuroendocrine systems in learning and behavior. The main disciplines included in this project are: immunocytochemical localization and radioimmunoassay techniques, microchemistry, electrophysiology, electronmicroscopy and clinical observations which are mainly performed in collaboration with various university clinics in Amsterdam.

The project is represented in the FUNGO projects nrs. 13-35-07, 13-35-04 and 13-35-10.

Theme 1: The sites of production, transport and release of vasopressin, oxytocin and related hormones

Various hypophysial and hypothalamic hormones among which vasopressin and oxytocin, influence behavioral processes. Transport of these peptide hormones from the hypothalamic cell bodies

to the brain regions where they affect behavior could occur via the liquor or, more probably, by direct transport via peptidergic fibers.

To study these possibilities, vasopressin and oxytocin pathways are specifically localized by immunohistochemical techniques using the unlabelled antibody enzyme method and purification of the first antiserum. It was found that vasopressin containing fibers of the suprachiasmatic nucleus (SCN) fan out into all directions and reach the organum vasculosum lamina terminalis, nucleus periventricularis and probably the lateral septum and median eminence. In the paraventricular (PVN) and supraoptic nucleus (SON), bipolar and multipolar cells were frequently observed with fibers projecting to the median eminence as well as to extrahypothalamic areas. Fibers from the PVN and probably from the SON reach the septum, hippocampus and stria terminalis. Via the stria terminalis, fibers reach the medial amygdala and hippocampus where oxytocin and vasopressin containing fibers could be visualized. From the PVN many oxytocin containing fibers reach the stria medullaris. Via the nucleus periventricularis and stria medullaris, fibers reach the lateral habenula where vasopressin but also oxytocin containing fibers could be demonstrated. From the lateral habenula, vasopressin and oxytocin fibers reach the longitudinal fasciculus. In addition, fibers could be demonstrated in the pineal stalk and in the border of the subcommissural organ. Fibers from the PVN and SON extend as far as the medulla oblongata where fibers under the area postrema could be seen. Punctuate perineural structures in the lateral habenula seem to indicate that on these places the hormones are released to exert an influence on brain function. This is in good agreement with the observations that lesions in these areas caused behavioral deficits that were impervious to vasopressin treatment. Thus, vasopressin and oxytocin could act as peptide neurotransmitters, which might be the basis of the behavioral effects.

The sites of production (SON, PVN, SCN) and release were in addition studied by means of immuno-electronmicroscopy. Using neurohypophysial tissue a study on the suitability of a number of fixatives, buffers, embedding media and a comparison between the two-step method and the unlabelled antibody enzyme method were completed. The optimal conditions found in this study enable sensitive and specific immunocytochemical localization of the hormones at the electronmicroscopical level. To bridge the gap between the light- and electronmicroscopy, a procedure was developed using semi-thin epon sections. After light microscopical orientation in the immunocytochemically stained

sections, a particular area can be selected for immuno-electron-microscopy from the same specimen. It was found that the hormones in the SON were present in granules of 140 nm, while vasopressin in the SCN was found in granules of about 95 nm. The PVN is presently under investigation. This finding might enable to define the source (SON, PVN *versus* SCN) of the neurohypophysial hormones that were found in the various brain areas (see before).

By means of radioimmunoassay (see below) vasopressin and oxytocin were demonstrated in the septum, amygdala, hippocampus, parafascicular nucleus, choroid plexus, organum vasculosum of the lamina terminalis and the subcommissural organ. These hormones could not be detected in the rat cerebellum, cortex and pineal gland. Pilot studies are in progress to examine whether the level of the neurohypophysial hormones in the brain area mentioned above correlates with behavioral parameters in the passive avoidance test (in collaboration with group IV).

The observation of extrahypothalamic hormone containing fibers raised the question whether the same cells would send their fibers to the neurohypophysis and to extrahypothalamic brain areas and whether hormone is indeed released in these areas. Electrophysiological techniques are used for this purpose, the more so since the capability of conducting action potentials is thought to be a prerequisite for hormone release. Until now, however, only a few PVN cells which were antidromically identified from the neurohypophysis, could also be stimulated from the stria terminalis. Even less cells showed a positive collision test, indicating spontaneous action potentials to be conducted from the PVN to the stria terminalis. Although, in principle, action potentials from PVN cells running into extrahypothalamic structures can be shown this way, "electroanatomy" thus still seems to be a very elaborative technique.

In order to correlate the different firing patterns of neurosecretory cells to their possibly different hormonal content, it is tried to combine electrophysiological and immuno-histochemical localization techniques. HRP, injected into the PVN area was found to be taken up by nerve cells and fibers. It will be attempted to label this way the neurosecretory cell that was recorded from and stain subsequently its hormonal content. In order to facilitate the label technique, a ventral approach to the HNS was learned. It is hoped that in this way electrophysiological phenomena can be correlated to the structure and hormonal nature of HNS cells and to enrafle also existing ques-

tions on recurrent and collateral inhibition and excitation. For the HRP and ventral approach techniques K. Boer visited the physiological laboratory of Prof. J.J. Dreifuss (Geneva).

Theme 2: Radioimmunoassay

Specific and sensitive assays are essential for many aspects of this project. Radioimmunoassays for vasopressin, oxytocin, vasotocin and α -MSH (developed in collaboration with Dr. A.J. Thody, New Castle, U.K., who obtained a ZWO-fellowship) are therefore running in our institute. Problems with extraction of these hormones from body fluids were solved. The detection limit for extracted samples is 1-2 pg and the specificity is well defined. For the many results obtained with these assays, the reader is referred to the various themes and the literature references. In addition, samples of many other laboratories and clinics are assayed; Pharmacology, V.U., Amsterdam; Rudolf Magnus Institute, Utrecht; Veterinary Hospital, Utrecht) St. Franciscus Hospital, Rotterdam, and Dept. of Obstetrics and Gynaecology, Univ. of Amsterdam). In collaboration with the latter clinic, an extensive study is running on the possible involvement of neurohypophysial hormones in the ethiology of toxæmia of pregnancy. The presence of vasopressin in human cerebrospinal fluid was confirmed in samples which were kindly donated by Dr. J. Vos (Amsterdam Univ. Clinic, Dept. of Neurology). In addition, collaborative radioimmunoassay studies are performed together with Dr. A. Tixier-Vidal (Paris), Dr. A. Reinharz (Geneva) and Dr. B.T. Pickering (Bristol).

Theme 3: Neurohypophysial hormones in development

Fetal endocrine systems might produce hormones that are different from those operating in adult organisms. It is *e.g.* thought that the fetal brain would make arginine-vasotocin instead of vasopressin and oxytocin and that vasotocin would remain in some adult brain structures (the pineal and subcommissural organ). Therefore, antibodies were raised against arginine-vasotocin (kindly donated by Drs. H. Greven, Organon) and a sensitive and specific radioimmunoassay was developed. The presence of arginine-vasotocin in mammalian material could, however, not be confirmed. It was not found in the rat pineal gland or subcommissural organ, in the fetal sheep or rat pituitary, in human amniotic fluid (20 weeks of pregnancy) or maternal and fetal blood at term. The material found in the subcommissural organ of young rabbits seems to be caused by cross reaction of

the large amount of vasopressin.

Neurohypophysial hormones have important effects on brain function as appears from their effects on behavior. Whether or not they may also be involved in brain development is not known. A pilot study is therefore performed in collaboration with group I on the brain development of rats, being homo- or heterozygous for diabetes insipidus. The homozygous rats showed retarded body growth, a reduced brain weight, DNA and protein content of the brain. Dendritic outgrowth in the cortex is currently determined (see group I).

Theme 4: Neurohypophysial hormones in reproduction

In order to establish the role of oxytocin in the physiology of parturition, three experiments have been performed that follow the previous work, in which it was demonstrated by means of electrical stimulation of the pituitary stalk, that the hypothalamo-neurohypophysial system (HNS) has the potency to induce labour and to accelerate its course. Moreover, a chronically increased release of neurohypophysial hormones before and during parturition was indicated on the basis of the mean firing rate of antidromically identified paraventricular neurones, that was higher during these stages than during the oestrous cycle. In the first experiment it was tested with continuous 6 Hz pituitary stalk stimulation, whether a small increase in firing frequency of HNS-neurones, like that recorded during parturition, results into an increased neurohypophysial hormone release. Oxytocin plasma levels, as measured by radioimmunoassay, indeed increased gradually during the 30 min stimulation period and declined afterwards. The physiological significance of this increase was demonstrated subsequently in one-day post-partum rats by the registration of uterine contractions, that were elicited by the same stimulation frequency after a delay of 6-9 min during the first stimulation period or sooner during the next ones. During the half hour non-stimulation intervals, uterine contractions disappeared or decreased. In the second experiment it was investigated, by measuring oxytocin plasma levels in the course of gestation, whether the release of neurohypophysial hormones already increased before the expulsion of pups, as appeared from the electrophysiological studies. This would be in contrast with the generally accepted idea that these hormones are increasingly released only during labour. Yet, oxy-

tocin plasma levels appeared to increase gradually from the onset of gestation on, and to rise more dramatically during the last day(s); they did not drop to basal levels immediately post partum.

In the third experiment the physiological effect of elevated oxytocin plasma levels on the initiation and the course of parturition was studied in homozygous Brattleboro rats, displaying diabetes insipidus (d.i.), that do not produce vasopressin, but have elevated oxytocin plasma levels, and for control in heterozygous and back-crossed homozygous dominant Brattleboro's, that do not display d.i.. D.i. rats started earlier to deliver than their controls did. Furthermore, the distribution of onsets of parturition in d.i. rats did not show the characteristic peak-like clustering around the median onset and was not obviously depressed during the dark period like in their controls or in Wistar rats. As far as the course of delivery is concerned, d.i. and heterozygous Brattleboro rats deliver at a faster rate than the homozygous dominant controls.

Concluding, it can be stated that these and previous experiments show that oxytocin is involved in the initiation and the course of spontaneous delivery of the rat.

Theme 5: α -MSH and related compounds

In human and rat fetuses, intrauterine growth was found to depend on the fetal brain and pituitary. Rat experiments revealed that the intrauterine growth acceleration on day 19 of pregnancy was induced by endogenous fetal α -MSH. In order to see whether α -MSH could be involved in intrauterine growth also in the human, its presence had first to be established. Therefore specific immuno-histochemical localization techniques were developed for α -MSH and ACTH. Immunofluorescence studies showed that in the human pituitary, α -MSH was present not only in the intermediate lobe of the fetus, but also in the "intermediate zone" of the pituitary of children and adults. In the course of development the ratio of α -MSH to ACTH-containing cells changes, however, in favor of the ACTH cells. Whether or not the same holds true for the circulating levels of these hormones is now under investigation.

Because of the stimulating action of α -MSH on fetal body growth, a pilot study has been performed on its action on brain development. Antibodies against α -MSH were injected s.c. directly into the fetus at day 19 of pregnancy. At day 21 of pregnancy not only fetal body weight appeared to be decreased but also

fetal brain weight, protein and lipids. Total DNA was not affected. α -MSH might thus be involved in nerve cell differentiation rather than in multiplication.

By means of immunohistochemical localization techniques, α -MSH-like compounds were found also to be present throughout the adult nervous system: (1) in the cerebrum mainly in association with myelinated structures, in the choroid plexus and pineal gland, (2) in the cerebellar basket cell fibers, (3) in neurons of the medulla oblongata, (4) in nerve fibers and motoneurons in the spinal cord, and (5) in neurites and cell bodies of the dorsal root ganglion cells. No staining was obtained with antibodies against ACTH 1-39 or 1-24. The α -MSH-like compounds are localized predominantly in neurons, and are probably synthesized within the nervous system. This idea is supported by immunoelectronmicroscopy, which revealed the presence of these compounds in the endoplasmatic reticulum of dorsal root ganglion cells.

Theme 6: Specificity in immunohistochemical localization techniques

In order to enable specific localization of hormones, characterization and quantification of cross reaction is necessary. Immunofluorescence techniques have been developed earlier for this purpose. For immunoperoxidase, quantitative procedures are in development in collaboration with C.W. Pool (Dept. of Zoology, Univ. of Amsterdam).

In tissue sections also unknown related peptides can be stained (see e.g. α -MSH-like compounds: are they different from α -MSH?). Therefore a procedure has to be developed by which the nature of the stained peptides could be identified. Isoelectric focussing of material from the nervous system on slab gels and staining with antibodies afterwards, was regarded as the most promising approach. Gel sections can be processed in immunocytochemistry similar to tissue sections which would enable characterization of the compounds stained in the tissue.

Equipment in which micro slab gels of 1.5 mm thickness could be electrophoresed at 5 °C was constructed for this purpose. Equilibrium electrofocussing of synthetic peptide hormones could be reached within 1 hour using a 7% polyacrylamide gel containing 4% Ampholine pH 3.5-10 and 12% sucrose at a voltage of up to 750 V. Radioactive ¹²⁵I-labelled oxytocin having an isoelectric point of pH 6.9 was used as a marker. Subsequently the pH-gradient was measured using a surface pH electrode where-

after the gel was frozen in Freon-12 for cryostat sectioning. A series of fixation procedures was tested, using ^{125}I -oxytocin as marker. However, whenever a water immersion solution of fixatives (aldehydes, carbodiimides, triazines) was used the oxytocin was washed out immediately. Only vapour fixation at 25 to 80 °C with aldehydes appeared to be successful in insolubilizing the hormone using paraformaldehyde. Up to 80% of the hormone remained in the polyacrylamide gel sections. The same recovery was reached by immersion for 2-4 hours in 2% formaldehyde dissolved in acetone. The subsequent immunohistochemical staining procedure as applied on tissue sections is now worked out.

Theme 7: Pineal hormones (Dr. P. Pevet and Prof. J. Ariëns Kappers (advisor)).

Mammals have evolved neuroendocrine mechanisms which ensure under natural conditions that their young are born during a restricted period of the year, permitting so their survival. The pineal is an intermediary between the environment and such reproductive processes. In this framework the pineal gland of mammals living under natural conditions was studied. The ultrastructure of the pinealocytes of some mammals such as bat, hedgehog, mole, mole-rat has been studied during the different parts of their annual sexual cycle. From the ultrastructural observations it can be concluded that at least two different types of secretory processes involved in the synthesis of peptidic/proteinaceous compounds, are present in mammalian pinealocytes: one is characterized by the production of granular vesicles by the sacculus of the Golgi apparatus, while the other is characterized by the presence of material originating directly from the cisterns of the granular endoplasmic reticulum. In our opinion this process is involved in the production of active pineal principles showing an antigonadotropic effect. In those species in which two populations of pinealocytes are present both secretory processes are specific of a population. In species showing only one population of pinealocytes (mole, hedgehog, rat) both types of secretory processes occur. In this case, however, we do not know whether each cell can show both processes or whether some are functionally different. In each of the studied naturally blind mammals (the mole, the mole rat and the golden-mole) one of these two secretory processes is hyperdeveloped.

This investigation was performed in collaboration with M.A.

Kuyper (R.S. Africa), Prof. Dr. E. Nevo (Israel), Dr. P.A. Racey (Scotland), Dr. M. Saboureau (France) and Dr. A.M. Voûte (Utrecht).

The pineal of various non-mammalian vertebrates contains functional photoreceptor elements which do not occur in adult mammals. Studies on the phylogenetic development of the pineal from a photoreceptor- to a secretory-organ led to the theory that the 'pinealocytes of mammals belong to a sensory cell line evolved from the sensory pineal photoreceptors. The transformation of a photoreceptor cell into a secretory pinealocyte during phylogenetic development allows for the presence of intermediate cell types found in the pineals of chelonians, lacertilians and birds which have been termed secretory rudimentary photoreceptor cells or pseudosensory cells. An electron-microscopical investigation of the pineal gland of two adult mammals (the mole and the noctule bat) has revealed the presence of some peculiar ciliary derivatives, similar to the club-shaped outer segment of rudimentary photoreceptor cells in the pineal organ of non-mammalian vertebrates. The pinealocytes of these two mammals can be thus classified in several morphological types, one of them displaying morphological features resembling those of rudimentary photoreceptor cells. This investigation was performed in collaboration with Prof. Dr. J.P. Collin (France) and Dr. A.M. Voûte (Utrecht).

Considering that the pinealocytes did evolve from photoreceptor cells, it seemed of interest to study the capacity of HIOMT to synthesize melatonin and/or 5-methoxytryptophol in the eyes of a mammal possessing an atrophied and rudimentary visual system under natural conditions. The activity of HIOMT in both eyes of the mole (*Talpa europæa*) was 2-10 times higher than in the pineal, indicating that in a mammal; an extra-pineal biosynthesis of methoxyindoles occurs. The results show, in addition, for the first time that the pineal is not necessarily the organ in which most of this compound is synthesized. The production, in different organs, of methoxyindoles, might help in understanding their paradoxical endocrinological effects described in the literature.

The identification of the nature, the sites of production and release of various neuropeptides which are supposed to be present in the mammalian pineal and subcommissural organ (especially α -MSH-like compounds vasotocin-like compounds, vasopressin, etc.) is currently in progress using radioimmunoassay and immunohistochemical localization techniques on the light and

electronmicroscopical level. This work will form core for further integration of Pevet's work into group III.

Papers read

Boer, G.J. "Biochemie in de neurosecretie". Post-academische cursus "Neuroendocrinologie", August 8-12, N.I.H., Amsterdam, 1977.

Boer, K. "Inleiding in de electrofysiologie van het HNS" (+ demonstraties) op de post-academische cursus "Neuroendocrinologie", August 8-12, N.I.H., Amsterdam, 1977.

Boer, K. "Effect of low frequency stimulation on hormone release" en "Electroanatomic identification of paraventricular-stria terminalis pathways". Roundtable meeting, Hemingford Gray, England, July 23-27, 1977.

Boer, K. "Het effect van laagfrequentie prikkeling van de hypofysesteel op de achterkwabhormoonafgifte bij de rat". 5e Vergadering van de Sectie Zenuwstelsel van de Ned. Ver. van Fysiologie en Farmacologie, Erasmus University, Rotterdam, December 16, 1977.

Buijs, R.M. Licht- en electronenmicroscopisch immunocytochemisch onderzoek aan de GTH cellen van de Black Molly. Intra- en extrahypothalame vasopressine en oxytocine bevattende banen in de rat. Afdeling Vergel. Endocrinologie, Zoölogisch Laboratorium, State University Utrecht, August 26, 1977.

Buijs, R.M. Intra- en extrahypothalame vasopressine en oxytocine bevattende banen in de rat. Vergadering van de Nederlandse Vereniging voor Endocrinologie, Utrecht, November 29, 1977.

Dogterom, J. "Radioimmunoassay van achterkwabhormonen". Post-academische cursus "Neuroendocrinologie", August 8-12, N.I.H., Amsterdam, 1977.

Pevet, P. and Kuyper, M.A. Etude ultrastructural des pinéalo-cytes de la Taupe dorée. Congrès Société Française de Microscopie Electronique. Nice, May 31-June 5, 1977.

Pevet, P. and Kappers, J. Ariëns. (Poster) Secretory processes in the mammalian pineal gland. An ultrastructural identification. Acta endocrinologica Congress. Lausanne, June 20-25, 1977.

Pevet, P. and Karasek, M. (Presented by Dr. M. Karasek). The consideration on the nature of the mammalian pineal active compounds. An ultrastructural contribution. 9th Congress of Polish Society of Endocrinology Poznan, June 23-25, 1977.

- Pevet, P. and Kappers, J. Ariëns. Secretory process differing from that producing granular vesicles in the mammalian pinealocytes: An ultrastructural identification. XXVIIe International Congress of Physiological Sciences. Paris, July 18-23, 1977.
- Pevet, P. and Kuyper, M.A. Granular vesicles in the mammalian pineal gland, their importance in the golden-mole. International Symposium on the Pineal Gland. Jerusalem, November 13-17, 1977.
- Swaab, D.F. Vragen rond synthese, transport, afgifte en functie van neurofysehormonen. Vakgroep Farmacologie, Medische Faculteit, University of Leiden, January 21, 1977.
- Swaab, D.F. Immunolocalisatie van hormonen in hersenen en hypofyse. Werkgroep Coupe Histochemie van de Nederlandse Vereniging voor Celbiologie, Lab. voor Pathologische Anatomie, Wilhelmina Gasthuis, Amsterdam, February 4, 1977.
- Swaab, D.F. Functional anatomy of the hypothalamus. Boerhaave cursus "Clinical and Experimental Aspects of Neuroendocrinology", Leiden, March 31-April 1, 1977.
- Swaab, D.F. Lopend neuroendocrinologisch onderzoek op het Nederlands Instituut voor Hersenonderzoek. Vakgroep Farmacologie (Prof. Dr. P.G. Smelik) Free University, Amsterdam, February 28, 1977.
- Swaab, D.F. The foetal brain and pituitary in intrauterine growth and parturition. V. International Symposium of Obstetrics and Gynaecology "Current Trends in Obstetrics and Gynaecology", Barcelona, May 26-28, 1977.
- Swaab, D.F. The influence of the fetal brain and pituitary on growth. Symposium "Paediatrics and Growth", Fifth Unigate Paediatric Workshop, London, May 30-31, 1977.
- Swaab, D.F. Fetal neuroendocrine mechanisms in development and parturition. 10th International Summer School of Brain Research, Amsterdam, July 11-15, 1977.
- Swaab, D.F. Algemene inleiding Neuroendocrinologie. Post-academisch onderwijs cursus "Neuroendocrinologie", in samenwerking met de Koninklijke Nederlandse Akademie van Wetenschappen, Nederlands Instituut voor Hersenonderzoek, Amsterdam, August 8-12, 1977.
- Swaab, D.F. Seminar "peptide hormone localization in the brain". College de France Groupe de Neuroendocrinologie cellulaire (Dr. A. Tixier-Vidal), Paris, November 28, 1977.
- Swaab, D.F. "Neurohistologie in historisch perspectief". Histologisch Laboratorium, Free University, Amsterdam, December 22, 1977.

- Van Leeuwen, F.W. Specificity in immunocytochemistry, Workshop on "section-histochemistry" of the Netherlands Society for Cell Biology, Amsterdam, February 4, 1977.
- Van Leeuwen, F.W. Optimalization of ultrastructural immunocytochemistry of neurohypophysial substances. 18e Federatieve Vergadering van Medisch Biologische Verenigingen, Leiden, April 13-15, 1977.
- Van Leeuwen, F.W. Immunoelectronmicroscopic demonstration of neurohypophysial peptides. Seminar at the Department of Zoology from the University of Amsterdam, Amsterdam, June 10, 1977.
- Van Leeuwen, F.W. "Immunolokalisatie van hypofyse-achterkwab-hormonen". Post-academische cursus "Neuroendocrinologie", August 8-12, N.I.H., Amsterdam, 1977.
- Van Leeuwen, F.W. Evaluation of fixatives, two staining procedures and specificity criteria in the localization of vasopressin and oxytocin in the neurohypophysis of the rat. Workshop on "immunoelectron microscopy" of the Netherlands Society for Biology, Amsterdam, September 14, 1977.
- Van Leeuwen, F.W. Immunoelectronmicroscopic methods for the optimal and specific localization of vasopressin and oxytocin in rat hypothalamo-neurohypophysial system. Annual Meeting of the Dutch Society of Electron Microscopy, Leiden, November 10-11, 1977.

Working visits abroad

- Boer, K.: Prof. Dr. J.J. Dreifuss, Lab. de Physiologie. Univ. de Genève, November, 1977.
- Pevet, P.: Prof. Dr. J.P. Collin (Lab. de Biologie Cellulaire, Poitiers, France), January 5-6 and March 21, 1977.
- Pevet, P.: Prof. Dr. E. Nevo (Biological Laboratories, Haiffa, Israel), November 18, 1977.
- Swaab, D.F.: Prof. Dr. K. Dierickx (Laboratorium voor Embryologie en vergelijkende Histologie, Rijksuniversiteit Gent), Gent, March 10-11, 1977.
- Swaab, D.F.: Dr. A. Tixier-Vidal (College de France, Groupe de Neuroendocrinologie), Paris, November 28, 1977.
- Van Leeuwen, F.W.: Prof. Dr. K. Dierickx (Laboratorium voor Embryologie Gent), Gent, March 10-11, 1977.

Students and guestworkers

- Buijs, R.M. (student biology): a) Immunolocalization of extrahypothalamic neurosecretory fibers in the rat. b) Immunoelectronmicroscopical demonstration of gonadotropic hormones

in Black Molly.

- Cransberg, K. (student biology): Effect of low-frequency stimulation of the neurohypophysis on hormone release and electroanatomy of extrahypothalamic neurosecretory fibers.
- Everts, M. (student biology): Immunolocalization on EM and LM level of hormones in the supraoptic and suprachiasmatic nucleus.
- Hiemstra, K. (student biology): The installation of a second electrophysiological registration unit and HRP labelling of neurosecretory cells.
- Jansens, P. (student biology): Immunolocalization of antibodies after intracerebroventricular injection.

Visitors

- Hobbelen, P. (Organon, Oss): to learn telemetric pressure measurements.
- Kronnie, G.K. te (Dept. Zoology, Amsterdam): Immunoelectron-microscopy of myosin and actin in the zebra fish.
- Schreuder-Van Gelder, R. and De Jong, H. (Dept. of Haematology, Wilhelmina Gasthuis, Amsterdam): Immunoperoxidase electron-microscopy on blood and bone marrow cells.
- Schuiling, G.A. (Dept. of Experimental Endocrinology, R.U., Groningen): to learn intra-mammary pressure measurements.
- Van Eys, G. (Dept. Zoology, Nijmegen): Immunocytochemistry of ACTH and α -MSH in *Tilapia mossambica*.
- Vet, B.J.C.M. de (Dept. Exp. Internal Medicine, Wilhelmina Gasthuis, Amsterdam): to learn chronic EEG repitration.
- Wilkin, G.P.: MRC Laboratories, Developmental Neurobiology Unit, Carshalton, Surrey, England.
- Woodhams, P.L.: MRC Laboratories, Developmental Neurobiology Unit, Carshalton, Surrey, England.

Attended conferences

- Boer, G.J.: 18e Federal Meeting, Leiden, April 13-15, 1977.
- Boer, G.J.: Symposium "Mechanisms, Regulation and Special Functions of Protein Synthesis in the Brain". Amsterdam, August 29-30, 1977.
- Boer, G.J.: Colloque International "La Biologie Cellulaire des Processus neurosécrétoires hypothalamiques", Bordeaux, September 6-10, 1977.
- Boer, G.J.: IIIrd European Round Table on Neuroendocrinology. Noordwijk aan Zee, September 23-25, 1977.
- Boer, G.J.: Symposium "Unity in Biochemistry". Amsterdam,

- October 20-21, 1977.
- Boer, G.J.: Symposium "Moleculaire Neurobiologie", Z.W.O., Den Haag, October 25, 1977.
- Boer, K.: 10th International Summer School of Brain Research. "Maturation of the Nervous System", Amsterdam, July 11-15, 1977.
- Boer, K.: Round Table in Hemingford Gray "Electrophysiology of the hypothalamus", July 23-27, 1977.
- Boer, K.: Symposium "Iontophoresis and transmitter mechanisms in the mammalian nervous system", Cambridge, July 28-30, 1977.
- Boer, K.: Symposium "Mechanisms, Regulation and Special Functions of Protein Synthesis in the Brain", Amsterdam, August 29-30, 1977.
- Boer, K.: Colloque International "La Biologie Cellulaire des Processus Neurosécrétoires Hypothalamiques", Bordeaux, September 6-10, 1977.
- Buijs, R.M.: Neural hormones and reproduction. Third brain-endocrine interaction symposium Würzburg, July 26-29, 1977.
- Dogterom, J.: Colloque International "La Biologie Cellulaire des Processus Neurosécrétoires Hypothalamiques", Bordeaux, September 6-10, 1977.
- Dogterom, J.: "Symposium, Mechanisms, Regulation and Special Functions of Protein Synthesis in the Brain", Amsterdam, August 29-30, 1977.
- Dogterom, J.: 10th International Summer School of Brain Research. "Maturation of the Nervous System", Amsterdam, July 11-15, 1977.
- Pevet, P.: Congrès Société Française de Microscopie Electronique. Nice, May 31-June 5, 1977.
- Pevet, P.: Acta endocrinologica Congress. Lausanne, June 20-25, 1977.
- Pevet, P.: 9th Congress of Polish Society of Endocrinology, Poznan, June 23-25, 1977.
- Pevet, P.: XXVIIe International Congress of Physiological Sciences. Paris, July 18-23, 1977.
- Pevet, P.: International Symposium on the Pineal Gland. Jerusalem, November 13-17, 1977.
- Swaab, D.F.: Werkgroep Coupe Histochemie van de Nederlandse Vereniging voor Celbiologie, Lab. voor Pathologische Anatomie, Wilhelmina Gasthuis, Amsterdam, February 4, 1977.
- Swaab, D.F.: Boerhaave cursus "Clinical and Experimental Aspects of Neuroendocrinology", Leiden, March 31-April 1, 1977.
- Swaab, D.F.: V. International Symposium of Obstetrics and Gynae-

- cology", Barcelona, May 26-28, 1977.
- Swaab, D.F.: Symposium "Paediatrics and Growth" Fifth Unigate Paediatric Workshop, London, May 30-31, 1977.
- Swaab, D.F.: 10th International Summer School of Brain Research, "Maturation of the Nervous System", Amsterdam, July 11-15, 1977.
- Swaab, D.F.: Symposium "Mechanisms, Regulation and Special Functions of Protein Synthesis in the Brain. Amsterdam, August 29-30, 1977.
- Swaab, D.F.: IIIrd European Round Table on Neuroendocrinology. Noordwijk aan Zee, September 23-25, 1977.
- Swaab, D.F.: Symposium "Moleculaire Neurobiologie" Z.W.O., Den Haag, October 25, 1977.
- Swaab, D.F.: Symposium "Onderzoek en Onderwijs in de Celbiologie: Toekomst, Heden en Verleden". Free University, Amsterdam, December 22, 1977.
- Van Leeuwen, F.W.: 18e Federatieve Vergadering van Medisch Biologische Verenigingen, Leiden, April 13-15, 1977.
- Van Leeuwen, F.W.: Third Symposium on Brain-Endocrine Interaction "Neuronal Hormones and Reproduction". Würzburg, W. Germany, July 26-29, 1977.
- Van Leeuwen, F.W.: Annual Meeting of the Dutch Society of Electronmicroscopy, Leiden, November 10-11, 1977.
- Van Leeuwen, F.W.: 10th International Summer School of Brain Research. "Maturation of the Nervous System", Amsterdam, July 11-15, 1977.

Miscellaneous

- Boer, G.J.: Member of the organizing committee of the Dutch-British Endocrine Meeting to be held in 1978 at Maastricht.
- Boer, G.J.: Course and Examinations of "Qualification of Radiation Protection" (J.A. Cohen Institute, Leiden and the Ministries of Social Affairs, Public Health and Milieu Hygiene). June 20, 1977.
- Boer, G.J.: Organization together with the other members of the group the Post Academic Course "Neurosecretion" held at the Institute under auspices of the Royal Academy, August 8-12, 1977.
- Boer, G.J.: Coordinator of the animal house, until September, 1977.
- Boer, G.J.: Head of the radioisotope laboratory of the Institute from April, 1977.
- Boer, K.: Editor serie Breinverkenningen in Intermediair.

Boer, K.: 1 x referee for J. Endocrinology.

Boer, K.: Coordinator of the drawing room.

Dogterom, J.: 1 x referee for J. Endocrinology.

Pevet, P.: Secretary-treasurer of the European Pineal Study Group, May, 1977.

Pevet, P.: Vice-chairman of the session "Comparative sexual endocrinology" during the XXVIIth International Congress of physiological Sciences, Paris, July 18-23, 1977.

Pevet, P.: Vice-chairman of the session "Cytophysiology of the Pineal" during the International Symposium on the Pineal Gland. Jerusalem, November 13-17, 1977.

Swaab, D.F.: Member committee for thesis J. Dogterom.

Swaab, D.F.: Integrated lecture for 3rd year of Medical School University of Amsterdam on "Growth and Development of the Brain".

Swaab, D.F.: Examined M.A.G. de Boer (student biochemistry), April 25, 1977.

Swaab, D.F.: Vice-president of Dutch Soc. for Endocrinology, May 1977.

Swaab, D.F.: Secretary of the Dutch Committee of the International Brain Research Organization.

Swaab, D.F.: Member of the advisory board of Acta Endocrinologica and of the Journal of Neural Transmission.

Swaab, D.F.: Leader of a Fungo Project (13-35-07).

Swaab, D.F.: Referee for Acta Endocrinologica (5x) and for the Journal of Endocrinology (2x).

Swaab, D.F.: Member of the organizing and editorial committee, 10th International Summer School of Brain Research "Maturation of the Nervous System", Amsterdam, July 11-15, 1977.

Van Leeuwen, F.W.: Chairman working group "Immunoelectronmicroscopy" and organized a workshop for the 40 members, at our Institute for this group, September 14, 1977.

IV. DEVELOPMENT AND CORRECTABILITY OF BEHAVIOR

Participants

Dr. N.E. van de Poll (psychologist, 100%)

Dr. J.P.C. de Bruin (biologist, 100%)

Drs. H.G. van Oyen (psychologist, 100%)

vacancy

V. Nolten (technician, 90%)

T. Dijkstra (technician, 100%)

Hormonal and environmental factors, acting during early stages of development predetermine to a certain extent structure and function of the central nervous system in adulthood. In this way somatic as well as behavioral aspects of an organism are affected and the position of an individual in its physical and social environment as well as its possibilities to adapt to changes are predetermined up to a certain level. The presence or absence of testicular hormones during perinatal development are now generally thought to accomplish changes in such diverse aspects of behavior as sexuality, aggression and maternal behavior as well as in activity, emotionality and some aspects of learning. An analysis of sex-differences of behavior in normally developed individuals in this respect contributes more to our understanding of the role of endocrine factors in brain development.

The effect of social deprivation or specific social stimulation during development is assumed to interact with the hormonal phenomena. An integrated study of these two factors, together with a detailed analysis of behavior in adulthood and research aimed at the localization of the underlying neural substrate, further contributes to the concepts of maturation and adaptation of the nervous system. By determining critical periods in development and the rigidity of the occurring behavioral changes, information is gathered for the understanding of individual characteristics and adaptability of behavior of the adult organism.

The project is represented in the FUNGO workgroup "Development of Brain and Behavior", in the project group "Aggression" of the Ethological working group of BION, and in the Comparative and Physiological Psychological working group of Psychonomy.

The project is subdivided into four themes:

1. Developmental aspects of sexual and aggressive motivation.
2. Sex-differences and hormonal factors in the development of

aggression, sexuality and learning.

3. The influence of social stimuli upon the development of sexually dimorphic patterns of behavior (not studied in 1977).
4. The neural substrate underlying sexual behavior, aggression and learning.

During 1977 research has been focussed on themes 1, 2 and 4. Some experiments were carried out in collaboration with group III, "Interaction between the nervous system and hormones during maturation and adaptation" on the Brattleboro rat. These experiments on hormonal factors in the development of learning are mentioned within theme 2.

Research on telencephalic functions in the Siamese fighting fish (*Betta splendens*) has been completed and resulted in the thesis of J.P.C. de Bruin (September 1977 - University of Amsterdam) (see also Progress Report 1976, p. 40-41). In *Betta splendens* nest building, sexual and aggressive behavior were described both in a qualitative and quantitative way. Considerable attention was devoted to the measurement of aggression. The nuclear cell masses of the telencephalon and its fibre connections were described, while schematic drawings at representative transversal levels were designed to enable a topographical representation of lesions sites. The effects of ablation of the telencephalic hemispheres and small lesions in the dorsal telencephalic area on the above mentioned behaviors were studied. The decrease in aggressive and sexual behavior caused by complete forebrain ablation is concordant with the concept of the teleost telencephalon as a non-specific facilitator of lower brain centres. The impairment of nest building following ablation of the telencephalon shows that it has probably integrative properties as well. The results of small telencephalic lesions are discussed in terms of a regional function specialization of the *area dorsalis pars dorsalis* and *pars medialis* with respect to aggressive behavior. The area exerts both facilitating and inhibiting influences on aggression. Based on neuro-anatomical and neuroethological evidence it is presumed that fibers originating from this area project upon certain parts of the hypothalamus, thus modulating the expression of aggressive behavior.

During 1977 Vera Nolten has spent most of her time assisting in the completion of the research of Drs. A.P. van der Meché and Drs. B. Bermond (section Ethophysiology). The spatial re-organization of the working group, which required extensive

planning and rebuilding, was started during the last month of 1977 and will be finished early 1978.

Theme 1: Developmental aspects of sexual and aggressive motivation

During pre- and postnatal development gonadal hormonal factors partly determine sexual and aggressive motivation which is reflected specifically in the interrelationship of masculine and feminine sexual behavior and of sexual and aggressive behavior underlying masculine and feminine motivation in male rats. An estrogen-progesterone-treated group of sexually inactive male rats and of sexually exhausted males showed lower levels of lordosis as compared to the intact sexually active males. The present work is aimed at an analysis of the interrelationship of sexual and aggressive behavior patterns observed in aggressive behavior tests of S-3 rats confronted with female Wistars (see Progress Report 1976). A further statistical analysis was accomplished with the cooperation of Ir. K. Kuypers (Computer department) and Drs. H. van Dis (Clinical Psychology, University of Amsterdam). Mutual influences of specific components of aggressive and sexual behavior in a confrontation of two female rats determining the interaction are indicated. These were partly determined by factors in the partner (hormonal treatment, dominance). This subject will be investigated in experiments using sequence analysis of the diverse parameters and by experiments aimed at the development of parameters for more specific analysis of preference and reinforcing aspects of social behavior.

Theme 2: Sex differences and hormonal factors in the development of aggression, sexual behavior and learning

Aggression

Earlier results on masculine and feminine responses in males and females in interaction with organizing effects of testosterone were published and extended. In order to establish stable and reliable levels of masculine and feminine responses induced by estrogen and testosterone treatment in groups of females in lesion experiments (theme 4), several control groups were tested in oil conditions. It was found that feminine responses are completely dependent upon hormonal treatment (estrogen or testosterone are both effective) whereas comparison of these groups with oil treated ovariectomized animals indicated that in our test conditions and with the dosages used,

masculine responses are not dependent on estrogen or testosterone treatment. These results, in addition to earlier experiments (Van de Poll and Van Dis, 1977), suggest the importance of stimulus factors in this test situation (receptivity of the stimulus females).

Gonadal hormones present during early stages of development are generally thought to accomplish developmental changes in the CNS which affect aggression in adulthood. These results are mainly based on work with mice. In order to establish the basic levels of aggressive behavior in female rats that can be qualitatively and quantitatively compared in later stages experimental work was started with female S-3 rats (see Progress Report 1976). Sexual and aggressive behavior were studied after TP or oil administration and the females were tested against Wistar females that were treated similar with TP or oil. All animals were ovariectomized before the experiment. Analysis of the results revealed that:

1. Substantial levels of aggressive behavior occur in these animals, even in the least aggression-provoking situation: ovariectomized non-treated females against similar Wistar females.
2. Aggression appeared to depend both, upon treatment of the experimental female and of the partner: oil-treated females were more aggressive when tested against testosterone-treated stimulus females: testosterone treatment of the S-3 female also increased aggression when these animals were tested against a stimulus female, not treated with testosterone.
3. When both animals were treated with testosterone, a general increase in social activity was seen: sexual behavior was increased both, in the experimental and the stimulus female, but aggression increased also.

In order to find further coherence between the different parameters and to improve scoring methods in future research a discriminant analysis was applied on this material, which is aimed at combining different parameters in such a way that the differences between the four conditions are maximalized, and can give an indication about the nature of the parameters of special relevance. It is indicated that sexual parameters are among the most prominent in discriminating the four conditions of this experiment.

Sexual behavior

Earlier observations showed that testosterone treatment of

adult females caused a long term inhibition of feminine sexual behavior suggesting the possibility of "organizing" effects of the hormone in adulthood. Groups of females treated for 16 days with testosterone- and oil-treated controls, therefore, were tested for feminine as well as for masculine responses in different stimulus conditions after an intermediate period of non-treatment. Normal levels of feminine responses were observed with the dosages used; masculine behavior, however, appeared to be facilitated.

Learning

The study of psychosexual differentiation and hormonal influences on learning in the rat was mainly focussed on aversively motivated learning. Age, shock intensity and hormonal factors were investigated as to their relation with the sex differences found in this learning task.

Two identical test cages for step-through avoidance learning were built by the mechanical workshop according to descriptions in literature and indications from the Rudolf Magnus Institute (Utrecht). Pilot studies aimed at standardization of the procedure were performed successfully. A high significant sex difference was found in a first experiment. Males showed far more inhibition (longer response latencies) on the final trial, twenty-four hours after the aversive stimulus (an electric shock delivered through the grid floor). The interaction of sex differences in the performance of this learning task and age was investigated in an experiment in which young adult and adult male and female rats (60 and 140 days old) were studied. Besides sex and age, shock-intensity was varied in this experiment as the unconditioned reaction to shock varies according to sex and age. The results of this experiment showed again a significant difference between male and female rats in passive avoidance, both for the 60 days old and for the 140 days old groups. It could be established, moreover, that for the 140 days old rats the percentage of animals showing complete inhibition of the response increased monotonically with the shock intensity, whereas for the 60 days old rats highest inhibition was found at intermediate shock intensity. Resulting in an inverted U-shaped relation between shock intensity and inhibition. As from the data obtained thusfar no conclusive evidence could be derived that female rats acquire this task, the duration of the interval between shock and test-trial is investigated at this moment in order to create test conditions enabling

females to master this task. In this experiment both gonadectomized and sham-operated animals are used to elucidate the contribution of circulating gonadal hormones to the sex differences in passive avoidance performance that were found.

To establish the relative importance of activational effects of circulating gonadal hormones and organizational effects of these hormones during development males and females were treated with either testosterone estrogen, progesterone or oil and tested for passive avoidance learning. Schedules of hormonal treatment were chosen to ensure that the animals were in comparable hormonal conditions both during shock and test trials. An additional group of males and females was treated with estrogen and progesterone as to differentially induce non-estrus and estrus state in the learning and test trial. These experiments showed that gonadectomy in adulthood reduces sex differences in passive avoidance learning whereas these differences can be restored with gonadal hormones. A complete abolishment of sex differences, however, was found after differential induction of estrus in the test trial only. It is suggested, therefore, that state dependency is involved as one of the important mechanisms affecting passive avoidance learning, differentially in males and females. This hypothesis, together with the possibility that sex differences in pituitary-adrenal functioning are involved in aversively motivated learning will be investigated. A pilot study on effects of experimental manipulation of perinatal gonadal hormonal stimulation on passive avoidance learning is in progress.

The study of sex differences in appetatively motivated learning is focussed on aspects of acquisition and longterm memory of spatial discrimination tasks (Progress Report 1976). As a consequence of the physical removal of the experimental set up to the main building and the necessity of more specific methods for analysis of the data, attention has been concentrated on statistical analysis (partly performed at SARA) and the development of an interface connecting the Grason Stadler programming system directly to the Interdata computer of the Institute. Installation of a program control system, new connections between the programming system registration units and operant conditioning chambers together with direct interfacing with the computer (to be completed in 1978) will improve the experimentation possibilities. Much attention has been paid to the solving of registration problems inherent to the available apparatus and specific learning tasks that are

studied. Analysis of the distribution of responses within the experimental sessions thereby will give better insight in the processes underlying sex differences in spatial discrimination learning (see Progress Report 1976). Technical advises were obtained from Ir. K. Kuypers (Computer department), H. Overdijk (Electronic workshop) and Ir. P.P. Loesberg.

In collaboration with the "Interaction between the nervous system and hormones during maturation and adaptation", experiments were performed on Brattleboro rats. Memory deficiency of homozygous rats caused the lack of vasopressin has been reported in literature. Passive avoidance learning was studied in male and female homo- and heterozygous Brattleboro rats and males of two different ages (65 and 125 days). It was hypothesized that retardation in development of the homozygous Brattleboro's might be involved in the findings reported in the literature. No differences, however, were found in the 65-day old groups whereas in contradiction to the expectation 125-old homozygous animals showed more response inhibition - suggesting better retention of the task than heterozygous animals of the same age. No marked sex differences were found either in homozygous or in heterozygous Brattleboro rats.

Theme 3: The neural substrate underlying sexual behavior and aggression

Area praeoptica and hypothalamus anterior

Earlier experiments established the involvement of the medial preoptic-anterior hypothalamic continuum in masculine and hormone-induced feminine sexual behavior. It was indicated that a specific part of this continuum, structures near the junction of the medial preoptic area and the anterior hypothalamus, are essential for the occurrence of masculine sexual behavior. It could be shown in addition that lordosis was facilitated by lesions bilaterally damaging anterior hypothalamic structures. Recent literature indicated that hormones might be acting on this brain levels organizing the neural substrate underlying masculine and feminine sexual behavior and pointing to different neuroanatomical connections of these two parts of the diencephalon with other (limbic) brain areas involved in sexually dimorphic behavior. This stimulated further experimentation on this line in females. It was established that, as in males, masculine responses are inhibited by lesions at the junction of the medial preoptic and anterior hypothalamic area.

However, feminine responses in these females appeared to be drastically reduced by lesions in both areas, in contrast to that in males. This might be an indication of behavioral consequences of structural differences in this part of the brain between the sexes brought about by the presence of gonadal hormones during development. Further experimentation aimed at improvement of the neuroanatomical data are in progress. In order to use more sensitive tests for the behaviorally responsiveness, different groups of stimulus females are used in which the levels of receptivity are varied by hormonal manipulation.

Praefrontal cortex

The praefrontal cortex, defined as the projection area of the nucleus dorsomedialis thalami, is involved in a variety of behavioral functions. Of special interest for our research are its possible functions with regard to social behavior (aggression and sexual behavior) and the performance of certain learning tasks, which exhibit sexual dimorphism. During 1977 a theoretical basis has been laid for research on praefrontal cortex functioning in rodents (*Rattus norvegicus*) which will follow two different approaches.

Literature data indicative that the praefrontal cortex of the rat might be distinguished anatomically functionally into two subfields, a medial and an orbitofrontal (sulcal) region. The aim of our study is to investigate the functions of both regions in the regulation of intraspecific aggression, sexual behavior and certain learning tasks, in which a sexual dimorphism exists. Both adult males and females will be studied.

It is known that the functional development of the praefrontal cortex continues into adolescence. This "delayed" functional development of the praefrontal cortex provides the possibility of studying the effects of lesions also during development. There are strong arguments for the hypothesis that the praefrontal cortex exhibits a sexual dimorphism during development, possibly extending into adulthood. Using various morphological parameters the structure of the praefrontal cortex during its development will be studied by comparing males and females of different age groups. When differences between the sexes can be assessed, the role of gonadal hormones will be studied as the possible causal determinant of structural sexual dimorphism. This research will be carried out in coöperation with group I (Adaptability of the nervous system of adult organisms).

Students

- M. de Boer (Biochemistry) "Effects of Medial Preoptic-Anterior Hypothalamic Lesions on Sexual Behavior of Female Rats".
- P. Janssens (Biology) started research on α -MSH in workgroup III in collaboration with our workgroup.

Papers read

- Bruin, J.P.C. de - Regional specialization in the forebrain of teleost, *Betta splendens* Regan. 18e Federatieve Vergadering van Medisch-Biologische Verenigingen. Leiden, April 1977.
- Bruin, J.P.C. de - The role of the telencephalon in the regulation of aggressive behavior in the Siamese fighting fish (*Betta splendens*). Association for the Study of Animal Behaviour. Durham, U.K., June 1977.
- Bruin, J.P.C. de - Telencephalon, aggressive and reproductive behavior in the Siamese fighting fish, *Betta splendens* (poster presentation), XVth International Ethological Conference. Bielefeld, W. Germany, August 1977.
- Oyen, H.G. van - Sexe-verschillen in appetetief gemotiveerd leren. Wgm. Verg. en Fysiol. Psychol. (Psychonomie). Nijmegen, December, 1976.
- Oyen, H.G. van - Passieve avoidance en gonadale hormonen. Psychofysiologie. Utrecht, November 1977.
- Oyen, H.G. van and N.E. van de Poll - Acquisition and retention of alternation performance of male and female rats. 18e Federatieve Vergadering van Medisch-Biologische Verenigingen. Leiden, April 1977.
- Poll, N.E. van de - Hormonale en neurale factoren in sexueel, agressief gedrag en leren in de rat. Farmacologie, V.U. Amsterdam, March 1977.
- Poll, N.E. van de and H. van Dis - The effect of dexamethasone on masculine sexual behavior in rats (poster presentation). 18e Federatieve Vergadering van Medisch-Biologische Verenigingen. Leiden, April 1977.
- Poll, N.E. van de, J.P.C. de Bruin, H. van Dis and H.G. van Oyen. Gonadal hormones and the differentiation of sexual and aggressive behavior and learning in the rat. 10th Summer School of the Netherlands Institute for Brain Research. Amsterdam, July 1977.

Visitors

- Dr. J.A. Gray (Oxford, England); Dr. H.H. Swanson (Birmingham,

England); Dr. R.F. Drewet (Durham, England); Dr. M. Figler (Baltimore, Md, U.S.A.).

Working visits

Bruin, J.P.C. de en N.E. van de Poll - Vakgroep Verg. Fysiol. Psychologie, Nijmegen, April 1977.

Bruin, J.P.C. de - Farmacologisch Laboratorium der V.E. Amsterdam, December 1977.

Oyen, H.G. van and N.E. van de Poll to Dr. J.L. Slangen. Psychologie, Utrecht (regularly).

Oyen, H.G. van and N.E. van de Poll to Ir P.P. Loesberg, Bennekom, May 1977.

Oyen, H.G. van and N.E. van de Poll to Dr. B. Bohus. R.M.I., Utrecht, May 1977.

Poll, N.E. van de and H.G. van Oyen - Farmacologie V.U., Amsterdam, March 1977.

Poll, N.E. van de to Dr. K. Slob and Dr. J. Baum. Endocrinologie, Rotterdam, November 1977.

Conferences attended

J.P.C. de Bruin, H.G. van Oyen and N.E. van de Poll: 18e Federatieve Vergadering van Medisch-Biologische Verenigingen, Leiden, April 1977; Meetings of the projectgroup "Aggression" working group Ethology (BION); Autumn conference working group Ethology (BION) Zeist, December 1977; Meetings of the working group Comparative and Physiological Psychology (Psychonomy); Summer School, Netherlands Institute for Brain Research, Amsterdam, July 1977.

J.P.C. de Bruin and H.G. van Oyen: Meeting of the working group "Molecular bases of behavior" (FUNGO). Utrecht, October 1977.

H.G. van Oyen and N.E. van de Poll: Spring conference Psychonomy. Arnhem, May 1977.

J.P.C. de Bruin: Summer conference Association for the Study of Animal Behavior. Durham, U.K., June 1977; XVth International Ethological Conference, Bielefeld, W. Germany, August 1977; Meetings of the "Nederlandse Dierkundige Vereniging"; "Verhaart" Meeting of neuroanatomy. Amsterdam, December 1977.

N.E. van de Poll: Meetings of the working group "Development of Brain and Behavior" (FUNGO); ZWO Symposium "Molecular Neurobiology. Den Haag, October 1977.

Miscellaneous

Oyen, H.G. van. Collaboration in the post-doctoral course
neuroendocrinology - see working group III.

Poll, N.E. van de. Co-editor of "Maturation of the Nervous Sys-
tem, Progr. in Brain Res., Elsevier, Amsterdam (In Press).

Poll, N.E. van de. Member of the Dobberke Foundation for Compa-
rative Psychology.

V. NEUROPHYSIOLOGICAL ASPECTS OF MATURATION AND ADAPTATION OF THE BRAIN

Participants

vacancy (leader)

Drs. C.V. de Blécourt (M.D., 100%)

Ir. K. Kuypers (engineer, 100%)

vacancy (scientific staff)

R. Nooy (technician, 100%)

vacancy (technician, 100%)

For the function of nerve cells, electrical activity is essential. This group will study the neurophysiological aspects of the nervous system during maturation and adaptation. Of course the program of this group will strongly depend on its future leader for which there is a vacancy at present. An account can thus only be given of the computer department and the completion of the Cerebral Blood Flow project. Since the departure of P.A. de Groot at the beginning of the year, a second vacancy has emerged. Kuypers has functioned as acting groupleader.

Computer department

The department uses an IBM-1130 and an Interdata M-70 computer. This year the Interdata has been expanded with the necessary peripherals to replace the IBM-1130. A 10 MB discdrive, a card reader, a chainprinter, 32 KB core memory and two CRT video display units have been connected to the Interdata. The digital tape unit and the plotter, which are part of the IBM-system, can now also be connected to the Interdata. Several projects have been undertaken or adapted this year:

The conversion from IBM to Interdata. Programs written in the machine language of IBM have to be converted to machine language of Interdata. Converted are a driver for the digital tape unit and subroutines for driving the plotter. Other programs have to be converted in the future.

The implementation of a multi-task system. With the arrival of a discdrive for the Interdata it was also possible to implement the OS-16-MT2 multi-task system. This means that the system has to be converted from a single user to a multi-user environment. This has made possible the connection of more than one experiment to the computer at the same time, without blocking it for other purposes, which is necessary *e.g.* during measurements of dendrites.

Computer-aided scanning of dendrites. The program for data acquisition has been further developed and converted to the multi-task system. One of the features added is that of error conversion during the scanning of dendrites. Programs for the analysis of the collected data have also been made. For instance a procedure has been developed to calculate the surface of the cell soma in the sections. Further an interface routine has been constructed between the data bank of the coordinates of the dendrites and the already existing programming package for analysis of segment lengths of dendrites of groups of animals.

Whyte system. Other statistical capabilities have been added to the system. The spearman rank correlation and factor analysis on frequency, latency and duration and on the data of the following matrix have been implemented. The calculation of the following matrix has been improved.

Grason-Stadler system. A start has been made this year with the construction of interfaces between the Grason-Stadler system, used by workgroup IV and the Interdata computer by the electronic workshop. At the same time the development of the corresponding data acquisition program has been started.

Routine programs. The programs for RIA, rCBF, FLOWMP and the programming packages for EEG analysis (Fast Fourier Transform and Aperiodic Interval Amplitude Analysis) have been used daily for the analysis of data.

The relationship between EEG and cerebral blood flow (C.V. de Blécourt)

From comparison of the clearance curves of ^{133}Xe and $^{99\text{m}}\text{Te}$ (a non-diffusible tracer) it was concluded that part of the injected ^{133}Xe left the brain without having been diffused into the brain tissue. The extensive venous plexus behind the orbit, that has direct communications with the intra-cerebral arterial circulation can be responsible for this phenomenon. This finding led us to re-analyse 200 previous CBF measurements. The results were, however, similar to those described in 1975. No increase in CBF was found during intermittent photic stimulation (IPS); in the resting situation, however, there was a significant correlation between EEG- and CBF parameters.

EEG's were analysed not only with analogue broadband frequency analysis (ABFA) but also with Fourier analysis (FFT) and aperiodic amplitude interval analysis (AAIA), with the assistance of Ir. J. Smith (Laboratory of Medical Physics, University of Amsterdam).

The following EEG parameters were tested ($\bar{x} \pm$ s.d.) (resting situation). ABFA: % delta (40.1 ± 5.2) and % theta (31.4 ± 3.9). FFT: % delta (48.4 ± 10.4), % theta (35.9 ± 8.9), the delta-theta median (*i.e.* the frequency at which the power in the (delta + theta) band is divided into two equal parts) was 3.1 ± 0.6 , the whole spectrum median (3.7 ± 0.7) and the dominant frequency (2.5 ± 0.9); AAIA: from this method only the mean interval value (msec) was tested (118 ± 24).

All of them, except for the mean interval value (AAIA) yielded significant coefficients of correlation ($p < 0.01$) with one or more of the following CBF-parameters (expressed in ml/g/min): flow mean weighted (FMW) (0.93 ± 0.19), flow slow (FS) (0.43 ± 0.09) and flow mean stochastic (FMS) (0.87 ± 0.22). The highest correlations were obtained between the delta-theta median and subsequently the FMW (0.86 , $p < 0.0005$), FS (0.79 , $p < 0.0005$) and FMS (0.79 , $p < 0.0005$).

Another series of experiments was performed this year, in which not only IPS of low intensity but also of a higher one was used. Analysis is still in progress.

Papers read

- De Blécourt, C.V. Artificial respiration in the rabbit for EEG-rCBF studies. 18th Dutch Federative Meeting, Leiden, April 1977.
De Blécourt, C.V. Artificial respiration in the rabbit for EEG-rCBF studies. 27th Internat. Congr., Internat. Union Physiol. Sci., Paris, July 1977.

Attended conferences

- De Blécourt, C.V. to: the 8th Cerebral Blood Flow symposium: Cerebral function, metabolism and circulation. Copenhagen, June 1977; The 9th Internat. Congress of EEG and Clinical Neurophysiology, Amsterdam, September 1977.

Participants

Drs. A.P. van der Meché (biologist, 100%)

Drs. B. Bermond (Psychologist, 100%)

In this project the main interest is focussed on brain and behavior studies. It deals with the function of telencephalic and diencephalic areas in memory (theme 1) and aggression (theme 2).

Theme 1: The neural and chemical substrate for learning and memory in the goldfish (*Carassius auratus* L.)

This theme has been completed and the results are condensed in the thesis of Van der Meché. This thesis describes the behavior of goldfish in a shuttle-box. Both spontaneous barrier crossing in a shuttle-box and the effects of telencephalon ablation on learning behavior and spontaneous barrier crossing are discussed.

It appears that goldfish, after an initial latency period, regularly cross the barrier spontaneously in the shuttle-box. Following a subsequent confrontation with the shuttle-box, this latency time is shortened and the crossing frequency becomes stabilized after 2 to 3 days. This behavior is discussed in terms of the habituation concept. The repeated crossings are neither caused by a desire to escape from a restricted area nor by searching for a more stimulus enriched environment than the bare shuttle-box compartment. We propose that the crossing behavior is maintained by a positive reinforcer. We witnessed a shortening of the latency time after a single crossing during a second confrontation. This results in a one-trial learning process with a positive reinforcer which is very useful for research in which, up till now, only a negative reinforcer was available. It became clear that the latency time during the first confrontation was not caused by reduced activity.

Active avoidance conditioning is a learning procedure for which the shuttle-box is most commonly used. Constant individual differences in learning capacity were established; avoidances during the trial period occur as well as crossings during the intertrial period. These crossings increased in frequency during the beginning of the conditioning. Once a certain level was attained, they remained constant even though the learning curve declines under the influence of the extinction procedure. The intertrial crossings are an expression of the general activity

of the goldfish in the shuttle-box and, as such, can appear during the trial period and be registered as a so-called avoidance. A connection exists between the number of avoidances and the number of intertrial crossings.

Observations indicate that intertrial crossings do occur during the light period. It is, therefore, not unreasonable to consider the total amount of intertrial crossings per time-unit to be part of the avoidance session-score. A method is provided to correct the avoidance score for intertrial crossings in order to obtain the net result of the proper learning process. It is further shown that active fish, as measured by their level of intertrial crossings, show a faster learning progress than less active ones.

Following telencephalon ablation retention of the avoidance conditioning is impaired, although a tendency for a partial saving of the response is present. Both acquisition and extinction are unimpaired by telencephalon ablation, however, both spontaneous and intertrial barrier crossings disappear. The indications are discussed that the difficulty to cross the barrier is important in shuttle-box behavior. Since active fish learn the avoidance response faster, and since intertrial crossings disappear following telencephalon ablation, the failure to find a difference in learning rate between fish with and without their telencephalon is not concordant with our expectation. Although active fish learn the response faster, following telencephalon ablation they remember the response worse than less active fish.

Theme 2: Neural and hormonal regulation of aggressive and sexual behavior in the S_3 rat (*Rattus norvegicus*)

Behavioral results and histological data of previous experiments have been (re)analyzed, and will be published in the form of a thesis early in 1978.

The different analyses provided two important conclusions:

1) Effects of castration and subsequent androgen treatment upon intermale aggressive behavior in rats. Although some investigators claim that castration leads to a decrease or disappearance of aggressive behavior, and hormone replacement therapy tends to reinstate it, the literature is very conflicting about this issue. To tackle this problem, the data of a castration-testosterone propionate (TP) replacement experiment were analyzed, both in a conventional way (by means of Mann Whitney U-test) and with the aid of a behavioral sequence analysis. The results

of these analyses demonstrated that TP treatment of castrated rats enhances, on the one hand, the level of aggressiveness in the treated castrates while, on the other hand, it decreases the level of aggression-provoking behavior of the non-treated non-castrated stimulus males, and increases the level of aggression-inhibiting behavior in these last-mentioned animals. The difference in behavior of the non-treated intact stimulus males (those used for the TP-treated castrates compared with those used for the non-treated castrates) is the result of a difference in the organization of the behavioral interactions between the castrate and his opponent. It was demonstrated that the stimulus males used for the TP-treated castrates, when compared with those used for the non-treated castrates, tend to react to mildly aggressive acts of the TP-treated castrates significantly more often by surrendering themselves completely, and less often by aggression-provoking acts.

Further analysis of the data indicated that this difference in behavior of the non-treated intact stimulus males (the behavior of the stimulus males used for the TP-treated castrates in comparison with that of the stimulus males used for the non-treated castrates) is not the result of the difference in levels of aggressiveness demonstrated by the two groups of castrates, but the result of a change in physical characteristics of the TP-treated castrates, in response to the hormone treatment, probably a change in pheromone-production.

The fact that the two effects of androgen treatment stated in the above-mentioned conclusion tend to counteract one another with regard to the amount of aggressive behavior shown during testing, explains why the literature on this subject is so conflicting.

2) The effects of implantation of testosterone-propionate pellets in the neighbourhood of the medial preoptic-hypothalamus anterior region. In the Progress Report of 1974, the conclusion based upon results of lesion experiments, stated that the medial preoptic-hypothalamus anterior region (MPOA-AH) can be functionally dissociated in at least two areas: a) a frontal part fulfilling a facilitating function in the regulation of inter-male aggressive behavior, and b) a more caudal part, fulfilling a facilitating function in the regulation of male sexual behavior.

In the Progress Report of 1975, additional lesion results were mentioned which further supported the conclusion stated above. Further analysis of these lesion results showed that the "aggressive subpart of the MPOA-AH" is anteriorly bordered by plate 19

and caudally by plate 21 of the König and Klippel atlas of the rat brain, while the "sexual MPOA-AH subpart" is bordered by plates 23 and 26. It was then hypothesized that androgens find their site of action for the stimulation of intermale aggression in the first-mentioned subpart of the POA-AH, and for the stimulation of male sexual behavior in the second-mentioned MPOA-AH subpart. This last hypothesis was finally tested in an experiment in which testosterone-propionate pellets were implanted in the neighbourhood of the two MPOA-AH subareas mentioned in puperal castrated S_3 rats. The behavioral and histological results of this experiment are now analyzed, and they confirm the hypothesis stated above. Only bilateral implants within a range of 1000 μ to the first-mentioned MPOA-AH subarea induced intermale aggressive behavior, while only those within a range of 1000 μ to the second-mentioned MPOA-AH subarea induced male sexual behavior.

The research of this theme forms part of the activities of the National Aggression Working Group, coordinated by Dr. P.R. Wiepkema, Zoological Laboratory, University of Groningen.

Papers read

Bermond, B. Castration and androgen treatment in S_3 rats: effects on aggressive behavior in the test males (treated) and aggression provoking behavior in the stimulus male (untreated). 18e Federatieve Vergadering voor Medisch Biologische Verenigingen, Leiden, April 1977.

Bermond, B. Effect of lesions in the preoptic area on aggressive and sexual behavior of the male S_3 rat. XVth International Ethological Conference, Bielefeld, 1977.

Van der Meché, A.P. Spontaneous barrier-crossing of goldfish (*Carassius Auratus L.*) in a shuttle-box: its use as a one-trial learning process with positive reinforcement. 18th Dutch Federative Meeting, Leiden, April 1977.

MONOAMINERGIC MECHANISMS IN RAT BRAIN: BIOCHEMICAL ASPECTS AND EFFECTS OF CHRONIC ADMINISTRATION OF TRICYCLIC ANTIDEPRESSANT DRUGS

Participant

Drs. M. van Wijk (biochemist, 100%)

The main, strongly interrelated, purposes of this project are: 1) To establish the effects of tricyclic antidepressant drugs (TADS) on several aspects of monoaminergic function in the CNS, especially after chronic administration (cf. Progress Report 1976), 2) to contribute, by developing new experimental models, to a better understanding of the basic biochemical organization of monoaminergic neurotransmission in the CNS.

So far, our interest has mainly been focussed on serotonin (5-HT) metabolism. To obtain a general idea about chronic effects of several TAD on 5-HT metabolism, measurements of turnover of this neurotransmitter and levels of TAD in whole rat brain have been performed in 1976. Part of 1977 was spent to some supplementary control experiments, the results of which did not alter the previous conclusions, which were briefly, that, although levels of TAD were much higher after chronic than after acute treatment, 5-HT turnover was similar after the two dosage schedules. Therefore the relative inertness of monomethylated TAD towards serotonergic neurons seemed for the most part maintained after longterm administration. For these turnover measurements the probenecid technique was used, which, however, in view of recent evidence, might influence cerebral 5-HT synthesis itself. Therefore, the effect of probenecid on 5-HT formation was investigated by means of aromatic decarboxylase inhibitors. These experiments did reveal, in addition to the known blockade of the transport of 5-hydroxyindole acetic acid, a moderate stimulatory effect on 5-HT synthesis. Presently, more sensitive and convenient semi-automatic methods to determine 5-hydroxyindoles are in progress. These techniques will be applied to evaluate the biochemical consequences of stimulating ascending serotonergic pathways in rat brain.

This project is at present supervised by Dr. J. Korf (Biological Psychiatry, State University of Groningen), and is partly performed at the Department of Biological Psychiatry and partly at the Netherlands Institute for Brain Research.

Papers read

- M. van Wijk and J. Korf. Levels of tricyclic antidepressant drugs and metabolism of 5-hydroxytryptamine in rat brain after acute and chronic treatment. 18th Dutch Federative Meeting, Leiden, April 13, 1977 (poster).
- M. van Wijk and J. Korf. Probenecid-induced accumulation of 5-HIAA and levels of tricyclic antidepressants in the rat brain after acute and chronic treatment. 6th International Meeting of the International Society for Neurochemistry, Copenhagen, August 25, 1977 (poster).

ELECTRONICAL WORKSHOP

Participants

J. Overdijk (technician, 100%)

R. Nooy (technician, for the time being 100% in this workshop)

Microiontophoresis stimulator (K. Boer)

A 5-unit constant current stimulator was built for the purpose of ejecting compounds by a multibarrel micropipette during recording of single unit activity. Nano- to microamperes current (positive as well as negative) can be maintained also alternatively and can be constantly measured. The stimulator can be driven by an external source. Electrical artifact problems during recording still exist, but will be tackled soon.

Electronic counter (K. Boer)

A digital pulse counter was built, counting up to 9999 pulses (of less than 1 msec duration) without a practical rate limit. The display can be arrested while counting continuous, and can be set at zero if required. It serves to calculate mean firing frequencies of recorded nerve cells during the experiment, and to check the number of stimulation pulses given during the experiment.

Temperature-controlled specimen holder for tissue culture (M.A. Corner)

Construction of a specimen holder (operation not step-wise) which is electronically controlled to prevent artifacts due to switch-gearing of the heating element during recording. A test-model has been designed, a final version of which will be built in 1978.

Beveling apparatus (M.A. Corner)

Design and construction of an apparatus for beveling the fine tips of glass micropipettes (in order to facilitate intracellular recordings from nerve cells) in collaboration with the mechanical workshop).

Agitator for development of miniature films (A. Potjer)

An electronically controlled unit which controls an electro-motor and is provided with two electronically operating time recorders, one of which indicates the intervals between rotation of the development tank (adjustable from 1-100 sec) while

the other controls the total time, to be indicated by a buzzer.

Alarm system (D.F. Swaab)

An alarm system was constructed for temperature control of 6 deep-freeze boxes for storage of low-temperature preparations. The system controls both, mains current for the unit and temperature in the boxes. In case of failure of the mains supply and/or rise of temperature in the boxes above -10°C , a control bulb at the relevant box and a centrally located siren will become operative.

Grason-Stadler system (N.E. van de Poll)

1. Rebuilding and renewal of the setup of the operant conditioning chambers and Grason-Stadler programming system, and installation of the program control system in the main building of the institute.
2. Development and construction of a module to be used for delivery of audio signals with adjustable intensity, and duration in the operant conditioning chambers. The module is controlled by the Grason-Stadler programming system.
3. An interface allowing for the data of the operant conditioning chambers and Grason Stadler programming system, to be introduced into the Interdata computer of the institute for further analysis is being constructed. The system was developed in collaboration with Ir. K. Kuypers (computer department). The data (32 bits) are transmitted to an interface (receiving part) at the computer in such a way that changes of the data result in transmission.
To test this interface, a 16 bit parallel display has been built which at the same time serves as a control for additional parallel bit lines. The display is made up of 16 light emitting diodes and a 16-bit buffer.

Assistance was given to the computer department (repair and modification in connection with the change over to a new computer system). Additional activities were carried out such as repair and routine maintenance of apparatus in the various departments and the building of 3 temperature-controlled heating elements (K. Boer, M.A. Corner).

MECHANICAL WORKSHOP

Participants

A.W. Kamstra (technician, 100%)

E. Moes (technician, 100%)

An enumeration of some large projects developed in this department is given below. Electronical aspects of these projects were developed in the Electronical Workshop.

a. (H. Romijn)

1. Rotating table for the permeation of specimens with embedding material.
2. Storage chest for electronmicroscopic grid boxes.
3. Receptacle for specimen holders.

b. (N. E. van de Poll)

Two identical step-through test cages were built to be used for testing passive avoidance learning according to descriptions in the literature.

c. (G.J. Boer)

1. Microelectrophoresis/electrofocussing equipment was developed for protein separation on polyacrylamide slab gels. Special holders were made in which the slab gels can be electrically isolated from the cooling electrode solution.
2. Special light box for observation of protein-stained polyacrylamide gels.

d. (K. Boer)

Several modifications were performed of the electrophysiological recording setups. The David Kopf stereotact for small animals was provided with a third electrode holder that was made to move into three directions. In this way, two stimulation electrodes and a recording electrode can be manipulated independently.

For the second setup, the rabbit stereotact, which was previously adapted for rats, was improved, viz. by making one electrode holder movable in three dimensions. Moreover, a new head holder was manufactured on top of a resting plate that can be fixed into the old stereotact. The head holder enables recording from the supraoptic nucleus by the ventral approach through the mouth cavity, which facilitates iontophoretic application of compounds to the supraoptic neurones, as well as possibly the technique of intracellular recording. This device can be used independently for the preparatory operations under the microscope, or can be replaced by the former head holder of the stereotact for the more usual registration tech-

nique.

In the above-mentioned as well as in the stereotact resting plate, provisions are made to contain a new heating plate, made after the design of that for the first setup (by H. Overdijk).

Next to these devices, a small Faraday cage was built for the second setup, that protects the electrical registrations against 50 Hz noise.

e. (M.A. Corner)

The following projects were completed this year on behalf of Workgroup II:

1. A vibrating knife for making fine slices of central nervous tissue for culture in vitro.
2. Modifications of a dry-heat oven for tissue culture purposes, including a variable motor for slowly rocking the culture dishes, and a gas-mixture control system for controlling the atmosphere inside the oven.
3. Design and construction of an apparatus for beveling the fine tips of glass micropipettes (in order to facilitate intra-cellular recordings from nerve cells, in collaboration with the Electronical Workshop).

f. (D.F. Swaab)

1. Storage trays (synthetic fabric) for storage of small tubes containing antibodies, samples, etc. for the immuno-procedures.
2. A Halasz-knife was prepared from sharpened stainless steel. This knife is used for stereotactic deafferentiations in the brain, after which immunocytochemical stainings are performed.
3. A machine was made (synthetic fabric) in which tubes can rotate. It is used for radioimmunoassay, purification of antibodies and incubation of agarose beads.

g. (J. Dogterom)

1. For the radioimmunoassay, three plastic racks were made to immerse extraction tubes in a waterbath (each rack containing 32 tubes) during evaporation of solvent by a nitrogen stream. These racks had to be made according to a model of a nitrogen spreading device of glass.
2. For the isolation of well defined brain nuclei, 6 steel punch pipes of different sizes were made.

h. (K.C. Hodde)

A transportable storage unit was devised for the very hygroscopical critical point- or freeze-dried material, prepared for

scanning EM. The conditions to be met were: smallest possible air volume, to contain as little moist as possible - easy access to specimen for very quick (un)loading - visual control of state of dryness.

The unit is a plexiglass tube, length 25 cm, diameter 4 cm, closed with discs and O-rings. One disc is glued to a mid-horizontal partition which carries the specimen stubs. The remaining 1/4 space is occupied by a metal gauze cylinder containing silica-gel. A tube mounted in the center allows a rod, fastened to the specimen carrier to go through, passing to the outside through a hole drilled in the disc on this side. A nut screwed on, presses both lids firmly shut.

Carried out were, in addition:

- chassis and front panels for apparatus in the electrical workshop;
- common repair, modification and adaptation of various apparatus such as photographic equipment, centrifuges, microscopes, shakers, γ -counter, etc. and routine maintenance and repair of micro-scissors, micropipettes, etc.

Seminars given at the Institute in 1977

- February 9 - Dr. David Hopkins (Dept. Neuroanatomy, Med. Faculty, Erasmus University, Rotterdam): The development of motor control in the rhesus monkey.
- March 29 - Prof. J.R. Wolff (Dept. Neuroanatomy, Max-Planck Institute for Biophysical Chemistry, Göttingen): Quantitative studies on the distribution and development of synapses in the visual cortex of the rat.
- April 20 - Dr. J. Parnavelas (Dept. of Anatomy and Embryology, University College, London): Neurons and their synaptic organization in the visual cortex of the rat.
- May 17 - Dr. J.A. Gray (Dept. of Experimental Psychology, University of Oxford): Electrophysiology and behavioral functions of the septo-hippocampal system.
- May 18 - Drs. J. de Groen (Dept. Clinical Neurophysiology, Academic Hospital, Leiden): Periodieke slaapverschijnselen in de humane pathologie: casuïstiek en achtergronden.
- July 25 - Prof. K. Ruf (McGill University, Montreal, Canada): The physiology of puberty in the rat.
- August 26 - Dr. H.P. Rees (Gainesville, U.S.A.): Autoradiographic studies with a ^3H -ACTH₄₋₉ analog in the rat brain.
- September 1 - Prof. S.P.R. Rose (The Open University Milton Keynes, U.K.): Neurochemical correlation of early experience and learning.
- September 2 - Dr. H. Hillman (University of Surrey, Dept. of Human Biology and Health): The chemistry of epilepsy, studied in vivo and vitro.
- September 12 - Dr. H.H. Swanson (Dept. of Anatomy, University of Birmingham, England): The social control of fecundity in Mongolian gerbils.
- September 22 - Dr. M.R. Issidorides (Psychiatry Dept., University of Athens, and Neurology Dept., Mt. Sinai Medical School, New York): Protein-rich bodies of substantia nigra and locus coeruleus in Parkinsonian and in normal brains.
- September 28 - Prof. G.S. Dawes, F.R.S. (Nuffield Inst. for Medical Research, University of Oxford, England): The development of breathing movements before birth.
- September 29 - Dr. Tj.B. van Wimersma Greidanus (Rudolf Magnus Institute, University of Utrecht): Neuropeptiden (ACTH, MSH, vasopressine) en gedrag.
- November 2 - Dr. T.D. Kernell (University of Amsterdam): De graduatie van zenuwcelactiviteit.

November 23 - Dr. P. McConnell (Dept. of Anatomy, University of Birmingham, England): The effects of starvation on cerebellar development.

December 21 - H. Hermans (Head Voorlopige Dienst Wetenschapsvoorlichting K.N.A.W.): Wetenschapsvoorlichting.

PUBLICATIONS

- Baker, R.E. Lack of behavioral reversals in skin-grafted *Lymnodynastes*. *IRCS Med. Sci.* 5, 436 (1977)
- Baker, R.E. Synapse selectively in somatic afferent systems. In: *Maturation of the Nervous System*, M.A. Corner *et al.* (eds.), *Progress in Brain Research*, Vol. 48. Elsevier, Amsterdam, in press.
- Baker, R.E. and M.A. Corner. Development of cutaneous afferent connexions in frogs: an experimental analysis. *Differentiation*, in press.
- Baker, R.E., M.A. Corner and W.A.M. Veltman. Cutaneous receptive field enlargement following skin-grafting at several stages in the frog. *Brain Res. Bull.* 2, 475-477 (1977).
- Baker, R.E. and A.Ph.J. Richter. Development of dorsal root III afferent fibres in postmetamorphic juveniles of the frog, *D. pictus*. *Neurosci.* 3, 271-273 (1977)
- Baker, R.E., W.A.M. Veltman and M.A. Corner. Effect of cutaneous stimulation on the development of misdirected wiping reflexes in skin-grafted *D. pictus*. *Developm. Psychobiol.* 10, 299-304 (1977).
- Bermond, B. Hormonen en agressief gedrag. In: *Agressief gedrag, oorzaken en functies*, P.R. Wiepkema & J.A.R.A.M. van Hooff (eds.), p. 291. Bohn, Scheltema en Holkema Wet. Uitgeverij, Utrecht (1977).
- Bermond, B. Castration and androgen treatment in S_3 rats: effects on aggressive behaviour in the test males (treated) and aggression provoking behaviour in the stimulus male (untreated). *Abstr. 18e Fed. Vergadering van Med.-Biol. Ver.*, Leiden, April, p. 134 (1977).
- Bermond, B. Effect of lesions in the preoptic area on aggressive and sexual behaviour of the male S_3 rat. *Abstr. XVth International Ethological Conference*, Bielefeld, August 23-31 (1977).
- Boer, G.J. and K. Boer. De klierfunctie van de hersenen. *Neurosecretie 1. Intermediair* 32 (1977).
- Boer, K. and W.L. Bakhuis. De rol van de hersenen. *De Syllabus no. 7*, pp. 179-183 (1976) (not mentioned in 1976 Progress Report).

- Boer, K. and G.J. Boer. Waterhuishouding en reproductie. Neurosecretie 2. Intermediair 33 (1977).
- Boer, K. and G.J. Boer. Nobelprijs voor onderzoekers hormonen. Volkskrant 10 december, p. 26 (1977).
- Boer, K. and J.W.L. Noltén. Paraventricular unit activity during labour in the rat: absence of silent cells and increase in continuously firing cells. J. Endocr. 72, 70P-71P (1977).
- Boer, K. Electrofysiologie van het HNS. P.A.O.-klapper, hoofdstuk X.
- Boer, K. Hersenwerk. Intermediair 45 (1977).
- Boer, K. and G.J. Boer. De Nobelprijswinnaars 1977: Yalow, Guillemin en Schally. Intermediair 49 (1977).
- Boer, K. and J.W.L. Noltén. Hypothalamic paraventricular unit activity during labour in the rat. J. Endocr. 76, 155-163 (1978).
- Buijs, R.M., D.F. Swaab, J. Dogterom and F.W. van Leeuwen. Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Cell Tiss. Res. 186, 423-433 (1978).
- Buijs, R.M., D.F. Swaab, J. Dogterom and F.W. van Leeuwen. Intra- en extrahypothalamische vasopressine en oxytocine bevattende banen in de rat. Ned. T. Geneesk., in press.
- Corner, M.A. Sleep and the beginnings of behavior in the animal kingdom - studies of ultradian motility cycles in early life. Progr. Neurobiol. 8, 279-295 (1977).
- Corner, M.A. Towards an evolutionary interpretation of sleep rhythms: a comparative study of early spontaneous motility patterns. In: Sleep, 1976, W.P. Koella & P. Levin (eds.), Karger, Basel, 1977, p. 233-235.
- Corner, M.A. What sort of neural networks are responsible for early behavior patterns in vertebrates. Proc. Internat. Union Physiol. Sci. (Paris) 12, 626 (1977) Abstr.
- Corner, M.A. Spontaneous motor rhythms in early life - phenomenological and neurophysiological considerations. In: Maturation of the Nervous System, M.A. Corner *et al.* (eds.), Progress in Brain Research, vol. 48. Elsevier, Amsterdam, in press.
- Corner, M.A., R.E. Baker and W.A.M. Veltman. Receptive fields of cutaneous mechanoreceptive neurons in the frog, *D. pictus*, following skin transplantations at larval stages. Brain Res. Bull. 2, 393-395 (1977).
- Corner, M.A., R.E. Baker and W.A.M. Veltman. Experimental studies on the development of cutaneous afferent reflex connections in frogs. Proc. 5th Internat. Neurobiol. Symp. (Magdeburg), in press.

- Corner, M.A., R.E. Baker, N.E. van de Poll, D.F. Swaab and H.B.M. Uylings. (eds.) *Maturation of the Nervous System. Progress in Brain Research*, vol. 48. Elsevier, Amsterdam, in press.
- Corner, M.A., H.J. Romijn and A.Ph.J. Richter. Synaptogenesis in the cerebral hemispheres of the chick embryo. *Neurosci. Lett.* 4, 15-19 (1977).
- Corner, M.A., W.A.M. Veltman and R.E. Baker. Topography of cutaneous mechanoreceptive neurons in thoracic spinal ganglia of the frog. *Proc. Internat. Union Physiol. Sci. (Paris)* 13, 148 (1977) Abstr.
- De Blécourt, C.V. Artificial respiration in the rabbit for EEG-rCBF studies. Abstr. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 142 (1977).
- De Blécourt, C.V. Artificial respiration in the rabbit for EEG-rCBF studies. *Proc. Internat. Union Physiol. Sci. (Paris)* 13, 168 (1977) Abstr.
- De Bruin, J.P.C. Agressief gedrag bij vissen. In: *Agressief gedrag, oorzaken en functies*. P.R. Wiepkema & J.A.R.A.M. van Hooff (eds.), p. 258. Bohn, Scheltema en Holkema Wet. Uitgeverij, Utrecht (1977).
- De Bruin, J.P.C. Regional specialization in the forebrain of a teleost, *Betta splendens Regan*. Abstr. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 137 (1977).
- De Bruin, J.P.C. Telencephalon, aggressive and reproductive behaviour in the Siamese fighting fish, *Betta splendens*. Abstr. XVth International Ethological Conference, Bielefeld, August 23-31 (1977).
- De Bruin, J.P.C. Telencephalon and behavior in teleost fish, a neuroethological approach. In: *Comparative Neurology: The Telencephalon*, S.O.E. Ebbesson (ed.). Plenum Press, New York, in press.
- Dogterom, J., C.M.F. van Rheenen-Verberg, Tj.B. van Wimersma Greidanus and D.F. Swaab. Vasopressin and oxytocin in cerebrospinal fluid of rats. *J. Endocr.* 72, 74P-75P (1977).
- Dogterom, J., Tj.B. van Wimersma Greidanus and D.F. Swaab. Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: Measurements in plasma and CSF of intact and hypophysectomized rats. *Neuroendocrinology* 24, 108-118 (1977).
- Dogterom, J., Tj.B. van Wimersma Greidanus and D. de Wied. Vasopressin in cerebrospinal fluid of man, dog and rat. *Am. J. Physiol.*, in press.

- Hodde, K.C., A. Miodonski, C. Bakker and W.A.M. Veltman. Scanning electronmicroscopy of microcorrosion casts with special attention on arterio-venous differences and application to the rat's cochlea. In: IITRI Scanning Electron Microscopy, vol. II, O. Johari (ed.), pp. 477-484 (1977).
- Janse, C., F.W. van Leeuwen, H. van Swighem and T.A. de Vlieger. Receptive fields of primary touch sensitive neurones in the freshwater snail. Proc. of the Royal Academy of Sciences, Series C, Biological and Medical Sciences 80, 97-104 (1977).
- Miodonski, A., K.C. Hodde and C. Bakker. Raster-Elektronenmikroskopie von Plastik-Korrosion-Präparaten: Morphologische Unterschiede zwischen Arterien und Venen. BEDO/1976, G. Pfefferkorn (ed.), in press.
- Pevet, P., J. Ariëns Kappers and A.M. de Voûte. The pineal gland of nocturnal mammals. 1) The pinealocytes of the bat (*Nyctalus noctula*, Schreber). J. Neural Transm. 40, 47-68 (1977)
- Pevet, P. On the presence of different populations of pinealocytes in the mammalian pineal gland. J. Neural Transm. 40, 289-304 (1977).
- Pevet, P., J. Ariëns Kappers and A.M. Voûte. Morphological evidence for differentiation of pinealocytes from photoreceptor cells in the adult noctule bat (*Nyctalus noctula*, Schreber). Cell Tiss. Res. 182, 99-109 (1977).
- Pevet, P. The pineal gland of the mole (*Talpa europaea*, L). IV. Effect of pronase on material present in cisternae of the granular endoplasmic reticulum. Cell Tiss. Res. 182, 215-219 (1977).
- Pevet, P. and M. Karasek. Are the pineal active compounds of mammals proteinaceous in nature? An ultrastructural contribution. Ann. Med. Sect. Pol. Acad. Sci., Abstr., in press.
- Pevet, P. and M.A. Kuyper. Etude ultrastructurale des pinéalo-cytes de la Taupe dorée. Biol. Cell. 29, 10a, Abstr. (1977).
- Pevet, P. and J. Ariëns Kappers. Secretory processes in the mammalian pineal gland: an ultrastructural identification. Acta Endocr. Suppl. 212, 157, Abstr. (1977).
- Pevet, P. and J. Ariëns Kappers. Secretory process differing from that producing granular vesicles in the mammalian pinealocytes. An ultrastructural identification. Proc. Internat. Union Physiol. Sci. (Paris) 13, 594 (1977) Abstr.
- Pevet, P. and M. Karasek. The consideration on the nature of the mammalian pineal active compounds. An ultrastructural contribution. (In polish). 9th Congr. Pol. Soc. Endocr., Poznań, June 23-25 (1977) Abstr.

- Pevet, P. and M.A. Kuyper. Granular vesicles in the mammalian pineal gland, their importance in the golden mole (*Ablusomus hottentotus*). Internat. Symp. on the Pineal Gland, Jerusalem, November (1977) Abstr.
- Romijn, H.J., M.T. Mud and P.S. Wolters. Electron microscopic evidence of glycogen storage in the dark pinealocytes of the rabbit pineal gland. *J. Neural Transm.* 40, 69-79 (1977).
- Romijn, H.J. Elektronenmicroscopisch onderzoek aan de epiphysis cerebri *in vitro* van het konijn. De stimulerende invloed van noradrenaline op de secretoire activiteit van het Golgi systeem. *Ned. T. Geneesk.*, in press.
- Romijn, H.J., M.T. Mud and P.S. Wolters. A pharmacological and autoradiographic study on the ultrastructural localization of indoleamine synthesis in the rabbit pineal gland. *Cell Tiss. Res.* 185, 199-214 (1977).
- Romijn, H.J. Endocriene secretie in de epiphysis cerebri van het konijn. Een elektronenmicroscopisch onderzoek. *Ned. T. Geneesk.*, in press.
- Swaab, D.F., J. Dogterom, F.W. van Leeuwen and K. Boer. Arginine-vasotocin in the foetal rat pituitary gland. *J. Endocr.* 72, 4P-5P (1977).
- Swaab, D.F., K. Boer and W.J. Honnebier. The influence of the fetal hypothalamus and pituitary on the onset and course of parturition. In: *The Fetus and Birth*, Ciba Foundation Symp. 47, 379-400, J. Knight and M.O.'Connor (eds.), Elsevier, Amsterdam-New York (1977).
- Swaab, D.F. Functional anatomy of the hypothalamus. Abstr. Boerhaave Committee for postgraduate education "Clinical and Experimental Aspects of Neuroendocrinology", Leiden (1977).
- Swaab, D.F., C.W. Pool and F.W. van Leeuwen. Can specificity ever be proved in immunocytochemical staining? Letter to the Editor. *J. Histochem. Cytochem.* 25, 388-391 (1977).
- Swaab, D.F. The Netherlands Institute for Brain Research. *NUFFIC Bull.* 21 (2), 13-20 (1977).
- Swaab, D.F., F.W. van Leeuwen, J. Dogterom and W.J. Honnebier. The fetal hypothalamus and pituitary during growth and differentiation. *J. Steroid Biochem.* 8, 545-551 (1977).
- Swaab, D.F. and M. Visser. A function for α -MSH in fetal development and the presence of an α -MSH-like compound in nervous tissue. In: *Melanocyte Stimulating Hormone: Control, Chemistry and Effects*, F.J.H. Tilders, D.F. Swaab & Tj.B. van Wimersma Greidanus (eds.), pp. 170-178. Karger, Basel (1977).
- Swaab, D.F. Hersenen en Ouderdom. Boekbespreking "Aging" vol. 1, *Clinical, Morphological and Neurochemical Aspects in the Aging*

- Central Nervous system, H. Brody, D. Harman & J.M. Ordy (eds.), Raven Press, New York, 1975, 221 pp., voor het Vakblad voor Biologen 21, 372 (1977).
- Swaab, D.F. and K. Boer. The role of the fetal brain and pituitary in intrauterine growth and parturition. Proc. Vth Internat. Symp. of Obstetrics and Gynaecology: Current trends in Obstetrics and Gynaecology, Barcelona, in press.
- Swaab, D.F., G.J. Boer, K. Boer, J. Dogterom, F.W. van Leeuwen and M. Visser. Fetal neuroendocrine mechanisms in development and parturition. In: Maturation of the Nervous System, M.A. Corner *et al.* (eds.), Progress in Brain Research, vol. 48, Elsevier, Amsterdam, in press.
- Swaab, D.F. and B. Fisser. Immunocytochemical localization of α -MSH-like compounds in the rat nervous system. Neurosci. Lett. in press.
- Tilders, F.J.H., D.F. Swaab and Tj.B. van Wimersma Greidanus. (eds.) Melanocyte Stimulating Hormone: Control, Chemistry and Effects. Karger, Basel (1977).
- Uylings, H.B.M. Optimization of diameters and bifurcation angles in lung and vascular tree structures. Bull. Math. Biol. 39, 509-520 (1977).
- Uylings, H.B.M., K. Kuijpers and W.A.M. Veltman. Environmental influences on the neocortex in later life. In: Maturation of the Nervous System, M.A. Corner *et al.* (eds.), Progress in Brain Research, vol. 48, Elsevier, Amsterdam, in press.
- Uylings, H.B.M., K. Kuijpers, W.A.M. Veltman and M.C. Diamond. Dendritic outgrowth in the visual cortex of adult rats induced by environmental conditions. Abstr. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 24 (1977).
- Van De Poll, N.E., J.P.C. de Bruin, H. van Dis and H.G. van Oyen. Gonadal hormones and the afferentiation of sexual and aggressive behavior and learning in the rat. In: Maturation of the Nervous System, M.A. Corner *et al.* (eds.), Progress in Brain Research, vol. 48, Elsevier, Amsterdam, in press.
- Van De Poll, N.E. and H. van Dis. Hormone induced lordosis and its relation to masculine sexual activity in male rats. Horm. Behav. 8, 1-7 (1977).
- Van De Poll, N.E. and H. van Dis. The effect of dexamethasone on masculine sexual behavior in rats. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 329 (1977) Abstr.
- Van De Poll, N.E., H. van Dis and B. Bermond. The induction of mounting behavior in female rats by p-chlorophenylalanine. Eur. J. Pharmacol. 41, 225-229 (1977).

- Van Der Meché, A.P. Spontaneous barrier-crossing of goldfish (*Carassius auratus L.*) in a shuttle-box: its use as a one-trial learning process with positive reinforcement. Abstr. Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 294 (1977).
- Van Dis, H., M.A. Corner, R. Dapper and W. Hanewald. Consistency of individual differences in the human EEG during quiet wakefulness. *Electroenceph. clin. Neurophysiol.* 43, 574 (1977).
- Van Leeuwen, F.W. and D.F. Swaab. Specific immunoelectronmicroscopic localization of vasopressin and oxytocin in the rat neurohypophysis. *J. Endocr.* 72, 62P (1977).
- Van Leeuwen, F.W. and D.F. Swaab. Specific immunoelectronmicroscopic localization of vasopressin and oxytocin in the neurohypophysis of the rat. *Cell Tiss. Res.* 177, 493-501 (1977).
- Van Leeuwen, F.W. and D.F. Swaab. Specific immunocytochemistry of neurohypophyseal peptides. *Ultramicroscopy* 2, 136 (1977).
- Van Leeuwen, F.W. Immunoelectronmicroscopic demonstration of neurohypophyseal hormones. *J. Histochem. Cytochem.* 25, 240 (1977).
- Van Leeuwen, F.W. and D.F. Swaab. Optimization of ultrastructural immunocytochemistry of rat neurohypophyseal substances. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 278 (1977).
- Van Leeuwen, F.W. Immunoelectron microscopic visualization of neurohypophyseal hormones: evaluation of some tissue preparations and staining procedures. *J. Histochem. Cytochem.* 25, 1213-1221 (1977).
- Van Leeuwen, F.W. Immunoelectron microscopic methods for the optimal and specific localization of vasopressin and oxytocin in rat hypothalamic-neurohypophyseal system. *Ultramicroscopy*, in press.
- Van Oyen, H.G. and N.E. van de Poll. Acquisition and retention of alternation performance of male and female rats. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 25 (1977).
- Van Wijk, M. and J. Korf. Levels of tricyclic antidepressant drugs and metabolism of 5-hydroxytryptamine in rat brain after acute and chronic treatment. Abstr. 18e Fed. Vergadering van Med.-Biol. Ver., Leiden, April, p. 436 (1977).
- Van Wijk, M. and J. Korf. Probenecid induced accumulation of 5-HIAA and levels of tricyclic antidepressants in the rat brain after acute and chronic treatment. Abstr. 6th Meeting Internat. Soc. Neurochemistry, Copenhagen, August, p. 490 (1977).
- Van Wijk, M., J.-J. Meisch and J. Korf. Metabolism of 5-hydroxytryptamine and levels of tricyclic antidepressant drugs in rat

brain after acute and chronic treatment. *Psychopharmacology* 55, 217-223 (1977).

Visser, M. and D.F. Swaab. α -MSH in the human pituitary. In: *Melanocyte Stimulating Hormone: Control, Chemistry and Effects*, F.J.H. Tilders, D.F. Swaab and Tj.B. van Wimersma Greidanus (eds.), pp. 42-45, Karger, Basel (1977).

THESES

Dogterom, J. The Release and Presence of Vasopressin in Plasma and Cerebrospinal Fluid as measured by Radioimmunoassay; Studies on Vasopressin as a Mediator of Memory Processes in the rat. University of Utrecht, February 2, 1977.

Uylings, H.B.M. A Study on Morphometry and Functional Morphology of Branching Structures, with Applications to Dendrites in Visual Cortex of Adult Rats under Different Environmental Conditions. University of Amsterdam, June 1, 1977.

Smith, J. Quantitative EEG Analysis of the Developing Chick. University of Utrecht, June 4, 1977.

Bakhuis, W.L. The Causal Organization of Climax Behavior in the Domestic Fowl (*Gallus domesticus*). University of Groningen, September 16, 1977.

De Bruin, J.P.C. Telencephalic Functions in the Behaviour of the Siamese Fighting Fish, *Betta splendens* Regan (*Pisces, Anabantidae*). University of Amsterdam, September 21, 1977.

Delta Institute for Hydrobiological Research

Progress Report 1977

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Institute for Hydrobiological
Research, Vierstraat 28,
4401 EA Yerseke, The Netherlands

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 biological changes to be expected as results 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 Ysselmeer in 1932, extensive biological research was carried out by a group of fishery biologists, members of botanical and zoological societies and academic staff, under the direction of Dr. H.C. Reedeke between 1920 and 1950. 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 co-ordinated effort to study the problems from various angles. After an exploratory phase, in which qualitative distribution of biota was to be studied from an ecological point of view, research was initiated, in order to elucidate the causal background of the changes observed.

The institute was erected under the name "Division Delta-Research of the Hydrobiological Institute", with the object to stress its affiliation to the Hydrobiological Institute at Nieuwersluis. As both institutes grew and matured the difference between the studies carried out in both of them gradually became apparent and in 1968 it was decided to change both names into their present form.

The institute is located at Yerseke on the Oosterschelde, the sea-arm to be semi-closed in the last stage of the s.c. "Delta-plan".

The exploitation of the institute is financed by means of funds allotted to the Academy, by the Ministry of Education and Science.

The institute had in 1977 a staff of personnel of 58 including 12 scientists (Table 1 a) and 2 members paid by the A.D.Z.. Additionally guestworkers, students and trainees took part in the programme of the Institute (Table 1 b).

(Lay-out, figures & plates: M.E. v.d. Boomgaard-Pekaar, J.A. v.d. Ende, A.A. Bolsius and R.H.G. Kleingeld).



Plate I: Dr. Karel Frederik Vaas was born on October 29th 1911 at Arnhem and studied biology at Leyden, where he passed in 1936 his final examination in botany (Prof. Dr. L.G.M. Baas Becking), zoology (Prof. Dr. C. van de Klaauw and Dr. H.P. Wolvekamp) and microbiology (Prof. Dr. A. Kluyver, Techn. Univ. Delft). In 1938 he was awarded the degree of doctor of philosophy after defending his thesis on "Studies on *Bacillus megatherium* de Bary". He worked for a short time at a high school in Wassenaar, teaching biology, and departed to Buitenzorg (now Bogor), Indonesia, to take up a post as a Fishery Consultant in the Section of Inland Fisheries of the Department of Agriculture. During World War II he was in the Dutch Colonial Army and took part in the short actions on Java, was taken prisoner by the Japanese Army and went through various Prisoner of War Camps in Java, Siam and Birma, working on the Birma-Siam Railway.

After a one-year leave of recuperation in the Netherlands, during which period he visited a number of Hydrobiological Stations in his home country and abroad, he returned to Java, as the Head of the Laboratory of Inland Fisheries, Bogor, and later carrying out research work in an advisory position. In Java he has been engaged in research on lakes, reservoirs, eradication of noxious weeds, transport of life fish fry by air, the newly immigrated fish species *Tilapia mossambica* (Ikan mudjair) and the production and consumption of natural food in carp ponds, the latter work in cooperation with his wife, A. Vaas-van Oven.

In 1958 he and his family returned to the Netherlands where he assumed the responsibilities of director of the newly created Section for Research in the Deltaic Area of the Hydrobiological Institute of the Royal Netherlands Academy of Arts and Sciences, and since 1968 the Delta Institute for Hydrobiological Research. He was director until his retirement on November 1st 1976 but remained until February 1st 1977 attached as advisor to the institute.

On the photograph Dr. Vaas is wearing the decoration of officer in the order of Oranje-Nassau, handed over by the Mayor of Reimerswaal (Yerseke) on behave of H.M. Queen Juliana.

BIBLIOGRAPHY OF DR. K.F. VAAS

- 1938; *Studies on the growth of Bacillus megatherium* de Bary. Thesis Leiden pp. 85.
- 1941; *Het Rawa complex te Tjampurdarat als visserij-object* (with J.J. Schuurman). *Landbouw*, 17: 13-55.
- 1947; *Iets over de Zoetwatervisserij op Java, Borneo en Celebes*. *Hand. Hydr. Ver.*
- 1947; *Biologische inventarisatie van de Binnervisserij in Indonesie*. *Landbouw*, 19: 1-23.
- 1947; *Over de betekeris van de zoetwaterflora als visvoedsel op Java*. *Vakbl. Biol.*, 27: 51-58.
- 1948; *Over het gebruik van visvijvers bij de reiniging van afvalwater in de tropen*. *Landbouw*, 20: 331-348.
- 1949; *On the ecology and fisheries of some javanese freshwaters* (with J.J. Schuurman). *Gen. Agr. Res. St. Buitenzorg, Java*. no. 97: 1-60.
- 1949; *On the ecology of some small lakes near Buitenzorg, Java* (with M. Sachlan). *Hydrobiologia*, 1: 238-250.
- 1950; *Bemesting van binnenwateren*. *Chronica Naturae*, 106: 1-3.
- 1951; *Notes on the Water hyacinth in Indonesia and its eradication by spraying with 2,4-D*. *Comm. Gen. Agr. Res. St. Bogor, Indonesia*. no. 120: 1-59.
- 1951; *Preliminary report on air transport of live fish in sealed tins under oxygen pressure*. *Proc. Indo-Pacific Fish. Council. Sec. II. S2/8*, pp. 10.
- 1952; *Methoden en uitkomsten van biologisch onderzoek naar economische bevissing in zee en zoetwater*. *Landbouw*, 24: 65-138.
- 1952; *Studies on Tilapia mossambica Peters (ikan mudjair) in Indonesia* (with A.E. Hofstede). *Contr. Int. Fish. Res. St.* no. 1: 1-88.
- 1952; *Notes on, and possibilities of fertilization of fish ponds in Indonesia*. *Proc. Nat. Inst. Sc. India*, 18: 1-11.
- 1952; *Zoet- en brakwatervis als voedingsmiddel in Indonesia*. *Landbouw* 24: 223-254.
- 1952; *Merkwaardige lagere kreeften*. *De Trop. Nat.*, 32: 131-133.
- 1952; *Notes on fisheries exploitation of the artificial lake Tjiburuj in West Java* (with M. Sachlan). *Contr. Gen. Agr. Res. St. Bogor*. no. 128: 1-22.
- 1952; *Fisheries in the lake district along the rivers Kapuas in West Borneo*. *Proc. Indo-Pacific Fish. Council. Sec. II. S2/10*, pp. 10.
- 1953; *Een Indonesische Bladpoot-kreeft*. *De Trop. Nat.*, 33: 45-48.
- 1953; *On the ecology and fisheries of some inland waters along the rivers Ogan and Komring in south-east Sumatra* (with M. Sachlan & G. Wiraatmadja). *Cont. Fish. Res. St.* no. 3: 1-32.
- 1954; *Enige aspecten van de tropische limnologie* (with A. Vaas van Oven). *Vakbl. Biol.* 34: 86-94.
- 1954; *Over de zweemblaas der vissen*. *M.I.A.I.* no. 4, 5, 6. pp. 176-186.
- 1954; *On the nutritional relationship between plankton and fish in Indonesian ponds*. *UNESCO Symp. Bangkok*. pp. 1-8.
- 1954; *Limnological studies on diurnal fluctuations in shallow ponds in Indonesia* (with M. Sachlan). *Proc. int. Ass. theor. appl. Limnol.*, 12: 309-319.
- 1955; *Cultivation of common carp in running water in west Java* (with M. Sachlan). *Proc. Indo-Pac. Fish. Council. Sec., II*: 1-10.
- 1957; *Some applications in seafisheries of methods used in inland fisheries*. *Berita Perikanan*, 9: 10-12.
- 1957; *Differences and similarities between culture and capture operations in inland fisheries*. *Curr. Aff. Bull. IPCF*, 19: 1-5.
- 1959; *Studies on the production and utilization of natural food in Indonesian carp ponds* (with A. Vaas-van Oven). *Hydrobiologia*, 12: 308-392.
- 1961; *De taak en het arbeidsveld van de Afdeling Delta-Onderzoek van het Hydrobiologisch Instituut te Yerseke*. *Vakbl. Biol.*, 41: 1-8.
- 1961; *Das Arbeitsfeld des Hydrobiologischen Institutes, abteilung Delta-Untersuchungen, in den Niederlanden*. *Int. Rev. ges. Hydrobiol.*, 46: 292-295.
- 1962; *Lophius piscatorius L., de Zeeduivel*. *Het Zeepaard*, 22: 112-113.
- 1963; *Annual report of the Delta division of the Hydrobiological Institute of the Royal Netherlands' Academy of Sciences for the years 1960-1961*. *Neth. J. Sea Res.* 2: 68-76.
- 1963; *Hydrobiologisch onderzoek in de Zeeuwse en Zuid-Hollandse wateren*. *Visserij Nieuws*, 16(7): 181-183.

- 1964; Annual report of the Delta division of the Hydrobiological Institute of the Royal Netherlands' Academy of Sciences for the year 1962. *Neth. J. Sea Res.*, 2: 284-292.
- 1964; De zwarte grondel, *Gobius niger*, in het Veerse Meer. *De Levende Natuur*, 67: 243-245.
- 1965; Annual report of the Division Delta-Research of the Hydrobiological Institute of the Royal Netherlands' Academy of Sciences for the year 1963. *Neth. J. Sea Res.*, 2: 605-614.
- 1965; De afdeling Delta-Onderzoek van het Hydrobiologisch Instituut der Kon. Ned. Akademie van Wetenschappen. *Het Zeepaard*, 25: 35-41.
1965. De afdeling Delta-Onderzoek van het Hydrobiologisch Instituut der Kon. Ned. Akademie der Wetenschappen. *Contactbl. Oecol.*, 1(2): 5-11.
- 1966, 1967, 1968a, 1968b; Annual report of the Division Delta-Research of the Hydrobiological Institute of the Royal Netherlands' Academy of Sciences for the years 1964, 1965, 1966 and 1967. *Neth. J. Sea Res.*, 3: 164-178; 3: 488-503; 4: 110-122 and 4: 268-281.
- 1966; Lakes in Dutch Reclamation Schemes. In: *Man-Made lakes*, ed. by R.H. Lowe McConnel, pp. 119-128, Academic Press, London.
- 1966; Resultaten van de visvangst in de Oosterschelde tijdens het S.W.G.-kamp 24-31 aug. 1964. *Het Zeepaard*, 26: 100-102.
- 1968; De visfauna van het Estuariumgebied van Rijn en Maas. *Biol. Jaarb. Dodonaea*, 36: 115-128.
- 1968; Een zee-engel (*Squatina squatina* (L.)) uit de Oosterschelde. *Het Zeepaard*, 28: 8-9.
- 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977; Annual report of the Delta Institute for Hydrobiological Research for the year 1968-1976. *Institutes of the Royal Netherlands Academy of Sciences Progress Report*.
- 1970; Studies on the fish fauna of the newly created Lake near Veere, with special emphasis on the plaice (*Pleuronectes platessa*). *Neth. J. Sea Res.*, 5: 50-95.
- 1970; Waterverontreiniging veroorzaakt door anorganische en organische kwikverbindingen. *Milieuhygiëne in Zeeland*, 1(3): 15-16.
- 1971; Biologisch onderzoek in het Veerse Meer. *Veerse Meergids*, 4(17): 12.
- 1971; Oil ravages the Biesbosch. *Mar. Poll. Bull.*, 2: 51-52.
1971. Thermische verontreiniging. *Vakbl. Biol.*, 6: 146-152.
- 1971; Water als koelmiddel bij de opwekking van elektriciteit. *Bull. Ver. Milieuhygiëne Zeeland*, 2(3): 8-9.
- 1972; Het Delta Instituut voor Hydrobiologisch Onderzoek te Yerseke. *Contactbl. Oecol.*, 8: 1-4.
- 1972; Een zeekarper in de Oosterschelde. *Het Zeepaard*, 32: 42-44.
- 1973; De rol van de vissen in het aquatische milieu. (Lezing cursus Limnologie van de Hydrobiologische Vereniging, Amsterdam, 1973). Yerseke, Delta Instituut voor Hydrobiologisch Onderzoek Interne verslagen 1973-8 pp. 21.
- 1975; Immigrants among the animals in the Delta-area of the SW-Netherlands. *Hydrobiol. Bull.*, 9: 114-119.
- 1975; with A.G. Vlasblom & P. de Koeijer; Studies on the Black Goby (*Gobius niger*, *Gobiidae*, *Pisces*) in the Veerse Meer, S.W. Netherlands. *Neth. J. Sea Res.*, 9: 56-68.
- 1977; with B.G. Otten, Zuidelijke immigranten in de Zeeuwse dierenwereld. *Natura*, 74: 32-35.

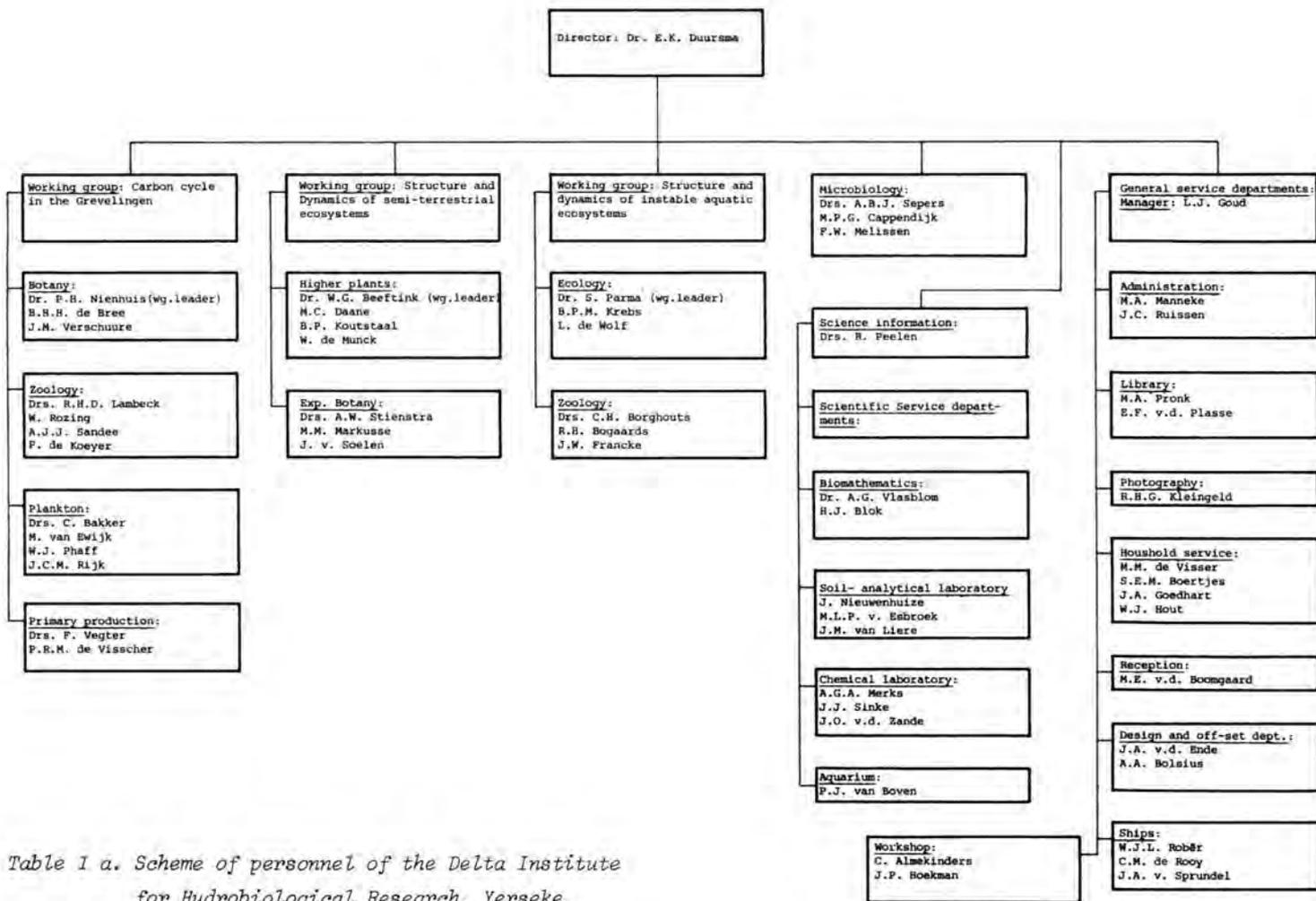


Table 1 a. Scheme of personnel of the Delta Institute for Hydrobiological Research, Yerseke.

Table 1 b

Guest workers

Drs. U.H. de Graaf (fish investigations), Ir. J.W. Rijstenbil (oxygen budgets), Drs. I.J. Weeber (biological classification), Drs. M.E. Siemelink (bio-modelling), R. Luyendijk (biological-assistant).

Students

M.J. Allan, J. Goedbloed, P. Valentijn, J. Koniuszek, P. Elenbaas, N. Tramper, E. Daemen, H. Sips, G. Pellikaan, M. Langeveld, J. Stellingwerf, N. Joanknecht, L. Meuleman, H. v.d. Ster, H. Dorenbosch.

Trainees

L. v.d. Velde, A.M. v.d. Repe.

INTRODUCTION

E.K. Duursma

The research carried out by the Delta Institute for Hydrobiological Research at Yerseke is directed to the study of aquatic and semi-terrestrial systems in the south-west Netherlands Delta of the rivers Rhine, Meuse and Scheldt connected to the North Sea. The studies have been started in 1957 with detailed surveys of flora and fauna and their reactions on the changes which occurred due to the closing of a number of sea-arms with dams (Fig. 1).

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 long periods. It is thus possible to carry out ecosystem studies under a variety of environmental conditions. The last years and also in 1977 these studies have been focussed on the functioning of estuarine and lagoon systems in general and those of the S.W.-Netherlands in particular. The results are considered essential for the environmental agencies in charge of the management policy of these waters.

This research was concentrated around a number of central themes on which the investigations were carried out by working groups (see also Table 1 a). The themes considered (i) the "Carboncycle in the Grevelingen", (ii) the "Structure and Dynamics of the Salt-marsh Ecosystem" and (iii) the "Structure and dynamics of instable aquatic ecosystems". The progress results of these three working groups are presented in this order under the project codes G, S and B, respectively.

Additionally a number of separate subjects have been studied by individual scientists and assistants. Their results are presented at the end of this report under the project code A.

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

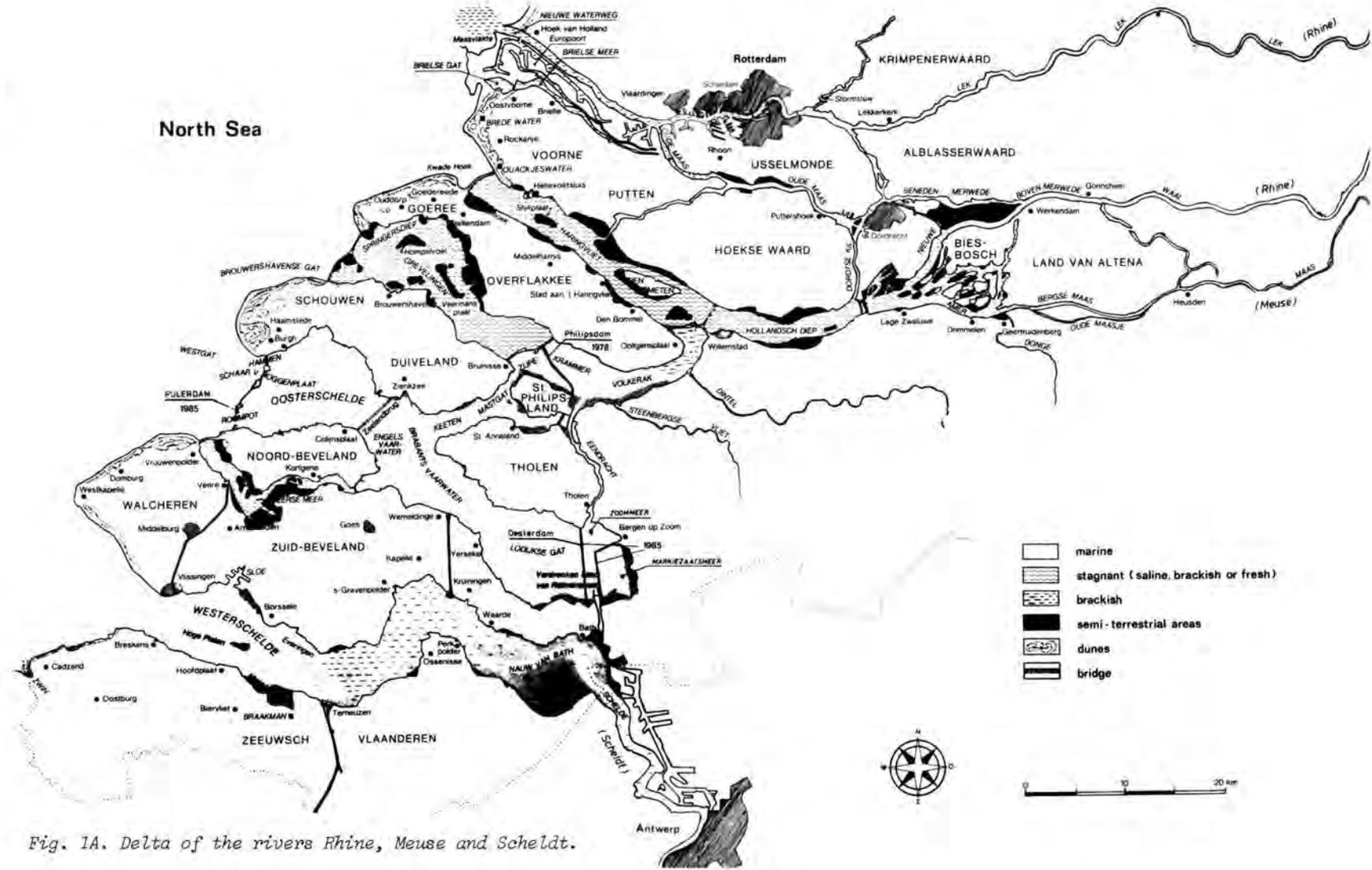


Fig. 1A. Delta of the rivers Rhine, Meuse and Scheldt.

Hydrological changes in the estuaries and sea-arms of the SW-Netherlands as a consequence of the construction of embankment dams.

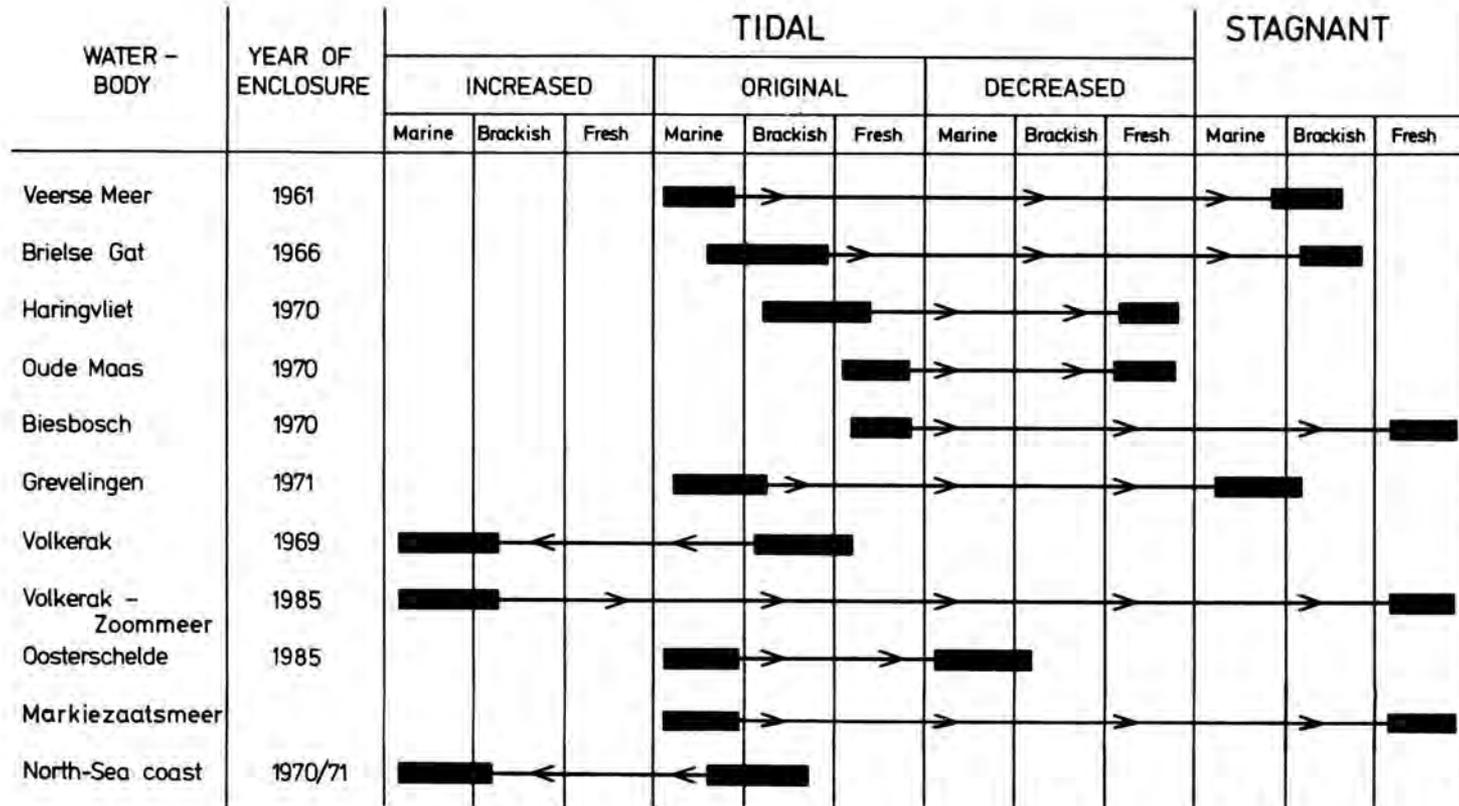


Fig. 1B. Hydrological changes due to the "Delta Plan".

working group projects and the more individually carried out projects for a period of five years. Reference is made to the workingplans 1977-1982 and 1978-1983 of the institute.

For general information, the ecological conditions for the area of the S.W.-Netherlands are presented previous to the progress results of the working groups.

Anonymous 1976 - Werkplan 1977-1982, W.P.-02. Delta Institute for Hydrobiological Research, Yerseke, Neth., pp. 36.

Anonymous 1977 - Werkplan 1978-1983, W.P.-03, Ibid. pp. 53.

GENERAL ECOLOGICAL CONDITIONS IN 1977

R. Peelen

Temperature

The winter period of 1977 was characterized by elevated average temperatures: January 3.3°C (normal 2.9°C); February 5.0°C (normal 3.0°C), March 7.0°C (normal 5.2°C). The other months of the year were all subnormal while the spring and summer were cool, with during April-June -1.0°C and July-September -0.7°C lower average temperatures than normal.

Solar radiation

For Vlissingen the radiation quantities of 1977 were higher than the normal values given for the station the Bilt except in August. However with regard to the passed year 1976 the radiation quantities in April and May, June, July and August were in general lower except for the first decade in June. This comparison applies in so far that 1976 was an exceptional year with fine weather (see Fig. 2).

Rainfall

In comparison with the normal rainfall in Vlissingen the following characteristics can be noted: January equal and February half as much rain as normal. March, April, May and June slightly more rain than normal. July was dryer than normal. August was slightly wetter than normal and September was dry with less rainfall than one third below normal (Table 2).

Windspeed

The windspeed measurements in meters/sec are presented in Table 2. January was more quiet than average, February was nearly average and March, April and May were more windy than average; June and August were again quieter than average and July and September equal to average.

River discharges

The water discharge of the rivers Rhine and Meuse varied this year slightly around the normal average yearly value which is for the Rhine at Lobith $2200\text{ m}^3/\text{sec}$ (near the German border) and for the Meuse at Lith $330\text{ m}^3/\text{sec}$. No daily measurements were available of the Scheldt, which river has an average yearly value of $95\text{ m}^3/\text{sec}$. Discharges with regard to the normal values are

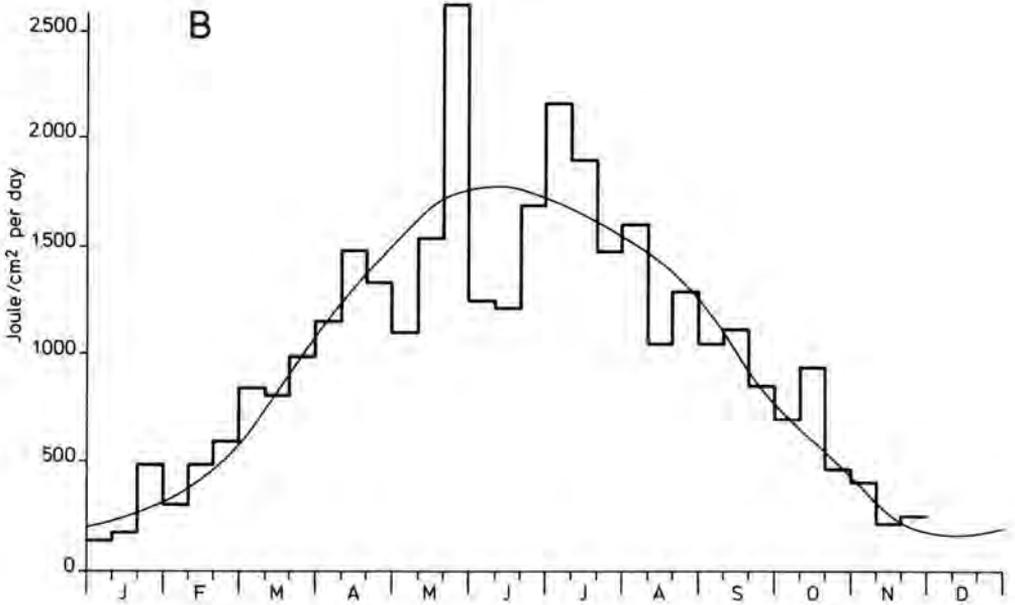
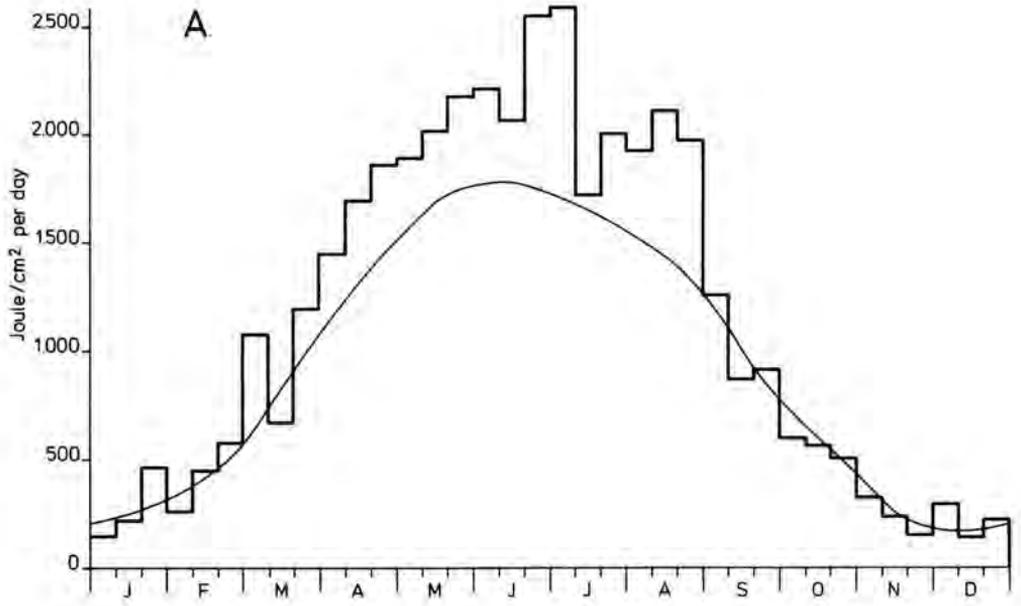


Fig. 2. Solar radiation at Vlissingen (block diagramme) for 1976 (A) and 1977 (B). The smooth curve represents the mean solar radiation at the Bilt, determined by the Royal Netherlands Meteorological Institute.

given in Table 2.

Only at the end of February there is some melting water in the rivers and the discharge reached three times normal values, followed by a period of normal discharges in the Rhine. In the corresponding period the discharge of the Meuse was lower than normal from May till October. A very low discharge of $1/14 \times \text{normal}$: $23 \text{ m}^3/\text{sec}$ was measured on October 14th. Yet a similar discharge in that time of the year is not extremely rare. In dry years sometimes no water flows over the barrage at all for a period of some days.

The managing of the locks in the Haringvliet is strongly related to the discharges of Rhine, Meuse and Scheldt and to the isohaline pattern in the mouth of the Rotterdamse Waterweg, in the mouth of the Haringvliet and in the mouth of the Western Scheldt. All locks in the Haringvliet were opened for a short time on 24th and 21th February, when discharges respectively of 6268 and 6174 m^3/sec at Lobith and 1009 and 1015 m^3/sec at Lith, were measured.

In order to stop salt intrusion in the Hollands Diep, fresh water was introduced into the Volkerak at an amount of $25 \text{ m}^3/\text{sec}$. From February 1th onwards it was raised to $50 \text{ m}^3/\text{sec}$. This was done in order to study the freshening when the "Philipsdam" and the Oysterdam in Krammer and Eastern Scheldt will be completed. This $50 \text{ m}^3/\text{sec}$ includes the discharges of the Dintel and the Steenbergse Vliet. Whether there will be a follow-up of this experiment with $100 \text{ m}^3/\text{sec}$ freshwater into the Volkerak is not yet decided. Calculations are being carried out in order to evaluate the eventual reaction of the Oosterschelde system on a similar operation as regards the flora and fauna of this waterbody.

Technical works by the Department of Roads and Waterways

1. A part of the Scheldt-Rhine shipchannel connection near the Philipsdam was deepened with $\frac{1}{2}$ m. The complete gully is now at the required depth for the waterway.
2. Works on the buildingpit for a shipping lock near the Philipsdam is in progress.
3. A buildingpit for the shipping lock in the Oysterdam will be started the coming year (South of the island of Tholen).
4. The possibility of a shipping lock in the Eastern Scheldt dam near the North Sea is being studied.
5. New stone coverages have been placed on the compartmentsdam in the Krammer and Eastern Scheldt. Mine stones have been used.
6. Eventual closure of the Spui is being considered. A disadvantage of such a dam will be a longer residence time of the water in the Hollands Diep-Haringvliet and thus the possibility of blooms of blue-green algae. Recent experiences however, do not point in that direction.
7. In the Brouwersdam the refreshing lock will be operational in May. This will be of influence on the biocoenosis of the Grevelingen.

At present the chlorinity of the Grevelingen varies from 12.42^o/oo in May till 14,05^o/oo in June. Usually the seawater outside the dam varies between 17 and 18^o/oo Cl⁻. Intake of this North Sea water into the Grevelingen might give salt stratifications in addition to the present thermo-stratification in spring, summer and autumn in the pits of Scharendijke and den Osse. These pits are respectively 52 and 38 m deep. With large intakes of North Sea water it is possible that the zone of more saline water will reach in the Brouwershavense Gat of 20 m, and in the Grevelingen and Delta gully of respectively 26 and 10 m depth.

Table 2. Temperature, rainfall, windspeed and riverdischarge.

	Temperature ° C			Rainfall mm	
	measur.	normal	deviat.	measur.	normal
January	3.3	2.9	+ 0.4	60.9	61.9
February	5.0	3.0	+ 2.0	68.9	44.8
March	7.0	5.2	+ 1.8	52.9	40.8
April	7.2	8.4	- 1.2	56.1	41.9
May	11.8	12.1	- 0.3	49.7	44.1
June	13.8	15.3	- 1.5	39.1	48.9
July	16.8	17.2	- 0.4	38.8	69.4
August	16.3	17.4	- 1.1	77.2	66.1
September	14.8	15.5	- 0.7	21.6	72.8
October	13.0	11.5	+ 1.5	70.2	38.8
November	8.0	7.3	+ 0.7	139.5	75.2

	windspeed m/sec		river discharge x normal	
	measur.	normal	Rhine	Meuse
January	6	7	½ x	2/3 x
February	6.5	6.5	2 x	2 x
March	4	3.5	½ x	½ x
April	4.5	3.5	1 x	2 x
May	6	5	1 ⁺ x	½-1 x
June	5	5.5	1 ⁻ x	½-1/3 x
July	6	6	1 ⁻ x	1/3-½ x
August	2.5	3.5	1 ⁻ x	1/3-1/5 x
September	5.5	5.5	1 - ½ x	1/3-1/6 x
October	6	5.5	½ x	1/3-1/14x
November	9	6.5	1-1½ x	1-2 x
December			1 x	1-2/3 x

References

Maandelijks overzicht der weersgesteldheid K.N.M.I., 74e jaargang.

Rijkswaterstaat afvoeren in m^3/sec van Rijn en Maas

Personal communications of Rijkswaterstaat (Department of Roads and Waterways).

WORKING GROUP "CARBON CYCLE IN THE GREVELINGEN"

INTRODUCTION P.H. Nienhuis

The southwestern part of the Netherlands has been shaped by the North Sea and by three large rivers, Rhine, Meuse and Scheldt. In 1953 about 1800 people were killed by an extreme storm flood, inundating more than half of the inhabited area. In order to avoid repetition of the flooding disaster, it was decided to close the main estuaries by high dikes. The Grevelingen was one of those estuaries, before 1971 in connection with the river Rhine and with the North Sea. In 1971 a dam closed the mouth of this estuary, excluding influences both from the rivers and from the sea: and the saline lake Grevelingen was created.

In 1972 it was decided to set up an ecosystem study centred around the cycling of organic matter in the Grevelingen. The study of the carbon cycle was chosen since the structure and the functioning of an ecosystem is supported by energy which is transmitted via carbon fluxes through the food chains. The working group consists of a team of 25 biologists, chemists and assistants. The group covers a number of aspects of the organic matter cycle, viz. the primary productivity measurements of phytoplankton by ^{14}C , biomass estimations of phytoplankton, microphytobenthos and macrophytes. Within the broad scope of the secondary producers attention is paid to zooplankton biomass, and biomass and production of macrozoobenthos. Further, studies are performed on meiozoobenthos and benthic and pelagic fishes. In relation to the efforts on production level, up to now, only little attention is paid to decomposition and mineralisation processes. Fig. 3 shows a box model of the Grevelingen carbon cycle. The different projects of the working group have been numbered from G 1 to G 10. Project G 11 comprises our efforts to build an ecological model of the Grevelingen carbon cycle.

Active members of the working group are T. Aldenberg, (Delta-service, Ministry of Roads and Waterways, Middelburg; modelling), Mrs. L. Arkesteijn (Agricultural University, Wageningen; phytoplankton), C. Bakker (plankton), B.H.H. de Bree (phytobenthos), E.K. Duursma (water-sediment interactions), Miss U.H. de Graaf (fishes), R.H.D. Lambeck (macrozoobenthos), A.G.A. Merks (chemical processes), P.H. Nienhuis (phytobenthos; leader of the working group), A.B.J. Sepers (mineralisation), M.E. Siemelink (eelgrass model), B. Steinmetz (Ministry of Agriculture and Fisheries; fisheries), F. Vegter (primary production phytoplankton), A.G. Vlasblom (mathematician), K. Willems (University Gent, Belgium; meiozoobenthos).

CARBONCYCLE IN LAKE GREVELINGEN

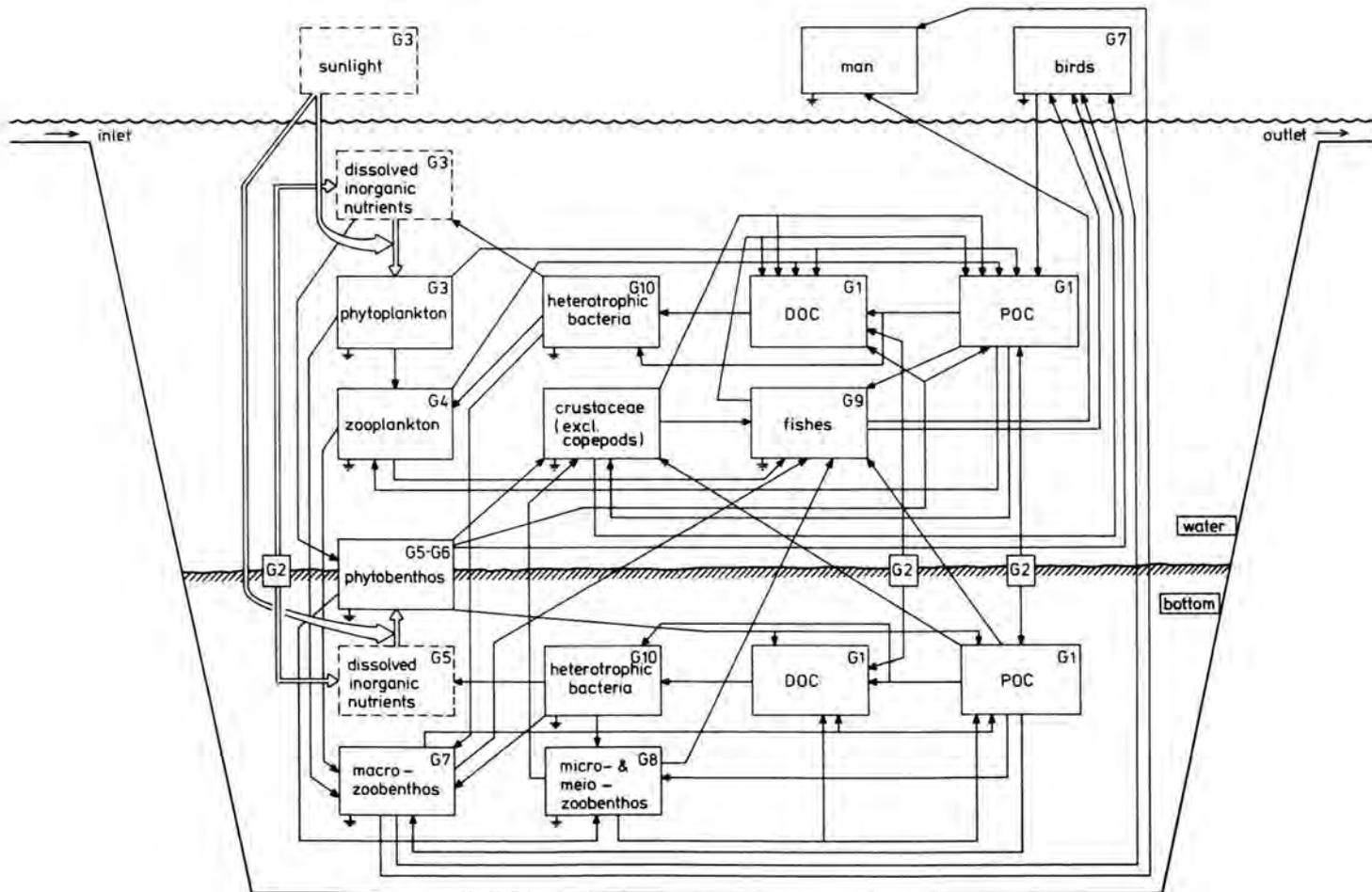


Fig. 3. Carboncycle in the saline Lake Grevelingen.

PARTICULATE AND DISSOLVED ORGANIC CARBON IN THE WATER COLUMN AND IN THE SEDIMENTS (G 1)

A.G.A. Merks

ATP may act as biomass indicator for living organisms in the watercolumn and in the bottom. Chlorophyll is used to measure phytoplankton standing stock. Preliminary data were gathered for the conversion factors between organic carbon and ATP and organic carbon and chlorophyll-A. On account of the extremely large range of the data these conversion factors will be studied in more detail in 1978.

Chlorophyll-A measurements in the water column were performed in a few selected stations. In order to get some insight into the spatial distribution of phytoplankton biomass a Turner fluorometer was used on board the MS "Marris Stella" for continuous registration of chlorophyll-A concentrations. The results show a non significant relation between the fluorometer data and the actual chlorophyll concentrations in the water mass. Scanning with only one fluorometer in Lake Grevelingen can be used to locate pigment concentrations; it cannot be used to measure quantities of chlorophyll-A in an accurate way.

Little progress was made in measuring dissolved organic carbon concentrations in water and sediment, owing to the fact that the TOC-sin analyser, bought in 1976, showed still serious failures. It was determined that the conservation of frozen water samples showed extremely high DOC-values due to contamination of the screw cups of the bottles used.

INTERACTIONS ON THE WATER SEDIMENT INTERFACE (G 2)

E.K. Duursma, P.J.v.Boven, P. de Koeyer, J. Nieuwenhuize & H.J. Sips.

A start has been made on the investigation of bioturbation by shrimps in a laboratory experiment and by the natural benthic fauna in the bottom of the Grevelingen. In both cases grinded gravel is used as sediment tracer. The mixing of gravel with deeper sediment caused by bioturbation is determined.

The laboratory experiment with shrimps showed a penetration of gravel into the sand with a "diffusion" less than the molecular diffusion of chloride. This is valid for an experimental shrimp population per unit surface of less than is usual in our estuaries and lagoons. The pilot experiment in the Grevelingen with gravel showed a stronger mixing of the top layer of the sediment. More refined experiments are in course with coloured sand.

THE ROLE OF PHYTOPLANKTON IN THE CARBON CYCLE (G 3)

L. Arkesteijn, C. Bakker, C. Meijs, R. Peelen, F. Vegter, J.C.M. Rijk & P.R.M. de Visscher.

Miss C. Meijs continued her work on the causes of the decline of the bloom of *Skeletonema costatum*, a dominant spring phytoplankter in Lake Gre-

velingen. She studied the lysis of *Skeletonema* cells during the process of dying off in cultures. The toxic effects of lysigenous products were analysed in light and dark cultures and under low nitrate concentrations. The presence of lysigenous compounds hampers growth significantly, but it is not likely that this process also operates in nature. The concentrations of *Skeletonema costatum* in Lake Grevelingen during the spring bloom of 1976 was 10^3 cells per ml at a maximum. This concentration is far too low to demonstrate effects of autotoxins on the deterioration of the population. Miss Meijs stopped her work in May 1977. Her successor is Mrs L. Arkesteijn.

The plankton environmental parameters of Lake Grevelingen in 1977 can be characterized as follows: chlorinity had values between 12.4 and $14^{\circ}/\text{oo}$. Dissolved orthophosphate fluctuated between 0.21 and 0.58 mg $\text{PO}_4\text{-P/l}$. Nitrate reached its maximum value at the end of January viz. 0.25 mg $\text{NO}_3\text{-N/l}$ and dropped to non-detectable values in May and June. The highest ammonia value was measured in February, 0.25 mg $\text{NH}_3\text{-N/l}$. Ammonia dropped to zero in June (one out of a series of weekly measurements). Secchi-disc transparency amounted to 7 m previous to the spring bloom, and 2-2,5 m during the blooms in spring and summer.

Lake Grevelingen is a relatively shallow water basin (mean depth = 5.3 m). Thermic stratification, connected with anaerobic conditions occurs only temporarily in the deeper gullies. Peelen, who left the working group as an active member in 1977, measured the oxygen content in the 45 m deep gully near Scharendijke during the period 1971-1975 (Klomp & Peelen, 1976). In 1975 stratification was demonstrated (Fig. 4) during the period June to September with oxygen saturation percentages below 5 in the water layer below 25 m.

The results of the quantitative phytoplankton investigations of Bakker deviated from those of 1976. The flagellates *Cryptomonas* spp. dominated the spring bloom with a standing stock of more than three times that of 1976. Two peaks were observed in March and April, with 2000 and 4000 cells/ml respectively, and concomitant chlorophyll-A maximum values of 8 and 20 mg/m^3 . The diatom *Skeletonema costatum*, dominant in spring 1976, was almost completely absent during March and April 1977. Phytoplankton peaks in summer were characterized by the diatoms *Chaetoceros* spp., *Rhizosolenia setigera*, *Ditylum brightwelli*, *Nitzschia seriata* and *Coscinodiscus centralis*. Especially *Chaetoceros debilis* and *C. affinis* appeared in larger numbers (100-2000 cells/ml).

References

- Klomp, R. & R. Peelen, 1976. Natuurlijke en kunstmatige destratificatie in de diepe gedeelten van het Grevelingenmeer. *H₂O*, 9: 106-112.

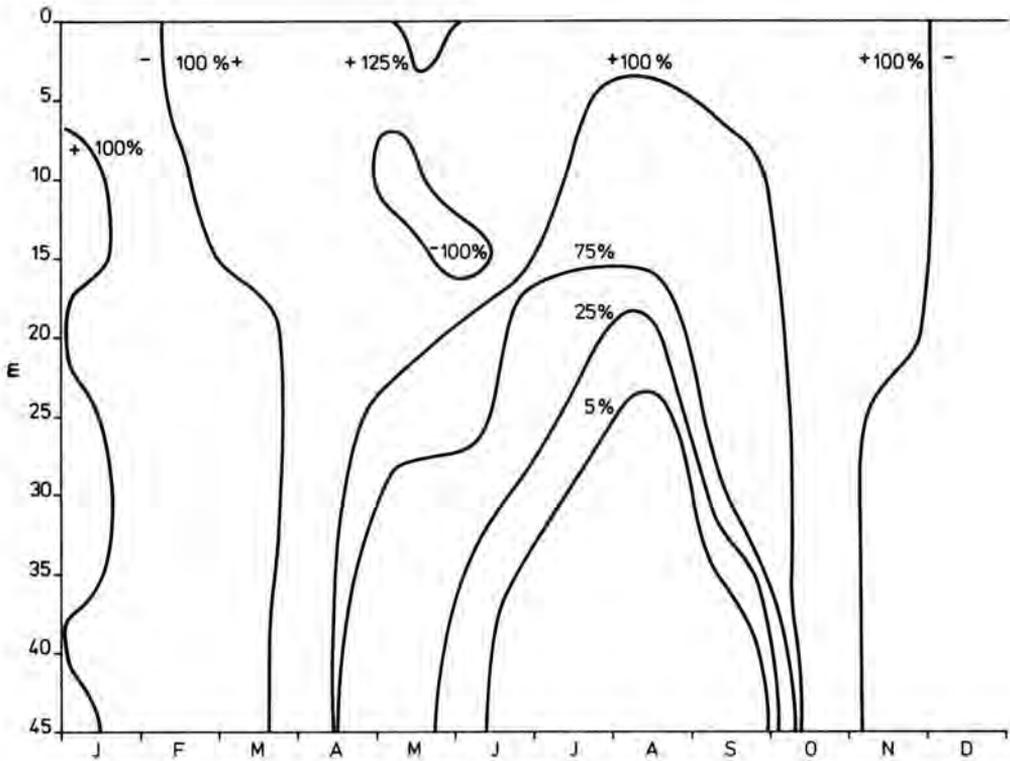


Fig. 4. Oxygen saturation percentages in a gully near Scharendijke in 1975.

PRIMARY PRODUCTION MEASUREMENTS IN THE GREVELINGEN (G 3)

F. Vegter & P.R.M. de Visscher.

One of the principal questions of the working group considers the organic carbon which is delivered to the basin by primary production. To answer this question we started in 1976 a programme on particulate phytoplankton production and excretion measurements.

The *in situ* ^{14}C method outlined by Steemann Nielsen (1952) and modified by Fogg (1958) was used. After an incubation period of 5 hours the samples (50 ml) were filtered by suction (180 mm Hg). The filters were placed immediately in a vial filled with scintillation solution. The filtrate together with the washwater of the filters was collected in a 100 ml bottle. An aliquot of the filtrate (5 ml) was acidified (pH = 2) in a scintillation vial with HCl and air bubbled until all the $^{14}\text{CO}_2$ had disappeared. Instagel (10 ml) was added. All countings were performed in a scintillation counter Isocap Mark II of Nuclear Chicago.

In 1976 the influence of the suction pressure on the excretion of labeled organic compounds by phytoplankton, mainly dominated by diatoms, was checked

Several incubated samples were filtered with different suction pressures between 240 and 0 mm Hg. From 240 mm Hg to 100 mm no decrease in radioactivity of the phytoplankton on the filter could be detected. At about 50 mm Hg a raise of radioactivity on the filter could be detected which was compensated by a decrease of radioactivity in the filtrate before it was air bubbled. On account of the *in situ* productivity measurements in 1977 a repeated check of the filtercorrection will be necessary during a flagellate bloom.

The relation between particulate production during increasing incubation time and the amount of radioactivity in the filtrate was also studied during 1976. It appeared from these experiments that the increase of radioactivity of the particulate production and the concomitant production of dissolved organic compounds did not always run linear with time. Some curves of both parameters reached a certain level; these curves were S-shaped, which is in contrast with the results of Fogg *et al.* (1965). A time lag was observed between the curve of the particulate production and the curve of the filtrate production, which is in agreement with the results of Saunders (1972).

In 1977 particulate organic carbon production of the phytoplankton as measured with the ^{14}C -method, on board the MS "Jan Verwey" at the anchor station G 11, amounted to $84 \text{ g C.m}^{-2}.\text{yr}^{-1}$. This value is about half that of the previous year. This erroneous drop in particulate productivity must be ascribed to the strongly increased share of the flagellates (*Cryptomonas* spp.) in the phytoplankton stock. These vulnerable phytoplankton cells break down during the filtration process. Even at extremely reduced under-pressure it was observed that the photosynthetic products of the flagellates run through the filters. It is therefore obvious that notwithstanding the considerable standing stock of flagellates in spring a peak in particulate productivity could not be observed. Combined extracellular production, including the dissolved photosynthetic compounds, and particulate production did show a maximum in spring, coinciding with a flagellate bloom. Total productivity (extracellular and particulate) amounted to 172 g C.m^{-2} in 1977.

References

- Fogg, G.E., 1958. Extracellular products of phytoplankton and the estimation of primary production. *Rapp. proces-ver. Reun. Cons. Perm. Explor. Mar.* 144: 56-60.
- Fogg, G.E., C. Nalawajko & W.D. Watt, 1965. Extracellular products of phytoplankton photosynthesis. *Proc. R. Soc. London, Ser B* 162: 517-534.
- Saunders, G.W., 1972. The kinetic of extracellular release of soluble organic matter by phytoplankton. *Verh. Internat. Verein. Limnol.* 18: 140-146.
- Steemann Nielsen, E., 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. *J. Cons. int. Explor. Mer.* 18: 117-140.

C. Bakker, M. van Ewijk-Rosier, W.J. Phaff & J.C.M. Rijk

G 11, years ago chosen as a representative anchor station, is the most frequently used sampling station in Lake Grevelingen, where weekly measurements of phytoplankton biomass and productivity are performed. Since studies on the zooplankton biomass and the interactions between zooplankton and phytoplankton were added to the programme, it was found out, on the basis of a statistical analysis of a large number of samples, that the station G 11 is representative for the Grevelingen as a whole, with regard to holoplanktonic species, *viz.* rotifers. The distribution of meroplanktonic species, *viz.* polychaet larvae, however, is determined by the presence of shallow areas, and the channel in which G 11 is situated shows an erratic picture of these zooplankters.

Bakker calculated standing stocks of copepods, polychaet larvae (*Pygospio*, *Polydora*, *Harmothoë*) and larvae of balanoids and gastropods. The results are fairly well comparable with those of 1976. He paid some attention to the plankton assemblage in eelgrass fields and regularly found medusae of a *Gonionemus* species, obviously restricted to that subsystem.



Plate II. *Synchaeta vorax*, Rousselet; length 250 μ m.

Rotifers of the genus *Synchaeta* represent an important component of the Grevelingen zooplankton. In early spring *S. vorax* Rousselet is the dominating species, followed by *S. triophthalma* Lauterborn in May. Development of *S. vorax* blooms was studied in relation to prevailing environmental factors of temperature and available food. Cryptomonad flagellates must be the main phytoplanktonic food for *Synchaeta*, because other algal species were very scarce during spring 1977. This conclusion on the basis of field data, is supported by results of Pourriot (1965) who obtained dense *Synchaeta* cultures on Cryptomonads as single food.

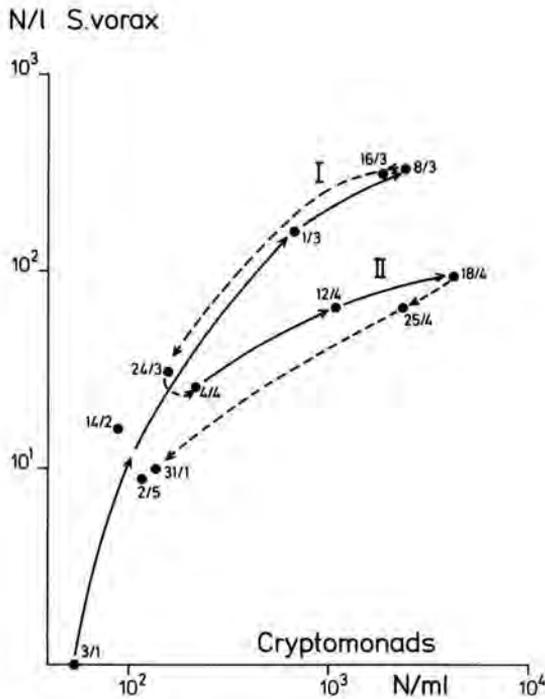


Fig. 5. Relation of *Synchaeta vorax* densities to *Cryptomonas* numbers during spring 1977.

Fig. 5 demonstrates a conspicuous synchronous increase and decrease of *Synchaeta* and *Cryptomonas* densities. The chronological sequence in the curves is indicated by arrows. The first *S. vorax* peak (I, 8/3: 330 ind./l) was reached when *Cryptomonas* amounted to 2500 cells/ml. The second *S. vorax* peak, however, was lower (II, 18/4: 95 ind./l) at a nearly two times higher *Cryptomonas* level (4300 cells/ml). Some other parameters (table 3) may explain this situation, showing that *Cryptomonas* densities ran parallel with the chlorophyll-A quantities, but not with the primary production intensity

(data of Vegter and de Visscher): the lower *Cryptomonas* peak demonstrated approximately a two times higher activity than the higher *Cryptomonas* peak, temperature conditions being the same. Moreover, biomass data of zooplankton show that other forms than *Synchaeta* (Cirriped and Copepod nauplii, Polychaet larvae) increased during the second period, presumably at the cost of the same algal stock.

Table 3. Environmental factors and zooplankton during the 1st and 2nd *S. vorax* maximum in Lake Grevelingen (spring 1977).

period	I (1/3 → 8/3)	II (4/4 → 18/4)
Water temperature °C	6 + 8	7 + 8.5
Cryptomonads (N/ml)	690 + 2500	220 + 4300
Chlorophyll-A (µg/l)	3.8 + 8.2	1.5 + 20.2
¹⁴ C-production (mg C/m ² day)	80 + 160	45 + 75
<i>Synchaeta vorax</i> (N/l)	160 + 330	23 + 95
<i>Synchaeta</i> biomass (wet wt. mg/l)	238 + 487	43 + 180
biomass of other zooplankton (")	71 + 61	80 + 130

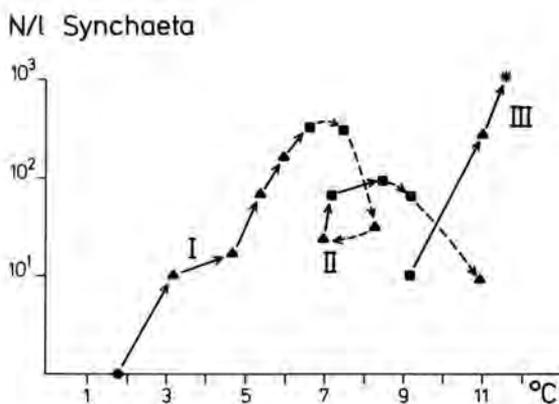


Fig. 6. Relation of *Synchaeta* spp. densities to water temperature during spring 1977. Solid lines: increase of rotifer densities; broken lines: decrease. I, II: 1st and 2nd spring maximum of *S. vorax*; III: maximum of *S. triophtalma*. Parallel *Cryptomonas* densities are: ● : < 10⁵/l; ▲ : 10⁵-10⁶/l; ■ : > 10⁶/l; * : > 10⁹/l of U-algae.

In Fig. 6, the relation between *Synchaeta* and temperature is demonstrated. As in 1976, positive influence of increasing temperature was only found in the range of 2-7°C. *S. vorax* densities were significantly ($P < 0.01$) cor-

related with both temperature ($r = 0.973$) and *Cryptomonas* numbers ($r = 0.947$). When temperature rose above 10°C *S. vorax* was succeeded by *S. triophthalma*, the latter species grazing definitely down the *Cryptomonas* algal stock, but presumably feeding on μ -algae too during the last stage of its exponential increase (Fig. 5, III).

Positive correlations of rotifer development with temperature and food algae were earlier established by Edmondson (1965) for fresh water rotifers in summer. Studies on population dynamics of Grevelingen *Synchaeta* blooms are being continued, using the methods of this author (Edmondson 1965, 1968).

References

- Edmondson, W.T., 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. *Ecol. Monogr.* 35(1): 61-111.
- Edmondson, W.T., 1968. A graphical method for evaluating the use of the egg ratio for measuring birth and death rates. *Oecologia* 1: 1-37.
- Pourriot, R., 1965. Recherches sur l'écologie des Rotifères. *Vie et Milieu*, suppl. no. 21. : 1-224.

PIGMENT CONCENTRATIONS IN BOTTOM SEDIMENTS OF LAKE GREVELINGEN (G 5)

P.H. Nienhuis, B.H.H. de Bree & J.M. Verschuure

Production and biomass of organic matter in the bottom sediments of Lake Grevelingen are unknown. Therefore in 1976 a program was initiated in order to gather information on a number of relevant parameters, *viz.* particulate organic carbon, dissolved organic carbon, pigments and interstitial nutrient concentrations. ^{14}C measurements of benthic productivity are in preparation. Pigment concentrations were determined in 1977 to provide an estimate of the standing stock of microalgae living on and in the sediments of Lake Grevelingen.

Sediment samples were taken at weekly intervals, at 4 sandy localities, with a perspex coring tube. Per locality 10 cores were taken and sliced in 1 cm pieces. The slices were mixed per layer and analysed for functional chlorophyll-A and phaeopigments both spectrophotometrically (Lorenzen, 1967) and fluorometrically (Strickland & Parsons, 1968), as described by Merks (1976). Extraction of pigments was done by an MSK homogenizer. Routine pigment analyses were performed with a Turner Fluorometer.

The top cm of the soil almost always contained the highest amount of pigments. Chlorophyll-A concentrations decreased sharply over the soil profile, but in most cases a small amount of chlorophyll-A was to be found down to a depth of 10 cm into the sediment. In reworked sediments irregular pigment profiles could be observed.

Figs. 7 and 8 show the seasonal fluctuation of chlorophyll-A and phaeopigments in the top cm of the sediment at station Herkingen, a sandy site in an eelgrass field, at a depth of 2.5 m, and at station Archipel a more exposed

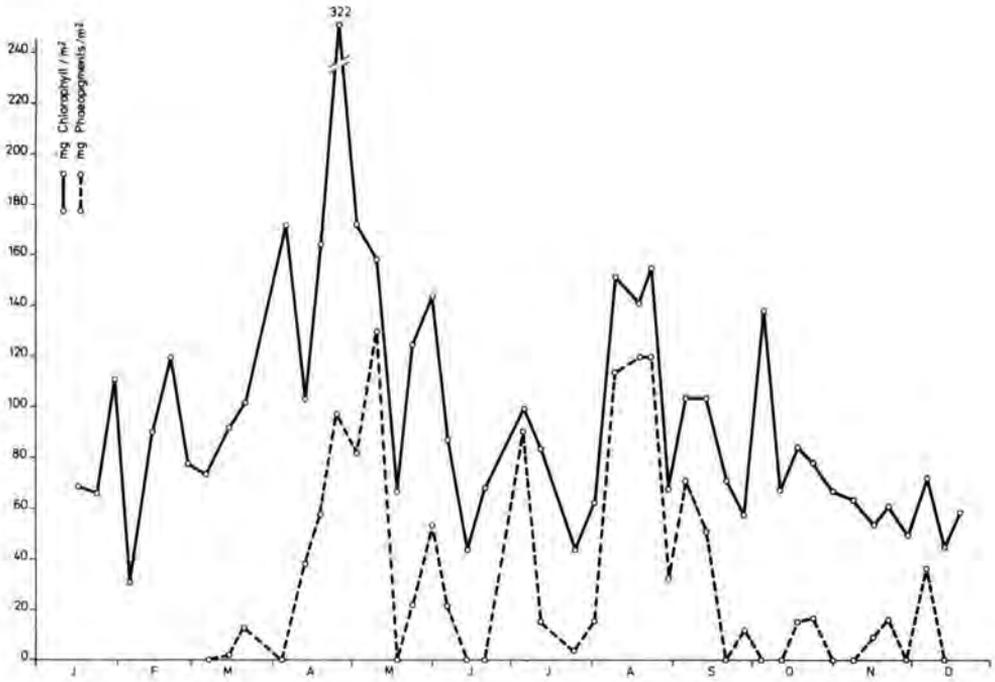


Fig. 7. Pigment concentrations in the bottom sediment (0-1 cm) of Station Herkingen in lake Grevelingen in 1977.

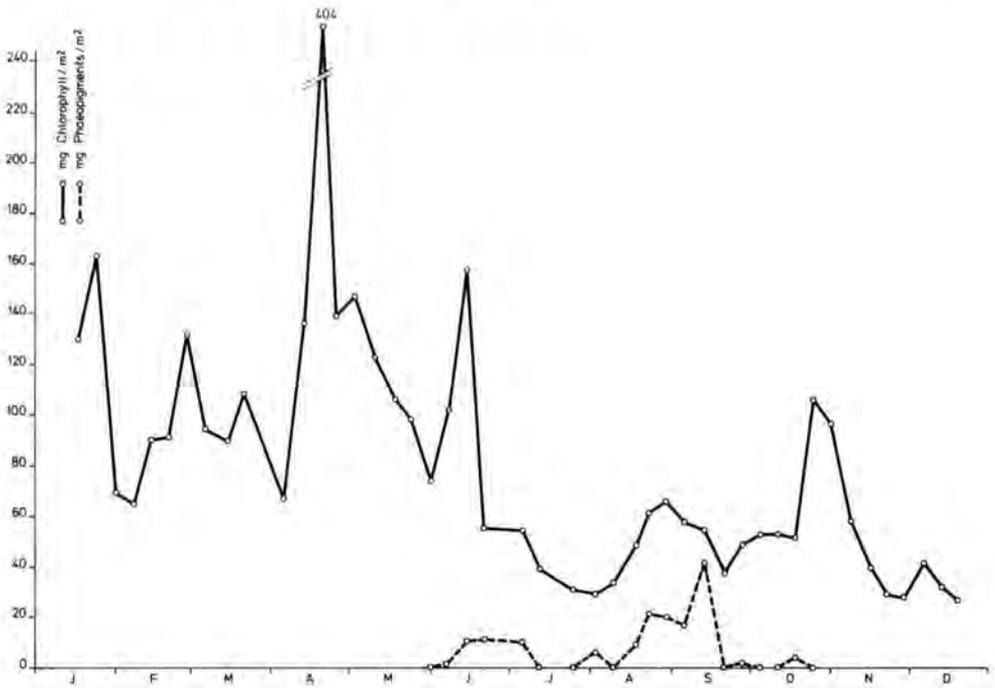


Fig. 8. Pigment concentrations in the bottom sediment of station Archipel in lake Grevelingen in 1977.

sandy site without eelgrass growth, at a depth of 3 m. A clearcut seasonal trend could not be observed. Both stations showed a significant bloom at the end of April (332 and 404 mg chlorophyll-A/m²). Herkingen showed a slight late summer peak, which was completely lacking at the Archipel.

Chlorophyll-A data at Herkingen fluctuated between 30 and 322 mg/m², with an average value of 105 mg/m². Phaeopigments fluctuated between 0 and 130 mg/m² with an average of 32 mg/m². Chlorophyll-A data at Archipel fluctuated between 30 and 404 mg/m², with an average value of 91 mg/m². Phaeopigments fluctuated between 0-40 mg/m² with an average of 4 g/m².

At a depth of 7 m below water level chlorophyll-A concentrations were strongly reduced. Chlorophyll-A fluctuated between 20 and 70 mg/m²; no seasonal trends could be observed. The amount of phaeopigments was relatively high in a 7 m deep bottom sediment. It fluctuated between 0 and 65 mg/m² with an average value of 16 mg/m².

The problem whether the presented pigment data provide enough information on the standing stock of the benthic micro algae will be studied in the near future. The high concentrations of the phaeopigments offer the suggestion that sedimentation of phytoplankton might be involved.

References

- Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments, Spectrophotometric equations. *Limnol. Oceanogr.* 12(2): 343-346.
- Merks, A.G.A., 1976. De meting van chlorophyll-a en phaeopitine in oppervlaktewater en in de bodem. Delta Inst. Hydrobiol. Onderz. Yerseke. Rapporten en Verslagen nr. 1976-4: 1-30.
- Strickland, J.D.H. & T.R. Parsons, 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Canada* 167: 1-311.

CONSUMPTION OF EELGRASS IN LAKE GREVELINGEN DURING THE GROWING SEASON (G 6) P.H. Nienhuis & E.T. van Ierland.

Production and ecology of *Zostera marina* L. were studied in 1968 and 1973-1976, as well through standing stock estimations, as through the determination of biomass increases in permanent quadrats and the correlation of distribution patterns with ecological factors (Nienhuis & De Bree, 1977).

The significance of eelgrass increased strongly after the change of the Grevelingen from an estuary to a saline lake. At present the *Zostera* subsystem covers in summer one fourth of the lake area, in clearly restricted areas and it has an average primary production in the subsystem of roughly 100 g C.m⁻².yr⁻¹, with maximum values of approximately 250 g C.m⁻².yr⁻¹.

It is frequently stated in literature (see Kikuchi and Peres, 1973, for a review) that only a very small part of the eelgrass production is directly consumed by animals. By far the largest part of the eelgrass production apparently enters the detritus food chains. There are hardly any data avail-

lable in literature to check this information. Therefore an attempt was made to quantify the consumption of eelgrass in Lake Grevelingen during the growing season of 1976 (Van Ierland, 1977). Total consumption was estimated at 400 ton dry weight, *i.e.* approximately 1.3 g C/m^2 , calculated for the entire lake Grevelingen; 26% of this was consumed by birds and 74% by invertebrates (Table 4). Fishes were ignored under the assumption that their consumption was negligible. Estimation of bird consumption was based on data from the literature and on bird censuses by Staatsbosbeheer. More than half of the eelgrass consumption by birds was caused by the Mute swan; this is supposed to be a reliable estimate. The other half is mainly caused by the Mallard, but this needs further evidence. Estimation for evertebrates was based on field data and aquarium-experiments. *Idotea chelipes* consumed 90% of the total invertebrate's share, according to a rather unreliable estimation. Total consumption of eelgrass during the growing season was approximately 6% of the year production in Lake Grevelingen.

Table 4. Consumption of eelgrass during the growing season in 1976, expressed in ton(10^3 kg) dry weight.

	<i>Cygnus olor</i>	56.3	ton dw	14.1 %
	<i>Branta bernicla</i>	1.6	" "	0.4 "
	<i>Fulica atra</i>	3.3	" "	0.8 "
Birds	<i>Anas platyrhynchos</i>	35.6	" "	9.0 "
	<i>Anas penelope</i>	3.4	" "	0.9 "
	<i>Anas acuta</i>	2.9	" "	0.7 "
	<i>Anas clupeta</i>	0.05	" "	0.0 "
Inverte-	<i>Idotea chelipes</i>	264.7	" "	66.3 "
brates	<i>Littorina littorea</i>	31.1	" "	7.8 "
	Total consumption	398.95	ton dw	100 %

References

- Ierland, E.T. van, 1977. Een poging tot kwantificering van de zeegrasconsumptie in het Grevelingenmeer gedurende het groeiseizoen. Delta Inst. Hydrobiol. Ond. Studentenversl. D2-1977: 1-43.
- Kikuchi, T. & J.M. Peres, 1973. Consumer ecology group. In: P.McRoy (ed.) - Seagrass ecosystems: Research recommendations of the International Seagrass Workshop: 41-45.
- Nienhuis, P.H. & B.H.H. de Bree, 1977. Production and ecology of eelgrass (*Zostera marina* L.) in the Grevelingen estuary, The Netherlands, before and after the closure. *Hydrobiol.* 52: 35-66.

P.H. Nienhuis, B.H.H. de Bree & J.M. Verschuure

Production and ecology of *Zostera marina* L. in the Grevelingen were studied in 1968 and in 1973-1976, as well through the estimations of standing stock (Table 5), biomass increases in permanent quadrats, as through correlation of distribution patterns with ecological factors.

Table 5. Standing crop and production data for *Zostera marina* in the Grevelingen estuary, before and after the closure. A = area of *Zostera* beds (cover more than 5%); B = percentage of the Grevelingen area covered by *Zostera* beds; C = range of standing crop in g/m^2 ash-free dry weight in *Zostera* beds (1968 data partly based on assumptions, 1973 and 1975 data based on measurements); D = average standing crop in g/m^2 ash-free dry weight in *Zostera* beds (1968 data partly based on assumptions, 1973 and 1975 data based on measurements); E = average production in $g C/m^2$ yr in *Zostera* beds; F = average production in $g C/m^2$ yr in entire Grevelingen.

	1968	1973	1975
A (ha)	1200	1585	2764
B (%)	8.5	14.7	25.6
C (g/m^2)	33.3-75.0	4.0-278.8	4.5-287.0
D (g/m^2)	54.2	129.9	98.3
E ($g C/m^2$ yr)	50.4	120.8	91.4
F ($g C/m^2$ yr)	4.3	17.8	23.4

After the closure of the estuary the intertidal eelgrass population extended downwards to depths of 5 m, owing to the increased transparency of the water. Light is a limiting factor in the vertical distribution of the eelgrass population in the lake, as can be concluded from irradiance reduction.

After the damming up of the estuary the area occupied and the density of the eelgrass beds increased strongly. Eelgrass annual overground production, based on doubled maximum standing crop values in July-August, was estimated at $50 g C/m^2$ in 1968, $121 g C/m^2$ in 1973 and $91 g C/m^2$ in 1975 in the *Zostera* beds, which is $4 g C/m^2$ in 1968, $18 g C/m^2$ in 1973 and $23 g C/m^2$ in 1975 for the entire Grevelingen area of $108 km^2$. The net production figures in the *Zostera* beds, were based on short term changes in biomass in permanent quadrats during 1974 and 1975, both for the overground and the underground parts of the plants.

As a subsystem of the Grevelingen ecosystem the *Zostera* population is of large significance. It is clearly limited, in space, it covers one fourth of the lake, where it has an average primary production of roughly more than $100 g C/m^2$ yr. Eelgrass-field annual net production averages $60-340 g C/m^2$ yr

in various parts of the northern hemisphere and the Lake Grevelingen population fits well into this picture. It is the only significant population on the stretch of the continental coast of Europe from northern France to Denmark.

References

Nienhuis, P.H. & B.H.H. de Bree, 1977. Production and ecology of eelgrass *Zostera marina* L.) in the Grevelingen estuary, The Netherlands, before and after the closure. *Hydrobiologia* 52: 55-66.

THE ROLE OF MACROZOOBENTHOS IN THE CARBON CYCLE (G 7)

R.H.D. Lambeck, W. Rozing & L. de Wolf.

The 7 years sampling programme for macrozoobenthos production studies, started by Wolff, right after the closure of the Grevelingen, was completed. In order to analyse the changes in macrozoobenthos diversity started in 1971 a large number of 0.1 m^2 grabsamples were taken. Quantitative results will become available in 1978. P. Valentijn and J. Koniuszek joined this programme during a 9 month period. They studied the production of polychaetes and of macrozoobenthos in shallow water areas, respectively.

ABUNDANCE, BIOMASS AND PRODUCTION OF POLYCHAETES ON THREE STATIONS IN THE SALINE LAKE GREVELINGEN (G 7)

P. Valentijn

According to Wolff and De Wolf (1977) the biomass of polychaetes in the Grevelingen estuary, with the exception of *Arenicola marina*, was very low in comparison to that of the most important macrofaunal group, the molluscs. While the relative proportions of the bottom fauna did not change very much after the closure and *Arenicola marina* declined greatly in numbers, Wolff *et al.* (1976) decided that biomass and production of polychaetes could in comparison, also be neglected in Lake Grevelingen. The aim of this study was to check this by determining (1) the density and biomass of the different species, (2) the structure of the most abundant populations by analyzing length-frequency distributions and (3) the variations in these parameters during the study-period.

At three stations 10 samples, consisting each of 2 circular cores of 32 cm^2 cross section, were taken by Scuba-divers at approximately six-week intervals from March to October 1977. From each core the top 5 cm was removed from the rest (total length + 20 cm), preserved in formalin and washed in the laboratory through a 1 and 0.3 mm sieve combination. During the first sampling this procedure was used for the 5-20 cm bottomlayer. Since hardly any small polychaetes were present below 5 cm, from the second sampling-date on, this layer was washed aboard only through a 1 mm sieve. The residues were sorted, counted, dried during 3 to 5 days at 70°C , weighed, incinerated at 570°C

Table 6. Mean density, mean biomass (ash-free dry weight) and species composition at three stations in Lake Grevelingen for 5 sampling dates in the period March-September 1977. Numbers in brackets represent total numbers exclusive *Streptosyllis websteri*.

Station	Plaatje		Archipel		Vlije van Bruinisse	
	medium sand, 0-5 cm	depth 0.70-1 m 5 - ± 20 cm	fine sand 0-5 cm	depth. ca. 3 m 5 - ± 20 cm	fine sand + mud 0-5 cm	depth 0-4 m 5- ± 20 cm
\bar{N}/m^2	59,000 (45,500)	3090	44,500 (13,150)	1555	13,200 (13,200)	1585
$\bar{B} \text{ g}/m^2$	4.8	7.6	3.1	4.6	4.5	6.6
Number of species	17		20		23	
Abundant species	<i>Streptosyllis websteri</i>	<i>Capitella capita</i>	<i>Streptosyllis websteri</i>	<i>Capitella capita</i>	<i>Scoloplos armiger</i>	<i>Heteromastus filiformis</i>
	<i>Pygospio elegans</i>	<i>Scoloplos armiger</i>	<i>Capitella capita</i>	<i>Scoloplos armiger</i>	<i>Polydora limicola</i>	<i>Scoloplos armiger</i>
	<i>Capitella capita</i>	<i>Heteromastus filiformis</i>	<i>Scoloplos armiger</i>	<i>Heteromastus filiformis</i>	<i>Heteromastus filiformis</i>	<i>Capitella capita</i>
	<i>Scoloplos armiger</i>	<i>Arenicola marina</i>	<i>Pygospio elegans</i>	<i>Arenicola marina</i>	<i>Capitella capita</i>	<i>Neomphitrite figulus</i>
			<i>Anaitides maculata</i>			<i>Nephtys hombergii</i> (<i>Arenicola marina</i>)

for 2 h and weighed again to obtain ash-free dry weight.

In Table 6 some preliminary results are summarized. The sample size provides an adequate density and biomass estimate for the smaller species. Large polychaetes, notably *Arenicola marina*, *Neomphitrite figulus*, *Nephtys* species and *Harmothoe imbricata*, occur in too low numbers, thus permitting only a rough estimate.

Taking the length frequency of worms in order to establish any age structure in their populations, produced poor results. A good exception gives only *Scoloplos armiger* by showing a 1977 year class, which growth rate could easily be determined. Concerning *Pygospio elegans* there are strong indications that an a-sexual reproduction plays a significant role in the population in spring.

Interpretation of the results permits in the best case a rough estimation of the net production of the most abundant species. In this case it may be speculated that the ratio between the production and the mean biomass might be high for the smaller worms in comparison to the relatively slow growing, long living molluscs.

A comparison in species composition before and after the damming of the Grevelingen could be made with Wolff (1973) and Wolff and de Wolf (1977), presenting data before 1971. Remarkable is the actual appearance of the non-rare species *Kefersteinia cirrata*, *Microphthalmus screlkowi*, *Platynereis dumerilii* and *Polydora limicola*, being unrecorded in the former Grevelingen estuary. Other species declined very much in numbers or disappeared e.g. *Nereis diversicolor*, *Scolelepis squamata*, *Paraonis fulgens*, and *Pectinaria koreni*.

References

- Wolff, W.J., 1973. The estuary as a habitat. An analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse and Scheldt. Zoöl. Verhandelingen, Leiden, 126: 1-242.
- Wolff, W.J., Vegter, F., Mulder, H.G. and Meijs, T., 1976. The production of benthic animals in relation to the phytoplankton production. Observations in the saline Lake Grevelingen, The Netherlands. Proc. 10th Eur. Mar. Biol. Symp., Ostend, Belgium, 2: 653-672.
- Wolff, W.J. and de Wolf, L., 1977. Biomass and production of zoobenthos in the Grevelingen estuary, The Netherlands. Estuar. Coast. mar. Sci. 5: 1-24.

BIOMASS AND PRODUCTION OF MACROZOOBENTHOS ON THE "SLIKKEN VAN FLAKKEE" (G 7)
J.W.J. Koniuszek.

The research into biomass and production of the macrozoobenthos in the saline Lake Grevelingen takes place a.o. by sampling four permanent stations. The shallow-water station "Plaatje" is surrounded by deep water, while

Table 7. Number and biomass of macrozoobenthos on the "Slikken van Flakkee" April 1977 (mean values of 47 samples)

Species	year-class	number/m ²	mean weight per individual in mg ash-free dry weight	biomass/m ² in mg ash-free dry weight
<i>Cerastoderma edule</i>	1972	0.8	257.6	206.1
	1973	7.5	199.3	1494.8
	1974	21.7	109.0	2365.3
	1975	70.8	39.2	2775.4
	1976	85.8	9.6	823.7
	total			7665.3
<i>Angulus tenuis</i>	1971	1.7	38.9	66.1
	1972	1.7	29.1	49.5
	1973	20.8	17.0	353.6
	1974	74.2	12.0	890.4
	1975	65.0	6.3	409.5
	1976	30.8	1.2	37.0
	total			1806.1
<i>Mya arenaria</i>	total	4.2	86.3	362.4
<i>Scrobicularia plana</i>	"	75.0	12.4	930.8
<i>Mytilus edulis</i>	"	75.8	141.4	10718.1
<i>Macoma balthica</i>	"	1.6	79.5	127.2
<i>Mysella bidentata</i>	"	4.2	0.1	0.4
<i>Venerupis pullastra</i>	"	0.8	31.4	25.1
<i>Hydrobia ulvae</i>	"	695.0	0.6	417.0
<i>Littorina littorea</i>	"	7.5	59.0	442.5
<i>Nassarius reticulatus</i>	"	3.3	21.9	72.3
<i>Arenicola marina</i>	"	40.0	108.7	4348.0
<i>Nephtys hombergii</i>	"	26.7	45.3	1209.5
<i>Anaitides maculata</i>	"	27.5	1.9	52.3
<i>Eteone longa</i>	"	9.2	0.8	7.4
<i>Scoloplos armiger</i>	"	802.5	0.6	481.5
<i>Capitella capitata</i>	"	292.5	0.5	146.3
<i>Nereis succinea</i>	"	5.0	2.5	12.5
<i>Magelona papillicornis</i>	"	3.3	3.3	10.9
<i>Corophium spec.</i>	"	1397.5	0.3	419.3
<i>Gammarus spec.</i>	"	5.0	4.8	24.0
<i>Jassa falcata</i>	"	2.5	0.8	2.0
<i>Bathyporeia spec.</i>	"	15.0	0.4	6.0
<i>Idotea chelipes</i>	"	60.8	1.4	85.1

Total biomass of Molluscs	22.57 g ash-free dry weight/m ²			
" " " Polychaetes	6.27	"	"	"
" " " Crustaceans	0.54	"	"	"
total amount of biomass	29.38	"	"	"

most of the shallow-water areas (here defined as less than 1.5 m deep, in total ca. 30% of the lake) are formed by depth-gradients from the landborder to deeper water. In order to investigate whether "Plaatje" is representative for these areas, a macrozoobenthos biomass and production research has been carried out on the "Slikken van Flakkee", the most extensive shallow water area of Lake Grevelingen.

Besides the size, another advantage of the "Slikken van Flakkee" is that some production data from the estuarine period, when this area consisted of tidal flats, are available, which makes a certain comparison with the present stagnant situation possible.

In order to obtain production data and production-biomass ratio's for the most important species, during the period March-October 1977 three permanent stations, situated on 0.45, 0.75 and 1.05 m depth, were sampled at six week intervals. At every station 35 samples were taken each consisting of 3 circular cores of 51 cm² cross section and 25 cm deep. The samples were washed through a 1 mm sieve in the field, conserved by freezing and later on sorted in the laboratory. For a production estimate of the whole area, a biomass survey has been carried out along three transects at 150 m intervals, taking 5 cores of 51 cm² cross section for each sample. The results of this survey are shown in table 7.

The production will be calculated according to the method used by Wolff and de Wolf (1977), which is based on the method of Crisp (1971). During the sampling at the stations a few species have been found that are not mentioned in table 7: the polychaetes *Pholoe minuta*, *Plathynereis dumerilii*, *Heteromastus filiformis*, *Cirratulus cirratus*, *Kefersteinia cirrata*, *Eumidea sanguinea*, the bivalve *Petricola pholadiformis*, the gastropod *Retusa alba*; the amphipod *Microprotopus maculatus*, and the isopod *Jaera albifrons*.

The polychaetes *Pygospio elegans* and *Polydora spec.* were abundant but owing to the freezing of the samples it was not possible to use these species for biomass and production calculations.

References

- Wolff, W.J. and Wolf, de L., 1977. Biomass and Production of zoobenthos in the Grevelingen Estuary, The Netherlands. *Estuar. and Coast. Mar. Science* 5: 1-24.
- Crisp, D.J., 1971. Energy flow measurements, p. 197-279. In: *Methods for the study of marine benthos*. N.A. Holme and A.D. McIntyre (Eds.). IBP Handbook 15. Blackwell Scientific Publications, Oxford.

K. Willems & A.J.J. Sandee

Willems started his meiozoobenthos investigations in 1975. Up to now 25 random stations were studied, spread over Lake Grevelingen, and ranging from

Table 8. Distribution of predominant harpacticoid species. I = percentage of stations where the species was found; II = percentage of stations where the species belongs to the two predominant species

	I(%)	II(%)
<i>Canuella perplexa</i>	96	64
<i>Canuella furcigera</i>	60	32
<i>Halectinosoma gothiceps</i>	56	12
<i>Ameira parvula</i>	48	4
<i>Tachidius discipes</i>	44	12
<i>Longipedia minor</i>	40	4
<i>Stenocaris minuta</i>	16	12
<i>Paraleptastacus espinulatus</i>	16	4
<i>Enhydrosoma curticauda</i>	16	4

Table 9. Density and biomass of harpacticoids during the period 29 March-13 April 1976 in Lake Grevelingen (n = number of samples)

	average density/m ²	av. biomass g/m ²
Med. Sand (n = 3)	36,700(min. 29,500 max. 45,000)	0.058
Fine Sand (n = 10)	81,200(min. 6,500 max. 399,000)	0.14
Mud (n = 6)	44,200(min. 16,500 max. 80,500)	0.13

mud (grain size smaller than 75 μm) to medium sand (grain size 250-500 μm). Some results were published (Heip, Willems & Goossens, in press). Harpacticoids and Nematodes stand for 95% of the numbers of animals counted.

Twenty three species of Harpacticoidea were found in the 0-4 cm sediment layer. The distribution of the dominant species is shown in table 8.

In general, Harpacticoidea are restricted to the upper 2 cm of the sediment. Interstitial species like *Stenocaris minuta* and *Paraleptastacus espinulatus* only occur in stations with a marked oxidized sediment upper layer.

Densities of harpacticoids (Table 9) can be compared with those in the North Sea, but are smaller than those *viz.* in the Danish Waddensea and in the Bristol Channel. Dry weight was estimated for some quantitatively dominant species (Table 10).

Table 10. Average dry weight in $\mu\text{g}/\text{individual}$ of harpacticoid species in Lake Grevelingen (n = number of samples):

<i>Canuella perplexa</i>	♀ 4.9 (n = 11)
	♂ 3.9 (n = 11)
<i>Canuella furcigera</i>	♀ 5.1 (n = 2)
	♂ 4.8 (n = 2)
<i>Tachidium discipes</i>	♀ + ♂ 1.8 (n = 10)
<i>Harpacticus flexus</i>	♀ + ♂ 1.9 (n = 2)
<i>Enhydrosoma curticauda</i>	♀ + ♂ 0.9 (n = 4)

Data on densities and biomass of Nematoda in the upper 4 cm of sediment are summarized in table 11. No differentiation between species was made for this purpose.

Table 11. Density and biomass of nematodes in Lake Grevelingen (n = number of samples).

	average density/ m^2	av. biomass g/m^2
Med. Sand (n= 3)	239,000 (min. 92,500 max. 361,500)	(n=3) 0.21
Fine Sand (n=10)	1,114,800 (min. 96,500 max. 1,826,500)	(n=5) 0.53
Mud (n= 6)	778,600 (min. 133,000 max. 1,807,000)	(n=6) 0.59

R. Surkijn, a student of Willems, compared the meiöfauna in the sea-arm Oosterschelde with that in Lake Grevelingen, a former sea-arm. His comparison showed (a) no significant differences in either density or biomass for nematodes (0-4 cm sediment layer), and (b) no significant differences in either density or species richness for harpacticoids (0-2 cm sediment layer), Surkijn, 1977).

References

- Heip, C., K. Willems & A. Goossens. Vertical distribution of meiöfauna and the efficiency of the Van Veen grab on sandy bottoms. *Hydrobiol. Bull.* (In press).
- Surkijn, R., 1977. Vergelijking van de fauna van permanent ondergelopen slikken in de Grevelingen met bestaande slikken in de Oosterschelde. *RU Gent. Fac. Wetenschapp. Verhand. Licentiaat Wetenschapp. Dierkunde* pp. 1-56.

THE ROLE OF FISHES IN THE CARBON CYCLE; SMALL FISH SPECIES IN THE ECOSYSTEM OF LAKE GREVELINGEN (G 9)

U.H. de Graaf & P. de Koeyer.

Since fish-eating birds did increase in numbers after the Grevelingen estuary was transformed into a lake, it was thought that small fish might play a role in the new lake-ecosystem. Therefore in 1977 research was focused on species composition, and on the estimation of numbers and biomass of small fish. The research has been restricted to shallow waters (0.70-1.50 m) only. Fishing was carried out with an ottertrawl and successive hauls lasted about 2½ minutes. The whole lake was sampled in two days, twice a month. By measuring trawling-distance numbers per standard square meter were calculated.

The fishing efficiency of the ottertrawl has been estimated by using an enclosure and comparing the absolute number of fish in the enclosure with the number caught by the ottertrawl, just outside the enclosure. The absolute number of fishes in the enclosure has been calculated by the method of estimating population size by successive approximation according to Healey (1971).

The most regularly caught species are: *Gasterosteus aculeatus*, *Pomatoschistus microps*, *Pomatoschistus minutus*, Atherinidae, *Zoarces viviparus* and *Gobius niger*. The last two mentioned species did occur in very small numbers in the catches, usually less than 10 fishes during a two days fishing period. In the *Zostera*-fields more *Zoarces viviparus* were caught than elsewhere. *Gobius niger* did appear in great numbers in catches from deeper water (> 2 m). *Atherina* spec. is very irregularly represented and most frequently absent but, sometimes great numbers are caught in one haul; *Atherina* obviously is a shoaling-fish. *Zoarces viviparus*, *Gobius niger* and *Atherina* spec. have been excluded from estimates on number and biomass.

During the summer the numbers of *P. microps* and *P. minutus* together increased until August. At the end of July juvenile gobies appeared in the catches in great numbers. The biomass of the gobies was highest in September and decreased starting from the end of September. In accordance with the results reported by Healey (1971,1972), Hesthagen (1975) and Fonds (1971, 1973) low numbers of gobies are found in spring and again in autumn. *P. minutus* and probably also *P. microps* disappeared from the shallow waters into deeper water, when the water temperature dropped in October and November.

Gasterosteus aculeatus was numerous in the catches throughout the sampling months. In summer however their numbers were more evenly dispersed over the whole lake, while in autumn these fishes were almost exclusively found in the eelgrass beds. Their number and also their biomass was highest in June, while juvenile fishes appeared in the catches in the first half of July. From then on biomass was steadily decreasing.

Owing to their abundance in summer it is concluded that small fish species might play a role in the ecosystem of the lake, however, because of migration

into deeper water in autumn, the research should be supported by investigations in deeper water, in order to determine their precise role in the lake-ecosystem.

References

- Healey, M.C., 1971. The distribution and abundance of sand gobies, *Gobius minutus*, in the Ythan estuary. J. Zool. London 163: 177-229.
- Healey, M.C., 1972. On the population ecology of the common goby in the Ythan estuary. J. Nat. Hist. 6: 133-145.
- Hesthagen, I.H., 1975. Seasonal occurrence and length variation in the sand goby, *Pomatoschistus minutus* (Pallas), in the shore zone of the inner Oslo fjord. Norw. J. Zool. 23: 235-242.
- Fonds, M., 1971. The seasonal abundance and vertebral variation of *Pomatoschistus minutus* and *Lozanoi* (Gobiidae) in the Dutch Wadden Sea. Vie Milieu 22(suppl.): 277-386.
- Fonds, M., 1973. Sand gobies in the Dutch Wadden Sea *Pomatoschistus* Gobiidae, pisces). Neth. J. Sea Res. 6(4): 417-478.

THE ROLE OF HETEROTROPHIC BACTERIA IN THE CARBON CYCLE (G 10)

A.B.J. Sepers, M.P.G. Cappendijk

Sepers reported his results in combination with the data obtained in the Haringvliet under project A 1.

DEVELOPMENT OF AN ECOLOGICAL MODEL OR SUB-MODELS FOR THE GREVELINGEN ECOSYSTEM; THE *Zostera marina* MODEL (G 11)

M.E. Siemelink & J. Stellingwerf

As a subsystem of the Grevelingen ecosystem the population of the macrophyte *Zostera marina* is of paramount importance (Nienhuis & De Bree, 1977). It was therefore decided to build a production model of this eelgrass subsystem.

A simple and practical model without predicting value is the production-model of Petersen (1913); he stated that the yearly *Zostera* production is about two times the maximum standing crop. If one wants to predict future states of the subsystem, the model has to reflect the dependance on the most relevant parameters. The advantage of such a model is that it presents more insight about the *Zostera* production. It is also possible to predict the effect of actions of management, if these actions are correctly translated into parameters. A predicting model that meets the above mentioned needs was composed in Titus (1975) for the production of *Myriophyllum spicatum*, a freshwater macrophyte. Our *Zostera*-model is derived from this model.

In the *Myriophyllum spicatum*-model it is assumed that the plant always strives to a maximum leaf-area per square meter substratum. This theoretical maximum is mainly determined by the amount of incident light available and by

self-shading effects. When the maximum leaf-area is exceeded the plant will reduce his leaf-area by leaf-sloughing. Because the cover-degree of the plants is strongly variable, it can be found at decreasing light-amounts in some places that the maximum leaf-area is exceeded, followed by sloughing, and at the same time that growth continues in places where that maximum has not yet been reached. This impedes the determination of the significant parameters of the model.

Another problem is the finding of a good relation between input-facts and accuracy of output (simulation-result). Too much input-facts make the model intractable. Sensitivity-analysis (= variation of one parameter and comparing the different outputs) will give insight into the problem which parameters demand a high accuracy and which do not.

Making use of estimations on relevant parameters, facts from the litterature and facts collected by the working-group, the model is filled in. The first simulation will be executed in the end of November 1977 at the computer of the Agricultural University of Wageningen. The simulation-results compared with field-measurements will give information about the accuracy of our model. Of course the model will become more accurate when the estimated parameters can be measured in the field. Our next year's programme concerns photosynthesis, respiration and leaf-sloughing experiments.

References

- Nienhuis, P.H. & B.H.H. de Bree, 1977. Production and ecology of eelgrass (*Zostera marina* L.) in the Grevelingen estuary, The Netherlands, before and after the closure. *Hydrobiologia* 52(1): 55-66.
- Petersen, C.G.J., 1913. Om Baedeltangens (*Zostera marina*) Aarsproduktion i de Danske Farvande. In: H.P.E. Jungersen & E. Warming (eds.): *Mindeskr. Japetus Steenstrups Føds*, 9: 1-20 G.E.C. Gad, København.
- Titus, J. et al. 1975. A production model for *Myriophyllum spicatum* L. *Ecology* 56: 1129-1138.

WORKING GROUP STRUCTURE AND DYNAMICS OF THE SALT-MARSH ECOSYSTEM

INTRODUCTION W.G. Beeftink

The objective of this Working Group is to investigate structural and functional aspects of plant systems in the salt-marsh ecosystem in order to understand the structure and functioning of the ecosystem as a whole.

Because it is technically impossible to describe the ecosystem totally, the group started from the hypothesis that the phanerogamic vegetation ranks a central place in the structure and functioning of the whole system. From this point of view different plant systems (phytocenoses, succession series, species populations, plant - animal and host - parasite relations) will be se-

lected as key or indicator systems.

Stress and disturbances, such as those initiated by the Delta Works are very useful in providing an insight in black-box problems. Other impacts for this study might be pollution effects, such as those of oil, biocides, and heavy metals, as well as different forms of human exploitation.

The group is working on the research levels of vegetation dynamics, population dynamics and physiology of adaptation mechanisms for survival. The fields of activity are divided over the group members and integrated in combined research projects. External co-operation with university departments and institutes, national and abroad, will be intensified to arrange research projects of mutual interest. Some fields of co-operation which have been opened up at present are: the Botanical Laboratory of the State University of Utrecht for photosynthetic research in relation to production of halophytes, the Department of Biological Sciences of the Aston University in Birmingham for investigations on halophyte-soil fungi relations, and the Institute for Applied Physical Chemistry of the Nuclear Research Centre in Jülich (West Germany) for the storage of heavy metals in the salt-marsh ecosystem and its effects on ecophysiological mechanisms.

As with the other working groups the research is carried out within a number of projects. They are numbered with the code S. For a number of projects details are presented hereafter and amplified with the abstracts of the published articles or reports.

VEGETATION DYNAMICS IN THE SALT-MARSH ECOSYSTEM (S 1)

Prior to the study of vegetation dynamics it is useful to describe the floristic composition and structure of salt-marsh communities and their environmental conditions. In a chapter of the first volume of Elsevier's series "Ecosystems of the World" a survey has been given of what is known about the European coastal salt-marsh communities excluded the Mediterranean ones.

In order to test the objective value of the classical syntaxonomical treatment of relevées, a numerical processing of European *Spartina* relevées has been executed. Comparison of both approaches were carried out by miss W.G. Kortekaas (Catholic University, Nijmegen) and showed striking similarities. It remains to be seen whether such a result also will hold for communities of a more complex structure than those tested.

Human exploitation and management measures may have a severe impact on the structure and floristic composition of salt-marsh communities. An overall view of the forms of these human effects as well as the degree to which they have to be restricted from the viewpoint of nature conservation, has been given in a contribution to Wiley's volume "The Coastline".

An analysis of soil characteristics of the salt-marshes has been carried out with data sets obtained from several long-term measurements. In estuaries as well as in the marsh itself soil gradients can be recognized, the former

being partly related to gradients in the estuarine water-body.

Groundwater level and groundwater salinity of the salt marsh are highly influenced by the tides and by rainfall. M.C. Daane especially found these effects when the tidal influence is cut off or increased by barraging estuarine branches.

Other relationships between the marsh and the adjacent water-body have been studied by the students E.H.R.R. Lammens (Catholic University, Nijmegen) and M.J. van Eeden (Agricultural University, Wageningen). They investigated the flow of particulate organic matter between these two systems and found a net transport from the water-body towards the marsh.

W. de Munck and W.G. Beeftink continued their long-term investigations on about 400 permanent sample plots distributed over the whole estuarine area, in the same manner as in former years.

Disturbances of varying origin causing die-back in *Halimione portulacoides* and other salt-marsh dominants, induce unilinear succession series in which *Suaeda maritima*, *Aster tripolium* and *Puccinellia maritima* successively play a major role. This phenomenon had been communicated at the jubilee-symposium of Prof. Dr. V. Westhoff (Catholic University, Nijmegen), and will be published in *Vegetatio*.

This and other succession phenomena gave rise to the construction of a hypothetical model on the structure and functioning of plant species in relation to disturbances. This model was used in a paper presented at the First European Ecological Symposium in Norwich, G.B.

B.P. Koutstaal continued his investigations on the development of lichen communities on the nature reserve "De Middelpaten". His results, together with their implications for management in this reserve, were embodied in a paper presented at De Levende Natuur. Another research project on vegetational changes as a consequence of barraging from the sea, has been carried out by miss M. Suy (State University, Utrecht). Using air-photograph techniques she found a relationship between the vegetation patterns before and after barrage building.

The research programme, carried out by M.C. Daane and W. de Munck, on the influence of cultural practices of man on the salt-marsh vegetation is continued. The interim results were presented in a paper read at the Third Symposium of the International Working Group "Succession Research on Permanent Plots", held in Bialowieza, Poland. Grazing effects on salt-marsh vegetation by domestic animals have been studied by miss M. Suy.

Prof. dr. H.W. Nürnberg and Dr. M. Stoepler (Institute for Applied Physical Chemistry, Jülich) engaged in a co-operated research programme for an overall survey of the degree of heavy-metal contamination of the salt marshes in the Scheldt estuary compared with those bordering the Eastern Scheldt, the pathways these compounds take in the soil - plant - animal systems, and the

degree to which the ecosystem is damaged. A programme for sampling various soils and plant species in four salt-marsh areas has been developed. At Jülich 24 soil samples and 114 plant samples are analysed on heavy-metal concentrations, at Yerseke the additional soil analyses have been made.

Dr. J.C. Duinker (Marine Pollution Department, Netherlands' Institute for Sea Research, Texel) agreed to take part in a preliminary research on the content of persistent pesticides in halophytes, to check whether these may be a vector for abnormal behaviour in animals.

GROUNDWATER LEVEL AND GROUNDWATER SALINITY IN SALT MARSHES AND BEACH PLAINS IN RELATION TO TIDES AND RAINFALL (S 1)

M.C. Daane & W.G. Beeftink

The influence of tides and rainfall on groundwater level and salinity of the groundwater have been measured in two salt marshes and three beach plains of the SW Netherlands. In case of the salt marshes and of one beach plain the tidal influence has been changed considerably during the period of measurement as a consequence of barraging of either the water-body in which the area is situated, or -more indirectly- another estuarine branch of the Rhine-Meuse system.

In salt marshes intersected by many creeks the tidal influence upon groundwater fluctuations is evident up to a distance of 100 m from the channels penetrating into the sandy underground of the marsh. In such case the tidal effect can penetrate via the channels hundreds of metres far into the marsh, and can thus even have an influence on the groundwater level of adjacent polder areas by seepage underneath the sea dike.

In beach plains, however, creeks are usually very few or totally absent. Generally, sand deposits such as those occurring in beach plains, are more elevated with respect to the tides than clayey and silty deposits such as salt marshes. Therefore tidal influence upon the groundwater in beach plains is limited to only 20 m distance from the open water in spite of the much more sandy soil profile as compared with that of the salt marsh proper. Consequently, groundwater fluctuations in beach plains are much more related to rainfall than to tides.

So, a raise of the high-water level caused by barraging influences the groundwater level of a salt marsh more than that of a beach plain. For instance, the salt marsh of the Slikken van de Heen, where the flood-water has been raised to about 30 cm at an average in 1969, the groundwater level showed a raise of 15-50 cm. For the Kwade Hoek beach plain, however, a preliminary processing of an eight year's data set did not reveal any significant effect of a tidal raise of the order of 25 cm in 1970 exceeding seasonal fluctuations caused by rainfall.

Groundwater-salinity relations seem to be more complex as they may be influenced by local rainwater lenses of varying size in the soil water, oozing

down in the soil profile and persisting owing to specific-gravity differences. In the beach plains also underground drainage of freshwater from dune ridges over the saline groundwater after wet periods has been encountered

PATTERNS IN VEGETATION, FAUNA AND ENVIRONMENTAL FACTORS OF THE SALT-MARSH ECOSYSTEM (S 3)

The data collected in 1974 for pattern analysis in the Bergen op Zoom salt-marsh by W.G. Beeftink, Mrs. C.H. Borghouts and P.H. Nienhuis and collaborators, have been put on punched cards and magnetic tape in 1975/76. This year J.M.W. Louppen (Catholic University, Nijmegen) started with the data processing for which he uses the canonical correlation method of W.T. Williams (Pattern Analysis in Agricultural Science, Elsevier, 1976).

G.J. Buth and A. Groenendijk (State University, Utrecht) studied the influence of the age of polder areas on the structure of vegetation and environmental factors of salt marshes. Numerical analyses with the Biopat programme mapped out by Dr. P. Hogeweg (Department of Bioinformatica, State University, Utrecht) of soil and vegetational data of seven areas, gave the following preliminary results: The youngest embanked areas (from 1961 onwards) differ largely from the older ones in their floristic composition and structure, and these differences are mainly related to the degree of soil maturity. In the older areas (backwards to into the 15th century) structural differences do not seem to correlate with age, but with environmental dynamics induced by patterns in (soil) water-level fluctuations. Management as a variable factor has been excluded as much as possible in the areas of investigation selected.

POPULATION ECOLOGY OF HALOPHYTES (S 4 and S 5)

For population-dynamic studies in halophytes the working group has started with *Salicornia europaea* agg. and *Halimione portulacoides*, both obligate halophytes, but the first one mainly restricted to reduced substrates and the latter to aerated soils. For *Salicornia* a life-cycle scheme has been originated to define the single research projects for quantifying the limiting factors in life history (Fig. 9). The following investigations have been executed:

The students Mrs. M.J. Verhoef-Allan (Agricultural University, Wageningen) and P.F.M. Elenbaas (State University, Utrecht) studied seed populations in some tidal and non-tidal salt marshes. For that purpose B.P. Koutstaal and J. Nieuwenhuize developed a method for separating *Salicornia* seeds from other soil constituents. The students estimated the number and ratio of seeds of *Salicornia stricta* and *S. brachystachya* in different salt-marsh zones, as well as the degree of patchiness in the seed distribution. A fungus found on the seeds and preventing germination and establishment of seedlings could not yet be identified.

On the same sample plots B.P. Koutstaal, P.F.M. Elenbaas and H. Schat (Free

Population biology *Salicornia europaea* agg.

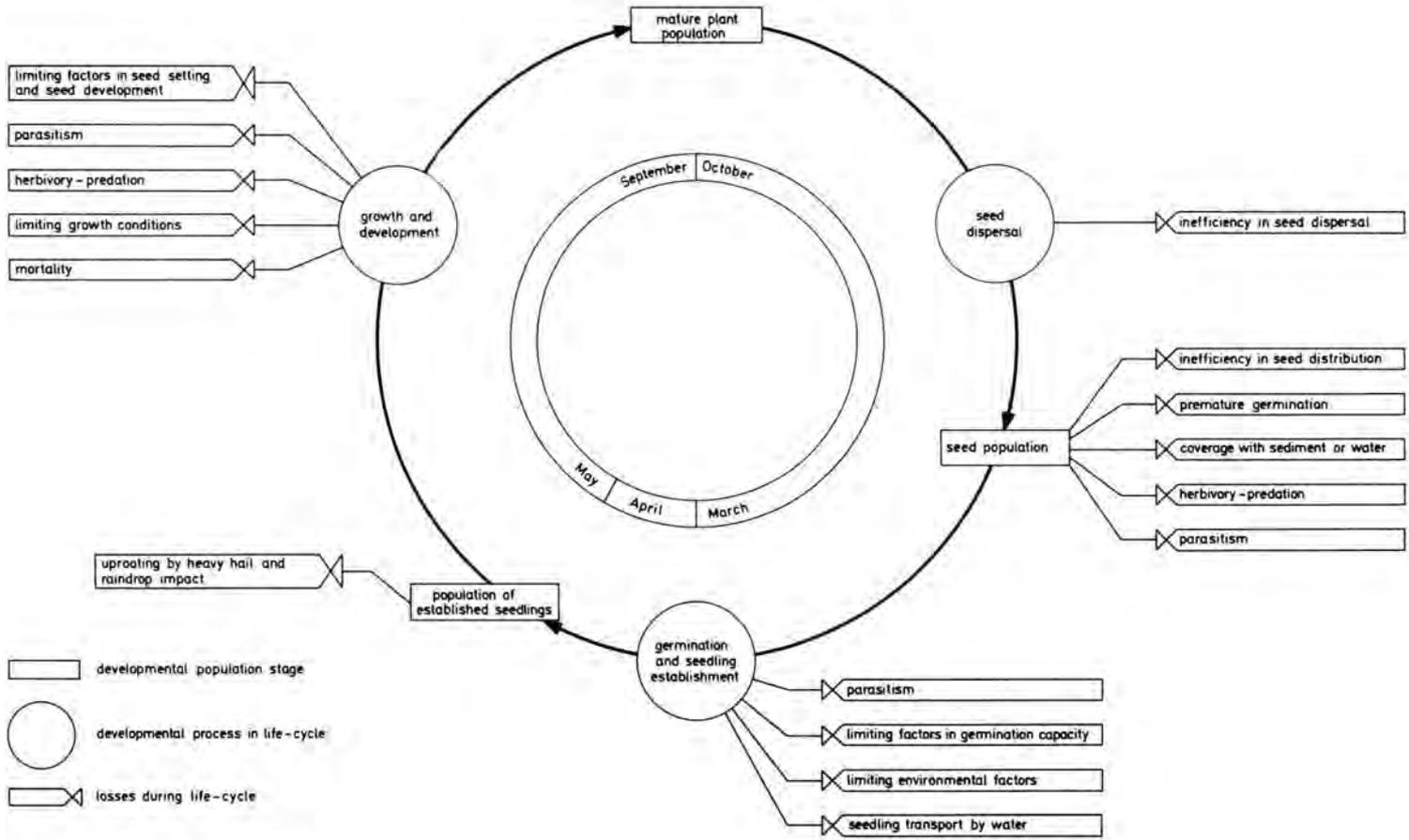


Fig. 9. Population biology of *Salicornia europaea* agg.

University, Amsterdam) investigated germination and mortality in *Salicornia*. These aspects of life history have been compared with the influence of the tides, of salinity and soil-moisture contents, and with the density of *Salicornia* and other salt-marsh species (For the Bergen op Zoom salt marsh).

W. de Munck started an investigation on plant-animal relationships, and found three grazing species: *Hydrobia ulvae* (Mollusca), *Lita* cf. *Salicorniae*, and *Coleophora salicorniae* (Lepidoptera), each with a characteristic grazing pattern. *Thrips funebris* (Thysanoptera) found in the holes formed by grazing of the former species is new for the Dutch fauna.

The study of *Halimione portulacoides* populations has been started by a preliminary investigation into the possibility of age determination on shoot development of the individual plants. This seems to be the case in populations of up to about 10-15 years. Older plants - and sometimes even younger ones - show more and more branches buried by sediments and rooted into the soil, interfering with or even totally washing out these characters for age determination.

POPULATION ECOLOGY OF *Salicornia europaea* agg. IN A TIDAL SALT MARSH (S 4 and S 5)

H. Schat

In 1976 a study of the population ecology of *Salicornia stricta* G.F.W. Meyer and *S. brachystachya* G.F.W. Meyer had been carried out in the salt marsh south of Bergen op Zoom bordering the inner end of the Eastern Scheldt. Of both species natality, mortality and plant weights were estimated at sample plots established along gradients from the mud flat up to the upper part of the marsh.

Data processing in 1977 gave the following results: Germination of both species is found between 110 and 280 cm above Dutch ordnance level (N.A.P.) (Mean High Water (MHW) level is 199 cm above N.A.P.; mean tidal difference is 387 cm). The highest densities in germination of *S. stricta* seeds occur just below or at MHW level, and those of *S. brachystachya* at about 40 cm above MHW level (Table 12; Fig. 10). Seedlings of *S. brachystachya* are very rare below the MHW level. The distribution patterns of seed seems to be by far the most important factor governing the patterns of seedling density.

Except in the highest parts of the range, mortality is lower in *S. brachystachya* than in *S. stricta*. Between 110 and about 150 cm above N.A.P. mortality is determined by washing out of seedlings by the tides. Where plant debris produced by *Spartina townsendii* had been left by the tides (this year from about 170 cm N.A.P. upwards) many seedlings were shaded out immediately after germination. Between about 185 and 200 cm above N.A.P. intraspecific competition governs the mortality rate, as it is positively correlated with density in this part of the range. Between 190 and 225 cm above N.A.P. shading out by *Aster tripolium* and *Puccinellia maritima* can also be an important fac-

Table 12. Germination, mortality rates and mean dry weights of annual *Salicornia* species in the salt marsh near Bergen op Zoom.

Level (cm + N.A.P.)	Germination (number/m ²)		Mortality %		Mean dry weight of full- grown plants (mg/ind)	
	<i>S.str.</i>	<i>S.brach.</i>	<i>S.str.</i>	<i>S.brach.</i>	<i>S.str.</i>	<i>S.brach.</i>
114	± 260	1	50	?		
126	± 40	0	50	?	2386*	1408
145	± 1175	1	28	?		
155	± 2500	1	10	?	368	519
190	± 5700	12	92	33	104	342*
195	± 14000	24	64	33	47	38
230	319	76	46	37		
236	945	396	64	54	72	430*
256	± 125	± 200	96	67		
260	± 60	± 85	88	95	* = significantly different	
294	± 500	± 95	100	100		
278	± 430	± 90	100	100		

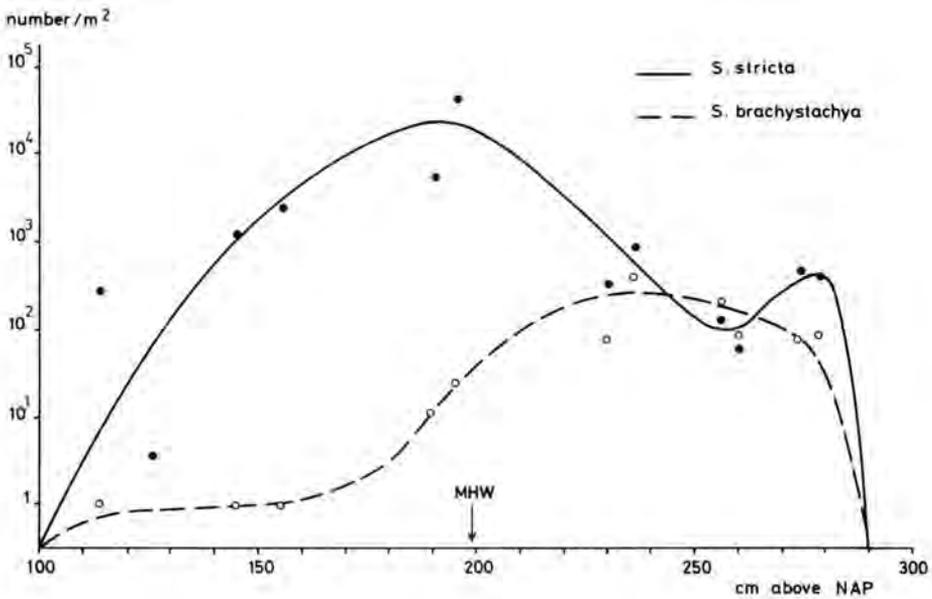


Fig. 10. Relation between seedling density in *Salicornia* species and the height of the marsh level.

tor. From 225 cm upwards water stress during neap tide periods is probably the master-factor for the increasing mortality rates.

Dry weight measurements on full-grown plants show that both species show maximal growth at the lower edge of the marsh. On higher levels plant weights decrease until a minimum is reached at about 195 cm above N.A.P., due to poor light conditions, intraspecific competition at high densities and/or to a high degree of waterlogging of the soil during ebb tides. After this minimum values a secondary maximum in dry weights occurs at about 210 cm above N.A.P., owing to a decreased density of the populations, and better light and drainage conditions. Above this level dry weights decrease definitively, probably because of increasing water-stress conditions. *Salicornia stricta* shows a relatively better growth in the lower part of the marsh, and *S. brachystachya* in the middle part.

The study also includes the germination ecology of other salt-marsh species which will be reported next year.

INVERTEBRATE HERBIVORY IN SALICORNIA EUROPAEA AGG. (S 4 and S 5)

W. de Munck & W.G. Beeftink

A preliminary investigation has been started in order to quantify the damage which invertebrate herbivory can cause to the production of biomass and to the propagules in *Salicornia europaea* agg.

In three tidal and three non-tidal salt marshes *Salicornia* plants were sampled. In these samples grazing patterns were found of *Hydrobia ulvae* (Mollusca), *Lita* cf. *salicorniae* E. Hering (Lepidoptera) and *Coleophora salicorniae* Wck. (Lepidoptera). *Hydrobia* grazing only occurs under tidal conditions. The number of grazed plants varied from 5-67%, and differed largely between the marsh areas investigated. There were no indications that grazing intensity differs between *Salicornia*'s growing on mud and in the marsh. Only the number of grazed internodes seemed to be smaller in the latter. Moreover, the grazing pattern on the stem tended to be lower to its base in the marsh than in the mud individuals.

Lita cf. *salicorniae* occurred more in non-tidal marshes (10-49% grazed plants) than in tidal ones (1-4%). The number of grazed internodes did not differ significantly between the tidal and non-tidal marsh areas investigated, but there are large differences between the marshes of each group (up to three fold on an average). It is not known whether this must be ascribed to differences in density of larvae per plant or to differences in grazing activity. The grazing pattern on the stem varied significantly over the marsh areas investigated, but not when they are grouped in tidal and non-tidal areas. Contrary to what had been found in *Hydrobia* the grazing pattern tended to be located lower in lower parts than in higher parts of the marsh.

Coleophora salicorniae was found in two tidal marshes. Its grazing pattern was evidently separated in space and time from that of *Hydrobia* and *Lita*. The grazing activity of *Lita* was concentrated in June-July, and that of *Co-*

Leophora in August-October. Consequently, the internodes grazed by *Lita* were situated lower in the stem than those grazed by *Coleophora*.

In the tidal marsh near Wilhelminadorp also *Thrips funebris* Bagnall (Thysanoptera) was found, finding shelter in the holes formed by grazing of the former species. This *Thrips* species is known from coastal plants in Germany and Great Britain, and is new for the Dutch fauna.

The authors are greatly indebted to the Department of Entomology of the Plant Protection Service (P.D.), Wageningen, for the identifications and to Mr. W.P. Mantel (Research Institute for Plant Protection, Wageningen) for confirming the *Thrips* identification.

ECOPHYSIOLOGY OF HALOPHYTES (S 6 and S 7)

Research on the ecophysiological level will be pointed at the physiological adaptation mechanisms of halophytes to the tides and related environmental factors. For that reason, preliminary experiments are carried out with the species selected for population studies on their reaction on different salinity, temperature and nutrient conditions. Besides that, an experimental design has been made by J. van Soelen for testing the tidal influences proper.

Miss A.W. Stienstra and J. van Soelen investigated the effects of different constant salinity and temperature conditions on the germination of *Salicornia* seeds. They found some indications to a larger plasticity in a *Salicornia stricta* population compared with that of a *S. brachystachya* population, occurring together in the same salt marsh.

J. van Soelen also examined the NaCl tolerance of *Salicornia brachystachya*. He found indications that either environmental conditions or the vitality of the experimental material, or both, have an influence upon the growth reaction expressed as stem length and dry shoot weight.

A field experiment with N and P fertilizers, carried out by A.W. Stienstra in *Salicornia* populations in a non-tidal salt marsh showed that nitrogen deficiency can be evident under such conditions. The rate to which this deficiency can be neutralized by fertilization, however, seems not only conditioned by the availability of phosphorus in the soil, but also to be density-dependent.

Data obtained for studying the relation between biomass and morphology of *Salicornia* collected in 1976 at the Slikken van Flakkee salt marsh will be worked out by A.W. Stienstra. A similar data set obtained in 1977 from two tidal and four non-tidal marshes to estimate the relation between growth and ion balance of *Salicornia* will be elaborated as well. In order to obtain a simple method for estimating the seed number of *Salicornia* plants, an investigation has been started by J. van Soelen and M.M. Markusse into the relation between seed production and the length of the spikes. Data processing will be finished next year with the help of the Biomathematical Division of the Delta Institute.

A.W. Stienstra studied the mineral composition of *Halimione portulacoides* leaves in the Stroodorpepolder salt marsh. She found that salt accumulation

in the leaves is partly compensated by succulence. No distinct correlation was noticed between soil salinity and the mineral contents in the leaves.

An experiment with *Halimione portulacoides* in 1976 demonstrated that plants grown up from seeds showed a rather large variability in shoot growth and leaf morphology. To eliminate this and the possibility of similar physiological variability, an experiment was set up to grow plants from cuttings. This method was most successful and most uniform with 0.5 n Hoagland solution, and appeared to be adequate for further experimental work. From this experience A.W. Stienstra and M.M. Markusse set up an experiment to test the growth response of *Halimione* to different constant salinity conditions. Optimal growth in roots, stems and leaves as well as ion contents of the leaves differed with respect to salinity. Leaves sampled in the field showed evident differences in their ion contents with age.

A GERMINATION EXPERIMENT WITH TWO ANNUAL *Salicornia* sp. (S 6 and S 7)

A.W. Stienstra & J. van Soelen.

The purpose of this experiment was to investigate the response of *Salicornia stricta typica* and *S. brachystachya typica* seeds to different salinity and temperature conditions. The experiment was carried out in March-April, 1976, with seeds collected in November, 1975 at the Slikken van de Heen salt marsh. The seeds were laid to germinate in petri dishes kept in temperature-controlled rooms. Every two days the NaCl solution was renewed.

The maximum germination percentage was estimated (Fig. 11) as well as the germination rate expressed as the Harmonic Mean Germination Day (HMGD) (Table 13). In both *Salicornia* species the germination rate increased when the temperature increased and the salinity decreased. At 10-30° C *Salicornia stricta* showed an increase of the germination rate at low salinities (2-4 ‰ Cl⁻). *S. brachystachya* only showed an increase at 5° C and 2 ‰ Cl⁻. The optimal environmental conditions for a high germination rate of *Salicornia brachystachya* were 25° C and 0 ‰ Cl⁻. For *S. stricta* these conditions were a variable temperature 25/15° C and a salinity of 0 ‰ Cl⁻. Light stimulated the germination rate of both species.

The percentage of germinated seeds of *Salicornia stricta* seemed greater in extreme situation (Fig. 11). This species had a higher percentage of germinated seeds than *S. brachystachya*. In the latter species the germination percentage also decreased at high temperatures and high salinities.

It is concluded that *Salicornia stricta* and *S. brachystachya* have a different germination pattern. The germination rate in *S. stricta* is lower at low temperatures and high salinities, but the percentage is higher than in *S. brachystachya*. Probably, due to this higher plasticity *Salicornia stricta* seems more adapted to spatial variety in the environmental factors investigated.

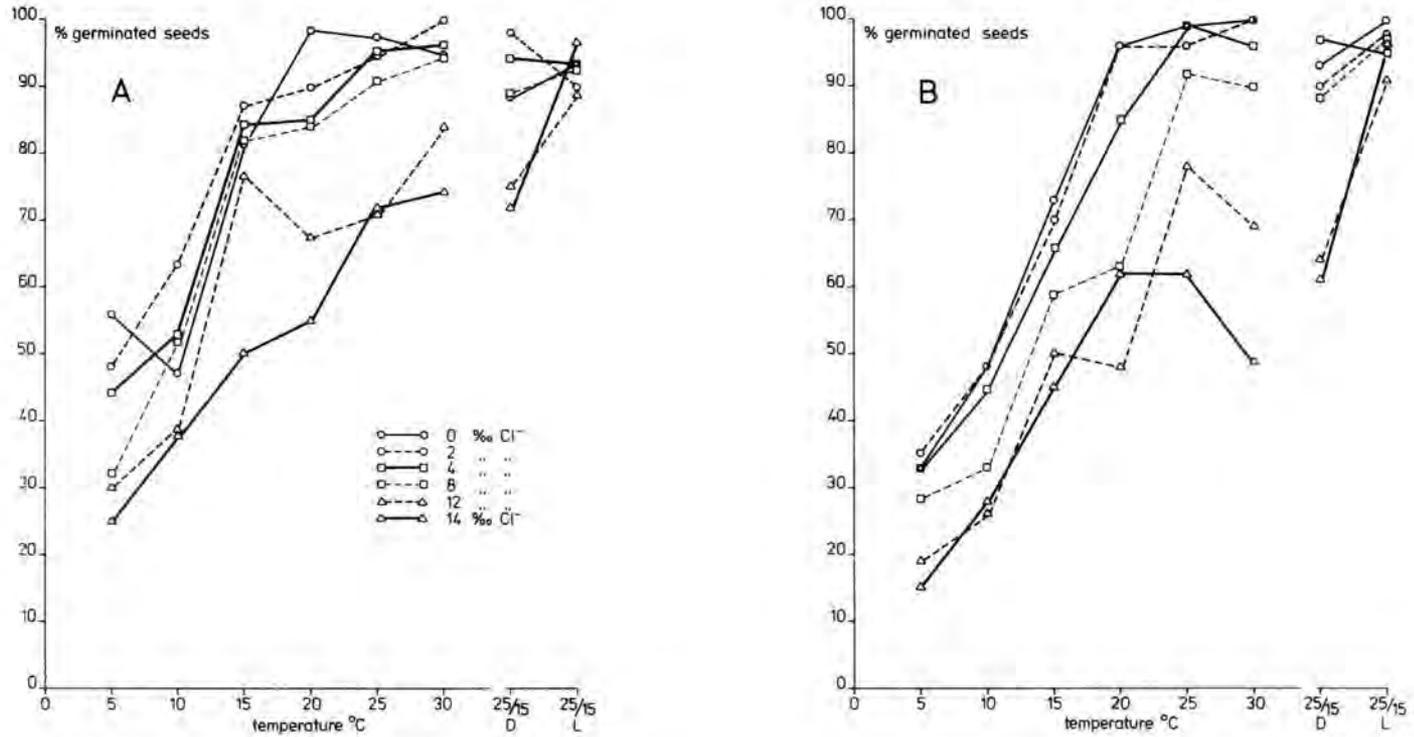


Fig. 11. Maximum germination percentage after 23 days at different temperatures and salinities. A. *Salicornia stricta typica*. B. *Salicornia brachystachya*. D = dark; L = light; 25/15 = day and night temperature interval.

Table 13. Harmonic Mean Germination Day (HMGD) of *Salicornia stricta typica* and *Salicornia brachystachya typica* seeds at different temperature ($^{\circ}$ C), salinity ($^{\circ}$ /oo Cl $^{-}$) and light conditions. D = dark; L = light.

$$\text{Formule HMGD} = \frac{\sum x}{\sum \frac{x t_i}{t_i}}, \text{ in which } x = \text{total number of seeds; } x t_i = \text{number of seeds, germinated after } t_i \text{ days; } t_i = \text{number of days, which are used to germinate.}$$

A. *Salicornia stricta typica*

Temp $^{\circ}$ C	5	10	15	20	25	30	25/15	25/15
$^{\circ}$ /oo Cl $^{-}$	L	L	L	L	L	L	D	L
0	10.3	11.1	4.6	2.8	3.8	3.0	3.5	2.1
2	10.9	7.5	4.1	3.0	2.9	2.6	3.1	2.3
4	11.0	10.4	4.3	3.4	3.1	2.9	3.7	2.2
8	15.0	9.7	5.2	3.6	4.1	4.3	4.6	2.3
12	17.7	14.6	6.3	5.0	6.0	4.6	6.3	2.7
14	20.8	12.6	9.4	6.3	5.5	5.6	5.6	2.8

B. *Salicornia brachystachya typica*

Temp $^{\circ}$ C	5	10	15	20	25	30	25/15	25/15
$^{\circ}$ /oo Cl $^{-}$	L	L	L	L	L	L	D	L
0	11.1	6.7	4.7	3.9	3.2	3.9	4.4	3.5
2	9.3	8.0	5.2	4.0	3.7	4.5	4.5	3.8
4	10.2	8.2	7.1	5.0	4.1	3.9	4.5	3.4
8	14.6	10.7	6.7	5.8	4.3	4.7	4.9	4.0
12	16.1	12.0	8.6	7.1	6.3	6.8	7.6	3.6
14	14.4	13.2	10.1	7.8	6.7	9.6	8.0	4.2

A FIELD EXPERIMENT WITH FERTILIZATION OF *Salicornia europaea* STANDS (S 6 and S 7)

A.W. Stienstra

The purpose of this experiment is to have a first impression of how far nutrient deficiency can influence growth of *Salicornia*, especially in non-tidal salt-marsh habitats.

The experiment was carried out in 1976 at two stations (Table 14 and 15) of the Slikken van Flakkee salt marsh in which also population-dynamic investigations were made. In station II (sample plot nr. 4) the *Salicornia* plants

Table 14. Mean biomass production of *Salicornia* in g dry weight/m² from four sample plots at two stations in the Slikken van Flakkee salt marsh.

Treatment	Station I				Station II			
	shoot	root	ratio	total	shoot	root	ratio	total
O	18.2	2.2	8.5	20.4	65.2	8.1	8.1	73.3
N	61.6	6.7	9.2	68.3	124.0	20.9	6.0	144.9
P	20.3	2.2	9.2	22.6	61.1	12.6	4.9	73.7
N + P	21.4	8.3	2.6	29.7	107.3	15.7	6.9	123.0

Table 15. Mean N and P contents of *Salicornia* in mg/g dry weight from four sample plots at two stations in the Slikken van Flakkee salt marsh.

Station I						
Treatment	N			P ₂ O ₅		
	shoot	root	ratio	shoot	root	ratio
O	15.8	7.9	2.0	6.0	2.2	2.7
N	14.8	7.6	1.9	4.3	1.9	2.3
P	13.5	6.7	2.0	5.1	1.9	2.7
N + P	14.2	7.4	1.9	5.5	2.4	2.3

Station II						
Treatment	N			P ₂ O ₅		
	shoot	root	ratio	shoot	root	ratio
O	33.1	6.5	5.1	6.8	1.7	4.0
N	21.8	6.9	3.2	5.1	1.9	2.6
P	34.7	5.9	5.9	7.4	1.8	4.1
N + P	18.8	6.9	2.7	5.8	2.1	2.8

grew larger and with a lower density than in station I (sample plot nr. 3). At both stations four sample plots were established, each with the following nutrient treatments added in May: (1) control, (2) 18.1 g NO₃⁻/m² as Ca(NO₃)₂, (3) 14.2 g P₂O₅/m² as Ca(H₂PO₄)₂, and (4) the gifts (2) and (3) combined. The single plots were 1 m² in size. In October the biomass of the total *Salicornia* vegetation in the plots was estimated, as well as its N and P contents. In that period also some soil parameters were analysed.

Soil analyses showed a slightly higher salinity in station II than in I. Soil moisture and total nitrogen contents were equal in October. It is therefore concluded that the N fertilizer was totally consumed and washed out at that time. Addition of P was reflected in evidently higher P contents in the P-treated plots (estimated with the P-Al method).

Biomass production was higher in all plots of station II compared with that of station I, in spite of lower plant densities (Table 14). Addition of N resulted in a threefold increase at station I, and in a twofold increase at station II. P addition did not give an evident higher total biomass production, and combined with N it seemed to suppress the N effect. Shoot/root ratios were generally higher at station I than at station II. There are indications that under limited growth conditions, such as at station I, root growth is mainly determined by nitrogen. Shoot-growth promotion by N, however, seems to be suppressed by P.

The higher biomass at station II is related to higher N contents in the shoots and to a low tendency to lower N contents in the roots (Table 15). Shoot/root ratios in N as well as in P contents were high in the control and P-fertilized plots, and low in the N-fertilized plots, mostly as a consequence of a relatively low N level in the shoots.

It is concluded that *Salicornia* can show evident nitrogen deficiency under non-tidal conditions. However, the extent to which this deficiency can be neutralized seems to be dependent on the availability of phosphorus in the soil, and on the density of the population.

GROWTH AND MINERAL COMPOSITION OF *Halimione portulacoides* UNDER DIFFERENT SALINITY CONDITIONS (S 6 and S 7)

A.W. Stienstra & M.M. Markusse

An investigation has been started to test the growth response of *Halimione portulacoides* to salinity. For that purpose a NaCl-tolerance experiment with Hoagland solution was carried out in the greenhouse, running from May to October, with plants grown up from seed. Growth was measured as dry weights of leaves, stems and roots, and mineral composition was estimated in the leaves, both at the end of the experiment. For comparison the ion contents in the field leaf samples were taken from plants growing on a creekbank levee in the Stroodorpepolder salt marsh.

The nutrient culture experiment suggests optimal growth of roots at a concentration of 0.1 n NaCl, of stems between 0.1 and 0.3 n NaCl, and of leaves at 0.3 n NaCl (Fig. 12), (0.1 n NaCl = 5.85‰ NaCl = 3.55‰ Cl⁻). Ion content analyses of the leaves show that the species accumulates Na up to concentrations of 0.3 n, and Cl up to 0.5 n NaCl. The K-, Ca-, and Mg-contents drop after the slightest addition of NaCl to minimal levels. Only K seems to re-increase towards the highest NaCl concentrations (Table 16A).

The ion contents in the leaves sampled in the field change with their age (Table 16B). At increasing age the Na and Cl contents increase. The K-content tends to decrease, and those of Ca and Mg to increase. Also the water content of the leaves increases with age, which means that salt accumulation is at least partly compensated by succulence. Salinity of the soil moisture fluctuates

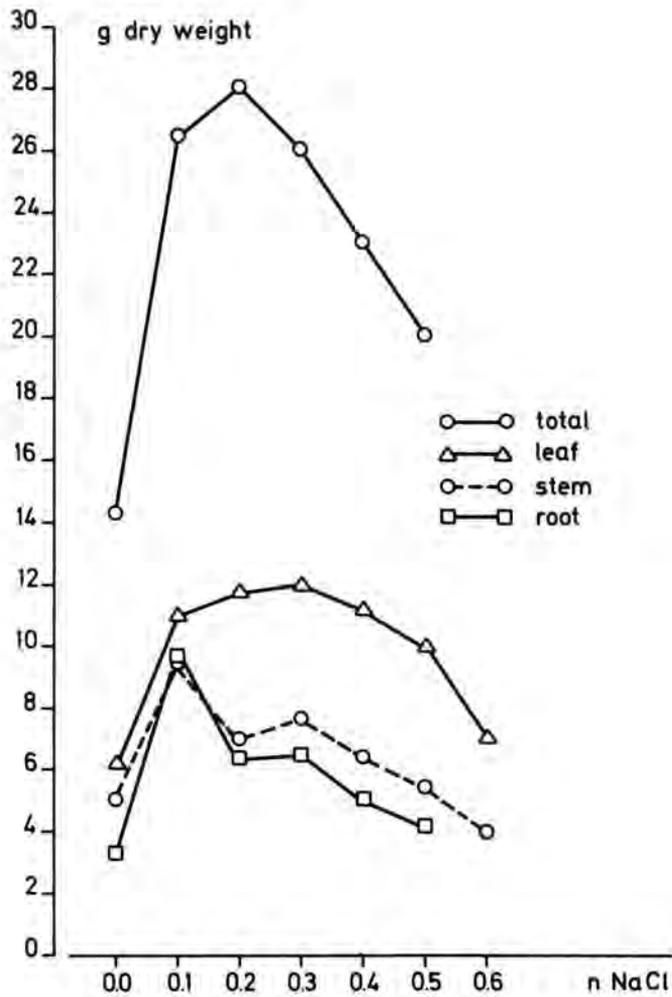


Fig. 12. Total dry weight production (g) of leaves, stems and roots of *Halimione portulacoides* from greenhouse experiments grown up at 0.0-0.6 n NaCl in 0.5 n Hoagland solution.

tuated between 0.3 and 0.6 n NaCl (average 0.5 NaCl) and so it ranged within the NaCl concentrations chosen in the experiment. This may be due to (1) the age difference between the plants sampled in the field and those used in the experiment, (2) the difference in substrate, (3) the much larger salinity fluctuations under field conditions, and (4) the generally much lower air humidity in the greenhouse with respect to that in the field.

Table 18. Mineral contents (mg/g dry weight) and water content (g/100 g wet weight) in the leaves of *Halimione portulacoides*. A. From plants grown up in a 0.5 n Hoagland solution at different NaCl concentrations. B. Collected in the Stroodorpolder salt marsh. a = young, not yet full-developed leaves; b = full-developed upper leaves; c = green lower leaves; d = yellow lower leaves. Date of sampling 31-7-1975.

A						
n NaCl	Cl	Na	K	Ca	Mg	watercontent
0.0	21.6	26	64.9	8.3	7.0	77.84
0.1	71.8	71	28.8	1.8	2.3	79.94
0.2	79.3	86	24.4	1.6	2.1	79.97
0.3	89.5	90	24.5	1.5	1.8	79.61
0.4	93.7	80	25.5	1.9	2.0	79.43
0.5	98.1	90	26.3	1.6	1.8	78.54
0.6	89.7	90	30.0	1.8	1.9	80.03
B						
	Cl	Na	K	Ca	Mg	watercontent
a	114.6	101	38.3	8.3	9.2	82.05
b	143.3	129	34.6	7.8	9.1	83.68
c	175.4	129	29.5	7.4	8.5	86.24
d	215.9	151	37.0	9.5	10.0	87.48

GROWTH OF *Salicornia brachystachya* UNDER DIFFERENT SALINITY CONDITIONS (S 6/7)
J. van Soelen

An investigation has been started to examine the NaCl tolerance of *Salicornia brachystachya*, G.F.W. Meyer. Seedlings grown close to the permanent quadrats (p.q.) nrs. 6 and 10 on the Slikken van Flakkee sand flat were cultivated in the greenhouse with a 0.5 n Hoagland nutrient solution supplemented with 0, 50, 150, 250, and 350 mMol NaCl per liter (100 mMol NaCl = 3,55^o/∞ Cl⁻). P.q. 10 is situated at the water edge of the area of investigation, and p.q. 6 is 350 m away in landward direction. The experiment was carried out

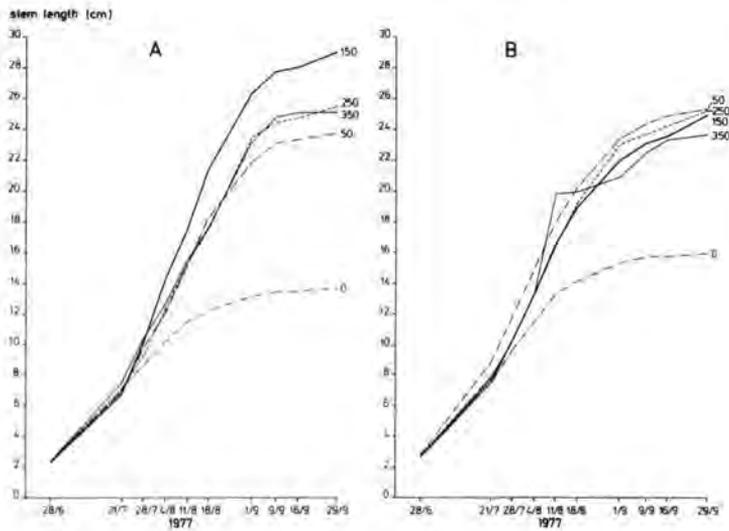


Fig. 13. Growth of *Salicornia brachystachya* originated from p.q. 6 (A) and 10 (B) and cultivated at 0.5 n Hoagland solution for different NaCl concentrations (mMol/L).

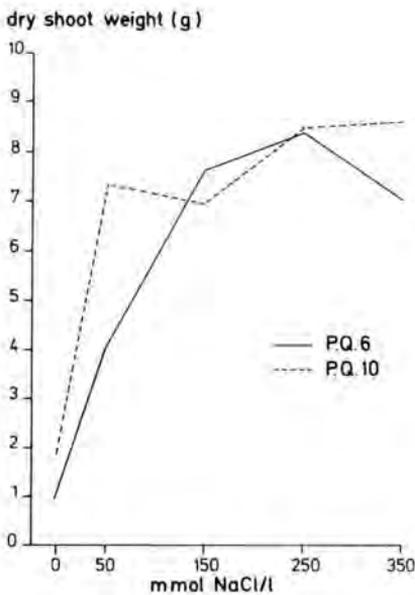


Fig. 14. Mean dry shoot weight of *Salicornia brachystachya* at the end of the growing period and cultivated for different NaCl concentrations.

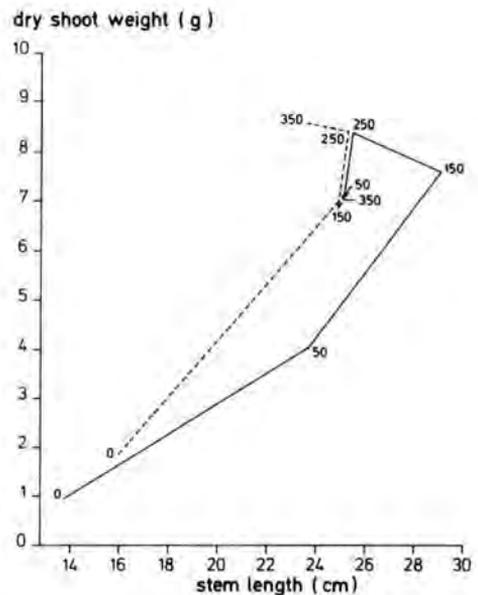


Fig. 15. Relation between stem length and mean dry shoot weight of *Salicornia brachystachya* cultivated for different NaCl concentrations (mMol/L).

from June to October, 1977, and has been started with eight plants per treatment.

The positive effect of salt on plant growth (Fig. 13), already known from Feekes (1936), seems to differ with the origin of the experimental plants. Plants grown close to p.q. 6 show optimal growth at 150 mMol NaCl, contrary to those collected from p.q. 10 where salinity concentrations did not differentiate stem lengths. This difference in response could be caused by the difference in soil salinity during germination and early growth in the field which was about twice in p.q. 10 compared with that in p.q. 6. Another cause could be the difference in vitality of the parent plants in both plots. Mortality of the plants under cultivation originated from p.q. 6 was higher than those originated from p.q. 10.

Biomass, expressed as dry shoot weight per plant, at the end of the experiment, proved to be maximal at higher salinity concentrations (250-350 mMol NaCl) than the salt concentration for optimal stem length (Fig. 14, 15), owing to higher ramification rates at these salinities. However, plants grown without NaCl were also more branched, but these ramifications remain very short. The control plants appeared to be easily attacked by *Coniothyrium* sp. and *Alternaria* sp.

References

Feekes, W. 1936. De ontwikkeling van de natuurlijke vegetatie in de Wieringermeerpolder, de eerste groote droogmakerij van de Zuiderzee. Ned. Kruidk. Arch. 46, 1-295.

A BOTANICAL EVALUATION OF A POLDER COMPLEX IN THE SOUTH PART OF THE ISLE OF ZUID-BEVELAND (S-general)

Agnes van de Vijver

In order to obtain an insight into the flora and vegetation of the polders in the Delta Area, the students miss J.G. Roeleveld, C. van der Kraan, P. Dieleman, C.C. Redeker, J.C.H. Peeters, K.V. Sykora and Mrs C.M.P. Sykora-Hendriks, J.W.M. Kuijpers, miss C.J. Smits and miss A. van de Vijver investigated several parts of the island of Zuid-Beveland. This project, which took several years, is finished now by the Delta Institute, and has been deputed for continuation to the Planological Department of the Province of Zeeland. The last two studies, carried out by miss A. van de Vijver, and dealing with the botanical diversity of the "Zak van Zuid-Beveland" have now been achieved. A summary is given here of the first one, the second to be following next year.

A botanical evaluation is given of 59 polders, varying from 4 to 391 ha with a total surface of about 6830 ha, situated as a joint complex in the south part of the isle of Zuid-Beveland. The date of embankment varies from

before 1250 to the beginning of the seventeenth century.

For the botanical evaluation inventories were used made by Sykora-Hendriks & Sykora (1973) of the polder dikes, and those of Smits (1974) and Van de Vijver (1977) of the polderland. These data have been processed with the evaluation method of Mennema (1973), which includes diversity (species richness), a floristic evaluation (including species rarity), a vegetational evaluation (comprising the degrees of vulnerability, rarity, and the degree to which the vegetation can be superseded), as well as the average rarity of all participating species together. The surface units chosen for this method were the km-quadrat (90 in total) and the polders as embankment unit (59 in total).

The km-quadrats with the highest evaluation values were found where saline conditions are present. Quadrats with wetlands and humid contact and stress zones were also estimated with a high botanical value, as well as those containing many hedges and brushwood combined with an adjacent vegetation of tall herbs. Highly evaluated km-quadrats coincide to a high degree with highly evaluated polders.

Soil profile, groundwater level and height above mean sea level were used as environmental parameters. However, there was no significant correlation between the diversity of these soil factors and the botanical evaluation. Also the expectation has not been confirmed that the quadrats with the highest soil diversity should coincide with those of the highest botanical evaluation.

Most quadrats in the area of investigation were estimated at the two lowest categories (I and II) of the national classification of botanical evaluation. Only two quadrats could be classed into category III. The categories IV and V were not represented. As from the national viewpoint about 70% of the quadrats belong to the categories I and II, the polder complex in question is somewhat below the average situation in the Netherlands.

The quadrats of the polders under investigation also show relatively low botanical evaluation values compared with those of quadrats from other polder areas, such as the Vijfheerenlanden, IJsselmonde and the "Groene Hart van Holland". These rather poor results may be real, but may also be caused by a more intense and more consequent application of the modern agricultural technics, such as the use of herbicides and heavy fertilization, in Zuid-Beveland. The cause may also be that the botanical inventories of these polders are still relatively incomplete compared with the other areas.

References

- Mennema, J., 1973. Een vegetatiewaardering van het stroomdallandschap van het Merkske gebaseerd op een floristische inventarisatie. *Gorteria* 6, 157-179.
- Smits, C.J., 1974. Floristisch en vegetatiekundig onderzoek van enkele polders in de Zak van Zuid-Beveland. Delta Institute for Hydrobiological Research Student Reports D2-1974, pp. 41.
- Sykora-Hendriks, C.M.P, and Sykora, K.V. 1973. De dijkvegetatie van Zuid-Be-

veland. Delta Institute for Hydrobiological Research Student Reports D1-1973, pp. 27.

Van de Vijver, A., 1977. De vegetatie van enkele polders in de Zak van Zuid-Beveland. Delta Institute for Hydrobiological Research Student Reports D5-1977, pp. 79.

WORKING GROUP STRUCTURE AND DYNAMICS OF INSTABLE AQUATIC SYSTEMS

INTRODUCTION S. Parma

The research theme of the Delta Institute is the study of the impact of human interferences on aquatic and semi-terrestrial ecosystems in estuarine and brackish regions. After an interference the ecosystem, having a distinct level of development, lowers its level of integration. Margalef (1968) connected these changes in system characteristics with the concept "maturity". An ecosystem in the pioneer stage of development has a low "maturity" and during the development of the system, when the interactions become more complicated, the "maturity" increases.

This ecological succession can be described by means of structural and functional ecosystem parameters. Research of this kind is usually situated in more "mature" ecosystems. Comparatively little is known of the pioneer stage of aquatic ecosystems, i.e. the stages with a low "maturity". This might be due to the rapid succession in the early stages, especially when the ecosystem is developing under rather constant environmental conditions. However, when an ecosystem is subjected to the effect of abrupt and unpredictable interferences it will remain constantly on a low "maturity" level. This makes it possible to study the structure and processes of the pioneer stages.

Small inland waters now are constantly subjected to these abrupt and unpredictable interferences. In our Delta region many ditches, small canals, creeks, cattle drinking pools, ponds, etc., are found where the environmental conditions are very inconstant. Unpredictable changes in salinity, temperature, oxygen content, discharge and water level, unexpected removal of shore vegetation and drainage of pesticides from agriculture, frequently occur. So our research team is mainly concerned with the study of structure and dynamics of these small inconstant systems.

The code B is used for the projects. Additional abstracts are presented in the section of published articles.

VARIATION IN SPACE AND TIME OF SOME ABIOTIC FACTORS IN INLAND WATERS OF ZEE-LAND (B 1)

The research programme in the "Adriaanpolder" ditch, measuring the diurnal vertical oxygen-, salinity-, chlorophyll- and plankton gradients was terminated for the time being and the results were used for a publication.

J.J. Sinke and J.O. van de Zande followed daily fluctuations in salinity and weekly fluctuations in major ions and nutrients in a ditch and a cattle drinking pool both in the Kaarspolder, S.-Beveland. The results are not yet elaborated and will give information on the short term variations of some abiotic factors. In the same localities Mr. W. Vermeulen (National Institute for Water Supply) took plankton samples. Also his data are in preparation.

Like in 1976 L. de Wolf took water samples in spring, summer, autumn and winter at 700 localities in the S.W.-part of the Netherlands, to determine salinity and water quality.

J.W. Rijstenbil started an investigation of the oxygen budget of some brackish water ditches. He was assisted by Miss L. van de Velde, who investigated the influence of wind velocity on the reaeration of a water mass. This project is executed in cooperation with the Provinciale Waterstaat van Zeeland (N. Oskam).

J.J. Sinke and J.O. van de Zande started a sampling programme of the physico-chemical characteristics of cattle drinking pools in the SW-part of the Netherlands. An investigation of the salinity, cations and ammonia of those pools on the island of Walcheren and in the Yerseke Moer was completed and the results will be prepared for an internal report.

P.F.M. Elenbaas (University of Utrecht) investigated the variation in space and time of some biotic and abiotic factors in some fresh- and brackish water ditches. These ditches were also selected on differences in type of sediment.

In collaboration with Dr. M. Marchand (Centre of Oceanography, Brest, France) some samples of *Palaemonetes varians* from the island of S.-Beveland were analysed on pesticides. There proved to be significant differences in the concentrations of lindane and of DDT between different parts of the island, presumably connected with variations in agricultural use of these chemicals.

CLASSIFICATION OF INCONSTANT BRACKISH WATER BIOTOPES BASED ON MACROFAUNAL SPECIES COMPOSITION (B 3 and B 4)

N. Tramper (Agricultural University, Wageningen) completed his study of the faunal composition of cattle drinking pools. His results are now ready for a mathematical cluster analysis.

I.J. Weeber started a similar investigation of ditches. Synchronously also physico-chemical data of the sampling localities were collected. The data will be used in one or several cluster analyses in order to obtain an optimal arrangement of the biotopes, based on their similarity in species composition.

R.H. Bogaards and J.W. Francke continued their investigation of the distribution of *Neomysis integer* in relation to salinity and morphometry of the locality. This year Zeeuws-Vlaanderen was sampled extensively. Moreover other macrofaunal components (esp. *Hemiptera*) were collected. The island of Schouwen was sampled four times at appr. 140 localities on salinity and macro-

fauna. The programme is finished now and the results are in preparation.

R. Luyendijk started an investigation to the distribution of waterbugs (*Hemiptera*) and waterbeetles (*Coleoptera*) in relation to some environmental characters, especially salinity. He already identified the *Hemiptera* collected during the above mentioned trips in Zeeuws-Vlaanderen and on Schouwen-Duiveland.

AUTECOLOGY AND POPULATION DYNAMICS OF SOME INVERTEBRATE SPECIES IN BRACKISH WATER HABITATS (B 5 and B 6).

B.P.M. Krebs continued his work on brackish water chironomids. He identified the material collected by N. Tramper and I.J. Weeber. Some taxonomical data were summarized. Moreover he wrote a report on the population dynamics of *Chironomus halophilus* and *Leptochironomus deribae* in the "Adriaanpolder" ditch.

J.A. Goedbloed (Free University, Amsterdam) investigated the salinity preference during oviposition of *Chironomus halophilus* and *C. salinarius* and the substrate preference of the larvae of the same species.

Mrs. M.J.M. Langeveld started observations to the influence of saline percolation on the diversity of the benthic fauna in the "Westgeul" (Zeeuws-Vlaanderen).

R.H. Bogaards, J.W. Francke and Mrs. C.H. Borghouts finished their investigations of extreme habitats in Schouwen. The salinity of these habitats returned from their extreme values in autumn 1976 to more normal concentrations and the recolonization proved to be a rapid process. The results are in preparation.

References

Margalef, R., 1968. Perspectives in ecological theory. University of Chicago Press, Chicago. pp. 112.

SALINITY AND WATER QUALITY OF THE INLAND WATERS IN THE SW-PART OF THE NETHERLANDS (B 1)

L. de Wolf

During the period 1976/1977 water samples were collected at three months intervals at appr. 700 localities on the islands of Schouwen-Duiveland, Walcheren, N.- and S.-Beveland and in Zeeuws-Vlaanderen. The samples were investigated on salinity, oxygen, pH and ammonium. Salinity was measured as conductivity and, because of the very good correlation, calculated as chlorinity. Ammonium was measured on an autoanalyser (Technicon AA2). The results are still being prepared but some general conclusions can be made:

- the island of Schouwen-Duiveland is generally very brackish (average chlorinity 5.5-16.5^o/oo). Only the dune area is fresh, while in the centre some β -mesohaline regions are present,

- the other islands (Walcheren, N,- and S,-Beveland) are mesohaline in general with some fresh and oligohaline regions situated along the dune coast and in the central, geologically older parts of S.-Beveland,
- Zeeuws-Vlaanderen has a large freshwater area in the pleistocenic southern part, while the coastal plain is oligo- or mesohaline,
- ammonium concentrations can be very high (over 10 mg/l $N-NH_3$ in winter), illustrating the polluted character of the polder ditches,
- oxygen can fluctuate considerably during the daylight period and its value as quality parameter is questionable.

The salinity maps of the different regions are being correlated with distribution of faunal and floral components.

A DEEP MICROGRADIENT WATER SAMPLER (B 1)

A.G.A. Merks

The sampling of supernatant bottom waters required an instrument able to sample at various short distances from the bottom floor. In particular for stagnant water sharp gradients of various components, like salinity, oxygen, phosphate, etc. might occur within distances of a few cm.

A number of samplers exist for this purpose, like the Jenkins Surface Mud Sampler (Mortimer, 1942) and the Maitland Tube (Maitland, 1969), which both have some disadvantages of price and disturbance at the sampling spot, respectively.

For this purpose a cheap and simple sampler has been constructed with help of L. de Wolf of the Institute, which is able to sample thirty 15-20 ml water volumes within a distance of 60 cm, vertically. Its construction is given on Plate III.

Sampling occurs after an accommodation period at the sampling site with the sampler fixed to a solid obstacle. Depending on the situation an accommodation time until 2 hours might be required (less in waters in which already a slight current is present).

An example of microgradients as observed in a ditch filled with some brackish water from bottom water seepage, is presented in figure 16.

The sampler, called "Merks Sampler" is commercialized by ATS, Mariastraat 42, The Hague, Netherlands

References

- Maitland, P., 1969. A simple corer for sampling sand and fine sediments in shallow water. *Limnol. Oceanogr.* 14: 151-156.
- Mortimer, C.H., 1942. The exchange of dissolved substances between mud and water. *J. Ecol.* 30: 147-201.



Plate III. View on Merks Sampler with J.J.Sinke.

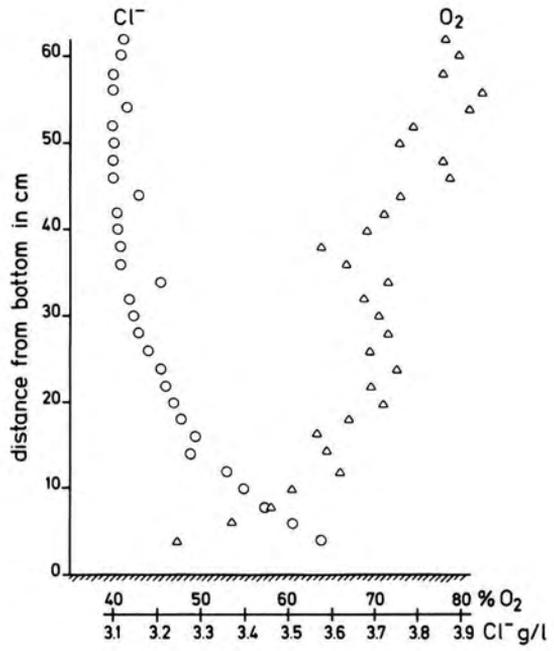


Fig. 16. Micro gradients of ‰ chloride and saturation of oxygen in a ditch.

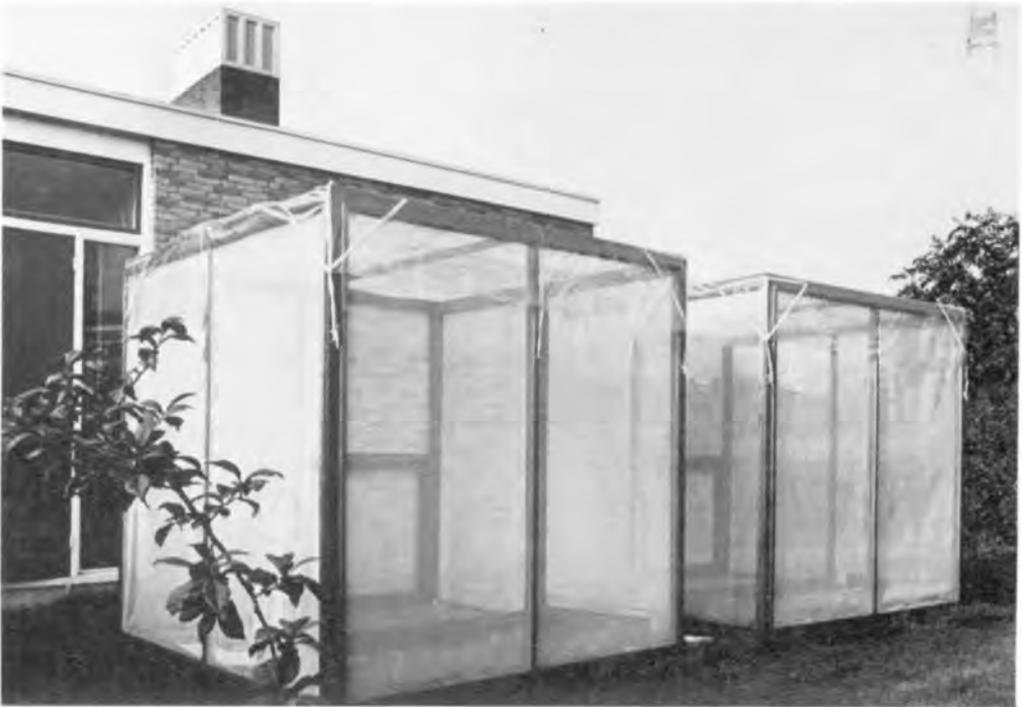


Plate IV. Outdoor cage for culturing *Chironomus salinarius* and *C. halophilus*

DIURNAL FLUCTUATIONS IN SALINITY, OXYGEN AND CHLOROPHYLL IN A SHALLOW BRACKISH DITCH (B 1)

S. Parma and C.H. Borghouts-Biersteker

In a series of observations we determined the extent of the diurnal variations in salinity, oxygen and chlorophyll in a ditch. The ditch (max. depth at the observation place 1,25 m) was free of macrophytes, polluted (maxima of N-NO_2^- : 0.6 mg/l, BOD_5^{20} : 40 mg/l) and brackish (variations in Cl^- : 0.6 - 5.2 $^{\circ}$ /oo). Due to water discharge the waterlevel can fluctuate over a range of 60 cm. The results showed that in this shallow and small waterbody:

- a salinity gradient can occur, having a permanent character and being stabilized against wind action. Disturbance of the gradient is possible through discharge of water, but a reestablishment of the gradient is a rapid process,
- a salinity gradient is closely accompanied by an oxygen gradient,
- the extension of both gradients is variable in space and time,
- the salinity gradient acts as a monimolimnion. Consequences of the presence of this monimolimnion may be:
 - + no participation of the sediment oxygen demand in the oxygen balance of the upper water layers and thus a less pronounced nightly minimum in the diurnal oxygen curve.
 - + rather high oxygen concentrations in the monimolimnion during periods with a high light penetrability, i.e. low chlorophyll content.

The presence of the gradients influences both the mud-dwelling fauna as well as the oxygen balance of the system. A study of these aspects is in progress.

THE INFLUENCE OF SALINITY GRADIENTS ON THE OXYGEN BUDGET OF A BRACKISH DITCH (B 1)

J.W. Rijstenbil

Brackish ditches in SW-Netherlands sometimes show a salinity gradient. This might be accompanied by differences in oxygen budgets of the upper and lower watermasses, dependent on the extent of the influence of the sediment.

Investigations on the oxygen budgets are in progress dealing with production and consumption of oxygen at different levels in the watercolumn; production and consumption by the sediments (Lucas & Thomas, 1970); diurnal pattern of dissolved oxygen (Lingeman *et al.*, 1975); exchange of oxygen at the air-water interface. In laboratory tests the influence of wind velocity on the reaeration constants was quantified (v.d. Velde, 1978).

Observations on two localities in S.-Beveland showed that salinity gradients are caused mainly by drainwater, running over brackish water (10 $^{\circ}$ /oo Cl^-). Between the two layers a rapid diffusion was noticed and the gradients disappear by exceeding an overall concentration of 8 $^{\circ}$ /oo Cl^- . In the summer, with only a small inflow of drainwater, the gradients were absent except during one observation period when we found chlorinities of 3.6 $^{\circ}$ /oo in the upper layer and 6.9 $^{\circ}$ /oo near the sediments. Table 17 demonstrates a situation of

absence and presence of a salinity gradient in the same ditch.

Our preliminary results do not show evidence that the budgets are influenced by salinity gradients (Table 18). The sediment oxygen demand is considerable and even a strong salinity gradient does not prevent the influence of the sediment on the upper water layer. At night only a strong reaeration can prevent anoxic conditions over the whole of the water column.

The situation may be complicated by a number of disturbances, like horizontal water movements, changes in waterlevel and animal activities (ducks). Sometimes the O_2 -production was very irregular and not proportional to depth and sunshine. We have no explanation for the occasional occurrence of an increase in oxygen at the mud-water interface during the midnightly period.

The oxygen budget studies will be continued in 1978 with a series of laboratory tests, eliminating the disturbing factors in nature.

Table 17. Absence and presence of a salinity gradient in a ditch on S.-Beveland

date	July 12/13	August 23/24
depth in cm	40	50
temperature in °C	22	26
Secchi value in cm	35	20
salinity gradient	absent	present
height of the upper water column (I)	25	35
height of the lower water column (II)	15	15
average chlorinity in I in ‰	9.6	4.2
average chlorinity in II in ‰	9.6	5.9

Table 18. Oxygen budgets of the upper (I) and lower (II) layer in a ditch in S.-Beveland in periods with and without salinity gradients (see also Table 17).

oxygen in mg/day m ²	upper layer		lower layer	
	no gra- dient	gra- dient	no gra- dient	gra- dient
exchange with atmosphere	+2848	+ 3802	-	-
daily oxygen production (water)	+5593	+14859	+3684	+ 491
daily oxygen demand (water)	-3690	- 3836	-1857	-1518
change of dissolved oxygen conc.	- 56	+ 3862	- 52	+ 941
sediment oxygen production	-	-	0	+ 307
sediment oxygen demand	-	-	-7272	-3553
deficiency (needed from layer I)	-	-	+5393	+5168
excess in layer I (overproduction)	-4807	-10963	-	-
rest(= probably loss by horizontal transport)	-	-	+ 586	-5798

References

- Lingeman, R., Flik, B. & Ringelberg, J., 1976. Stability of the oxygen stratification in an eutrophic lake. Verh. Int. Verein. Limnol. 19: 1193-1201.
- Lucas, A. & Thomas, N., 1970. Sediment oxygen demand in Lake Eries Central Basin. Hypoproject 5: 45-51.
- Velde, L. van de, 1978. Plankton en zuurstofhuishouding in brak slootwater. Students Reports, Delta Institute, in preparation.

OBSERVATIONS ON THE VARIATION IN SPACE AND TIME OF SOME BIOTIC AND ABIOTIC FACTORS IN FRESHWATER AND BRACKISH WATER DITCHES

P.F.M. Elenbaas (B 1)

In five locations, viz. one ditch with a sandy bottom, one ditch with a clay bottom, two ditches with peaty bottoms and one cattle pool, we determined a number of abiotic and biotic parameters along horizontal and vertical sections. The samples along the vertical section were taken with the "Merks" sampler (Merks, this report). The sampling stations in horizontal direction were located in the middle of the ditch, between macrophytes, along edges, etc. all within a distance of 10 meters. We determined nitrogen- and phosphorus compounds, chloride, oxygen, temperature and pH in water samples and nutrient content in bottom samples. Plankton counts were made only from surface- and from bottom water and were accompanied by chlorophyll measurements. This sampling programme was repeated at regular time intervals.

The programme, still in progress, must give an answer to the following questions: is a ditch chemically homogeneous and if not, along which sections do gradients exist; is there a seasonal periodicity at the different places and do these periodicities take place synchronously; is there a quantitative and qualitative influence of the type of substrate on the chemical composition of the water; what is the relationship between planktonic and chemical periodicity at the different places.

Preliminary results show a considerable increase in orthophosphate over very short vertical intervals (10 cm) in ditches with a dense vegetation of filamentous algae. Also over horizontal sections large differences in nutrient contents were observed.

IMPACT OF CHANGES IN QUALITY AND QUANTITY OF SEEPAGE WATER ON THE MACROFAUNAL DIVERSITY OF THE "WESTGEUL" (ZEEUWS VLAANDEREN (B 2)

Mrs. M.J.M. Langeveld

The "Westgeul", a cut-off side-arm of the Western-Scheldt, was originally influenced by saline seepage water. Since the salt-marshes situated in the tidal area just before the "Westgeul" are reclaimed, a change in seepage regime and quality can be expected. This might have consequences for the diversity of the fauna in the "Westgeul".

A research programme was planned in order to answer the following questions:

what changes in quantity and quality of the seepage do occur; what is the effect in space and time of this seepage on the chemistry of the "Westgeul"; what is the effect in space and time on the diversity of the macrofauna.

Since July we took monthly quantitative bottom samples with a Birge-Ekman grab on several places in a north-south transect. Moreover we determined chlorinity and oxygen, ammonia-, iron- and chlorophyll content from the free water zone. In October we established a number of permanent sampling plots in the semi-terrestrial zone of the creek. These plots will be phytosociologically investigated during the next decade. A series of ground water pipes was established in order to follow the chlorinity of the subsoil.

The study is still in progress, but some preliminary results can be presented, viz: the chlorinity of the creek is generally 12-13⁰/oo; ammonia content shows a north-south gradient from 0.3 till 0.06 mg/l; iron shows a north-south gradient from 3.7 till 0.7 mg/l; based on a similarity index, we noticed a distinct difference in the faunal composition of the northern and southern part of the creek.

The investigation is financially supported by Dow Chemical B.V., Terneuzen.

CLASSIFICATION OF INLAND WATERS (ESP. DITCHES) IN SW-NETHERLANDS BASED ON THEIR MACROFAUNAL SPECIES COMPOSITION (B 3)

I.J. Weeber

Forty localities, situated in Zeeuws-Vlaanderen and on the islands of S.-Beveland and Schouwen-Duiveland were sampled twice (March and July) in order to study the quantitative macrofaunal species composition. From a small number of species semi-quantitative data are available.

The larger part of the localities are ditches and small canals, but also some larger waterbodies are involved. The choice of the biotopes was defined by a large range in environmental factors, especially salinity.

In total we found about 250 taxa, mainly aquatic insects and their larvae. Oligochaetes were not taken into consideration. The results are in preparation.

The data will be used in one or several cluster analyses in order to obtain an optimal arrangement of the biotopes, based on their similarity in species composition. BIOPAT computerprogrammes will be applied (Dr. P. Hoggeweg, Utrecht).

Besides the biological investigation, we carried out a bimonthly physico-chemical sampling programme, viz. O₂, temperature, pH, Ca²⁺, Mg²⁺, Na⁺, K⁺, Cl⁻, P-PO₄ and N-NH₃. Only once the dissolved organic carbon content (DOC) of the water and the particulate organic carbon content (POC) of the substrate were determined. Moreover we noted data about: structure of the sediment, quantity of emerged and submerged macrophytes, depth of the ditch, loading with herbicides and extent of isolation.

By means of BIOPAT-computer programmes we will try to correlate these en-

Environmental factors with the "biological types".

RECOLONIZATION AFTER HYPERSALINITY IN SOME BIOTOPES ON SCHOUWEN-DUIVELAND (B 4)

Catharina H. Borghouts, R.H. Bogaards & J.W. Francke

Due to a prolonged dry period in summer 1976 the salinity increased tremendously in various small waterbodies. To study the effects of these hypersaline conditions and the following decrease in salinity on the biocoenosis, we started a sampling programme in a number of localities.

From July 1976 up to December 1977 we studied the changes in some chemical parameters, e.g. chlorinity (see Fig. 17) and their effects on the macrofauna at 11 stations on the island of Schouwen-Duiveland. The investigated waters are of two main types, viz. ditches belonging to the drainage polder-system and isolated waterbodies with varying intensity of seepage, called "inlagen".

At our ditch stations the salinities in 1976 were only slightly higher than in 1977; significant changes in the macrofauna composition were not observed. In most of the isolated water bodies the influence of the increased salinities proved to be more quantitative than qualitative.

Only the Suzanna Inlaag, where the chlorinity had increased to 160⁰/oo in 1976, the macrofauna totally disappeared. About 9 weeks after this maximum, when the chlorinity had dropped to about 23⁰/oo, the first animals reappeared, viz. larvae of Dolichopodids, *Chironomus salinarius* and Ceratopogonids. However, at the end of 1977 the species richness was still lower compared to the period preceding the extremely high salinities.

A number of species can resist extreme salinities. *Corophium spec.* and *Idotea chelipes* were found alive at chlorinities of 80⁰/oo. *Palaemonetes varians*, *Chironomus salinarius* (larvae) and *Ochtebius marinus* were present at a chlorinity of 50⁰/oo and *Abra tenuis* still at 40⁰/oo.

THE AUTECOLOGY AND LIFE HISTORY OF *Chironomus halophilus* KIEFF AND *Chironomus salinarius* (KIEFF) (DIPTERA, CHIRONOMIDAE) (B 5)

Angélique van der Schraaf & Corinne van Velzen

Chironomus salinarius and *C. halophilus* were both successfully cultured in captivity for some generations. We experimented with indoor cages (150 x 40 x 15 cm) placed in rooms of constant temperature (20⁰ C light-dark cycle of 15 - 9, 2300 lux and a relative humidity of 55%) and with outdoor cages (2 x 2 x 2 m) placed in the open air (Plate IV).

In the indoor cages the fecundity (% egg-laying females) of *C. halophilus* was higher (13%) than that of *C. salinarius* (4%). The natality (% vital eggs per eggmass) was about equal (80 and 90%) and the fertility (% eggmasses with vital eggs) proved to be the largest in *C. salinarius* (80 and 50%). The fertility decreased in the next generation.

In the outdoor cages the fecundity of *C. salinarius* proved to be the lar-

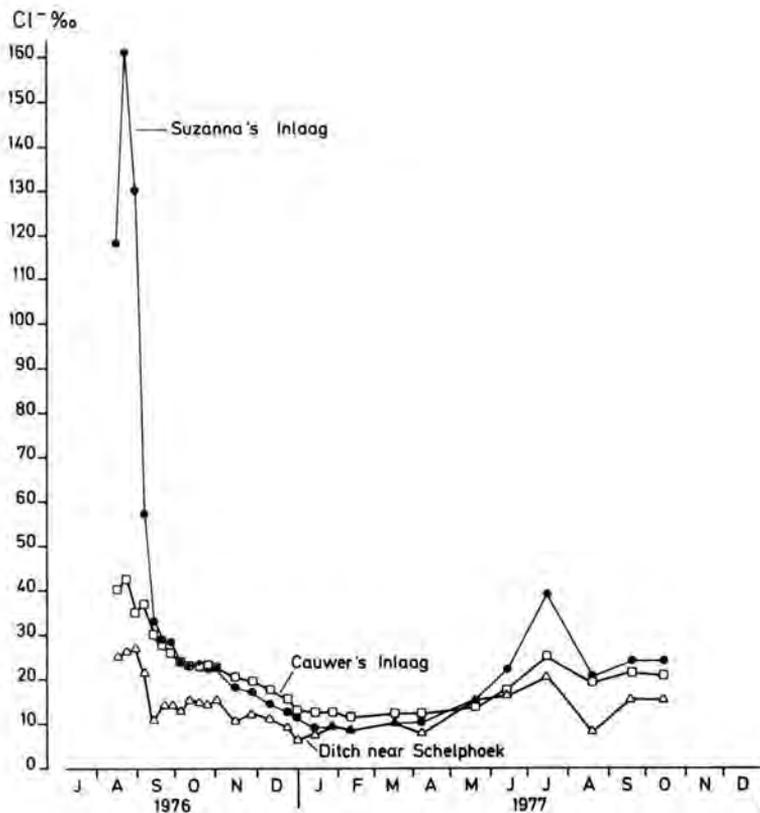


Fig. 17. Fluctuations in chlorinity at three stations on the island of Schouwen-Duiveland

gest (34 resp. 11%) while also the natality and fertility were much lower in *C. halophilus* (9.5 and 1%) than in *C. salinarius* (100% and 99%).

In outdoor cultures of *C. salinarius* the emergence of the adult and the oviposition were correlated with the highest measured temperature during daytime. These correlations were absent in outdoor cultures of *C. halophilus*. The differences between the highest measured temperature during daytime and lowest measured temperature during nighttime showed a strong correlation with oviposition and adult emergence of both species. In *C. halophilus* two taxonomical types can be distinguished showing differences in egg masses, larval morphometry, colour of the adults and larval developmental rate.

In cultures of both species we experimented with different sediments and chlorinities. The composition of the sediment (sand-silt mixtures) had no effect on the larval developmental rate, but an effect on the adult size was present. There are slight indications for an effect of the chlorinity on larval developmental rate.

ASPECTS OF THE AUTECOLOGY OF *Chironomus halophilus* KIEFF. AND *Chironomus salinarius* KIEFF. (DIPTERA, CHIRONOMIDAE) (B 5).

J.A. Goedbloed

Autecological data of *Chironomus halophilus* and *C. salinarius* frequently occurring in non-constant brackish waters, are required for a better understanding of their role in this type of ecosystem. Two aspects were studied, viz.

A. the preference of ovipositing females for differences in:

- contrast - entirely white trays/trays with dark bottom and white sides,
- water quality - water of the sampling site/diluted sea water of the same salinity,
- salinity - ten different salinities from 0 to 15.75⁰/oo Cl⁻ with intervals of 1.75⁰/oo.

B. the migration and sediment preference of the larvae, i.e.:

Investigation on migration of *C. salinarius* in the field: during a 24 hours period 500 liters water were sieved every 2 or 3 hours through a plankton net (mesh width 33 μm).

Sediment preference: sand/mud

Table 19. Numerical results of some experiments on the preference of adults (A) and larvae (B) of *Chironomus halophilus* and *C. salinarius*.

A. 1.		dark trays		white trays							
	<i>C. halophilus</i>	22		2							
2.		water sampling site		diluted sea water							
	<i>C. halophilus</i>	28		19							
	<i>C. salinarius</i>	27		24							
3. (‰ Cl ⁻)	1	2.75	3.50	5.25	7.00	8.75	10.50	12.25	14.90	15.75	
	<i>C. halophilus</i>	23	34	25	26	22	11	5	2	2	3
	<i>C. salinarius</i>	9	15	22	16	17	12	17	8	8	11
4.		0 larvae/m ²					15,000 larvae/m ²				
	<i>C. salinarius</i>	29					61				
		(the numbers represent the amount of deposited egg-masses)									
B. 2.		sand		mud							
	<i>C. halophilus</i>	74		88							
	<i>C. salinarius</i>	80		116							
		(the numbers represent the amounts of larvae)									

The results are not yet elaborated by means of statistics. For numerical results see Table 19. Some preliminary results can be discussed here.

A. Ovipositing females:

1. *C. halophilus* has a strong preference for trays with a dark bottom and white sides. This type of tray was used in all further experiments,
2. A preference for water of the sampling site was not demonstrable. So it seems that chemical factors in this water, other than salinity, do not influence the oviposition preference of both species,
3. Both species have a certain preference for chlorinities lower than $12.25^{\circ}/\text{oo}$. *C. halophilus* prefers chlorinities of $0-7^{\circ}/\text{oo}$, *C. salinarius* from $1.75-10.50^{\circ}/\text{oo}$. This difference in preference of both species could be related to the observation that eggs of *C. halophilus* deposited in chlorinities higher than $10.5^{\circ}/\text{oo}$ do not develop.
4. *C. salinarius* has a certain preference for trays already inhabited by larvae.
5. There is evidence that the females are not depositing their egg masses randomly throughout the experimental cage. There seems to be influence of light and of the presence of vertical components (like poles and walls).

B. About the larvae:

1. At least in *C. salinarius* no migration whatsoever could be observed, not even of the very mobile first instar larvae.
2. In both species there seems to be a certain preference for mud.

OBSERVATIONS ON A LARVAL CHIRONOMID POPULATION IN A BRACKISH DITCH (B 6)

B.P.M. Krebs

During the period April 1973-March 1975 we made biological and chemical observations in a brackish ditch (width appr. 11 m, max. depth 1.60 m) in the polder "Adriaan", N. Beveland.

The chlorinity of the surface water varied between 0.6 and $7.0^{\circ}/\text{oo}$ and showed a distinct seasonal periodicity (maximum in summer). The chlorinity of the top most 5 cm of the submerged mud in the deepest parts varied between 6 and $10^{\circ}/\text{oo}$, so the ditch was permanently stratified.

During the observation period we sampled larvae in regular time intervals and reared them till the adult stage. The following species, nearly obligatory restricted to the shallow edges of the ditch, were identified: *Chironomus salinarius* Kieff., *C. halophilus* Kieff., *C. annularius* Meig., *C. plumosus* L., *C. niger* Scharf, *Leptochironomus deribae* Freeman, *Glyptotendipes barbipes* Staeg., *Procladius choreus* Meig. and *Psectrotanypus varius* Fabr..

The finding of *L. deribae* is a valuable addition to the geographical distribution of the species. From the *Chironomus* species and *L. deribae* we collected taxonomical data.

Among these species the larvae of *C. halophilus* and of *L. deribae* were especially abundant. The population dynamics of these species were followed by means of Birge-Ekman samples, sieved over 0.3 mm mesh width. Fig. 18 shows

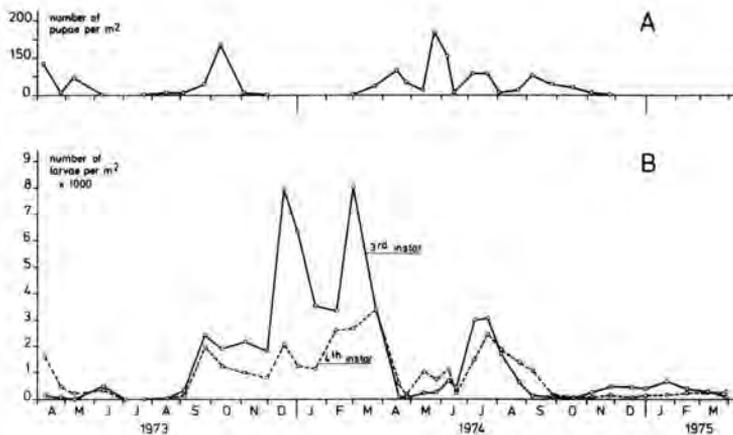


Fig. 18. Seasonal variation in density of 3rd and 4th instar pupae (A) and larvae (B) of *C. halophilus* in the "Adriaanpolder" ditch

the seasonal periodicity of the third and fourth instar larvae and the pupae of *C. halophilus*. A striking phenomenon is the large difference in larval density between 1974 and 1975 (3400 and 200 m² respectively). The number of generations was two in 1973 and presumably three in 1974. The larval density (3rd and 4th instar) of *L. deribae* reached a maximum of 1600 per m² in December 1973. During periods of increased rainfall (autumn 1974 and spring 1975) we observed a quick settlement of the species *C. annularius*. The larval densities rose till 2500 per m².

POPULATION STRUCTURE AND LIFE-CYCLE OF *Neomysis integer* (Leach) IN TWO TYPES OF INLAND WATERS (B 8)

Catharina H. Borghouts

During 2½ year the population structure and life cycle of *Neomysis integer* (Crustacea, Mysidacea) were studied in two types of inland water, viz. a large brackish-water lake (Lake Veere, 1800 ha, 8-12⁰/∞o Cl⁻) and a small water body (Den Inkel, 4.4 ha, 8⁰/∞o Cl⁻).

The animals were collected monthly using hand-operated nets. Length was measured after preservation to the nearest mm. Based on the degree of development five categories were distinguished, viz. juveniles, immature females, mature females, breeding females and males. Moreover from some extra samples the brood-size was determined.

The differences between the populations in Lake Veere and Den Inkel concerned mainly the length of the animals and the proportion of juveniles. The maximum length of *Neomysis integer* from Lake Veere was 19 mm and from Den Inkel 13 mm. The life-cycle of both populations does not seem to differ very

much. The over-wintering generations breed in April and May and produce the spring-generations. This spring generations grow and mature and start breeding in June-July. Breeding is continued in Lake Veere up to September and in Den Inkel up to August. The animals born in autumn grow up and overwinter. Although in winter mature females were present in both populations, no breeding females were found. It seems that in winter there is no breeding of any importance.

There proved to be a relationship between brood-size and body length. Because of differences in body length of the females in the two populations, also the brood-size differed considerably.

THE "KREEK BIJ WESTKAPELLE" (B 9)

R.H. Bogaards, J.W. Francke & S. Parma

Within the scope of a description of the inland waterbodies in S.W. part of the Netherlands we made morphometrical, physico-chemical and biological observations in the "Kreek bij Westkapelle". The creek (= kreek) was formed in October 1944 by a bombardment of the Royal Air Force, causing a huge gap in the sea-wall. Almost the entire island of Walcheren was flooded. Through tidal currents and dredging a funnel-shaped channel was formed behind the gap. After closing the dike one year later, the creek was left intact.

The maximum depth is 16,65 m, the volume $1,5 \times 10^6 \text{ m}^3$ and the surface area 33,9 ha. Roads and weirs divide the creek into three parts. The retention time of the water in the main creek is approximately one year.

Monthly figures of surface water chlorinity, analysed in the period 1957-1961 show a decrease from $10.0^{\circ}/\text{oo}$ to $7.75^{\circ}/\text{oo}$. In the period 1961-1977, however, the chlorinity was rather constant, ranging from 6.75 - $7.75^{\circ}/\text{oo}$.

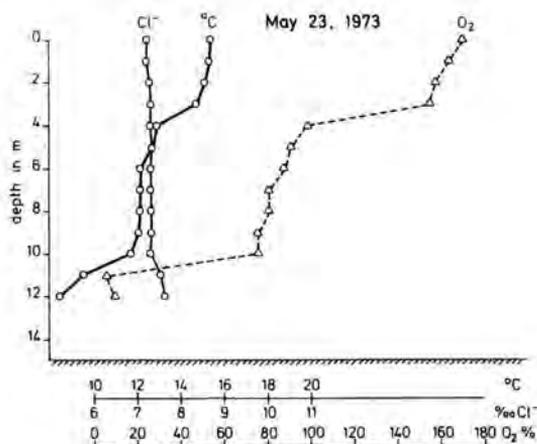


Fig. 19. Temperature-, salinity- and oxygen stratification in the "Kreek bij Westkapelle".

Data from a vertical section in the deepest depression of the creek show the presence of both a thermocline and a halocline (Fig. 19). The halocline is not permanently present and may be attributed to seepage (Fig. 20).

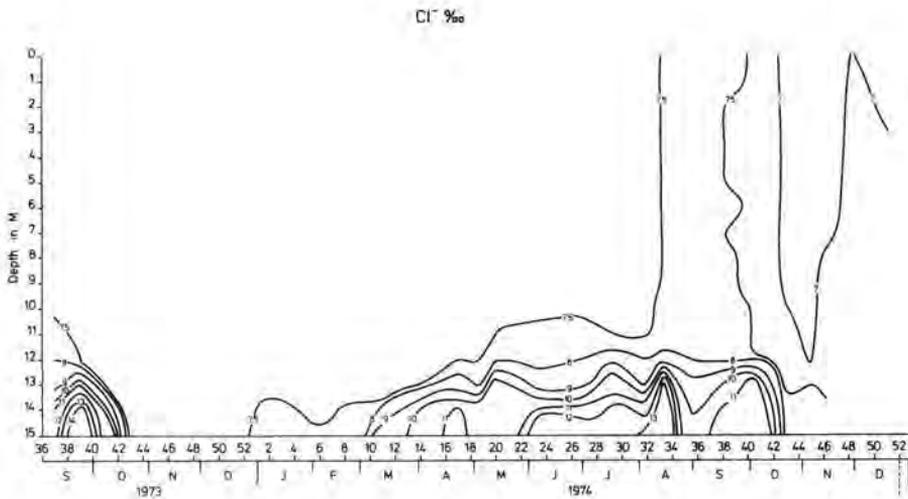


Fig. 20. Isohalines in the "Kreek bij Westkapelle" during the period September, 1973-December, 1974.

Nutrient contents (P and N) are high, presumably caused by the discharge of the mechanically cleaned effluent of a sewage plant. Phytoplankton biomass in summer may be considerable in view of the maximal chlorophyll concentrations of 160 mg/m^3 .

An extensive qualitative survey in 1977 of the littoral, benthonic and nec-tonic macro-organisms shows a typical brackish water fauna. This fauna is restricted to the permanently oxygenated shallower areas.

Comparing our data with older records, available from the literature (e.g. Bakker, 1950; van Benthem Jutting, 1946) and our Institute, we noticed the disappearance of marine species, like *Mytilus edulis* L. and *Mya arenaria* L.

References

- Bakker, D., 1959. De inundaties gedurende 1944-1945 en hun gevolgen voor de landbouw. V. De flora en fauna van Walcheren en andere inundatiegebieden tijdens en na de inundatie. Versl. Landbouwk. Onderz. 56(17): 1-40.
- Benthem Jutting, W.S.S. van, 1946. Marine organisms in the island of Walcheren (Netherlands) during the inundation October 1944-October 1945. Archief Zeeuws Genootschap Wetensch. '44-'45: 1-7.

Coastal meromictic lakes are known from e.g. Northern Europe (Holten, 1965; Lindholm, 1975, Strøm, 1962), but were never found in the Netherlands. The Ritthem creek system (near Vlissingen, island of Walcheren) was formed in 1944 through erosion of the sea after bombardments of the dike by the Royal Air Force. When the dike was repaired the creek was managed as a nature reserve. The morphometry of the creek is rather complicated with several separate depressions of maximally 8 m depth. The total area is 14.4 ha and the total content $0.3 \times 10^6 \text{ m}^3$.

In some of the depressions we measured chlorinity, temperature, oxygen and sulfide. There proved to be a very distinct salinity stratification with an extensive anoxic water mass below 2-3 m. In this anoxic part we found the exceptionally high sulfide concentrations of 620 mg/l. The stratification was very stable throughout the season and in winter even an inverse temperature gradient was present (see Fig. 21).

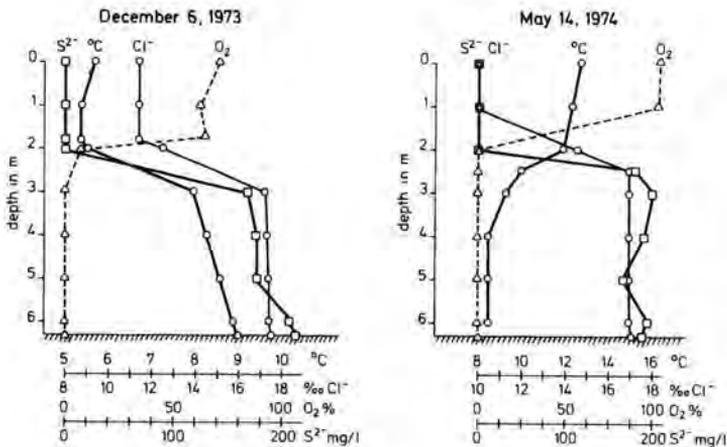


Fig. 21. Stratification of chlorinity, temperature, oxygen and sulfide in the creek system near Ritthem (island of Walcheren)

A similar phenomenon was found in a creek near Veere (island of Walcheren) with a corresponding history (bombardment in 1944, erosion and restoration). Area and volume are not yet calculated but we sounded a maximum depth of 15 m. Figure 22 shows a stable salinity stratification with high sulfide concentrations in the anoxic part of the creek (over 300 mg/l) and a halocline between 1 and 2 m.

Obviously old seawater, perhaps mixed with seepage water, is trapped in the lower level of these two creeks, creating a permanent stratification. We have here the first example of true meromixis in the Netherlands.

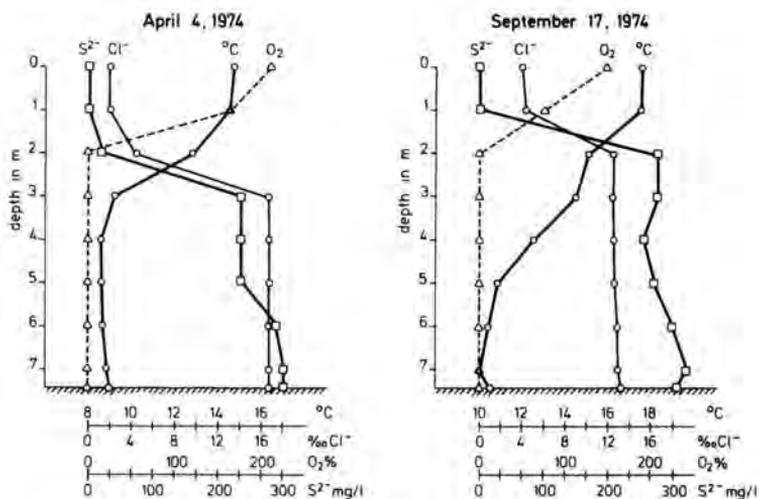


Fig. 22. Stratification of chlorinity, temperature, oxygen and sulfide in the creek system near Veere (island of Walcheren).

We have planned a physico-chemical and biological investigation of this type of ecosystem.

References

- Lindholm, T.J., 1975. Coastal meromixic lakes on Åland (S.W. Finland) *Aqua Fennica* 1975: 24-40.
- Holtan, H., 1965. Salt water in the bottom layers of two norwegian lakes. *Nature* 207: 156-168.
- Strøm, K., 1962. Trapped seawater. *New Scientist* 13: 384-386.

MISCELLANEOUS RESEARCH (A-PROJECTS AND ADDITIONAL INFORMATION)

MEASUREMENT OF DENSITY AND ACTIVITY OF HETEROTROPHIC MICROBIAL POPULATIONS (A1)

A.B.J. Sepers, M.P.G. Cappendijk & F.W. Melissen

In studying ecosystems dealing with nutrient cycles or energy flow, it is necessary to assess the role of the microbial population. This can be achieved by the determination of the density and the activity of the microbial population. As a part of the research into the aerobic degradation of amino-acids, a comparison has been made between ATP-levels, oxygen consumption rates and the maximum uptake rate, V_{max} , as determined by the technique described by Hobbie & Crawford (1969).

For ATP analysis 50 ml of water was filtered through membrane filters with

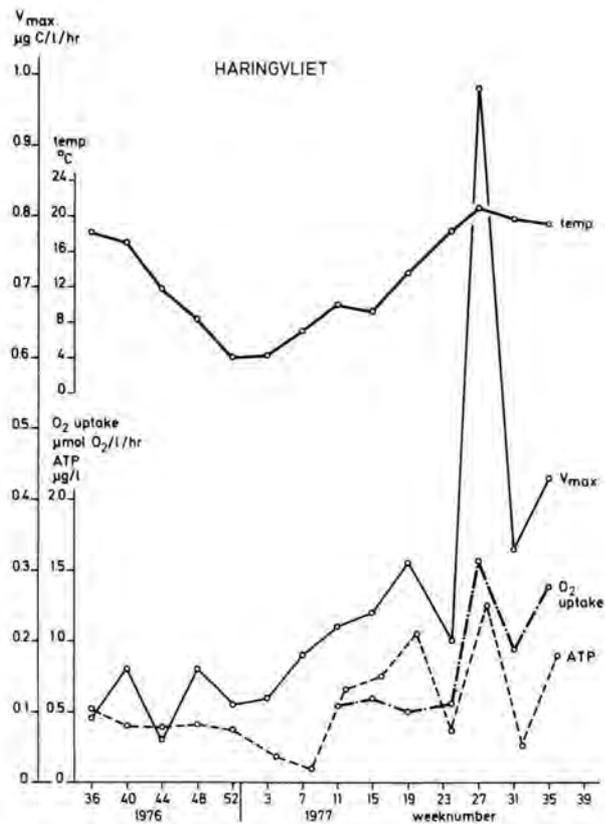


Fig. 23. Haringvliet ATP-level, oxygen uptake rate, V_{max} -values and temperature from August 1976 till September 1977.

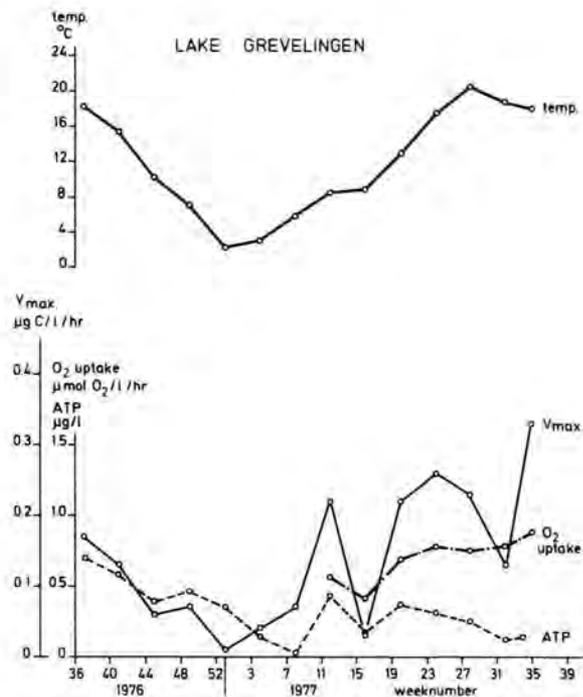


Fig. 24. Lake Grevelingen ATP-level, oxygen uptake rate, V_{max} -values and temperature from August 1976 till September 1977.

a pore size of 0.2 and 3.0 μm . ATP was extracted by boiling in TRIS-buffer for 5 minutes. The ATP content of the extract was measured by the luciferin-luciferase reaction. Initially it was assumed that the difference in ATP content between the residues on the 0.2 and 0.3 μm filter would be a parameter of bacterial biomass. Frequently no difference in ATP levels in both extracts could be demonstrated, which is probably due to adhesion of bacteria to particulate matter. Moreover filtration of laboratory cultures through 3.0 μm membrane filters and subsequent determination of the ATP level of the residues showed that a remarkably high percentage (circa 70%) of the bacteria was retained by the 3.0 μm filter. In the measurements discussed below, 3.0 μm membrane filters were used. Thus, the measured ATP levels represent the biomass of 70% of the bacteria in addition to the biomass of phytoplankton, smaller zooplankton and fungi.

Oxygen uptake rates were determined by measurement of the concentration of dissolved oxygen in glass stoppered bottles immediately after sampling and after incubation during 24 hours at the in situ temperature (in triplicate). Dissolved oxygen was measured by the Winkler titration method. Maximum uptake rates (V_{max}) were determined according to the method described by Hobbie & Crawford (1969), using a mixture of amino-acids as the substrate (obtained by acid hydrolysis of algal protein; The radiochemical Centre Amersham CFB 25). In figure 23 and 24 the values of the ATP-level, oxygen uptake rate and V_{max} for a one year period are summarized for the Haringvliet and Lake Grevelingen, respectively. The Haringvliet is a fresh water body with high nutrient levels; Lake Grevelingen is a saltwater lake with a mesotrophic character.

As could be expected the V_{max} -values of the Haringvliet are higher than the V_{max} -values of Lake Grevelingen over nearly the whole period. Oxygen consumption rates differed only in summer and ATP levels in spring and summer. The parameters of the Haringvliet show comparable trends, especially during the summer period when the highest activities were found. This may be interpreted as an indication for the correlation between these parameters. In Lake Grevelingen the values of the measured parameters fluctuate less, probably due to the more constant environment and lower nutrient levels. For this environment a correlation between these parameters cannot be established.

From the oxygen measurements carbon mineralisation rates can be calculated assuming a conversion factor of 0.31 (Vollenweider, 1969). These calculated carbon mineralisation rates have been compared with the mineralisation rates expressed by the V_{max} -value. Only 1.7-6.3% of the mineralisation rate calculated from the oxygen uptake rate is represented by the V_{max} -value.

In our experiments respiration has been measured, not only of bacteria but of phytoplankton and zooplankton too. V_{max} -values indicate the maximum uptake rate which depends on the density of the microbial population, capable of utilizing the offered substrate. The actual uptake rate is a fraction of the V_{max} -value and is determined by the substrate concentration in situ. Assuming

that a high percentage of the oxygen uptake, as measured by our procedure, is bacterial, then the conclusion is justified that the applied mixture of amino acids is not the appropriate model substrate for the measurement of total carbon mineralisation. Supposing that the same conclusion holds for single organic substrates, a better reflection of the carbon mineralisation rate is obtained by the use of a more complicated mixture of organic compounds (including sugars, amino acids, organic acids etc.) as the substrate.

References

- Hobbie, J.E. and C.C. Crawford, 1969. Bacterial uptake of organic substrate, new methods of study and application to eutrophication. *Verh. Internat. Verein. Limnol.* 17: 725-730.
- Vollenweider, R.A., 1969. A manual on methods for measuring primary production in the aquatic environment. I.B.P. Handbook no. 12, Blackwell Sci. Oxford pp. 213.

AUTOMATIC RECORDING OF BACTERIAL DENSITIES BY LIGHT ABSORPTION (A 1)

F.W. Melissen

The control of the bacterial densities in continuous and batch cultures can be carried out by measuring the light absorption of the suspension in a colorimeter. A programme-unit was constructed in order to automatise this analysis and thus to obtain optimum control over the cultures.

The working procedure consist of four steps: i) the introduction of the liquid into the colorimeter, ii) the printing of the light absorption extinction, iii) the reset of the colorimeter and iv) the printing of the zero (reset)value of the colorimeter.

The programme-unit contains three time-clocks with the following settings: K1 circling time 2 hours, K2 circling time 1 hour and K3 circling time $\frac{1}{2}$ hour. Each time-clock can be switched on separately through the switches S2, S3 and S4. The clocks are regulated by switches S5-S16, and their functioning is indicated with a control lamp (see fig. 25). The materials used are: S₁-S₁₆ : On/off switches 220V - 3A, L₁-L₁₆ : Neon lamps 220V, K₁ : Cycle timer AZW 11 - 2 Fanal, K₂ : Cycle timer AZW 11 - 3 Fanal, K₃ : Cycle timer AZW 11 - 4 Fanal, Z: Fuse 250V - 3A.

The procedure is as follows (Fig. 26): i) Valve 1 opens and the peristaltic pump 1 starts to pump the liquid (sample) into the colorimeter cuvet. ii) Valve 1 closes and the peristaltic pump 2 stops. The recorder connected with the colorimeter, starts to print the colorimeter values. iii) Recorder stops, valve 2 opens and the peristaltic pump 2 starts to pump distilled water into the colorimeters cuvet. The colorimeter gives the zero value. iv) Valve 2 closes and the peristaltic pump 2 stops; the recorder starts to print the colorimeter reset values.

The programme-unit is equally suitable for other types of automatic recor-

ding of liquid densities measured by light absorption.

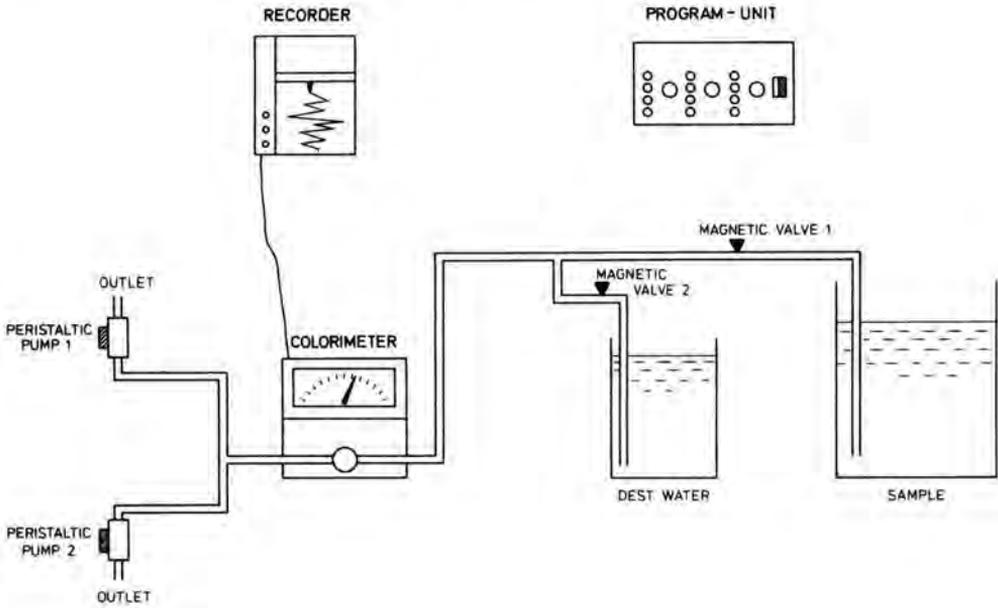


Fig. 25. Programme-unit for automatic light absorption measurements

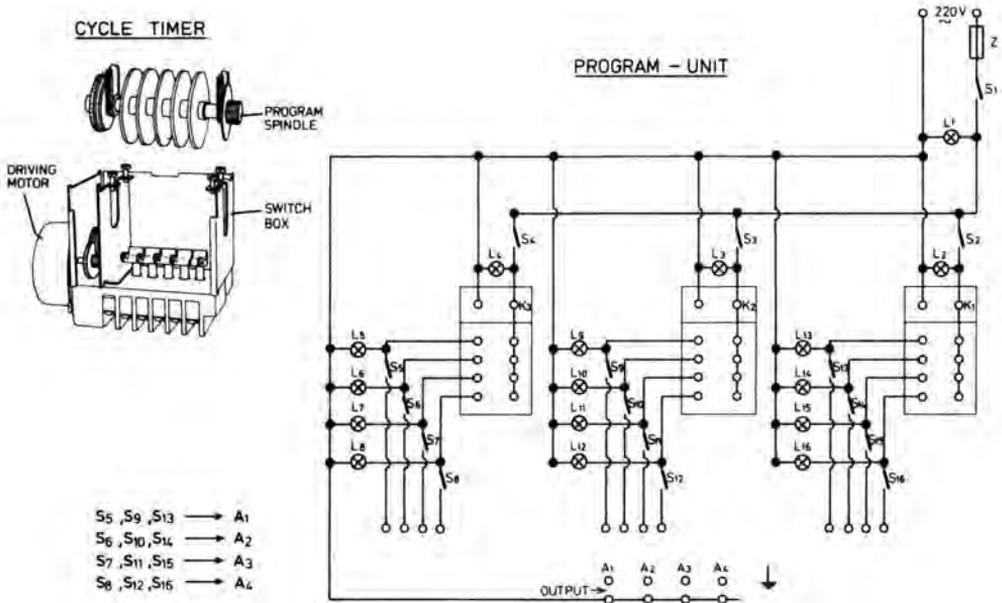


Fig. 26. Scheme of the automatic colorimeter unit

P. de Koeyer, P.J. van Boven & E.K. Duursma

Pilot experiments have been set up with brown shrimps in order to determine their influence on the mixing of the top sediment layers in which they normally dig themselves in. Five healthy shrimps of a size of 5 cm were placed in trays of 17.8 x 11.5 cm containing 6 cm of sand, covered with 2 mm of equal sized gravel and 10 cm of sea water. The shrimps were left without nutrition in order to avoid excessive agitation upon feeding. Incidental dead animals were replaced by healthy ones. At intervals of one week a complete tray with shrimps and sediment was deep frozen. From each tray four cores were taken and the gravel concentration determined in 2½ mm sections.

The mixing of the gravel with the sand was considered as an apparent diffusion of gravel into the bottom by bioturbation. Thus the percentage of the gravel could be plotted and calculated according to the model of diffusion with an instantaneous source used by Duursma and Hoede (1967). The apparent diffusion coefficient, so determined was $0.54 \times 10^{-7} \text{ cm}^2/\text{sec}$ ($\pm 0.05 \times 10^{-7} \text{ cm}^2/\text{sec}$) which is a hundred times lower than the coefficient of molecular diffusion of chloride in interstitial water of marine sand (Duursma and Bosch, 1970).

The number of five shrimps used in the experiment, which is equivalent to 240 per m^2 is relatively high as compared with the estimated maximum concentration of 0-2/ m^2 in the Grevelingen and 11/ m^2 in the Oosterschelde.

References

- Duursma, E.K. and C. Hoede, 1967. Theoretical, experimental and field studies concerning diffusion of radioisotopes in sediments and suspended solid particles of the sea. Part A: Theories and mathematical calculation. *Neth. J. Sea Res.*, 3: 423-457.
- Duursma, E.K. and C.J. Bosch, 1970. *Ibid*, Part B: Methods and experiments. *Neth. J. Sea Res.*, 4: 395-469.

THE PREPARATION OF COLOURED SAND TRACERS (A 16)

J. Nieuwenhuize & H.J.J. Sips

In order to obtain a clear insight into rate and nature of bioturbation in Lake Grevelingen and the Oosterschelde, two hundred kilograms of sediment were coloured with a fluorescent paint. The staining-process used by Wilkens (1969), was chosen as a starting-point in the development of our method.

The sediment, collected from the trial-areas, consists almost completely of quartz sand, with an average grain-size of 250 μm , and a maximum grain-size of 600 μm . Fluor-Fire-Red paint from Visprox, Haarlem was applied.

From several experimental trials it was concluded that the best tainting procedure for this sand was: i) 50 kg sand is washed with 5% HF and tapwater. The wet sand is subsequently mixed with 1 kg of paint, 2 l of water and 120

ml of ethyl-alcohol. A too low amount of water keeps the sand too dry for homogeneous mixing while a too large water content separates the paint from the sand. The alcohol serves as temporary solubilizer of the paint thus improving homogeneous tainting. It additionally brightens the colour. The mixing process is carried out for 2 hours in an ordinary concrete-mixer.

For the fixation of the paint on the sand grains a careful drying procedure is required at 50° C. Higher temperatures should not be used in order to avoid loss of colour (Wilkens, 1969). The drying procedure can take place on plates in temperature-controlled stoves. After drying, slight grinding is required to break up the formed aggregates of sand.

As presented in Fig. 27 the grain-size composition of sand has slightly changed owing to the tainting process, however, negligibly so, as far this will influence its applicability for bioturbation experiments.

Two methods have been tested for the determination of tainted sand concentrations in mixtures with original sand: i) visual counting under the microscope and ii) extraction of the paint in acetone and subsequently the determination of its colour in a colorimeter. There seems to be a reasonable agreement between these two methods (Fig. 28). The second method is, however, less reliable than the first one when tainted sand grains become eroded.

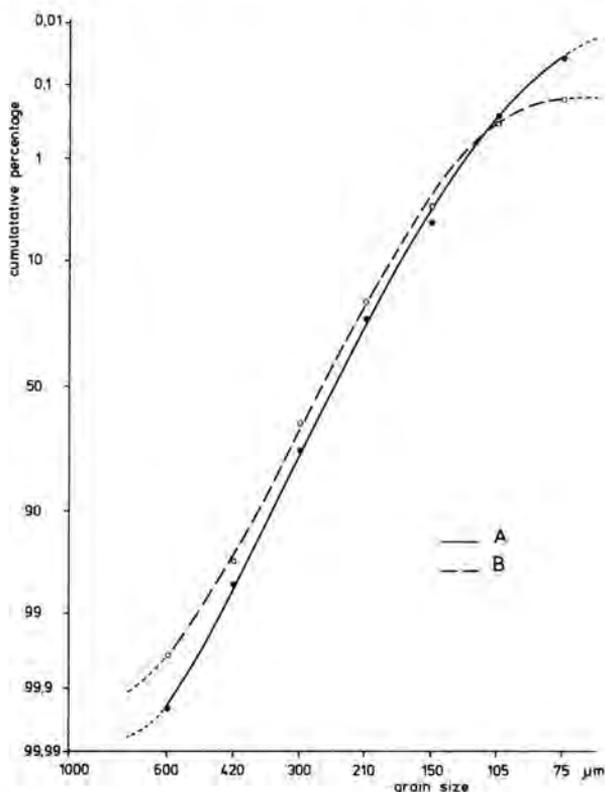


Fig. 27. Grain-size distribution of the sand before (A) and after (B) being tainted with Fluor-Fire-Red.

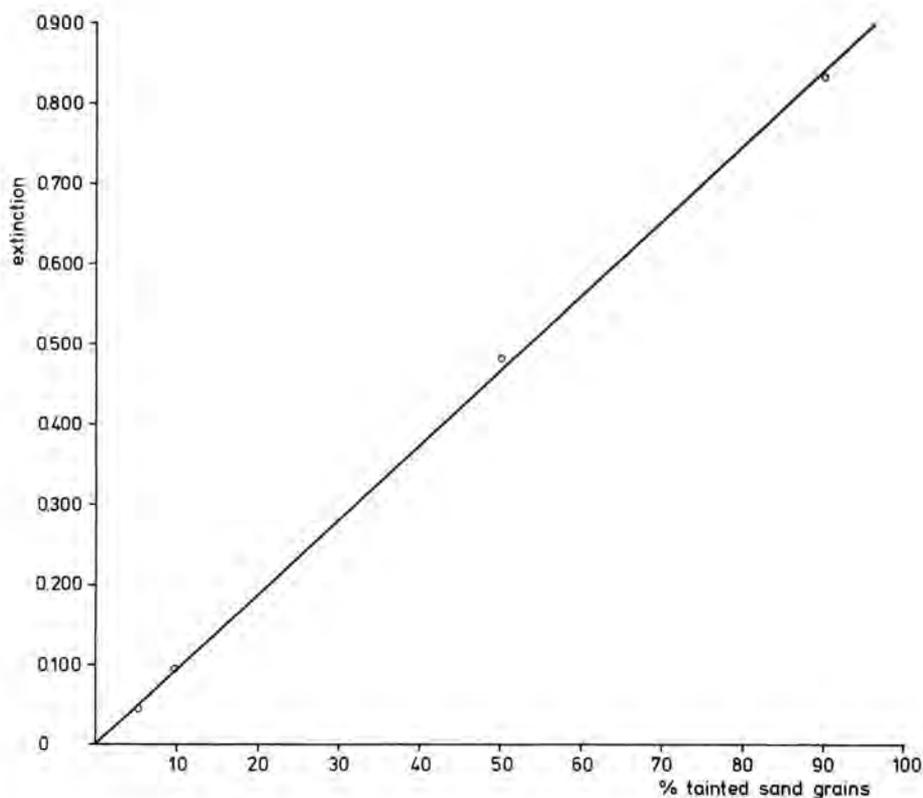


Fig. 28. Relationship between the percentage of coloured sand grains in sand and the extinction at 532 nm of 2.5 g mixture extracted with 10 ml acetone.

References

- Wilkens, D.H., 1969. Meting van bodemtransport met tracers. sectie 48. 13. Instructieblad No. 9.

A NON-VALID VISUAL METHOD TO DETERMINE ORGANIC MATTER IN SOILS

J.M. van Liere & J. Nieuwenhuize

For project S 3 a great number of dried soil samples from salt-marshes had to be analysed for organic matter (976 samples). From these 108 have been analysed using the wet oxidation method (Hofstee and Fien, 1971). The rest was to be classified by its colour on the content of organic matter. The colour scale of Munsell (Anonymous, 1954) has been applied and tested with the same 108 samples for which the organic matter content was determined.

As demonstrated in Fig. 29, the soil-colour scale of 9 groups is not related to the % humus of the soil samples used, thus the visual method can not be used as a reliable index for our soil organic matter samples.

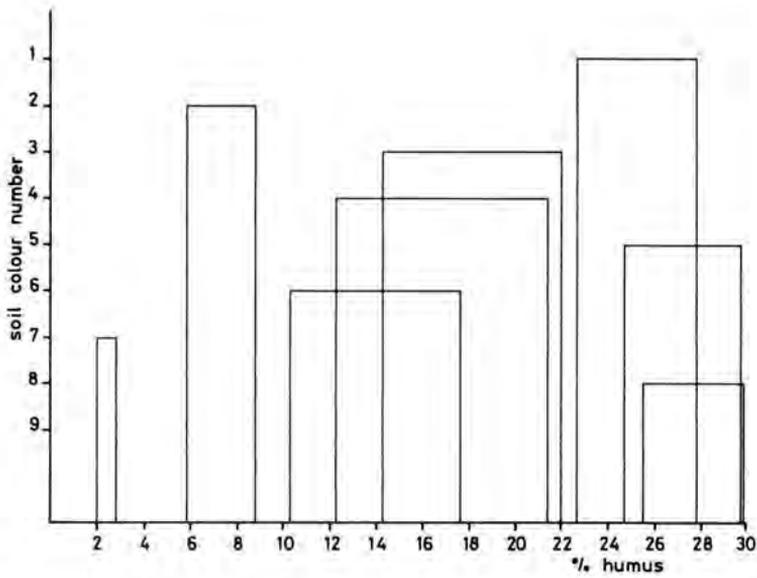


Fig. 29. Relationship between the Munsell colour scale for soils with the percentage of humus, determined by wet oxidation.

References

- Anonymous, 1954. Munsell Soil Color Charts, Munsell Color Comp. Inc. Baltimore, pp. 21.
- Hofstee, J. & H.J. Fien, 1971. Analysemethoden voor grond, gewas, water en bodemvocht. Rijksdienst van de IJsselmeerpolders, Kampen, I-52.

- Beeftink, W.G. The structure of salt-marsh communities in relation to environmental disturbances. Proc. First Eur. Ecol. Symp. Norwich. Blackwell, London (Δ-166).
- Beeftink, W.G., M.C. Daane, W. de Munck & J. Nieuwenhuize. Aspects of population dynamics in *Halimione portulacoides* communities. Vegetatio. (Δ-160).
- Beeftink, W.G., M.C. Daane, W. de Munck & J. Nieuwenhuize. Vegetation dynamics in the salt marshes as a consequence of some traditional cultural practices of man. Phytocoenosis, Warsaw. (Δ-165).
- Borghouts, C.H. Population structure and life-cycle of *Neomysis integer* (Leach) (Crustacea, Hycidacea) into tupes of inland waters. Verh. Int. Ver. Limnol. (Δ-157).
- Duursma, E.K. Migration in the seabed, a complex phenomenon. JOA-session on Benthic Processes. Publ. Texas A & M Univ.
- Duursma, E.K. & A. Hanafi. Pesticides investigations for indonesian brackish-water fish and shrimp culture III: Distribution of chlorinated pesticides in environmental samples. Bull. Shrimp. Cult. Res. Centre, Ind.
- Duursma, E.K. & A. Hanafi. Some aspects of pesticides in aquatic environment. Thalassia Jugoslavica.
- Hanafi, A. & E.K. Duursma. Pesticides investigations for Indonesian brackish-water fish and shrimp culture II: Phosvel. Bull. Shrimp. Cult. Res. Centre, Ind.
- Heip, C., K. Willems & A. Gossens. Vertical distribution of meiofauna and the efficiency of the Van Veen grab on sandy bottoms. Hydrobiol. Bull. (Δ-156).
- Koutstaal, B.P. & H.J.M. Sipman. De korstmossen van de Middellplaten. De Levende Natuur. (Δ-158).
- Lambeck, R.H.D. De leeftijdsverhoudingen en andere populatiegegevens van Rotganzen (*Branta B. bernicla* L.) in het Oosterschelde-Veerse Meer gebied seizoen 1976-1977. Watervogels. (Δ-155).
- Nienhuis, P.H. Dynamics of benthic algal vegetation and environment in Dutch estuarine salt-marshes, studied by means of permanent quadrats. Vegetatio. (Δ-163).
- Nienhuis, P.H. An ecosystem study in Lake Grevelingen, a former estuary in SW Netherlands. Kieler Meeresforschungen. (Δ-164).
- Nienhuis, P.H. & B.H.H. de Bree. Morphological variability in *Rhizoclonium riparium* (Roth) Harv. (Chlorophyceae: Cladophorales) under natural and experimental conditions. Proc. 8th Internat. Seaweed Symp., Bangor, 1974. (Δ-137).
- Parma, S. & C.H. Borghouts-Biersteker. Diurnal fluctuations in salinity, oxygen and chlorophyll in a shallow brackish ditch. Verh. Int. Ver. Limnol.
- Rijstenbil, J.W. De zuurstofhuishouding van brakke sloten. (De invloed van een zoutgradiënt op de zuurstofbalans). Rep. Delta Inst. 1978-2.

Vlasblom, A.G., J.H.B.W. Elgershuizen. Survival and oxygen consumption of *Prawnus flexuosus* and *Neomysis integer* and embryonic development of the latter species in different temperature and chlorinity combinations. Neth. J. Sea Res. (Δ -167).

Vlasblom, A.G., K.F. Vaas & W. Rozing. The osmotic concentration of the blood plasma of plaice (*Pleuronectes platessa*) from three habitats of different salinity. Neth. J. Sea Res. (Δ -161).

PUBLISHED ARTICLES AND REPORTS IN 1977

(Δ -139 = publication number of the Delta Institute).

WORKING GROUP "CARBON CYCLE IN THE GREVELINGEN"

Ierland, E.T. van, 1977. Quantification of seegrass consumption in Lake Grevelingen during the growing season (In Dutch). Stud. Rep. Delta Inst., 1977-2, pp.43. An attempt was made to quantify the consumption of Eelgrass (*Zostera marina* L.) in Lake Grevelingen during the growing season. The total consumption was estimated at about 4×10^5 kg dry weight. i.e. approximately 1.3 g C/m^2 from which 26% was consumed by birds and 74% by invertebrates. Fishes were ignored under the assumption that their consumption was negligible. The estimation of the consumption by birds was based on data from the literature and on bird censuses by Staatsbosbeheer. More than half of the eelgrass consumption by birds was caused by the Mute swan. The other half may be caused by the Mallard, but this needs further evidence. Estimation for invertebrates was based on field data and aquarium-experiments. *Idotea chelipes* consumed probably 90% of the total invertebrate's share. The total consumption of eelgrass during the growing season was only approximately 6% of the year production in Lake Grevelingen. This is in good agreement with literature data. Thus the largest part of the eelgrass enters the detritus foodchains.

Mulder, H.G., 1977. Some critics on the Rand-model (In Dutch). Rep. Delta Inst., 1977-1, pp. 4. Some critics are presented on the applied boundary conditions rather than on the applicability of the H-theorema. Care is needed with small biological systems for which the conditions might not be sufficient to describe the system thermodynamically.

The Rand-model is essentially a "classical" linear equilibrium model, only different in the way of giving solutions. These solutions have no practical value, thus management decisions cannot be based on this model.

Nienhuis, P.H., 1977. Three thousand hectares of eelgrass in Lake Grevelingen: the Wadden Sea in the beginning of this century (In Dutch). Zeeuws Nieuws over natuur, landschap, milieu 3(3): 6-11. After the closure of Lake Grevelingen in 1971 an eelgrass (*Zostera marina*) population has developed which covers now about 3000 ha of the lake area. The population is comparable with the eelgrass beds in the Wadden Sea (N.Netherlands) in the beginning of this century. In 1932 a "wasting disease" destroyed the Wadden Sea stands and they never recovered since then.

Nienhuis, P.H., 1977. Changes in the primary productivity in the Grevelingen after the closure in 1971 (In Dutch). Vakbl. Biol. 16(57): 271-274. (Δ -159). The pool of particulate organic matter in the Grevelingen estuary was fed from various sources. The amount of organic carbon from the North Sea, entering the estuary as detritus equalled the *in situ* primary production. After the closure of the estuary the import of organic matter from the North Sea was completely cut off. Overall yearly production of the phytoplankton was not notably influenced by the closure, notwithstanding the large changes in

environmental conditions. The significance of the phytobenthos production increased considerably.

Nienhuis, P.H. & B.H.H. de Bree, 1977. Production and ecology of Eelgrass

(*Zostera marina* L.) in the Grevelingen estuary, the Netherlands, before and after the closure. *Hydrobiologia* 52: 55-66. (Δ-146). Production and ecology of *Zostera marina* L. were studied in 1968 and in 1973-1975, both through standing stock estimations, biomass increases in permanent quadrats, and correlation of distribution patterns with ecological factors. After the closure of the estuary the intertidal eelgrass population extended downwards to 5 m below lake level, probably owing to the increased transparency of the water, the area occupied, and the density of the eelgrass beds increased strongly. Eelgrass annual overground production, based on doubled maximum standing crop values in July-August, was estimated at 50 g C/m² in 1968, 121 g C/m² in 1973 and 91 g C/m² in 1975 in *Zostera* beds, and 4 g C/m² in 1968, 18 g C/m² in 1973 and 23 g C/m² in 1975 for the entire Grevelingen area. A minimum estimate of net production in *Zostera* beds at a depth of 0.50-0.75 m, based on short term changes in biomass in 2 permanent quadrats in 1974 and 1975, was 40.5 g C/m² yr for overground parts and 12.7 g C/m² yr for underground parts.

Vegter, F., 1977. The closure of the Grevelingen estuary: its influence on phytoplankton primary production and nutrient content. *Hydrobiologia* 52:

67-71. (Δ-147). The Grevelingen was cut off from the sea in May 1971, and changed into stagnant Lake Grevelingen. After the closure nitrate concentrations decreased to extremely low values (less than 2 μgat NO₃-N/l). Ammonia concentrations varied between 10-30 μgat NH₃-N/l, comparable with the situation before the closure. Phosphate concentrations fluctuated between 1-2 μgat PO₄-P/l in the estuarine phase, and increased to 10 μgat after the closure, presumably caused by decomposition of biological material and release of phosphates from the bottom. Phytoplankton primary production was not markedly affected by the damming up, and amounted to 175 g C/m² in 1971.

Wolff, W.J., 1977. A benthic foodbudget for the Grevelingen estuary, the Netherlands, and a consideration of the mechanisms causing high benthic secondary production in estuaries. In: "Ecology of Marine Benthos", ed. by Bruce

C. Coull, pp. 267-280. University of South Carolina Press. (Δ-132). An annual food budget for the zoobenthos of a tidal estuary of 140 km² in The Netherlands is constructed. Although this budget is partly based on several questionable assumptions, the following observations seem reliable. Primary production *in situ* and import of organic detritus from the coastal sea appear to be the most important food sources. Detritus imported from salt marshes or other terrestrial systems is relatively unimportant.

This type of food budget, which also has been found in the Dutch Wadden Sea, is very different from the benthic food budgets of American estuaries where the benthos depend primarily on salt marsh or mangrove detritus and primary production *in situ*.

The mechanisms causing high benthic secondary production in estuaries are reviewed and categorized into three types (1) those in which the supply of dissolved nutrients from various sources causes high primary production; (2) those with supply of particulate organic matter from various sources to estuarine waters; and (3) those in which the shallow nature of the estuary causes rapid sinking of particulate organic matter, as well as rapid transport of particulate organic matter by turbulent diffusion.

Wolff, W.J., A.J.J. Sandee & L. de Wolf, 1977. The development of a benthic ecosystem. *Hydrobiologia* 52: 107-115. (Δ-150). The development of a benthic ecosystem on a sandy bottom at 3 m depth in the saline Lake Grevelingen, The Netherlands, is described. The development started after the closure of the former Grevelingen estuary and was followed for 4½ years. Ecosystem attributes studied were biomass, secondary production, production/biomass ratio,

type of food chains, species diversity, pattern diversity, size of organisms, type of life-cycles, and r- and K-selection. The results are discussed in relation to E.P. Odum's (1969) theory of ecosystem development.

Wolff, W.J. & L. de Wolf, 1977. Biomass and Production of Zoobenthos in the Grevelingen Estuary, the Netherlands. *Estuarine and Coastal Marine Science* 5: 1-24. (Δ -139). The Grevelingen estuary has an area of 140 km² with 4 km² of salt marshes and 55 km² of tidal flats. The tidal range is 259 cm, salinity about 15.0‰ Cl⁻. Biomass and annual production of the five quantitatively most important benthic animals, viz. *Littorina littorea*, *Hydrobia ulvae*, *Cardium edule*, *Macoma balthica* and *Arenicola marina* were determined at six stations. Combined with an assessment of the benthic biomass in the whole estuary, this enabled us to make an estimate of the total production of these species. Data on the production of *Mytilus edulis* were derived from fisheries statistics. The total benthic secondary production for the whole estuary is estimated at 50.3-57.4 g ash-free dry weight m⁻² year⁻¹, expressed as somatic growth. The place of the zoobenthos in the estuarine foodweb is discussed.

WORKING GROUP "STRUCTURE AND DYNAMICS OF THE SALT-MARSH ECOSYSTEM"

Beeftink, W.G., 1977. The coastal marshes of Western and Northern Europe: an ecological and phytosociological approach. In, "Wet Coastal Ecosystems", V.J. Chapman (Ed.). Amsterdam, Elsevier, 109-155. Within the scope of David W. Goodall's series "Ecosystems of the World" an overall view has been given of the biota of the West- and North-European salt marshes including the Atlantic coasts of the Iberian Peninsula. After defining the concept of salt marsh six types of salt-marsh formations have been characterized: estuarine, Wadden, lagoonal, beach plain, bog and polderland type.

The abiotic component of the ecosystem is mainly characterized by (1) texture and structure of the soil profile, (2) water régime and salinity, and (3) the processes governing the nutritional state. In the first aspect both sedimentation and erosion are involved as well as reworking of eroded materials in new salt-marsh formations. Sedimentation of allochthonous, mainly mineral materials occurs as well as deposition of mostly autochthonous plant debris. The water régime is contrasted by tidal and non-tidal (temporarily stagnant) conditions. Salinity is governed by flooding, rainfall and evapotranspiration. Brackish water submergence, known from benthic organisms in the transition zone between the North Sea and the inner Baltic, is also found in salt-marsh plants under estuarine conditions.

The nutritional state is only partly and very incidentally known. Mud flats and low marshes have generally no nutrient deficiencies, but their extreme environment (anaerobic, saline) demands special adaptations from the plants. High marshes show more differentiation owing to lower flood frequencies and local accumulation of plant debris washed onto the marsh. Different forms of phytogenic interrelationships in the fields of symbiosis, epiphytism and parasitism have been discussed, and a list of some host-parasite relationships found in the S.W. Netherlands and derived from literature is given.

The body of the publication is formed by a description of the European salt-marsh communities, especially their floristic ecology, composition and distribution. Subsequently, the following communities have been dealt with in this manner: *Zostera*-, annual *Salicornia*-, *Spartina*-, and *Scirpus maritimus* communities of the lower salt marsh, communities of the upper salt marsh, cattle tracks, enclosed areas, etc., therophytic communities in the storm-flood zone, and communities growing on plant debris washed ashore. Floristic characterization is illustrated by synthetic tables of relevés from the S.W. Netherlands and the Swedish west coast. Zonation and dynamic trends in vegetation are discussed with data from the literature and from long-standing investigations carried out on sample plots in the S.W. Netherlands.

Studies on the ecology of salt-marsh algae are relatively few in comparison with those of phanerogams. The reason is that algal ecologists usually shrink

from the high methodological thresholds. After a period of pioneering algal investigations in Great Britain, especially Dutch ecologists paid attention to this part of the salt-marsh ecosystem.

European studies on the ecology of animals living in the salt marsh have been discussed briefly, considering five categories of animals: mammals, birds, arthropods, molluscs, and a number of meiofaunal groups, such as nematods, flatworms and protozoans. Vertebrate grazing seems to be limited by the tides. It is a very complex process, in which selectivity and intensity of grazing, treading and manuring share. The degree of consumption and the overall influence of grazing on halophytes is specified qualitatively for the S.W.-Netherlands.

In Europe the ecosystem approach in its proper sense is still in its infancy compared with North America. Only some aspects have been investigated incidentally, such as food relations between animals of the soil environment, production of phanerogamic vegetation, and distribution and turnover of organic matter and minerals.

Beeftink, W.G., 1977. Salt-marshes In, "The Coastline", R.S.K. Barnes (Ed.). London,

Wiley, 93-121. This paper is a part of R.S.K. Barnes' multi-authored book *The Coastline*, a contribution to its ecology and physiography in relation to land-use and management and the pressures to which it is subject. After defining the concept of salt marsh, its different types found at the European coasts and its geomorphology, some information is given on structural (zonation, dynamic relationships) and functional (trophic structure, material cycles, energy flow) aspects, as well as on environmental master-factors governing the salt-marsh feature.

The types of pressure on the salt marsh are classified into agriculture, open-cast mining, land reclamation and improvement for agricultural purposes, large-scale impoundment of coastal waters, pollution, recreation, industry and urban settlement, and education and field studies. These topics are dealt with separately. The most common agricultural use is grazing, but also hay-making and gathering plants and animals for food occur. Open-cast mining varies from cutting and removal of turf and sods, to winning of clay, leaving the marsh in great disorder for a long time. Measures for reclaiming involve different techniques for trapping silt and clay, using brushwood and earthen groynes and *Spartina* plantations. Soil maturing is promoted by drainage and embankment. Recently, the latter grew in size to large-scale impoundments damming estuaries, bays and sea-arms, and having a much wider impact on the coastal habitat than the former agricultural and civil-technical improvements. Still more elusive is pollution, as its source may be even remote and its effect is often stealthy. This threat is mostly caused by a complex array of litter, man-made detritus and chemical substances. From these the effects of urban sewage, agricultural wastes and fertilizer outwash have been dealt with, as well as those of oil-spillage, heavy metals, radioactive waste and thermal pollution. Attention is paid on the influence of old forms of industry, the modern harbour works and the present industrial and power plants. Finally, the influence of different forms of recreation and education including field studies are enumerated.

From the conservationist's point of view utilization of the coastal salt-marsh ecosystem is only acceptable as far as it has no harmful effect. From this starting point the salt-marsh still allows many human activities considered in the fields of education and scientific need, recreation and economic uses.

In order to obtain an ecological basis to management some methods have been described of gaining knowledge on pattern and process in vegetation and environment. Management purposes, however, are closely related to the policy pursued with respect to specific areas. For nature conservation the choice of that policy will depend on: (1) the need for conservation of species and communities, (2) the ecological possibilities of the area, and (3) the possibilities of managing the area. For that purposes criteria for an evaluation of the salt-marsh are drawn up, viz. extent, diversity, rarity, naturalness and replacement. The urgent need has been stressed of erecting

adequate data banks for vegetation, animals and habitat.

Finally, some management measures have been dealt with for conservation of salt-marsh biota against external and internal harmful influences. Pollution and introduction of species are considered as having the most important external human effects. The measures marked out for regulating internal influences are grazing, cropping and spraying, turf cutting and excavating, drainage, and no interference at all. The general design has to be that the salt-marsh should be managed in such a manner as to leave its standing biomass on a relatively low level, and its stability on a high level.

Beeftink, W.G., M.C. Daane, J.M. van Liere & J. Nieuwenhuize, 1977. Analysis of estuarine soil gradients in salt marshes of the Southwestern Netherlands with special reference to the Scheldt estuary. *Hydrobiologia* 52: 93-106.

(Δ-149). Macro- and microgradients in salt-marsh soils of the Scheldt estuary have been studied. The soil parameters used are clay, carbonate, organic matter, pH, nitrogen, potassium and phosphate. The hypothesis that estuarine soil gradients can be reflections of similar aquatic gradients is affirmed for two gradients. Soil gradients of upstream increasing organic matter and phosphate contents have been found, relating significantly to corresponding aquatic gradients caused by river pollution. In the same direction gradients of decreasing nitrogen and potassium contents occur. A macrogradient in carbonate content was not found. Some microgradients in clay and carbonate contents are described, and relevant mutual relationships between the soil parameters are characterized.

Koutstaal, B.P. & J. Nieuwenhuize, 1977. Quantitative determination of the seed of *Salicornia* species in soil. Report Delta Institute 1977-3. pp. 16.

In 1975 investigations had been started on the population biology of *Salicornia brachystachya*, G.F.W. Meyer, and *S. stricta*, G.F.W. Meyer. For the study of the seed populations a method was developed to isolate the seeds of these species from soil samples. A prerequisite was that the method should separate all seeds from the soil particles, and that after the treatment the seeds should be in a living and undamaged condition. For that purpose soil samples, 5 x 5 cm large and 1-1.5 cm deep, were taken in two marshes, one under tidal and the other under non-tidal conditions.

The following method gave the best results: the samples are placed on a 0.42 mm sieve (39.5 mesh) and the seeds are washed out with running water. If there are many pieces of algae, roots or other plant debris, the sample is first filtered with a 2 mm sieve (10-mesh). The residue consisting of seeds together with some organic material is transmitted on a filter paper, 16 cm diameter, fitted in a Büchner funnel. After vacuum filtration the seeds are counted by means of a stereomicroscope. Parallel lines sketched on the filter papers facilitate counting considerably. Organic materials can absorb up to 2-5% of the seeds available and samples very rich in roots up to about 20%.

The most important seed characteristics of *S. brachystachya* are that the seed dimensions are mostly less than those of *S. stricta*. The seeds of *S. brachystachya* are also more or less rounded at the side of the hilum because the radicle is as long as or shorter than the cotyledons, and the seed coat is thickly hirsute and greyish green to brown in colour. For *S. stricta* the seed dimensions are mostly more than those of the other species. Its seeds are more or less acute at the side of the hilum because the radicle is mostly longer than the cotyledons, and the seed coat is less thickly hirsute, dark brown in colour.

Lammens, E.H.R.R. & M.J. van Eeden, 1977. Flow of particulate organic matter between a salt marsh and an estuary in the southwestern Netherlands.

Stud. Rep. Delta Inst. D1-1977, pp. 41. A study has been made to determine the contribution of a salt marsh in the eastern part of the Eastern Scheldt to the nutrient status of this estuary. This marsh is intersected by two large creek systems. From the larger one an estimate has been made of the net transport by the tides of total suspended organic matter, the

primary production of the catchment area and the decomposition rate of its dominant plant species. Also the amount of detached plant debris washed ashore onto the marshes upper margin and in the direct surroundings has been estimated.

The minimal production above ground was calculated at 620 g dry weight/m² for 1975. From that amount 90% seems to be decomposed on the marsh itself, and 10% washed ashore by the tides. The transport pattern of particulate organic matter through the creek shows a net import of 57-66 tons over the whole year (300-340 g dry weight/m²). This means that not all estuarine salt marshes contribute to the productivity of their adjacent water bodies, as is suggested by Odum (1971) (Odum, E.P., 1971. Fundamentals of ecology (third ed.). Saunders, Philadelphia, pp. 574).

Stienstra, A.W., 1977. Mineral composition of *Halimione portulacoides* (L.)

Aellen leaves. *Hydrobiologia* 52: 89-91. (Δ-140). Mineral composition of *Halimione portulacoides* (L.) Aellen leaves was studied. The leaves accumulate salt, which is partly compensated by succulence. The ratio K/Na is less than 1. No distinct correlation between salt content in the soil and the mineral content at different stations could be found.

Suy, M., 1977. Vegetational changes in a salt marsh as a consequence of barraging from the sea and of grazing by cattle and goats. *Stud. Rep. Delta*

Inst. D4-1977, pp. 136. The area of investigation, Het Groene Strand near Oostvoorne, is situated in the northern part of the island of Voorne-Putten, just opposite the "Maasvlakte", the most western part of the Rijnmond industrial area. Until 1966 it was a tidal salt marsh partly enclosed by two curved dune ridges and surrounded by sea-water of about 10-15‰ Cl⁻. In spring 1966, however, the area was cut off from the sea by barraging the Brielse Gat in which the salt marsh is situated. The present investigation deals with the vegetational changes influenced by (1) the separation from the sea, and (2) the management measures introduced after barraging.

For studying the first aspect maps have been drawn of the vegetation patterns in 1954, 1961, 1967 and 1972 using aerial photo-interpretation and permanent sample-plot analyses. These maps show that before 1966 the salt marsh silted up remarkably, and had a clearly zoned vegetation pattern composed of few species. After barraging this vegetation died back, *Spartina townsendii* already within one year, and was succeeded by communities of halo-nitrophilous species. These communities occurred in a similar pattern as their precursors. After that, few glyco-nitrophilous species emerged generally surviving longer than the halo-nitrophilous ones. These species assemblages, too, show a similar distributional pattern as the original salt-marsh vegetation did. *Hippophae rhamnoides* originally only present on the dune ridges, expanded considerably.

From the management measures suitable for the salt marsh grazing is by far the most important one. Comparing grazed and ungrazed parts of the marsh in transects crossing grassland-shrub and grazed-ungrazed boundaries, it could be established that the vegetation is influenced in the following ways:

(1) grazing by cattle does increase the number of plant species and the heterogeneity of the grassland compared by non-grazing. Especially species characteristic of the alliance *Agropyro-Rumicion crispi* and communities of cattle tracks settled during grazing. Reed vegetation is preferred by cattle more than the other plant species available, and consequently it has been subdued after 1966 more than under ungrazed conditions. The expansion of *Hippophae rhamnoides* has hardly been retarded by cattle grazing.

(2) Grazing by goats was too short (3 years) to make any conclusions about its influence on the *Hippophae* expansion, although goats prefer this species more than cattle. Goats also showed more preference to *Sambucus nigra*, *Epi-lobium hirsutum*, *Cirsium arvense* and *Urtica dioica* than cattle. Therefore, goat grazing seems to be useful for suppressing heavy growth of tall herbs and prickly weeds. It is concluded that in such areas grazing is very suitable for regulating and differentiating the vegetation of salt marsh and low dunes after barraging the area from tidal influence.

Parma, S. & B.P.M. Krebs, 1977. The distribution of chironomid larvae in relation to chloride concentration in a brackish water region of the Netherlands. *Hydrobiologia* 52: 117-126 (A-151). Drainage ditches are a familiar and common habitat in the Netherlands. Until recently however they have been the focus of few ecological studies. Extensive studies on Walcheren, a brackish water region of The Netherlands, have shown that chironomid larvae form a major component of the animal communities in the mud. The spatial distribution of some of these species can be correlated with the chloride concentration of the water. Biometric data are included for the species *Chironomus salinarius*, *C. halophilus* and *C. plumosus*.

Schraaf, A. van der & C. van Velzen, 1977. The autecology and the life history of *Chironomus halophilus* and *Chironomus salinarius* - Diptera, Chironomidae. Stud. Rep. Delta Inst. D3-1977, pp. 107. *Chironomus salinarius* Kieff. and *Chironomus halophilus* Kieff. were successfully cultured in captivity for some generations. We experimented with indoor cages (150x40x15 cm) placed in rooms of constant temperature (20° C; L:D - 15:9, 2300 lux; rel. humidity 55%) and outdoor cages (2x2x2 m) placed outside the laboratory. In the indoor cages the fecundity (% egg-laying females) of *C. halophilus* was higher (13%) than that of *C. salinarius* (4%). The natality (% vital eggs per eggmass) was about equal (80 and 90%) and the fertility (% eggmasses with vital eggs) proved to be the largest in *C. salinarius* (80 and 50%). The fertility decreased in the next generation.

In the outdoor cages the fecundity of *C. salinarius* proved to be the largest (34 resp. 11%) while also the natality and fertility was much lower than in *C. halophilus* (0.5 and 1%) than in *C. salinarius* (100% and 99%). In outdoor cultures of *C. salinarius* the emergence of the adults and the oviposition were correlated with the highest measured temperature during daytime. These correlations were absent in outdoor cultures of *C. halophilus*. The difference between the highest measured temperature during daytime and lowest measured temperature during nighttime showed a strong correlation with oviposition and adult emergence of both species.

In *C. halophilus* two types can be distinguished showing differences in egg masses, larval morphometry, colour of the adults and larval developmental rate. In the cultures we experimented with different sediments and chloridities. The composition of the sediment (sand-silt mixtures) had no effect on the larval developmental rate, but an effect on the adult size was present. There are slight indications for an effect of the chloridity on larval developmental rate.

Freshly captured fourth instar larvae of *C. salinarius* were cultured to adults in chloridities from 0-15.2‰. No optimum value could be found. Fourth instar larvae of *C. salinarius* proved to have a slight preference for 7‰ Cl⁻. There are weak indications for a certain salinity preference of ovipositing females. A preference of the larvae for the highest present oxygen content cannot be excluded. A preference of the larvae for a special type of silt-sand mixture could not be proved. Some data about the life history of both species are summarized.

MISCELLANEOUS

Bakker, C., Comparative plankton investigations in a brackish lake (L. Veere) and a brackish tidal estuary (Westerschelde) in the SW Netherlands.

Hydrobiol. Bull. 11(1): 18-19. As the data, presented in this lecture, have been published elsewhere (see references), only a summary is given here.

A comparison was made between the plankton assemblages of the meso- and polyhaline ranges of Lake Veere and the Westerschelde estuary. Species composition, densities and seasonal distributions of the predominant planktonic organisms of the investigated brackish water ecosystems were quite diffe-

rent, depending on several other environmental factors than salinity.

Bakker, C., W.J. Phaff, M. v. Ewijk-Rosier & N. de Pauw, 1977. Copepod biomass in an estuarine and a stagnant brackish environment of the S.W. Netherlands. *Hydrobiologia* 52: 3-13. (Δ-141). In the mesohaline zone of the Westerschelde estuary *Eurytemora affinis* is the dominant copepod, demonstrating large biomass values nearly throughout the year. In the meso-polyhaline Lake Veere *Acartia tonsa* is abundant, mainly during summer. In spring a small population of *Eurytemora americana* is found.

The tidal estuary harboured far denser copepod populations throughout the year than the stagnant brackish lake water. The average yearly copepodid + adult biomass in the Westerschelde estuary was approx. 850 mg/m³ (wet weight), in Lake Veere approx. 130 mg/m³.

Temporarily low oxygen values did not influence negatively the copepod populations in the Westerschelde estuary.

The seasonal distribution of the dominant copepods in both areas is explained in the light of recent literature data.

Perennial *Eurytemora affinis* abundance in the Westerschelde estuary must be mainly caused by large concentrations of nannodetritus particles, bacteria included, throughout the year. *Acartia tonsa* in Lake Veere has to thrive mainly on nannophytoplankton.

Boaden, P.J.S., 1976. Soft meiofauna of a sand from the Delta region of the Rhine, Meuse and Scheldt. *Neth. J. Sea Res.* 10: 461-471. (Δ-136). A brief survey of sands in the Rhine Delta Area was undertaken in September 1967 and revealed a fairly diverse meiofauna. A list of the distribution of the Cnidaria, larger Tubellaria, Gastrotricha and Annelida is given and details of the sampling localities presented. The probable effect of changes in sediment and salinity on this fauna is discussed.

Boissevain, E.C. & S. Parma, 1977. How can the Eastern Scheldt be saved?

(In Dutch). *Natuur en Milieu*, 77/4, pp. 12-15. Recently the government decided to close the Eastern Scheldt by means of a perforated dam. In this way they tried to co-ordinate the claims for safety against superfloods and for nature conservation. A survey is given of new threats of the biological value like intensive recreation, power plants, skin diving, reduction of the tidal movement and heightening of the dikes.

Braber, L. & C.H. Borghouts, 1977. Distribution and ecology of *Anthozoa* in the estuarine region of the rivers Rhine, Meuse and Scheldt. *Hydrobiologia* 52: 15-21. (Δ-142). Data on the ecology and distribution in the estuarine region of the rivers Rhine, Meuse and Scheldt are given for the species of *Anthozoa*. These data have been correlated with several environmental variables, but especially salinity.

Duce, R.A. & E.K. Duursma, 1977. Inputs of organic matter to the ocean. *Mar.*

Chem. 5: 319-339. The first objective of this introductory paper is to summarize our present understanding of the quantities of total organic carbon produced in the ocean by photosynthesis and non-biotic photochemical reactions, and the amount entering the ocean from rivers, the atmosphere, and the sediments. In this overview it will become apparent that our knowledge of the primary mechanisms and processes involved with the input of organic matter to the ocean via rivers, the atmosphere, sediments, and *in situ* photochemical reactions is fragmentary and often completely lacking. This becomes critical when we attempt to estimate the input of certain naturally occurring organic compounds and some synthetic organic compounds, since their primary input to the sea is generally via the atmosphere, rivers, and dumping. Thus the second objective of this paper is to emphasize our need to understand the mechanisms involved in these other input processes and the necessity of developing field programs and mathematical models to evaluate the input of specific organic compounds via these pathways. Polychlorinated

biphenyls are used as examples of how necessary it is to understand these other input routes in order to evaluate the cycling of pollutant substances in the ocean.

Duursma, E.K., 1977. Effect of hydrostatic pressure on the diffusion of chloride in marine sediment. An in situ experiment. *Deep-Sea Research* 24:

1161-1166. An *in situ* diffusion experiment was carried out between the depths of 170 and 970 m, with a laboratory experiment as control, using Na^{36}Cl as radioactive tracer. Plastic tubes containing Mediterranean sediment of $38.9 \pm 0.8\%$ moisture content of 10 cm length were used with 1/2 cm seawater with ^{36}Cl as instantaneous source. The diffusion coefficients determined showed no pressure effect within the sample standard deviation of $0.7 \times 10^{-6} \text{ cm}^2\text{sec}^{-1}$ for a mean diffusion coefficient of $7.7 \times 10^{-6} \text{ cm}^2\text{sec}^{-1}$.

Hofstra, J.J. & A.W. Stienstra, 1977. Growth and photosynthesis of closely related C_3 and C_4 grasses, as influenced by light intensity and water supply. *Acta Bot. Neerl.* 26(1): 63-72. Growth of Indonesian C_4 and C_3 grasses was compared in field experiments in Indonesia and in growth chamber experiments in the Netherlands. Two C_4 species *Axonopus compressus* (Swartz) Beauv. and *Setaria plicata* (Lamk.) T. Cooke, were used and one C_3 species, *Oplismenus compositus* (L.) Beauv.

Both the field and the growth chamber experiments showed no large differences in relative growth rate between the C_3 and the C_4 grasses, grown at high light intensities, neither with a high, nor with a low water supply.

Net photosynthesis of the C_3 species was lower than that of the C_4 species at all growth and experimental conditions. Whenever this resulted in a lower unit leaf rate of the C_3 species, this disadvantage was overcome by formation of a larger leaf area.

At very low light intensities, the C_4 species *Axonopus* had a lower relative growth rate than the C_3 species *Oplismenus*. This may explain the absence of this C_4 grass in the deep shade.

The absence of the C_3 species in open areas cannot be explained by a lower relative growth rate of this species at high light intensities. Other factors as a low shoot/root ratio, a high soluble sugar content and a high initial growth rate may give C_4 grasses a high competitive value over C_3 grasses in open and dry areas. Some of these factors may depend indirectly upon the C_4 mechanism of photosynthesis.

Kortekaas, W.M., E. van der Maarel & W.G. Beeftink, 1976. A numerical classification of European *Spartina* communities. *Vegetatio* 33: 51-60. (Δ -140). The classical syntaxonomical treatment of the European *Spartina* communities as published in the series *Prodrome of the European plant communities*, is compared with the results of a numerical treatment, based on largely the same set of relevés. 576 relevés, selected from the total salt marsh data set were subjected to agglomerative clustering with relocation with the similarity ratio as similarity measure. The resulting numerical system was compared with the syntaxonomical hierarchy. The correlation between both systems is close. The numerical units are slightly more heterogeneous because no purification occurred, which implies relevés to be left out of consideration. One new syntaxon, *Spartinetum townsendii asteretosum tripolii*, could be suggested from the results of the numerical treatment. Perspectives for the development of a numerical syntaxonomy are stressed.

Koulman, J.G. & W.J. Wolff, 1977. The Mollusca of the estuarine region of the rivers Rhine, Meuse and Scheldt in relation to the hydrography of the area.

V. The *Cardiidae*. *Basteria* 41: 21-32. (Δ -154). Based on literature data and personal investigations it is concluded that *Cerastoderma edule* (L.) and *C. glaucum* (Poiret) are closely related, but distinct species. The haploid chromosome number of both species is 19. The distribution and ecology of the two species in the estuarine area of the rivers Rhine, Meuse and Scheldt are described in relation to environmental factors. *C. edule* lives nearly

exclusively in tidal environments, *C. glaucum* in areas with stagnant water. This in accordance with the hypotheses that *C. edule* cannot live in stagnant water, owing to a detrimental substance liberated by the living animals themselves, and that *C. glaucum* does not tolerate wave-action or a long period of emersion.

It is concluded that *C. edule* shows a correlation between mean rib number and mean salinity, whereas *C. glaucum* does not. Therefore Eisma's (1965) method for determining the salinity of fossil seas is modified.

Lambeck, R.H.D., 1977. Economical aspects of the management of wild geese

(In Dutch). Econ. Statist. Ber., 62 (No. 3115): 737-741. This paper is a summary of a part of an unpublished M.Sc. thesis from 1973. Destruction of natural habitats for certain purposes has so far been considered to be a diseconomy of the economical production. Thus, in contrary to cases where farmland, houses etc., are involved, no compensation has been paid. While nature area's are nowadays scarce too, this habit is not in accordance to the economic theory. However, determining the value of nature area's is very difficult. Many essential biological data are still lacking. Geese have a very strong relationship with man. Relatively spoken they are rather appropriate for an economical analysis. A survey of habitat preferences is given.

Based on the energetics of geese calculations on the required amount of food have been made. The carrying capacity of meadows in wintertime has been determined at 2000 Whitefronted Goose-days/hectare. Based on two assumptions of the influence of human disturbances on the use of a feeding area, a graphical relation between carrying capacity in goose-days/ha and the surface of the whole feeding area could be presented. This all gives us the opportunity to compensate quantitatively for the loss of habitat.

In Europe hardly any research has been carried out on hunting statistics and the expenditures of hunting and bird-watchers. To give an idea about the value of wild geese some American data (restricted to hunting) are given. Although the total hunting pressure in Europe and Northern America is more or less the same, the organization of and limits to hunting are quite different however.

A survey of Dutch data on costs of management and creation of new habitats is presented. Also the relations with agricultural interests are discussed.

In conclusion, for a cost-benefit analysis it is more or less possible to calculate the costs. As regards to the benefits of wild geese more research is necessary.

Lambeck, R.H.D., 1977. The occurrence of Brent goose *Branta bernicla* L. subspecies in the Dutch Waddensea area during spring 1976. (In Dutch). Limosa

50(3/4), 92-97. Nearly all Brent geese *Branta bernicla* visiting The Netherlands in the winter season belong to the nominate subspecies *B.b. bernicla*. Hitherto it had been assumed that the Pale-breasted Brent, *B.b. hrota*, was only a rare guest in this country. During a field study in the 1975/76 season on population ecology of *B.b. bernicla* in the Dutch Waddensea area, one or more *hrota*'s were seen regularly in the flocks. From 20 March on sightings were systematically recorded, till the end of May 21 *hrota*'s were seen. All birds were in adult plumage; presumably most are of sub-adult status. It seems likely that many different individuals were involved in view of the spread of places and dates in the observations. It can be concluded that probably several tens of *Branta b. hrota* visited The Netherlands that season. Possibly this is the rule every year.

One *hrota* (2 May 1976, saltings Linthorst-Romanpolder, province of Groningen) wore a yellow neck-collar. This individual had been ringed in the summer of 1975 on Bathurst Island, arctic Canada, and was also recorded in England (Lindisfarne) and one time (8 April, 1976) on the Dutch Island of Schiermonnikoog by A.K.M. St. Joseph. So not all *hrota*'s are necessarily from the Spitsbergen population.

There were two records of the Black Brant, *B.b. nigricans*. One on the Lauwerpolder saltings, province of Groningen (5 and 7 March) and the second one on the Island of Texel (30 April and 11 May). These constitute the third and fourth sightings of this subspecies in The Netherlands. The possibility

that these birds are escapes can, however, not been excluded.

Nieuwenhuize, J., 1977. Determination of $\text{NO}_3\text{-N}$ in plant extracts with the

Technicon Autoanalyzer. Rep. Delta Int., 1977-2; pp. 12. The method, described by Strickland and Parsons (1960), for nitrate determination in water was tested for plant extracts using the Technicon Autoanalyzer. Dried plant material (80°C) was extracted with distilled water (0.5 gr with 50 ml H_2O), which gave a brown-yellow coloured liquid. This liquid was introduced in the Technicon Autoanalyzer. The essential point was to use proper blanks of the reagent N-1-naphtyl-ethylene diamine dihydrochloride. Calibration was carried out with the extracts containing additions of KNO_3 . An accuracy of 2% could be obtained for the range of 0.1-1.0 mg $\text{NO}_3\text{-N/l}$. (Strickland, J.D.H. & T.R. Parsons, 1968. A Practical Handbook of Seawater analyses. Ottawa. Fisheries Research Board of Canada. Bulletin 167., 71-76).

Nijssen, H. & P. de Koeijer, 1977. The first record of *Trachinotus ovatus*

(Linnaeus, 1758) in the coastal waters of the Netherlands (Pisces, Perciformes, Carangidae). Bull. Zool. Museum Univ. Amsterdam, 6(7): 49-52. A live specimen of *Trachinotus ovatus* (Linnaeus, 1758) is reported for the first time from the coastal waters of the Netherlands.

Sepers, A.B.J., 1977. The utilization of dissolved organic compounds in aquatic

environments. Hydrobiologia 52: 39-54. (Δ -145). The uptake of dissolved organic matter by bacteria, phytoplankton and invertebrates is discussed referring to the literature. In natural waters the uptake of dissolved organic compounds appears to be primarily a bacterial process.

Stienstra, A.W., 1977. Photosynthetic activity and growth of some C-3 - C-4

plants at different light intensity. Report: Research during the period 1-7-1971 till 1-7-1973, Treub Laboratorium Bogor. WOTRO grant: W 86-14.,

pp. 34. The photosynthetic capacity was measured of three plants: *Setaria barbata* (C-4 plant), *Axonopus compressus* (C-4 plant) and *Oplismenus compositus* (C-3 plant). These measurements were done after they were grown up under semi-natural circumstances at different light intensities. More over the growth, the morphological development, the chlorophyll content and the stomata per unit leaf area were measured. From these experiments can be concluded: Both C-4 plants show a higher photosynthetic capacity at all light intensities than the C-3 plant. The three species show the same alteration in reaction of the photosynthetic system at different light intensities. Field observations show, that C-4 plants need for their growth a high light intensity and a high temperature. Under semi-natural circumstances *Oplismenus* can grow in full sunlight while *Setaria* and *Axonopus* can grow in the shade. In the shade *Setaria* and *Axonopus* are less productive than *Oplismenus* notwithstanding their high photosynthetic capacity. Under these experimental conditions the chlorophyll content and the morphology development do not show any correlation with the C-3 - C-4 photosynthetic system.

Vaas, K.F., Delta Institute for Hydrobiological Research, Yerseke, Progress

Report 1976. Institutes of the Royal Netherlands Academy of Arts and Sciences Progress Report 1976. pp. 166-178. (Δ -156). A survey is presented on the progress of the research carried out in the institute.

Vader, W., 1977. Habitat and distribution of *Perforatella rubiginosa* (Gastropoda, Pulmonata) in the freshwater tidal region of the Scheldt Estuary,

Belgium. Hydrobiologia 52: 2-28. (Δ -143). The amphibious land-mollusc, *Perforatella rubiginosa*, is a common, wide-spread and characteristic inhabitant of the freshwater tidal region of the river Scheldt and its tributaries in

NW Belgium. The species occupies a rather narrow zone in the uppermost intertidal area, which is characterized by large amounts of tidal drift covering the bottom, and usually by a luxuriant vegetation of tall, nitrophilous herbs, often with *Urtica dioica* dominant. Other characteristic molluscs of this zone are *Carychium minimum*, *Oxyloma pfeifferi*, *O. sarsi*, *Deroceras laeve*, *Vitrea crystallina* and *Zonitoides nitidus*, locally also *Nesovitrea hammonis*.

Vlasblom, A.G., S.J. Graafsma & T.A. Verhoeven, 1977. Survival, osmoregulatory ability and respiration of *Idotea chelipes* (Crustacea, Isopoda) from Lake Veere in different salinities and temperatures. *Hydrobiologia* 52: 33-38. (Δ-144). 1. An ecological and physiological study of *I. chelipes* from Lake Veere, The Netherlands, was made.

2. Both osmoregulatory capacity and survival decrease with increasing temperature as well as with decreasing salinity.

3. Respiration experiments suggest that the need of energy by osmoregulatory activity may be supplied at the cost of other physiological processes, at any rate at temperatures of 10° C and higher.

4. It may be expected that, if temperatures higher than 15° C and salinities lower than 8‰ coincide, the population of *I. chelipes* will be affected negatively.

Vijver, A. van de, 1977. The vegetation of some polders in the isle of Zuid-

Beveland. Stud. Rep. Delta Inst. D5-1977, pp. 79. In this report the botanical diversity is estimated in a complex of polders occurring in the isle of Zuid-Beveland. For that purpose botanical inventories and phytosociological records (relevés) have been made during two successive growing seasons (1972 and 1973): Within the area of investigation the following landscape components have been distinguished: arable land, field and road verges, bed and sides of ditches, grasslands, orchards, railways, creeks and ponds. It was impossible to classify the relevés simply according to the Braun-Blanquet system of phytosociology. Therefore, the species have first been classified into sociological groups on the level of alliance or order based on the present-day knowledge of phytosociology in the Netherlands embodied in Westhoff and Den Held (1969). For each landscape component the presence of these sociological groups has been estimated.

Comparing these data it appeared that also on this basis the landscape components do not differ largely in their vegetational composition. Obviously, modern agricultural and civil-technical practices interfere too much, levelling vegetational variety even in an old complex of polderland. As could be expected most species and the highest presence of the sociological group, characteristic of the *Chenopodietea* has been found in the relevés made in arable land. Fields with legumes were richer in those species than fields with cereals. The species characteristic for the *Arrhenatherion elatioris*, the *Molinio-Arrhenatheretea* and the *Agropyro-Rumicion crispi* were most represented in the relevés made on ditch-sides. No floristic difference could be found between the vegetation exposed North and that exposed South. Among the landscape components ditch-sides were most rich in species. Verges of arable fields appeared to be in a transitional situation between the vegetation of those fields and that of ditch sides. With the exception of a high presence of species characteristic for the *Lolio-Plantaginion* road verges resembled ditch-sides. Grassland relevés stood out by a large number of species characteristic of the *Agropyro-Rumicion crispi*. Saline grasslands showed many *Glauco-Puccinellietea* species. Floristic differences in grasslands were not related to their height with respect to ordnance level. Orchards showed especially species characteristic of the *Lolio-Plantaginion* and to lesser extent, that of the *Chenopodietea*. Railways were very variable in species composition because of the varied origin of the soil brought in from elsewhere and used for their construction. Creeks and ponds had many species of the *Phragmitetea* occurring with a high presence, and in ditch-beds the species group characteristic of the *Agropyro-Rumicion crispi*, was well-developed, besides that of the *Phragmitetea* species. (Westhoff, V. & A.J. den Held, 1969. *Plantengemeenschappen in Nederland*, Thieme, Zutphen, pp. 324).

Wolff, W.J., 1976. Distribution of Pantopoda in the estuarine area in the Southwestern part of the Netherlands. *Neth. J. Sea Res.*, 10: 472-478.

(Δ-152). The distribution of the pycnogonids *Nymphon rubrum*, *Achelia echinata*, *Phoxichilidium femoratum*, *Anoplodactylus petiolatus*, *A. pygmaeus*, *Pycnogonum littorale*, and *Callipallene brevirostris* in the estuarine area of the rivers Rhine, Meuse and Scheldt is described. Some observations on their salinity tolerance and the occurrence of food species are recorded.

Editor: Dr. Egbert Klaas Duursma

Director

Centraalbureau voor Schimmelcultures

Progress Report 1977

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HISTORY AND FUNCTION OF THE INSTITUTE

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

After Prof. Westerdijk retired in 1959, she 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 1977 the total number of strains maintained was 23.800, 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 carried out mainly in taxonomy of fungi. In the division of human and animal mycology, problems pertaining to this field are studied. Investigations into the chemistry of fungal metabolites are carried out in the biochemical department.

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

SCIENTIFIC STAFF (as from December 1st 1977).

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

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

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

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

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

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

Dr. G.W. van Eijk (biochemistry)

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

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

Dr. G.S. de Hoog (Dematiaceae)

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

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

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

Mrs. Drs. G. de Bruin-Brink (documentation)

Yeast Division.

Drs. L. Rodrigues de Miranda (Basidiomycetous yeasts)

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

D. Yarrow (Saccharomyces and related genera)

INTRODUCTION

The second International Mycological Congress, held in Tampa (Florida, U.S.A.) from 27th August to 3rd September 1977, offered a unique opportunity to meet foreign colleagues and to become familiar with the newest trends in mycology. Twelve staff members and a guest worker visited the congress with partly private financial support. Four invited papers were presented in organized symposia and there were also invitations to staff members to participate in four round table discussions. Four staff members presented seven posters in their special fields of study partially in co-operation with North American and British colleagues. These contributions to the congress required much preparation; the results of own scientific research were mostly presented and often illustrated by instructive microphotographs (many taken with the scanning electron microscope).

Two staff members joined a post-congress workshop in Kananaskis (Alberta, Canada) which dealt with the relationships between perfect and imperfect states in fungi. Two papers were given in the course of this workshop and there was active participation in all discussions.

1. DIVISION OF FUNGUS TAXONOMY

Dr. H.A. van der Aa

The study of type specimens of *Phyllosticta* is being continued. Collections were obtained from herbaria in Berlin (260 specimens), Stockholm (214 specimens), La Plata (75 specimens), Utrecht (65 specimens), Verona (30 specimens) and Siena (6 specimens). An increasing number of species have been excluded, most of which are newly established synonyms of well-known *Phoma* species, while others will have to be reclassified in *Asteromella*, *Phomopsis*, *Coniothyrium* etc.

New data on the genus *Phyllosticta* s.str. have also been collected, often in combination with observations on the spermatial and ascigerous states. On the holotype specimen of *Phyllosticta anonae* P. Henn. (B) the conidial state proved to be accompanied by well-developed ascomata of the so far undescribed *Guignardia* state. Dr. R. Schneider, Berlin, sent in a new isolate of *Phyllosticta capitalensis* P. Henn. which produced the previously unknown ascigerous state. A new species of *Phyllosticta* was isolated from leaves of *Macadamia integrifolia* collected by W. Nicolai on plantations in Guatemala. The species had rather large stromata, medium-sized conidia and was accompanied by a *Leptodothiorella* spermatial state, which was found both on the

plant and in pure culture. A strain of another *Guignardia* species from *Camellia japonica* leaves was obtained from H.J. Boesewinkel, Plant Health Diagnostic Station, Auckland. The fungus was identified as *Guignardia camelliae* (Cooke) Butler and proved to be the ascigerous state of *Phyllosticta theaecearum* van der Aa.

Single conidial isolates were made of *Phaeoramularia dissiliens* (Duby) Deighton, causing typical leaf spots on *Vitis vinifera* in Iraq. The colonies grew extremely slowly under various conditions, but after about 2 months at 25°C under UV light a macroconidial and an *Asteromella* spermatial state were formed. A perfect state has not been observed but these experiments proved that *Phaeoramularia* is closely related to *Cladosporium* and *Cercospora*, which also have *Asteromella* spermatial states and belong to *Mycosphaerella* life cycles.

In August and September diseased leaves of *Nymphaea alba* were collected on the lakes near Kortenhoef. The leaves showed numerous lesions with necrosis. A *Colletotrichum* species without setae was regularly isolated, but the disease seems to be caused by a combination of factors. The fungus had been described under several names in *Gloeosporium* and *Ovulariella* of which the type specimens were studied.

Dr. J.A. von Arx

The study of mycelial yeasts was continued, partly in co-operation with Drs. A.C.M. Weijman. The cultural and microscopical characters of all the available strains of *Dipodascus* and *Geotrichum* species were compared. The strain CBS 625.75, sent in for identification by Prof. J.S.F. Barker (Sydney, Australia), proved to represent an undescribed species of the genus *Dipodascus* with spreading colonies, dichotomously branched hyphae and asci containing 1 to 20 ascospores. In its cultural characters this fungus is close to *Endomyces geotrichum*, but this species differs by a slightly larger growth rate of the colonies, consistently 1-spored, spherical asci and larger, ornamented ascospores. Consequently this species and some other *Endomyces* species with *Geotrichum* conidial states, have been transferred to the genus *Dipodascus*. A number of species recently described as *Geotrichum* and *Trichosporon* proved to represent conidial cultures of some *Dipodascus* and *Hypophichia* species. A fungus isolated from gills of *Armillaria mellea*, which had hitherto been wrongly identified as *Endomyces decipiens*, proved to be a different species and was described as *Geotrichum armillariae*. A key was prepared for the delimitation of all accepted species of the genus *Dipodascus* and the *Geotrichum* conidial states.

In another study a number of strains of Gymnoascaceae were examined. Some recently described genera of this family proved to be incorrectly or insufficiently characterized. The genera *Petalosporus*, *Disarticulatus* and *Plunkettomyces* are regarded as synonyms of *Arachniotus* and *Gymnascella*, *Gymnoas-*

coides, *Macronodus*, *Tripedotrichum* and *Uncinocarpus* are reclassified as synonyms of *Gymnoascus*. The type species of the genus *Kuehniella* proved to be identical with *Arachnotheca albicans*. The genus name *Rollandina* is considered to be a "nomen confusum", its type specimen containing two different organisms: unknown structures and the ascomycete *Nannizzia fulva*, with its *Microsporium* conidial state. In a paper, which will be published in *Persoonia*, all the accepted genera of the Gymnoascaceae are briefly reviewed.

In co-operation with Prof. E. Müller (Zürich), the families of Ascomycetes were arranged in a system in which the respective conidial states are considered. Certain additional taxa, which may represent intermediates between the Sphaeriales and the Dothideales, were studied. All bitunicate Ascomycetes were classified in the latter order. Much time was devoted to classifying the genera of the Dothideales in more natural families, which can be partly delimited by different conidial states.

Dr. W. Gams

The greater part of 1977 was given to the preparation of texts on the taxonomy and ecology of selected soil fungi which will be published in a compendium of soil fungi by K.H. Domsch, W. Gams and H. Anderson. Some lengthy monographs were written of important human and plant pathogens, including species of *Arthroderma*, *Trichophyton*, *Coccidioïdes*, *Emmonsia*, *Verticillium*, *Sclerotinia*, *Botrytis*, *Sclerotium*, *Thanatephorus* and *Phymatotrichopsis*. In the course of this work SEM photographs were prepared to illustrate the treated *Chaetomium* species.

Studies on phialidic hyphomycetes included the comparison of some *Phialophora* strains sent by Th. Nilsson, Uppsala, with known species and the recognition of at least two distinct new species. Following a demonstration by S. Zweibel at the 2nd International Mycological Congress at Tampa, the CBS strains of *Ph. verrucosa* were reexamined and all found to belong to *Ph. americana* (Nannf.) Hughes, except for CBS 138.67, which is a proper *Ph. verrucosa* Medlar. Thus human-pathogenic strains occur in both these taxa which had generally been regarded as synonymous in the last decades. A considerable number of strains, obtained from Dr. G.J. Samuels, Auckland, New Zealand, including *Nectria* species with *Acremonium* conidial states and *Niesslia* species with *Monocillium* conidial states, were studied. For a demonstration at the 2nd International Mycological Congress at Tampa, SEM photographs were prepared with the assistance of Miss M. Nieuwstad, illustrating various mechanisms of conidial chain formation and phialides with solitary conidia. It could be shown that the connectives in *Penicillium* and *Torulomyces* are not comparable with the truncate conidial connections in *Acremonium* and *Fusarium*.

A key to the recognized species of *Mortierella* has been submitted for publication in *Persoonia*. Since it was noted that the species published by Linemann in 1936 had never been validly published (no Latin diagnoses), three

species were validated, while for some others younger names had to be adopted.

W. Verkerke (Cand. biol., University Amsterdam)

Under the supervision of Dr. W. Gams a fungal inventarization was made for the "Drie Organische Stofbedrijven", farms at Nagele in the Norht-East Polder. From each farm composite samples were twice taken in the wheat plots (previous crop potato), which were regarded as most representative of the whole management, and analysed by means of the soil-washing technique. On the basis of over 1000 isolates, it was concluded that the "Wisselweide" (with 2 years of grass ley in the rotation and with addition of stable manure) showed the greatest number of species (55), the "Klaverland" (mainly green manure) the least (42) and the "Kunstmestakker" (artificial fertilizers but no organic matter added) was intermediate. The same tendency in species diversity was consistently observed. Although the species found give little indication of the antiphytopathogenic potentialities in these soils, the presence of some species with known antifungal activities seems to parallel the above trend. Amongst some interesting species isolated, *Anixiopsis stercoraria* with the conidial state, *Chrysosporium keratinophilum*, was studied in detail in collaboration with Dr. R.A. Samson.

Drs. E.J. Hermanides-Nijhof

An article on *Aureobasidium* and allied genera was finished (Studies in Mycology No. 15). Five generic names, including *Kabatiella* are listed as synonyms of *Aureobasidium*: 14 species and one variety are recognised. Some morphologically similar species, represented by only one or a few herbarium collections, were treated as different species distinguished by minor morphological characters and host specificity. More study is needed to elaborate the possible synonymy of some of these taxa.

Strains of the genus *Kabatina* proved to be *Hormonema*-like in culture. The conidia arise in basipetal sequence from inconspicuous butts and are broadly ellipsoidal to reniform. On the natural substrate, however, acervuli develop which are composed of a dark basal stroma from which densely packed, erect, short-celled conidiophores arise. The apical, occasionally intercalary conidiogenous cells form conidia from their percurrently elongating apices. Since the genus *Hormonema* was explicitly described to cover only cultural states, the phenetically dissimilar genus *Kabatina* was retained to accommodate the stromatic states of the fungi concerned. More fungi will probably be found to produce a *Hormonema* state in culture (Luttrell, E.S., *Mycologia* 32: 530-536, 1940; Sutton, B.C. & Funk, A., *Can. J. Bot.* 53: 521-526, 1975).

A reorganisation of the collection strains of the genus *Fusarium* was started. Several strains maintained under incorrect names were reidentified. The strains of the *Gibbosum* and the *Martiella* sections were reorganised. In *Gib-*

bosum four species (*F. equiseti*, *F. compactum*, *F. acuminatum* and *F. longipes*) and in *Martiella* five species (*F. solani*, *F. coeruleum*, *F. javanicum*, *F. eumartii* and *F. caucasicum*) were recognised.

Dr. G.S. de Hoog

The first months of 1977 were devoted to the preparation of a manuscript on the black yeasts. For this study, undertaken in co-operation with Dr. P. Hogeweg (Utrecht), Dr. H.L.C. Meuzelaar (Amsterdam) and Drs. A.C.M. Weijman (Baarn), the results of a numerical taxonomical classification were compared with those of chemical "finger-printing" by means of pyrolysis-mass spectrometry of a sample of the organisms concerned. The chemical information was supplemented by qualitative data gained by gas-liquid chromatographical analysis. The principal traits of both independent sets of characters appeared to show similar tendencies. On the basis of this knowledge, 9 genera were distinguished, two of which were new and two validated. The genera contained a total of 37 species or form-species, and five varieties. Eleven new species were described, two of which were validated, and 20 new combinations and a new name were proposed.

In co-operation with Dr. G. Morgan-Jones (Auburn, Alabama), a new monotypic genus of black yeast-like fungi was described from *Cronartium* galls. It forms hyaline conidia on large denticles which are either scattered or in small groups on undifferentiated, partly rough-walled hyphae or on short hyphal protrusions.

A theoretical experiment was conducted by commencing with a classification as a taxonomic hypothesis, formulating its criteria of goodness, and testing it by means of a confrontation with the data. Since grouping of a particular set of data is dependent on (a) our criteria for the segregation of groups and (b) the kind of system we aim to construct, (a) and (b) being entirely anthropogenic, one is free to choose any system as long as the underlying reasoning is consistent, i.e. as long as the classification does not conflict with the pre-established criteria and aims. As an example, two systems of the Ascomycetes, constructed by Dr. J.A. von Arx (Baarn) and Dr. D. Malloch (Toronto, Canada), were compared. These systems appeared to be differently oriented; one being based mainly on similarity, the other mainly on phylogenetical suppositions.

For a study of *Cladobotryum*-like fungi growing on toadstools and moulds, cultures were grown on shredded agarics in plain agar. The genus *Cladobotryum* was found to be heterogeneous and was restricted to species with monoblastic retrogressive conidiogenous cells. *Mucrosporium* was redescribed for the polyblastic species *Cladobotryum dendroides*. The name *Sympodiophora* was replaced by the earlier synonym *Pseudohansfordia* and seven new combinations were proposed. In this genus the conidiogenous loci may each form one conidium or a basipetal chain or head as is also found in *Cladobotryum*; the fertile cells

are, however, polyblastic. The generic name *Helminthophora* Bon. was reintroduced for *Eurasina bondarzewiae* (= *Cladobotryum leptosporum*). New species of *Denticularia* and *Sporothrix* were described and a new genus was erected to accommodate *Gloeosporium aecidiophilum*.

C.A.N. van Oorschot, M. Phil.

The genus *Chrysosporium* is defined as having arthroconidia and aleurioconidia which are terminal, intercalary or lateral, occasionally formed in chains and which have broad, basal attachments. The available strains of *C. pannorum* (= *Geomyces pannorum* (Link) Sigler & Carmichael) are found to be rather heterogeneous in their colony characters and branching patterns. An interesting strain has been isolated from a soil sample from Columbia which appears to be intermediate between *C. meridarium* and *C. pannorum* because the arthroconidia measure $4.5 - 6.0 \times 3.0 - 4.5 \mu\text{m}$ as in *C. meridarium*, but the fertile hyphae show *C. pannorum*-type branching. Some new strains, most of which were kindly sent in by Dr. J.W. Carmichael, possibly belong to new taxa. Quite a number of strains described under the name *Chrysosporium*, produce blastoconidia and for those with ampulliform swellings the name *Myceliophthora* Cost. is available. *Chrysosporium luteum*, *C. thermophilum* and *C. fergusii* have been reclassified in *Myceliophthora* in a paper which is in print in Persoonia 9. Several species, such as *C. asperatum*, *C. parvum* (*Emmonsia parvum*), *C. parvum* var. *crescens* (*E. crescens*), *C. serratus* (conidial state of *Ctenomyces serratus*) and *C. dermatitidis* (conidial state of *Ajellomyces dermatitidis*) may be closely related to *Myceliophthora*. Other species appear to be unrelated to the *Chrysosporium*-complex and have to be excluded.

Type studies of all species described in *Chrysosporium* and related genera were initiated. For several species, such as *C. asperatum*, older epithets are available. Due to a lack of authentic material and insufficient descriptions and illustrations, the position of a number of species remains uncertain.

The type strain of the xerophylic *C. fastidium* has been found to produce smooth, brown bodies up to $45 \mu\text{m}$ in diameter. The strain is being cultured under various conditions in an attempt to ascertain whether these are actually poorly developed ascomata. Mating tests have so far proved unsuccessful. The species is compared to *Bettsia alvei*, a xerophylic fungus found on pollen in bee combs. The ascomata of *Bettsia alvei* resemble the bodies produced by *C. fastidium*. Its conidial state, *C. farineola*, has double-walled arthroconidia, which are often in chains, as well as thick-walled aleurioconidia and arthroconidia similar to those of *C. fastidium*.

Strains maintained in the CBS collection as *Briosia cubispora* and *Briosia microspora* have been studied with the light microscope and the SEM to clarify their mode of conidiogenesis and their taxonomic positions. The two strains of *Briosia cubispora* were often seen to have infertile portions between the arthroconidia as well as central septal wall excrescences, as previously

shown by Cole (Can. J. Bot. 53: 2983-3001, 1975) in another strain. The available strain of *Briosia microspora* did not show the infertile portions or clear excrescences and the primary wall tore at the septum, subsequently retracting slightly away from the swollen terminal part of the conidium.

Drs. A.J. van der Plaats-Niterink

The study of the genus *Pythium* was continued. In the group of species with filamentous sporangia, a number of isolates produced catenulate hyphal swellings but no oogonia. Matings with *P. catenulatum* were unsuccessful; this species is characterized by a rather high optimum temperature (30-35°C); below 25°C it shows a radiate growth pattern on potato-carrot agar, while a rosette pattern appears between 25 and 35°C. This phenomenon was also observed in some of the seven studied isolates of *P. middletonii*. *P. middletonii* Sparrow (1960) is based on *P. proliferatum* de Bary (1860) non Schenk (1859). These isolates agreed well with de Bary's slides of the type culture preserved in the British Museum of Natural History in London. The main difference was the more conspicuously aplerotic oospores in the type slides, though in the living cultures the oospores are always free from the oogonial wall. *P. multi-sporum*, *P. ostracodes* and *P. oedochilum* were studied for the group with proliferating sporangia.

In co-operation with Ir. I. Blok, Wageningen, research was done on *Pythium* species on lettuce (*Lactuca sativa*) and their pathogenicity. Two species were found to be important: *P. tracheiphilum* and a new *Pythium* species with characteristically ornamented oogonia.

A possibly heterothallic species with spherical sporangia, sent by Dr. O. Vaartaja (Canada), turned out to be identical with four previously studied isolates. Four of these produced some oogonia in single culture, while the presence of the fifth isolate was found to stimulate their production in combination with any of the other isolates.

In co-operation with Mrs. E. Ilieva (Bulgaria) the results of matings between various isolates of *Phytophthora nicotianae* and *P. capsici* were studied. Although crossings between A¹ and A² strains of these different species produced oogonia, the oogonia were of the *P. nicotianae* type in interspecific matings, and of the *P. capsici* type in intraspecific matings of the latter species. Interspecific matings took longer for oogonium production. While *P. capsici* has the optimum temperature for oogonium formation at 15°C, interspecific oogonia were best formed at 20°C.

Experiments with three sterile *Phytophthora* isolates from England resulted in the production of oogonia in crossings with *P. drechsleri*. The oogonia obtained did not resemble those of *P. drechsleri* but were more similar to those of *P. verrucosa*.

The taxonomic studies on the genus *Penicillium* were continued. Species of the *P. citrinum* series were reinvestigated and compared with other taxa producing a penicillus with a terminal whorl of metulae and flask-shaped phialides. Sclerotium-producing species such as *P. raistrickii*, *P. soppii* and *P. pedemontanum* were also investigated. A detailed study was carried out of the available strains of the human-pathogenic species, *P. marneffeii*. The type strain of this species is morphologically identical with *P. citrinum* and the synonymy of these two species is proposed.

The compilation of the *Aspergillus* species described since 1965 was completed. Eighty-six species were listed and their taxonomic status was critically examined. About 30 taxa are accepted, whereas the remaining names are rejected or considered to be doubtful. A manuscript was prepared with the results of this compilation.

Together with Dr. M.R. Tansey (Indiana University, Bloomington, USA) keys to the thermophilic and thermotolerant fungi were prepared. These keys form part of a guide to species of fungi which can grow and sporulate at 45°C. The lists with 55 species and the keys were presented as a hand-out to accompany the poster session at the Second International Mycological Congress at Tampa (USA).

The investigations on the entomogenous fungi, collected by Dr. H.C. Evans in South America, were continued. Numerous specimens from Ecuador and the Amazon region were examined and identified. The material contained many *Cordyceps* species which did not fit any known species. Ascospore isolations of several *Cordyceps* species grew successfully in pure culture. *Sporodiniella umbellata* Boedijn, a mucoraceous entomogenous species, was rediscovered together with its zygosporic state. This species was originally described from insect remains in Indonesia and appeared to be rather common on Membracidae on cocoa farms in Ecuador. A manuscript with a neotype designation and a re-description of the species was prepared and submitted.

In co-operation with Dr. G.L. Benny (University of Florida, Gainesville, USA) a scanning electron microscopy study of sporangiole development in the genera *Radiomyces* and *Hesseltinella* was conducted. A detailed SEM study of the spore development in *Cunninghamella* supported the light microscopic and transmission electron microscopic observations that this structure is a monosporous sporangiole.

Some new and simplified preparation techniques for preparing fungal specimens for scanning electron microscopy were tested in co-operation with Dr. J. A. Stalpers and Mr. W. Verkerke. From the experiments it was concluded that fungal specimens were well fixed in either 6% glutaraldehyde (minimum 5 h) or 1% OsO₄ (minimum 2 h). Rapid chemical dehydration using 2-methoxy-ethanol proved to be very useful and time-saving.

During the tenure of a NATO-Fellowship awarded by the German Academic Exchange Service (DAAD), several studies of species of *Penicillium* and *Byssoschlamys* were carried out together with Dr. R.A. Samson.

The reinvestigation of *Penicillium* species used in cheese fermentation was completed. *Penicillium caseicola* is regarded as a synonym of *P. camemberti* on the basis of its morphological characters. The green colour of the colony of *P. camemberti*, regarded as the main difference between the species by Raper and Thom (A Manual of the Penicillia, 1949), proved to be unstable on media with a high saccharose-concentration.

A study of the *Penicillium janthinellum* series was initiated. The colony characters and morphology of many strains from the CBS collection were compared on Czapek and malt extract agars at 25°C. Several strains did not show the typical divaricate branching pattern of conidiophores in the *P. janthinellum* series, but were similar to members of the *P. citrinum* series. This led to a revision and a compilation of the *P. citrinum* series. Special attention was paid to the differentiation of the species, *P. citrinum* and *P. steckii*. Biochemical as well as morphological studies were made. Using the Bacterial Spectrum Test (after Raper and Thom, 1949), the production of antibiotics was observed in 14 out of 20 strains. Further tests were made to identify the antibiotic substances by thin layer chromatography. In addition to citrinin in strains of both species penicillic acid, its precursor orsellinic acid and citreoviridin were also identified.

The development of ascomatal initials and asci of *Byssoschlamys fulva*, *B. nivea* and *B. verrucosa* was investigated by means of scanning electron microscopy. The ascomata of *B. verrucosa* developed from a single coiled initial. *B. fulva* and *B. nivea* usually showed two initials twisting around each other. The further development of croziers and asci was similar for all three species. The ascomata were rarely covered by a thin layer of interwoven hyphae.

E.S. Hoekstra (Cand. Biol., State University, Utrecht)

Under supervision of Dr. R.A. Samson a taxonomic study of the *Cordyceps* and *Torrubiella* specimens, collected by Dr. H.C. Evans in Ghana, was carried out. The *Cordyceps* material contained eleven species. *C. australis* appeared to be very common on the ant *Palathothyreus tarsatus*, while *C. unilateralis* was regularly found on formicine ants. Other *Cordyceps* species from Ghana are *C. dip-terigena*, *C. lutea*, *C. nutans*, *C. polyarthra*, *C. uleana*, *C. tuberculata* and *C. rubripunctata*. In the last species the *Hirsutella* conidial state was found on the same stroma. Two other *Cordyceps* species could not be identified as known species and they are proposed as new species. The *Torrubiella* specimens contained about 15 forms. *T. arachnophila* proved to be common on Arachnidae. On Coccidae eight separate forms could be distinguished but the identification

of each form proved difficult because the taxonomy of the genus *Torrubiella* is still unclear. On Formicidae four *Torrubiella* species were recognized, two of which were identical with *T. carnata* and *T. liberiana* and two probably represent undescribed taxa.

A. Al-Musallam, M. Sc. (Kuwait University, Kuwait)

Under supervision of Dr. R.A. Samson, a revision of the species of the *Aspergillus niger*, *A. ochraceus* and *A. flavus* groups was started and the CBS strains of the species were re-examined and compared with recent isolates. These species are of economical importance in view of their potential biomedical hazards in contaminated food and their industrial use for the production of, for example, organic acids and enzymes. The identification of the species proved to be difficult due to the existence of strains with intergrading characters. Characters such as colour, growth rate and sclerotium formation are variable and we expect that an extensive comparative study of numerous strains should reveal the significance of these characters. A bibliographic study of the original descriptions was done to determine the systematic position of old and doubtful species.

Dr. M.A.A. Schipper

A paper was prepared as the final part of a monograph of the genus *Mucor*, following the foregoing Studies in Mycology 4, 10 and 12 on "*Mucor hiemalis* and related species", "*Mucor mucedo*, *M. flavus* and its related species" and "*Mucor circinelloides*, *M. racemosus* and related species", respectively. It includes a genus delimitation, a general key to cultured species, descriptions and drawings of 22 species and 2 varieties not yet dealt with in the former parts of the study, including the new taxa *Mucor amphibiorum*, *M. variosporus* and *M. zychae* var. *linnemanniae*. The results of extensive mating experiments are discussed.

In addition, a monograph was prepared of the genus *Rhizomucor* with the three thermophilic species *R. pusillus*, *R. miehei* and *R. tauricus*. The genus *Rhizomucor* in the family Mucoraceae contains thermophilic species with poorly developed stolons and rhizoids and dark coloured sporangia which lack apophyses. The zygosporic stage is of the usual *Mucor* type: zygosporangia covered with blunt projections borne in the aerial mycelium between equal suspensors formed opposite to one another. The genus differs from *Mucor* by a maximum temperature for growth at or above 50°C and a minimum at or above 20°C, and by the occurrence of stolons and rhizoids. The material and methods employed were the same as those used in the study of the genus *Mucor*. *Rhizomucor* has been a cause of much confusion. Lucet & Costantin (C. r. hebd. Séanc. Acad. Sci., Paris 129: 1033, 1899) erected the section, *Rhizomucor* in the genus *Mucor* to include *Mucor* species with rhizoids. However, *Rhizomucor* was soon treated as a genus name.

Vuillemin (Les champignons parasites et les mycoses de l'homme, p. 41, 1931) accepted the genus *Rhizomucor* and gave a description of the taxon with arguments for synonymy of *Mucor pusillus* Lindt (Arch. exp. Path. Pharmac. 21: 272, 1886), *Rhizomucor septatus* (Bezold) Lucet & Costantin (Archs Parasit. 4: 362, 1901) = *Mucor septatus* Bezold in Siebenmann (Schimmelmycosen des menschlichen Ohres, p. 97, 1889) and *Rhizomucor parasiticus* Lucet & Costantin (1899).

Subsequently the genus was rejected by most students of the family Mucoraceae. Hesseltine & Ellis (Mucorales - in: The Fungi, 4B: 187-217, 1973), however, re-established the genus, expanding the description with the additional characteristics of thermophily, dark coloured sporangia without apophyses and zygospores of the usual *Mucor* type.

Drs. J.A. Stalpers

A manuscript with keys for the identification of wood inhabiting Aphylophorales in pure culture has been completed and will appear as Studies in Mycology 16. About 550 species classified in 15 different families appear in the keys which are generally dichotomous, being subdivided into diagnostic and descriptive sections. At the end of a description there appears a species code consisting of 96 characters or circumscribed parts of characters, including microscopic, macroscopic, physiological, ecological and genetic features. This code, which serves as a synoptic key, is suited to be transferred onto edge-punched cards.

There are seven main keys based on the distribution of the clamp connections, the presence of laccase and some special structures such as skeletal hyphae and binding hyphae. The following groups are distinguished:

- A. Clamps absent from the main hyphae of the advancing zone, with narrower and clamped branches. In other parts of the colony some wide hyphae with clampless septa may be present.
- B. Clamps present at all primary septa. Laccase present. Branched and/or unbranched skeletal hyphae and/or cuticular cells and/or interlocking hyphae present.
- C. Clamps present at all primary septa. Laccase absent. Branched and/or unbranched skeletal hyphae and/or cuticular cells and/or interlocking hyphae present.
- D. Clamps present at all primary septa. Laccase present. Skeletal hyphae, cuticular cells and interlocking hyphae absent.
- E. Clamps present at all primary septa. Laccase absent. Skeletal hyphae, cuticular cells and interlocking hyphae absent.
- F. Multiple clamps present.
- G. Not all primary septa with clamps. These may be completely lacking or ab-

sent from some septa, but not as described under A.

The systematic position of some species, which are similar in culture but classified in different genera or families in the taxonomic literature, is discussed. *Hapalopilus rutilans*, *H. croceus*, *Tyromyces amarus*, *T. fissilis*, *Poria salmonicolor* and *P. mutans* are closely related and should not be placed in three different genera. *Globifomes graveolens*, *Haploporus cytisinus* and *Trametes robinophila* are closer to *Ganoderma* than several other species placed in the Ganodermataceae. The separation of *Laricifomes officinalis* from *Fomitopsis* and of *Buglossoporus pulvinus* from *Piptoporus* is supported by cultural characters. *Peniophora gigantea* seems to be closer to *Meruliopsis* than to *Phanerochaete*.

2. DIVISION OF BIOCHEMISTRY AND PHYSIOLOGY

Dr. G.W. van Eijk

The crystalline substance present in cultures of *Heterobasidion annosum* (= *Fomes annosus*) CBS 169.28 and 567.67 (CBS Progress Report 1976) was also detected in the strain CBS 141.25. Recently Dr. D. Donnelly and co-workers at Dublin (Tetrahedron Letters, 651, 1977) detected a new toxic metabolite, fomannoxin, in addition to the fomannosin in *H. annosum*. In co-operation with Dr. Donnelly investigations are being carried out to ascertain whether a structural or biochemical relationship between the above crystals and these two compounds does exist. It was found that strain CBS 834.72, which does not form crystalline material in the medium, only produces minute amounts of the other 2 metabolites. CBS 141.25 produces large amounts of fomannosin and small amounts of fomannoxin. Evidence has been obtained from thin-layer chromatographic analysis (TLC) that both CBS 169.28 and CBS 567.67 form fomannosin. These results may indicate some biochemical relationship between the crystalline compound and fomannosin. Data obtained from physico-chemical experiments suggest that structural relationships are absent.

Using TLC and UV-visible spectrometry, 23 strains of *Penicillium citrinum* and *P. steckii* (see Dr. C. Eckardt in this report) were screened for the production of secondary metabolites. Several strains produced citrinin. Surprisingly the yellow-coloured substance was identified as citreoviridin in 3 strains of *P. citrinum*. The presence of citreoviridin in cultures of *P. citrinum* has not yet been reported. The compound is also produced by *P. pedemontanum* CBS 343.52, CBS 378.65, CBS 408.65, CBS 409.65, CBS 265.65 and CBS 108.66 and by *P. myczinskii* CBS 220.28, CBS 263.29 and CBS 253.31. Citreoviridin could not be detected in cultures of *P. myczinskii* CBS 348.61.

Two strains of *Hendersonula toruloidea* isolated from man form yellow crystalline material on malt agar. Two main pigments, A and B, were isolated in a pure state from cultures of these strains. The UV-visible spectra of both compounds were identical indicating the same chromophoric system. Further

study (MS, IR and NMR) revealed that B is identical with 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone ($C_{14}H_{14}O_5$), a substance already known as metabolite from *H. toruloidea*. A molecular formula $C_{16}H_{16}O_7$ was determined for A by exact mass measurements. An extra CO absorption at 1742 cm^{-1} in the IR indicated the presence of an ester group in the molecule. Careful examination of the NMR spectrum of A in comparison with that of B, enabled us to elucidate the final structure. The compound A was identified as 2,7-dimethoxy-5-hydroxy-6-(1-methyloxycarbonyl)ethyl-1,4-naphthoquinone. Since the molecule has an asymmetric C atom, it was interesting to investigate whether or not the pigment showed optical activity. A specific rotation $[\alpha]_{589}^{25} = -46.0^\circ$ (c 0.3 in $CHCl_3$) was measured.

A *Phoma* species, recently identified as *Phoma rubefaciens* Togliani by G.H. Boerema, produces a red pigment which was identified as cynodontin. It was the first *Phoma* species that we found to produce cynodontin in our study of pigment formation by *Phoma* species.

A paper concerning the usefulness of metabolite analyses in relation to fungal taxonomy was presented at a symposium on chemotaxonomy during the 2nd International Mycological Congress.

Drs. A.C.M. Weijman

The study of the taxonomic application of fungal carbohydrate constitution was continued and extended to 377 strains. A paper was prepared on carbohydrate composition and taxonomy of the genus *Dipodascus*. The carbohydrates released during acid hydrolysis of intact cells were studied by gas-liquid chromatographic analysis. In addition, cells were characterized by pyrolysis gas-liquid chromatography and pyrolysis mass spectrometry in co-operation with Dr. H.L.C. Meuzelaar of the FOM-Institute of Atomic and Molecular Physics (Amsterdam). The data obtained support the classification of *Dipodascus uninucleatus* in a separate genus, *Dipodascopsis*, as proposed by Batra (in press).

Some biochemical aspects were contributed to a taxonomic study by Dr. G.S. de Hoog dealing with black yeast-like fungi (Stud. Mycol. 15: 1-140, 1977). Convincing evidence was obtained supporting the exclusion of *Cryptococcus nigricans* from *Cryptococcus* and its accommodation in the genus *Phaeococcus*. The presence of xylose indicates the basidiomycetous nature of *Cryptococcus*, whereas the absence of this component and the presence of galactose points to the ascomycetous affinity of *Phaeococcus*.

A paper concerning the carbohydrate composition and taxonomy of the genera *Geotrichum* and *Trichosporon* is in preparation. In our opinion, *Geotrichum* has to be restricted to galactomannan-containing species of ascomycetous affinity, whereas *Trichosporon* is suitable for the accommodation of xylose-containing basidiomycetous fungi.

Another application of the intact cell approach is a contribution to the elucidation of taxonomic affinities of the Endogonaceae to other major fungal

groups. Due to the absence of distinct morphologic features (e.g. zygospores, oogonia, antheridia) some chlamydo-spore genera, such as *Glomus*, can be included in the Zygomycetes or in the Oomycetes (Pirozynski and Malloch, Biosystems 6: 153-164, 1975). Since Zygomycetes and Oomycetes differ greatly in cell wall composition (chitinous versus non-chitinous walls), the demonstration of chitin can indicate the proper classification of a given strain. All representatives of *Endogone*, *Glomus* and *Glaziella* generously supplied by Dr. J.W. Trappe, were highly chitinous. These findings were confirmed by pyrolysis mass spectrometry experiments. This study will be extended when material of other genera of the Endogonaceae becomes available.

A useful improvement was achieved with the introduction of blood serum flasks to the growing, harvesting, washing, lyophilizing and storing of fungal cultures. The time-consuming and laborious transfer of material in different pieces of glassware is avoided and all manipulations are executed in a single flask.

3. DIVISION OF MEDICAL MYCOLOGY

Dr. G.A. de Vries

For the study of keratinophilic fungi and actinomycetes 60 soil samples were collected in S. and E. Flevoland. By means of the hair bait method the following keratinolytic species were isolated; a new species of *Anixiopsis* which will be described in the near future, *Arthroderma quadrifidum*, *A. uncinatum*, *Chrysosporium keratinophilum*, *Ctenomyces serratus*, *Gymnoascus reessii*, *Trichophyton terrestre* and a *Trichophyton* of which the species name is not yet known. The thermophilic/thermotolerant flora of these soil samples were also investigated. They contained great numbers of actinomycetes, among which many strains of *Thermoactinomyces*. *Aspergillus fumigatus* was the most common thermotolerant fungus. The other fungi were *Thermomyces lanuginosus*, *Malbranchea pulchella* var. *sulfurea* and *Mucor* species. From soil samples collected near some hot springs in Iceland, only *Aspergillus fumigatus* was isolated.

For the study of the conidial states of *Petriella* and *Petriellidium*, some new strains were investigated and added to the collection of experimental strains.

Drs. G.C.J. Muts concluded his investigations on sugar and fatty acid patterns and the stereoisomeric form of diaminopimelic acid of the cell walls of actinomycetes from the CBS collection. The results were used as arguments for a reclassification of some species. A manuscript entitled "Biochemical analysis of *Nocardia galtieri* (Goret & Joubert, 1951) comb.nov. and some other actinomycetes" was submitted to the International Journal of Systematic Bacteriology.

Liver, lungs and kidneys of some mice, *Sylvaemus sylvaticus*, *Microtus arvalis* and *Mus musculus*, killed in a mouse trap at Baarn, were macroscopically

examined in order to see if they contained spherules of *Emmonsia*. This proved not to be the case. Cultures made of the viscera of *Sylvaemus sylvaticus* remained sterile.

Dr. Søren Andersen at Odense, Denmark, sent 3 strains of fungi for the study of the pathogenic mycoflora of diseased dolphin skin. One of them was identified as an unusual *Ulocladium* close to *U. radicinum* and *U. lanuginosum*. A subculture was sent to Prof. E.G. Simmons at Amherst, Mass., USA, for further study. It was suspected by Dr. Andersen to be the causative agent of tinea-like skin lesions in *Phocaena phocaena*. The etiologic significance of the other strains, *Mucor hiemalis* f. *corticola* and *Phoma glomerata*, was regarded as very doubtful.

In collaboration with Dr. Samson comparative cultural experiments were carried out using 2 *Penicillium steckii* strains and 1 *P. marneffeii* strain as test organisms. *P. marneffeii* appeared to differ from *P. steckii* by the production of a soluble red pigment, as well as by its ability to grow in a yeast-like form at 37°C on enriched media.

4. DIVISION OF YEASTS (LABORATORY OF MICROBIOLOGY, TECHNICAL UNIVERSITY, DELFT)

Drs. L. Rodrigues de Miranda

In the beginning of 1977 a strain of *Candida lusitaniae* was received from Israel, isolated from citrus-peel juice. As this substrate was so different from the substrates from which *Candida lusitaniae* is usually isolated (warm-blooded animals), the possibility was considered that a strain of the opposite sex had been isolated. Mixing of the type strain with the freshly isolated strain on Difco malt agar, resulted in the conjugation of the cells and the formation of ascospores. The spores, 2-4 per ascus, were oblong with obtuse ends; one end being wider than the other. The asci ruptured on maturation, liberating the ascospores which tended to agglutinate. The perfect state of *Candida lusitaniae* fits well in the genus *Kluyveromyces* and will be described as *Kluyveromyces lusitaniae*. All available strains of *Candida lusitaniae* mated with either the strain from citrus-peel juice or the type strain of *Candida lusitaniae*.

The assimilation patterns of all strains of the genus *Cryptococcus* in the collection were determined. The two varieties of *Cryptococcus albidus* var. *albidus* and var. *diffluens*, will be placed together. The criteria used to separate these varieties were differences in the assimilation of lactose (absent in *diffluens*), D-galactose, α-methyl-D-glucoside and melezitose. Five strains of the variety *albidus* either do not assimilate lactose or assimilate it very weakly. The assimilation of galactose, α-methyl glucoside and melezitose is variable in both varieties. All species of the genus *Cryptococcus* were supposed to be imperfect states of hemibasidiomycetous fungi.

Some doubts had arisen concerning the species *Cryptococcus lactativorus*. Electronmicroscopic pictures were therefore made of ultra thin sections of the cell wall which proved to have an ascomycetous structure. Mating experiments were started and cells with evagination and many conjugating cells were observed on Yeast-malt agar and Difco-malt agar. To date no ascospores have been observed. The assimilation of D-gluconic acid is being tested and will probably replace the assimilation of inositol as the delimiting criterion for the genus *Cryptococcus*.

Drs. M. Th. Smith

The revision of the genera *Hanseniaspora* and *Kloeckera* has been completed. Eighty-two cultures of *Hanseniaspora* and *Kloeckera*, representing twenty-nine species, were examined for their morphological and physiological characteristics. In co-operation with Dr. S.A. Meyer at the American Type Culture Collection, the DNA base composition of sixty-seven cultures was determined and DNA reassociation experiments were undertaken to evaluate the status of the different *Hanseniaspora* and *Kloeckera* species and, further, to establish the relationship between the perfect *Hanseniaspora* species and their imperfect *Kloeckera* states.

As a result of this study, six distinct groups can be recognized:

- a. The *H. guilliermondii* group comprising 10 sporulating cultures and one non-sporulating strain, the type strain of *K. apis*. The group is characterized by assimilation of 2-keto-gluconate and growth at 37°C. Sucrose, maltose and glycerol are not assimilated; sucrose is not fermented. Sporulating cultures produce (1-)4 hat-shaped ascospores per ascus. The average GC content of the group is 33.4%.
- b. The *H. occidentalis* group with 4 sporulating and 8 non-sporulating cultures (including the type of *K. javanica*). The group is characterized by assimilation of sucrose and glycerol and fermentation of sucrose. Maltose and 2-keto-gluconate are not assimilated; no growth occurs at 37°C. Sporulating strains produce 1-2 ascospores per ascus which are smooth and round with an equatorial ledge. The average GC content of the group is 35.5%.
- c. The *H. osmophila* group with one sporulating (the type strain of *H. osmophila*) and 7 non-sporulating cultures (including the type of *K. corticis*). The group is characterized by assimilation of maltose. Glycerol and 2-keto-gluconate are not assimilated; assimilation of sucrose is variable. Growth at 34°C is negative. The sporulating culture produces 1-2 round and warty ascospores per ascus. The average GC content of the group is 40.3%.
- d. The *H. uvarum* group with 27 sporulating and 5 non-sporulating cultures (including the type of *K. apiculata*). The group is characterized by assimilation of 2-keto-gluconate. Sucrose, maltose and glycerol are not assimila-

ted; sucrose is not fermented. No growth occurs at 37°C. Sporulating cultures produce 1-2, round, warty and/or smooth ascospores per ascus, with an equatorial or sub-equatorial ledge. The average GC content of the group is 34.0%.

e. The *H. valbyensis* group with 5 sporulating and 5 non-sporulating cultures (including the type of *K. japonica*). Sucrose, maltose, glycerol and 2-keto-gluconate are not assimilated. Sucrose is not fermented. No growth occurs at 37°C. Sporulating cultures produce 1-4, mostly 1-2, hat-shaped ascospores per ascus. The average GC content of the group is 29.9%.

f. The *H. vineae* group with 6 sporulating and 3 non-sporulating cultures (including the type of *K. africana*). The group is characterized by assimilation of maltose. Glycerol and 2-keto-gluconate are not assimilated; assimilation of sucrose is variable. Growth at 37°C is positive. The sporulating cultures produce 1-2 round and warty ascospores per ascus. The average GC content of the group is 40.2%.

Detailed data of this study will be published in a joint publication in Antonie van Leeuwenhoek.

A taxonomic study of the genus *Brettanomyces* and its perfect counterpart, *Dekkera*, has been initiated. Morphological and physiological characteristics and DNA base composition of various strains present in the CBS yeast collection have been examined.

D. Yarrow

The DNA base compositions of some strains of *Saccharomyces* and *Zygosaccharomyces* were determined. Two values reported by Yarrow and Nakase (Antonie van Leeuwenhoek 41: 81-88, 1975) could not be confirmed, viz. those of *Z. bailii* CBS 680 and *Z. mrakii* CBS 4218.

	T _m (°C)	mole % GC
<i>Saccharomyces dairensis</i>		
CBS 6904	84.5 ± 0.1	37.1 %
<i>S. transvaalensis</i>		
CBS 2186	83.3 ± 0.07	34.1 %
CBS 2248	83.4 ± 0.13	34.4 %
<i>Zygosaccharomyces bailii</i>		
CBS 680	86.7 ± 0.05	42.4 %
CBS 685	87.3 ± 0.14	43.9 %
CBS 749	87.0 ± 0.23	43.2 %
CBS 1085	87.2 ± 0.22	43.7 %
CBS 1096	87.2 ± 0.18	43.7 %
CBS 1097	87.2 ± 0.12	43.7 %

<i>Z. mellis</i>		
CBS 736	85.7 ± 0.17	40 %
<i>Z. bisporus</i>		
CBS 1082	87.3 ± 0.17	43.9 %
<i>Z. rouxii</i>		
CBS 678	85.9 ± 0.17	40.5 %
<i>Z. cidri</i>		
CBS 4575	86.5 ± 0.09	41.9 %
<i>Z. mrakii</i>		
CBS 4218	85.9 ± 0.05	40.5 %

The differences between the species *Zygosaccharomyces rouxii*, *Z. bailii* and *Z. bisporus* are not all clear. Van der Walt (in the Yeasts, 1970) separated *Z. rouxii* from the other two species by its ability to use maltose but reported that this property could be acquired by strains that had been identified with the other species. From this it appears that the use of maltose is not a suitable characteristic to separate these species. Van der Walt also recognized two varieties by their ability to grow on 60% (w/v) glucose, *Z. bisporus* var. *mellis* and *Z. bailii* var. *osmophilus*. The %GC of the DNA of strains of these two varieties falls within the range of *Z. rouxii* (40-41%) and is lower than the range for *Z. bailii* var. *bailii* (42.4-43.9%) and *Z. bisporus* var. *bisporus* (43.7-44.1%). Moreover the type and most strains of *Z. bailii* var. *bailii* do grow on 60% glucose, though usually somewhat more slowly than the strains of the variety *osmophilus*. *Z. bailii* has been reported to be highly resistant to preservatives such as benzoic and sorbic acids. There appears to be a correlation between DNA base compositions, high resistance to sorbic acid and cell size which allows recognition of three taxa, *Z. rouxii* (syn. *Z. bailii* var. *osmophilus* and *Z. bisporus* var. *mellis*), *Z. bisporus* and *Z. bailii*. This will be investigated further.

Attempts were also made to trace Barker's original strain of *Z. barkeri*, the type species of the genus *Zygosaccharomyces*. There were two possible candidates: a strain deposited by Beijerinck with the CBS and another deposited by Harden with the NCYC. Unfortunately there was insufficient information available to establish with certainty that either of these is Barker's strain, therefore a neotype will have to be chosen. From the description this species could be equally well identified with *Z. rouxii* or *Z. bailii*. However, Barker also reported that after prolonged cultivation on malt agar, the fermentation of malt extract became more vigorous. This observation possibly indicates that this strain was adapting to maltose, a phenomenon observed in *Z. rouxii* but

not in *Z. bailii*. Therefore the strain of *Z. rouxii* CBS 732 will be proposed as neotype of *Z. barkeri*.

PUBLICATIONS 1977

- Arx, J.A. von, 1977 -- On *Yukonia caricis* and some other Ascomycetes. -- *Revue Mycol.* 41: 265-270.
- Arx, J.A. von, 1977 -- Ascomycetes as Fungi imperfecti. -- *Abstr. 2nd int. Mycol. Congr.* p. 23.
- Arx, J.A. von, 1977 -- The undifferentiated ascus. -- *Abstr. 2nd int. Mycol. Congr.* p. 22.
- Arx, J.A. von, 1977 -- Notes on Gymnoascaceae. -- *Persoonia* 9: 393-400.
- Arx, J.A. von, 1977 -- Notes on *Dipodascus*, *Endomyces* and *Geotrichum* with the description of two new species. -- *Antonie van Leeuwenhoek* 14: 333-340.
- Arx, J.A. von, Rodrigues de Miranda, L., Smith, M. Th. & Yarrow, D., 1977 -- The genera of yeasts and the yeast-like fungi. -- *Stud. Mycol.* 13: 1-42.
- Benny, G.L. & Samson, R.A., 1977 -- The Radiomycetaceae (Mucorales). -- *Abstr. 2nd int. Mycol. Congr.* p. 51.
- Eijk, G.W. van & Roeymans, H.J., 1977 -- Cynodontin, the tetrahydroxyanthraquinone of *Curvularia* and *Drechslera* species. -- *Experientia* 33: 1283-1284.
- Eijk, G.W. van, 1977 -- The role of pigments in the determination and classification of fungi. -- *Abstr. 2nd int. Mycol. Congr.* p. 701.
- Evans, H.C. & Samson, R.A., 1977 -- *Sporodiniella umbellata*, an entomogenous fungus of the Mucorales from cocoa farms in Ecuador. -- *Can. J. Bot.* 55: 2981-2984.
- Evans, H.C. & Samson, R.A., 1977 -- Entomogenous fungi from Ecuador. -- *Abstr. 2nd int. Mycol. Congr.* p. 183.
- Gams, W., 1977 -- Delimitation of some phialidic hyphomycete genera. -- *Abstr. 2nd int. Mycol. Congr.* p. 215.
- Gams, W., 1977 -- Taxonomic problems in *Mortierella*. -- *Abstr. 2nd int. Mycol. Congr.* p. 216.
- Gams, W., 1977 -- A key to the species of *Mortierella*. -- *Persoonia* 9: 381-391.
- Hermanides-Nijhof, E.J., 1977 -- *Aureobasidium* and allied genera. -- *Stud. Mycol.* 15: 141-177.
- Hoog, G.S. de, 1977 -- *Rhinocladiella* and allied genera. -- *Stud. Mycol.* 15: 1-140.
- Hoog, G.S. de, 1977 -- The black yeasts and related Hyphomycetes. -- *Abstr. 2nd int. Mycol. Congr.* p. 133.
- Hoog, G.S. de & Hermanides-Nijhof, E.J., 1977 -- Survey of black yeasts and allied fungi. -- *Stud. Mycol.* 15: 178-222.
- McCoy, C.W., Kish, L.P. & Samson, R.A., 1977 -- Entomopathogenic Deuteromycetes. -- *Abstr. 2nd int. Mycol. Congr.* p. 428.

- Meyer, S.A., Brown, R.E. & Smith, M. Th., 1977 -- Species status of *Hanseniaspora guilliermondii* Pijper. -- *Int. J. syst. Bact.* 27: 162-164.
- Oorschot, C.A.N. van, 1977 -- The genus *Myceliophthora*. -- *Persoonia* 9:401-408.
- Samson, R.A. & Evans, H.C., 1977 -- Notes on entomogenous fungi from Ghana IV. The genera *Paecilomyces* and *Nomuraea*. -- *Proc. k. ned. Akad. wet., Amsterdam* 80: 128-134.
- Samson, R.A., Hadlok, R. & Stolk, A.C., 1977 -- A taxonomic study of the *Penicillium chrysogenum* series. -- *Antonie van Leeuwenhoek* 43: 169-175.
- Samson, R.A., Eckardt, C., & Orth, R., 1977 -- The taxonomy of *Penicillium* species from fermented cheeses. -- *Antonie van Leeuwenhoek* 43: 341-350.
- Samson, R.A., Jack Grisman, M. & Tansey, M.R., 1977 -- Observations on the thermophilous ascomycete *Thielavia terrestris*. -- *Trans. Br. mycol. Soc.* 69: 417-423.
- Samson, R.A. & Mahoney, D.P., 1977 -- *Talaromyces galapagensis* sp.nov. -- *Trans. Br. mycol. Soc.* 69: 158-160.
- Samson, R.A. & Stalpers, J.A., 1977 -- Preparation of fungal specimens for scanning electron microscopy. -- *Abstr. Najaarsverg. Ned. ver. Electronenmicroscopie* p. 65.
- Samson, R.A. & Tansey, M.R., 1977 -- Guide to thermophilic and thermotolerant fungi. -- *Abstr. 2nd int. Mycol. Congr.* p. 588.
- Schipper, M.A.A. & Arx, J.A. von, 1977 -- Status of collections of fungus cultures in Europe. -- *Abstr. 2nd int. Mycol. Congr.* p. 596.
- Smith, M. Th., Simione, Jr., F.P. & Meyer, S.A., 1977 -- *Kloeckera apis* st. nov., the imperfect state of *Hanseniaspora guilliermondii*. -- *Antonie van Leeuwenhoek* 43: 219-223.
- Stalpers, J.A., 1977 -- Similarities of cultural characters in several families of the *Aphylliphorales*. -- *Abstr. 2nd int. Mycol. Congr.* p. 630.
- Tio, T.H. & Vries, G.A. de, 1977 -- Subcutaneous *Zygomycosis* (*Phycomycosis*). -- *S.A. Mediese Tydskrif* 52: 77-78.
- Vries, G.A. de, 1977 -- Taxonomy of the pathogenic fungi: its multidisciplinary nature and current status. -- *Proc. 6th Congr. ISHAM* pp. 259-263 (1975).
- Vries, G.A. de, 1977 -- Contribution à la connaissance des champignons hypogés de la Belgique. -- *Lejeunia, n. Sér.*, 86: 1-16.
- Weijman, A.C.M., 1977 -- The application of Curie-point pyrolysis mass spectrometry in fungal taxonomy. -- In: C.E.R. Jones and C.A. Cramers (Ed.), *Analytical Pyrolysis*, pp. 225-233, Elsevier Sci. Publ. Co., Amsterdam.
- Weijman, A.C.M., 1977 -- Carbohydrate composition and taxonomy of the genus *Dipodascus*. -- *Antonie van Leeuwenhoek* 43: 323-331.
- Weijman, A.C.M., 1977 -- The application of cell-wall and intact cell analysis in fungal taxonomy. -- *Abstr. 2nd int. Mycol. Congr.* p. 726.

**Hubrecht Laboratory
International
Embryological Institute**

Progress Report 1977

Hubrecht Laboratorium,
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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 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 under Scientific Staff below).

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

The Laboratory houses the Central Embryological Library (collection of reprints covering the field 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.

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INTRODUCTION

The central theme of the research carried out at the Laboratory is *the origin of patterns of cellular differentiation*. This theme is being approached on three different *levels of biological organisation*: the morphogenetic, the cellular, and the molecular level. This report is therefore organised on the basis of these three levels of approach, represented by sections I-III.

In reading the report two things should be borne in mind: (1) there is always overlap between levels of organisation; (2) research areas are classed under a given level on the basis of the fundamental problems that are primarily being studied, not on the basis of the methods used; therefore, research placed under a particular level will often be found to use methods belonging in part to other levels.

I. MORPHOGENETIC LEVEL

This section describes research in which the *main approach* is aimed at the morphogenesis of the organism as a whole or of certain of its parts, even though much of the research is on eggs, blastomeres and specific cell types.

A. EMBRYOGENESIS IN AMPHIBIANS: ORIGIN AND ESTABLISHMENT OF EMBRYONIC AXES; ORIGIN OF PRIMORDIAL GERM CELLS

The two major foci of interest in this subsection are: (1) the analysis of regional differences in the fertilised egg and the cleavage and gastrula stages, in connection with the establishment of the future dorso-ventral and antero-posterior axes of the embryo and with pattern formation in the mesoderm; (2) the origin of primordial germ cells in anurans and urodeles as an expression of the pattern of differentiation in the early embryo.

i. The origin of bilateral symmetry in the egg

In the period between oviposition and first cleavage, pigment shifts in the superficial cytoplasm of the fertilised amphibian egg lead to the formation of the grey crescent and the consequent external symmetrisation of the egg. Internally, in the eggs of certain anurans a central region of "clear cytoplasm" is present shortly after fertilisation (see previous reports). Prior to first cleavage this cytoplasm is progressively displaced towards the future dorsal side of the egg (and is then called the "dorsal cytoplasm"). This is a constant phenomenon and is the first internal morphological sign of bilaterality. It may be related to the final bilaterality of the gastrula, which is foreshadowed by the establishment of dorso-ventral polarity in the endodermal yolk mass of the blastula (see introduction to sect. iii below).

1. *Cinematographic observation of an "activation wave" on the surface of the fertilised egg (*Xenopus laevis*)* (K.Hara, P.Tydeman)

Eggs were fertilised using a new method of local artificial insemination

(see sect. IV.1 below). The eggs were filmed simultaneously from above and from the side. Although the exact moment of sperm entry cannot be determined, at 5-8 min after the addition of sperm the first visible reaction appears on the films in the form of a light zone moving as a wave away from the sperm entrance point. This wave is called the "activation wave" (AW).

The AW travels to the opposite side of the egg at a speed of ca. 0.5 mm/min, which is ten times faster than the subsequently occurring "post-fertilisation waves" (PFWs) and "surface contraction waves" (SCWs) (see previous reports and sect. VI, refs. 7,8). After the passage of the AW the pigment cap of the egg contracts ("activation contraction"), the egg rotates under gravity, and the cap relaxes again before the PFWs occur. The AW most probably reflects the propagation of cortical granule breakdown.

2. *The correlation between surface phenomena and internal cytoplasmic displacements in the fertilised (monospermic) egg (Xenopus laevis)* (G.A. Ubbels, K.Hara, P.Tydeman)

The superficial pigment shifts involved in grey crescent formation and the concomitant internal cytoplasmic displacements are not necessarily causally related (see previous report, sect. I.A.i.2 and this report, ref.27). However, recent cytological observations correlated in time with separately made cinematographic observations show that the occurrence of the two "post-fertilisation waves" (PFWs, see ref.7) coincides temporally with changes in the orientation of fibrillar structures in the segregated cytoplasm (ref.29). Thus the two PFWs may in some way be connected with the internal cytoplasmic displacements. This is supported by the observation that eggs activated artificially by tap water or salt solutions reveal neither PFWs nor an eccentric displacement of the segregated cytoplasm. That a grey crescent is formed in such eggs suggests that the PFWs are not essential for external symmetrisation.

A study has recently been started combining cinematographic analysis with cytological and cytochemical analysis of eggs from the same batch developing synchronously.

3. *Pigment displacements in polyspermically fertilised eggs (Xenopus laevis)* (K.Rzehak, G.A.Ubbels)

It has been found that the superficial pigment displacements differ between monospermic and polyspermic anuran eggs. Whereas Herlant and Brachet (1910-1912) found that in *Rana temporaria* eggs fertilised by more than one sperm can be externally distinguished only during first or second cleavage, in *Xenopus* polyspermy can be recognised as soon as the darkly pigmented points of sperm entrance become visible, i.e. shortly after the initial "activation contraction" of the animal pigment cap (see previous report, sect. I.A.i.2 and this report, ref.27). A further local concentration of pigment then takes

place around each sperm entrance point. Since areas where the pigment accumulates are very often seen to be thrown into folds, we suggest that pigment concentration is connected with local contraction of the cortex. As the areas in which the pigment accumulates increase in size, the surface from which the pigment is shifted away becomes almost as light as the surface of the vegetal hemisphere.

In dispermically fertilised eggs the grey crescent is always formed opposite a point half-way the shortest distance between the two points of sperm entrance. In eggs containing more than two sperms the surface between the areas of pigment accumulation is usually so poor in pigment that the position of the grey crescent cannot be established with certainty.

4. *The possible factors involved in cytoplasmic movements in the fertilised egg (Xenopus laevis)* (G.A.Ubbels, K.Rzehak)

This work was started in 1975 (see report for 1975, sect. I.C.1) and partially continued in 1976 (see previous report, sect. I.A.i.3). Its aim is to test the hypothesis that in the anuran egg the entrance of the sperm evokes and/or activates a kinetic system that is responsible for the dorsal displacement of the "clear cytoplasm" (CC), and to study the kinetic system itself more closely.

In dispermically fertilised eggs, where the mitotic spindles tend to lie parallel to each other, the CC is always displaced to one side and comes to occupy a position opposite a point half-way the shortest distance between the two sperm entrance points, i.e. in the region where the grey crescent is formed in such eggs (see 3 above). Preliminary observations suggest that in trispermic eggs, where the long axes of the mitotic spindles often form a triangle, the CC is also displaced to one side and comes to lie opposite a point between two of the three sperm entrance points. In strongly polyspermic eggs (8-15 sperms), in which multipolar spindles are often found, the CC remains centrally located.

The chemical nature of the fibrillar structures present in the CC (see previous report, sect. I.A.i.4a), which are conceivably involved in its dorsal displacement, is being characterised further by means of fluorescent antibody techniques.

5. *Electron microscopy of the "dorsal cytoplasm" in the fertilised egg (Xenopus laevis)* (G.A.Ubbels, A.R.J.Bleumink)

This work is a continuation of that described in the previous report (sect. I.A.i.4b). At that time no fibrillar components could be demonstrated in the dorsal cytoplasm at 60 min post-fertilisation with the ultrastructural techniques used. The search for them is being continued at the same and earlier stages, using an improved preservation method which enhances contrast of sub-cellular structures.

The dorsal cytoplasm contains many cytoplasmic vesicles. Whether their presence is related with the formation of the asters and/or the mitotic apparatus is being investigated at 70 min post-fertilisation.

6. *The morphogenetic significance of the "dorsal cytoplasm" (Xenopus laevis)*
(J.H.Cleine)

This work is a continuation of that by R.T.M.Hengst described in the previous report (sect.I.A.i.5). Its purpose is to test whether the mitochondria and cytoplasmic vesicles present in abundance in the "dorsal cytoplasm" (see previous report, sect.I.A.i.4b) may have a morphogenetic function in the establishment of the dorso-ventral axis of the egg and embryo.

To this end pellets consisting of mitochondria and cytoplasmic vesicles (0.2-1.0 μ m in diameter) are obtained by fractional centrifugation of eggs and blastulae ranging from just after fertilisation to stage 7 (N.& F.). This material is then injected ventrally into uncleaved eggs at 30-60 min post-fertilisation. Only preliminary results have been obtained so far.

ii. The state of dorso-ventral polarisation in early cleavage stages

This work is a continuation of that described in the report for 1975 (sect. I.A.1; see also previous report, sect.I.A.ii.1a). Its aim is to ascertain whether there are morphogenetic differences between dorsal and ventral blastomeres, either in the animal or in the vegetative portion of the embryo, or in both. Such differences could be the expression of the internal bilaterality of the uncleaved egg (see i above) and could play a role in the establishment of the later dorso-ventral polarity of the endodermal yolk mass with respect to mesoderm induction (see introduction to sect.iii below).

1. *Dissociation and re-association of blastomeres of early embryos (Xenopus laevis)* (H.Takasaki, K.Hara)

Fertilised eggs were dejellied and placed in 1/15 M phosphate buffer solution (pH 7.8) with the vitelline membrane intact. Cleavage proceeded at the normal rate but the blastomeres lost their normal contacts. When the vitelline membrane was subsequently removed the embryo fell apart into single blastomeres. When the vitelline membrane was left intact and the embryos were returned to normal Holtfreter solution before the 64-cell stage, they developed into normal larvae. In contrast, blastomeres isolated after vitelline membrane removal at the 32-cell stage or earlier and re-associated in normal Holtfreter developed poorly due to early arrest of cleavage.

In order to obtain better development of blastomere recombinates, animal blastomeres isolated by the above method were re-associated with vegetative blastomeres isolated surgically, or *vice versa*. Such recombinates formed abnormal embryos of various kinds. The results are being analysed in Japan.

iii. Mesoderm induction; regional differentiation of mesoderm; neural induction

In the amphibians the mesoderm arises from the totipotent animal ("ectodermal") moiety of the blastula as a result of an inductive action emanating from the vegetative yolk mass (the future endoderm). The inductive capacity is strongest in the dorsal portion of the yolk mass and markedly declines in its lateral and ventral portions. This dorso-ventral polarisation of the presumptive endoderm accounts for the greater width of the induced mesodermal marginal zone on the dorsal side in the late blastula, and probably also for the regional pattern of mesodermal differentiation in later stages.

1. *The extension of mesoderm induction into the ectodermal cap of the blastula and early gastrula (Ambystoma mexicanum) (E.C. Boterenbrood)*

This work is a continuation of that described in the report for 1974 (sect. I.B.4). At that time experiments were performed to analyse the extent of mesodermisation of the animal moiety of the embryo at successive stages of mesoderm induction by the vegetative yolk mass. To enhance the differentiation of the mesoderm the dorsal halves of two isolated animal caps were fused together with their animal-vegetative axes in line, and cultured for 8-10 days. Embryos were used of stages 7 to 10⁻ (Hara & Boterenbrood, see ref.6).

The explants have now been analysed qualitatively. They show considerable differentiation of dorsal mesodermal structures. Apart from mesoderm, pharyngeal endoderm is also formed; it is abundant in explants of later stages but scarce or absent in explants of stage 7. In normal development the most vegetative part of the presumptive notochordal area in the marginal zone acquires differentiation tendencies for pharyngeal endoderm. This "endodermisation" is assumed to be the ultimate step in the process of meso-endoderm induction ("vegetalisation", Nieuwkoop & Ubbels, 1972). The above results suggest that it takes place already shortly after stage 7.

The quantitative analysis of mesoderm differentiation in these explants is in progress.

2. *The possible relation between the regional pattern of mesoderm differentiation and regional quantitative differences in mesoderm-inducing capacity in the endoderm (Ambystoma mexicanum, Triturus alpestris) (E.C. Boterenbrood, J.Narraway)*

This work is based on experiments carried out last year with T.W.Suryono (see previous report, sect. I.A.iii.2, third par.). The problem studied was whether purely quantitative differences in mesoderm induction can lead to regional differences in mesoderm differentiation. In last year's experiments the effective period of mesoderm induction was varied by making recombinates of the dorsal part of the vegetative yolk mass with ectoderm of successively

older gastrula stages, making use of the fact that the mesodermal competence of the ectoderm is known to disappear towards the middle of the gastrulation period.

The analysis of the recombinates has now shown that ventral mesodermal structures appear after a short effective period of induction, while longer periods of induction result in the additional formation of mesoderm which is increasingly more dorsal in character. This suggests that in normal development the dorso-ventral regionality of mesodermal differentiation may indeed be due to regional quantitative differences in mesoderm-inducing capacity in the endoderm.

Because in the *Ambystoma* material used the frequency of ventral mesodermal structures was too low to allow very clear conclusions, the experiments were repeated with material of *Triturus alpestris*, which is known to give better differentiation of ventral mesodermal structures under experimental conditions. These recombinates are being analysed.

3. *The effects of inhibitors of RNA and protein synthesis on neural induction in vivo (Ambystoma mexicanum) (M.Sala)*

Neural induction in amphibians is assumed to occur in phases, during which the inductive factors are first formed in the archenteron roof and then released stepwise to the overlying competent ectoderm. In this study actinomycin D and puromycin were injected, first into the blastocoel and later into the gastrocoel, at various times during the induction process (stages 10, 10½, 10¾ and 13½ H.). The gross morphological effects were recorded photographically and the embryos were fixed at regular intervals. They are being studied histochemically in Italy.

iv. The origin of the primordial germ cells

Research carried out in this Laboratory since 1973 (see previous report, sect. I.A.iv) has shown that the origin of the primordial germ cells (PGCs) in anuran and urodelan amphibians is fundamentally different. In the urodeles the PGCs do not arise from special cells in the endoderm but from common totipotent cells of the animal ("ectodermal") moiety of the blastula; they are a normal constituent of the mesoderm that is induced by the presumptive endoderm (see introduction to sect.iii above). An uninterrupted "germ line" such as is taken to exist in anurans is not found here. This finding has led to a renewed interest in the problem of germ cell origin. One aspect of this problem is whether the so-called germinal cytoplasm found in amphibian PGCs plays a role as a germ cell determinant.

1. *Time and mode of determination of primordial germ cells in urodeles (Ambystoma mexicanum) (P.D.Nieuwkoop; A.Sutasurya, Bandung)*

Last year three series of experiments were carried out in which the presump-

tive or definitive, ventral or lateral mesoderm of gastrulae and neurulae was explanted alone or combined with ventral endoderm as potential inductor (see previous report, sect. I.A.iv.1). A preliminary analysis of the experimental material in Bandung has now shown the following:

(1) The formation of PGCs as part of the induction of the entire mesoderm by the vegetative yolk mass is a precocious event that (at least in *Ambystoma*) is independent of later local inductive influences from the ventro-caudal endoderm. (2) In contrast, blood cell formation in the ventral mesoderm seems to be due to a local inductive effect of the ventro-caudal endoderm during late gastrulation and early neurulation. (3) Ventro-caudal endoderm does not seem to be able to neutralise the inhibitory effect of the notochordal anlage on PGC formation, which is thought in normal development to be responsible for the restriction of PGC formation to the ventral marginal zone.

2. *Formation of primordial germ cells in xenoplastic recombinates of anuran and urodelan material* (P.Michael)

To throw more light on the strongly divergent modes of origin of the PGCs in anurans and urodeles, experiments have been started in which animal, ectodermal caps of anuran blastulae are recombined with vegetative yolk masses of urodelan blastulae, and *vice versa*. According to our present hypothesis the former type of recombinant is expected to be sterile, while the latter will contain both anuran and urodelan PGCs. Material of several different anuran and urodelan species is being tested to find the most viable combinations.

3. *Book on primordial germ cells in the Chordates* (P.D.Mieuwkoop, A.Sutasurya)

This year our main scientific activity has been the writing of a monograph on PGCs in the Chordates at the request of Cambridge University Press. This considers both embryological and phylogenetic aspects of the subject. The manuscript is completed.

4. *The effect of UV-irradiation on the germ cell lineage (*Xenopus laevis*)*
(K.E.Dixon)

UV-irradiation of the vegetative hemisphere of anuran eggs or early embryos causes serious disruption to the germ cell lineage (see Züst and Dixon, 1977). We have addressed the question whether this disruption is due to specific effects on the germinal cytoplasm in the egg (the putative germ cell determinant) or to more general effects on embryogenesis.

4a. *Cinematographic observations on cleavage and gastrulation in irradiated embryos* (with K.Hara and P.Tydeman)

We envisage the possibility that UV-irradiation primarily disrupts morphogenetic processes of cleavage and gastrulation, and that this disruption only secondarily leads to sterility. Uncleaved eggs were irradiated from the veg-

etative side. Each egg was placed next to an unirradiated control egg and the pairs were filmed from above and below from first cleavage till the end of gastrulation. During the period of synchronous cleavage the vegetative blastomeres of irradiated embryos showed disturbances in cleavage furrow formation. The start of gastrulation was delayed and gastrulation was often temporarily blocked by extrusion of the yolk plug or by expulsion of the already invaginated endodermal mass.

The temporal cleavage pattern of the animal blastomeres was not affected during the period of synchronous cleavage. However, during the subsequent period of asynchronous cleavage the cleavage cycles were lengthened as compared with the control embryos.

Whether these morphogenetic disturbances are related to ultimate sterility is still unknown.

4b. Other studies on the UV effect

Eggs were irradiated in such a way that the region of the cytoplasm containing the putative germinal determinant was shielded from UV. The germ cell lineage of the resulting embryos will be compared with that of embryos developed from eggs irradiated in the usual way.

The effects of UV on cleavage could be mimicked by injections of a Ca^{++} buffer. The resulting embryos will be examined to see whether this treatment also causes disruption to the germ cell lineage.

These studies are being continued in Australia.

II. CELL-BIOLOGICAL LEVEL

This section describes research in which the main approach is aimed at the cellular aspects of development (cell ultrastructure, cell interactions, cell behaviour, etc.), even though most of the research has distinct morphogenetic implications.

A. EPITHELIAL-MESENCHYMAL INTERACTIONS IN MAMMALIAN ORGANOGENESIS

The pattern in which a branching epithelium develops, such as that of the salivary gland, lung or kidney, is characteristic for each organ and can be described in terms of points of branching and growth of the epithelium. It has long been known from organ culture experiments that the investing mesenchyme is essential for both growth and morphogenesis of these epithelia. Recombinates of epithelium and mesenchyme are being used to investigate branching morphogenesis *in vitro*.

1. *Transfilter tissue interaction in lung morphogenesis (Mus musculus)* (K.A. Lawson, W.A.M. van Maurik, J.G. Bluemink)

Intimate cell contacts between mesenchyme and epithelium and discontinuities in the basal lamina are present at the distal ends of the outgrowing lung pri-

mordium, i.e. in the region where new buds, and thus branch points, are being formed (see previous report, ref.1). The possibility that such contacts are necessary for branching morphogenesis is being examined by means of transfilter cultures, using Nuclepore filters (see previous report, sect.II.A.1).

1a. Fine structure of transfilter cultures

Epithelium responding transfilter to mesenchyme forms basal as well as lateral clefts. The clefts are lined with Alcian Blue-staining material (acid glycosaminoglycans) and frequently contain fibrillar (collagen) material. At the periphery of the clefts cytoplasmic processes traversing the filter from the mesenchyme can sometimes be found in intimate association with the epithelium where the basal lamina is discontinuous. Much fragmented cytoplasm is found in the clefts; such debris is never found in transfilter cultures where whole mesenchyme cells crawl through the pores (0.3 μm pore diameter). It is hoped to identify the origin of this material with an immunological technique.

1b. Correlation of epithelial response and cytoplasmic penetration of the filter pores

Epithelial response to mesenchyme transfilter is quantitatively reduced with decreasing pore diameter below 0.8 μm . Epithelium regularly forms clefts on 0.2 and 0.4 μm filters but only very occasionally on 0.1 μm filters. Epithelium is not maintained transfilter on 0.05 μm pore filters, although intact organ rudiments develop normally on these filters. Counts (in transverse sections) of the percentage pore openings containing cytoplasmic processes indicate that fewer of the mesenchymal processes penetrating the underside of the filter emerge at the upper side when this is covered with epithelium. Counts on the upper side of 0.2 and 0.1 μm filters are too low for reliability. Preliminary results from scanning electron microscopy show that (in the absence of epithelium on top of the filter) ca. 5% of the upper surface of 0.1 μm filters is covered by cytoplasmic extensions from mesenchyme cultured on the underside, as against ca. 12% when 0.2 μm filters are used. A method is being developed to examine the situation under epithelium on 0.2 and 0.1 μm filters, where complete penetration is expected to be very sparse or very localised.

2. Specificity of mesenchyme requirement for branching morphogenesis (*Mus musculus*, *Rattus norvegicus*) (K.A.Lawson, J.G.Stroop-Wijchman)

If the mechanism of branching morphogenesis is basically the same in different organs, a degree of interchangeability among mesenchymes of different organs could be expected. Although it was long held that there is a high degree of specificity in mesenchyme requirement for salivary, lung and kidney development, we have shown earlier that lung mesenchyme is able to support salivary epithelial development (Lawson, 1974) and that salivary mesenchyme can support lung epithelial development (see previous reports). Recombinates of both rat

and mouse material have now shown that both submandibular and lung mesenchyme are able to support growth and branching of the ureteric bud. However, no conditions have been found in which metanephric mesenchyme supports morphogenesis of salivary or lung epithelium.

As described in the previous report (sect.II.A.2), the ability of lung epithelium to develop in salivary mesenchyme has been exploited to investigate which tissue component controls the branching pattern of the epithelium. This experiment has been refined and repeated and the conclusion that the mesenchyme controls the timing and position of new branch points has been confirmed.

B. REGIONAL DIFFERENCES IN THE PLASMA MEMBRANE OF THE AMPHIBIAN EGG

Experimental evidence suggests that the distribution of cytoplasmic components in uncleaved, fertilised eggs of different animal groups may depend on the cortex, i.e. the plasma membrane and its associated structures (Dollander, 1961, Pasteels, 1964, Arnold and Williams-Arnold, 1974). Accepting the idea that a cortical pattern of information can be set up and maintained for some time in the form of an anisotropic organisation of the plasma membrane, it would be of interest to know whether regional differences in plasma membrane composition exist.

1. Freeze-fracture electron microscopy of the plasma membrane of the fertilised egg (*Xenopus laevis*) (J.G.Bluemink, L.G.J.Tertoolen)

1a. The particle pattern of the plasma membrane as related to animal-vegetative polarity

The aim of this study was to compare the animal and vegetative hemispheres of the fertilised, uncleaved *Xenopus* egg. Freeze-fracture electron microscopy of the plasma membrane shows that intramembranous particles (IMPs) range in size from ca. 50 to 200 Å, and that more IMPs are attached to the E-face than to the P-face (see also report for 1975, sect.V.2a and previous report, ref.2). IMP-free regions (diameter ca. 0.1 µm) on the tips of surface protrusions (E-face) are irregularly distributed and occupy ca. 8.5% and 2% of the total area in the animal and vegetative half, respectively. The overall IMP densities (E-face) of the animal (1934 ± 262 IMPs/µm²) and the vegetative hemisphere (1872 ± 229 IMPs/µm²) do not differ significantly ($P > 0.1$). The relative densities of IMPs of 16 different size classes were compared in seven animal and seven vegetative halves, counting ca. 10,000 IMPs in each hemisphere. For IMP sizes of ≤ 81 Å a significant difference ($P < 0.0005$) was found, more small IMPs being present in the animal half.

The complementary P-faces have many small pits (the "negatives" of the IMPs on the E-face) but only a small number of IMPs (ca. 100 IMPs/µm²). A comparison of P-faces obtained from the animal and the vegetative hemisphere showed

no difference in IMP density.

The results have been submitted for publication (see ref.17). The animal-vegetative anisotropy observed in the *Xenopus* egg may represent a phase-specific topographical pattern of surface components (cf. McMahon and West, 1977) which is under homeostatic control. Whether or not plasma membrane anisotropy is related to the distribution of cytoplasmic components is open to further investigation.

1b. Evidence for particle-associated filaments

In freeze-fracture electron micrographs of the plasma membrane the IMPs are not simple globules. Filament-like extensions give a spiny appearance to the profiles of many IMPs. Occasionally longer filaments (IMPf's) are seen to extend from an IMP or, more rarely, to run from one IMP to another. They are ca. 20 Å thick. This finding has been submitted for publication (see ref.16).

In the amphibian egg the plasma membrane is intimately associated with an intricate cortical filament network. The apposition of a microfilamentous network to the plasma membrane could provide the rigidity needed to withstand stress forces during morphogenesis and to maintain cell surface topography. Putative linkages between intrinsic membrane proteins and elements of the cytoskeleton have been suggested in the literature. The IMPf's might represent the thin elements of the anastomosing cortical network, drawn out from the cytoplasm during freeze-fracturing. Alternatively, they may be embedded in the hydrophobic core of the lipid bilayer. Whatever their nature, they are probably instrumental in creating long-range stability in the plane of the membrane.

C. MORPHOGENESIS AND PATTERN FORMATION IN CELLULAR SLIME MOULDS

The cellular slime mould *Dictyostelium discoideum* (Dd) has been chosen as a model system for identifying general rules for cell behaviour underlying morphogenesis and pattern formation. We are concerned with understanding the later (post-aggregation) development of this organism: development of the multicellular slug leads to the formation of a fruiting body containing a proportionate pattern of two cell types (stalk cells and spores).

1. Identification of rules controlling cell movement and cell contact (*Dictyostelium discoideum*) (A.J.Durston, C.Weinberger, S.Matsukuma, F.C.Vork)

Last year we developed a vital staining method (using neutral red) which enabled us to visualise movements of individual cells in later Dd structures (see previous report, sect.II.C.2a). This method, in conjunction with time-lapse filming, has permitted detailed observations of cell movement and cell contact during later Dd development (see ref.18).

All later stages show periodic waves of cell movement (period 3-10 min,

velocity about 50 $\mu\text{m}/\text{min}$). These resemble waves seen during the aggregation stage of Dd development. This suggests that two cell competences that control cell movement during aggregation (chemotaxis and signal relaying, both in response to 3',5', cyclic AMP) persist throughout later development (see previous report, introduction to sect.II.C.2). We are investigating this point further. The waves show specific geometries determined by developmental stage. They are important for all later morphogenetic movements and pattern formation. The Dd tip is a pacemaker for wave initiation throughout development. This accounts, in part, for its properties as an organiser.

Cells in all later stages are connected head-to-tail in files. This suggests that the so-called contact sites A, which connect cells end-to-end in the aggregation stage, also connect later cells. Cell files are oriented along the slug's long axis and account for certain polar properties of later stages.

Under certain conditions, neutral red is a specific stain for the prestalk cell (see 2b below). Slugs therefore often show an axial pattern of neutral red staining. We detected two interesting features with respect to this pattern. (1) Stained cells continually move backward from the anterior prestalk zone and accumulate in the rear end of the slug. We suspect, as did Bonner (1951), that this process leads to the formation of the (stained) rearguard zone of the slug. (2) Unstained (prespore) tissue is often paralysed. The details of paralysis suggest that this occurs due to a response to a super-threshold concentration (or other parameter) of a substance which is present as an axial gradient in the slug. Prespore cell paralysis plays a vital role in certain morphogenetic movements (e.g., rounding-up prior to fruiting body formation). It is also important in pattern formation. When the anterior prestalk zone is removed from a slug, the remaining posterior portion regulates and makes a new prestalk zone (and a new tip). During this process, stained (prestalk) cells, which were initially scattered throughout the unstained tissue, sort out and aggregate to form the prestalk zone. During sorting-out, prespore tissue is paralysed and the prestalk cells move periodically, as in aggregation. We are investigating the mechanism of prespore cell paralysis.

2. Identification of rules controlling cell differentiation (*Dictyostelium discoideum*) (A.J.Durston, F.C.Vork)

2a. Immunofluorescence studies (with S.K.Brahma, Lab. of Anat. and Embryol., State Univ. of Utrecht, and P.Poot)

We acquired an immunofluorescent staining technique which reliably distinguishes prestalk from prespore cells. A rabbit antiserum is prepared against freeze-dried *Dictyostelium mucoroides* spores (similarly as by Takeuchi, 1963). This binds to appropriately fixed prespore cells of Dd but not to prestalk cells. The prespore cells are then stained with FITC-goat anti-rabbit IgG. We are now employing this stain to investigate the control of cell differentiation.

2b. Ultrastructural studies (with J.G.Bluemink and W.A.M.van Maurik)

In continuation of the study mentioned in section II.C.3 of the previous report, we have found that the neutral red-staining organelle is an autophagic vacuole which is virtually cell type-specific for prestalk cells in old migrating slugs.

3. *The role of the cell cycle in later development (Dictyostelium discoideum)*
(A.J.Durston, D.O'Day, F.C.Vork)

Recent evidence shows that Dd cells continue to divide during later development (Zada-Hames and Ashworth, 1977). We have started experiments to determine if the cell cycle is related to differentiation in Dd. Our preliminary results suggest that it is.

4. *The relationship of pattern formation to axial gradients (Dictyostelium discoideum)* (A.J.Durston, F.C.Vork)

Last year we showed that tip formation in the slug is regulated by two axial gradients: a stable activating gradient and a labile inhibitory gradient (see previous report, sect.II.C.1 and ref.6). We have investigated the relationship between these gradients and the pattern of differentiation in the slug. Our findings suggest that the stable (activating) gradient relates to the pattern of differentiation; specifically, that it reflects the axial gradient in concentration of prestalk cells in the slug. The results have been submitted for publication (see ref.4).

III.MOLECULAR-BIOLOGICAL LEVEL

This section describes research in which the *main approach* is aimed at the broadly molecular aspects of development: macromolecular assemblies, regulatory molecules and ions, their interactions, and the attendant biophysical phenomena.

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

In this collaborative project the attention is being focussed primarily on the plasma membrane-cytoplasmic interactions which possibly play a role in the regulation of the cell cycle and thereby in the initiation of cellular differentiation. Murine neuroblastoma cells *in vitro* are being used as a model system for neuronal differentiation, which involves neurite formation.

1. *Plasma membrane-cytoplasmic interactions during the cell cycle and differentiation of neuroblastoma cells in vitro (clones Neuro-2A and N1E-115)*
(S.W.de Laat, P.T.van der Saag, J.G.Bluemink, W.H.Moolenaar, S.A.Neemans, H.P.M.Vijverberg, A.Feijen, M.M.Guarda, J.Struijk, L.G.T.Tertoolen, W.M. Vonk)

1a. The role of membrane potential and ion metabolism in the regulation of the cell cycle and differentiation

This work is a continuation of that described in the previous report (sect. III.A.1a). The measurements of the membrane potential and the intracellular K^+ activity in synchronised sparse cultures of Neuro-2A cells have been completed. The results described previously were confirmed. A start has been made with tracer-flux experiments, using ^{86}Rb as an indicator for K^+ transport. The main attention is now being focussed on the alterations in electrical membrane properties and Na/K metabolism induced by cell-to-cell contacts.

1b. The role of the microviscosity of the plasma membrane in the regulation of differentiation

This research is being carried out in collaboration with Dr.M.Shinitzky (Weizman Institute of Science, Rehovot, Israel).

The microviscosity $\bar{\eta}$ is a measure of the fluidity of the membrane lipid matrix. It is determined by measuring the rotational mobility of a lipid probe, 1,6 diphenyl-1,3,5 hexatriene, using fluorescence polarisation methods. Neuro-2A cells were induced to differentiate by adding 1mM dibutyryl cAMP + 200 μ gr isobutyl-1-methylxanthine/ml to the culture medium and $\bar{\eta}$ was measured at regular time intervals. It decreases during the first eight hours of differentiation, to reach a stable level about 25% below that observed during the growth phase. The decrease in $\bar{\eta}$ is apparent before gross morphological alterations can be observed. Treatment of the cells with lipid vesicles consisting of the saturated phospholipid dipalmitoyl phosphatidylcholine (DPPC) leads to a reversible increase in $\bar{\eta}$, while lipid vesicles prepared from the unsaturated phospholipid dioleoyl phosphatidylcholine (DOPC) has the reverse effect. Incubation of the cells with DPPC vesicles during the induction of differentiation inhibits the formation of neurites completely but reversibly. DOPC vesicles have no significant effect on neurite formation. It may be concluded that an increase in membrane fluidity is a prerequisite for morphological differentiation.

1c. Lateral mobility of plasma membrane components during the cell cycle and differentiation

This research was carried out by S.W.de Laat at Cornell University (Ithaca, N.Y., U.S.A.), in collaboration with Dr.E.L.Elson and Dr.J.Schlessinger (from the National Cancer Institute, Bethesda, Md.).

Membrane molecules can in principle show three types of motion: rotational, transversal and lateral. Quantitative measurement of the nature and rate of molecular movements will provide an indication of possible changes in the dynamic properties of the plasma membrane and a means to analyse the molecular mechanisms underlying such changes. The lateral mobility of membrane molecules can be studied by the technique of fluorescence photobleaching recovery

(FPR). The fluorescence intensity of a small area of fluorescently labelled membrane, defined by a focussed laser beam ($\phi \sim 2 \mu\text{m}$), is recorded. Fluorophores within this area are irreversibly photobleached by a short pulse of intense laser light. Transport rates and the nature of the transport process (diffusion, flow etc.) of unbleached fluorophores into the bleached region from the surrounding cell surface can be derived from the recovery of fluorescence measured in the bleached region with attenuated laser excitation.

Using FPR, the lateral mobilities of membrane lipids, glycolipids and proteins were determined during the cell cycle and differentiation of Neuro-2A cells. As probe molecules we used the lipid analogue 3,3'-dioctadecylindocarbocyanine iodide (diI), the fluorescein-labelled ganglioside GM1, and rhodamine-labelled rabbit antibodies against murine E1-4 cells. For cell cycle studies the Neuro-2A cells were synchronised by mitotic shake-off.

The two lipid probes (diI and GM1) show a qualitatively similar modulation during the cell cycle. The diffusion coefficient is minimal in mitosis, increases rapidly two to three-fold during the G_1 -phase, remains constant during the S-phase, and decreases again shortly before mitosis. Membrane proteins show a different behaviour. Like membrane lipids they exhibit a minimal diffusion coefficient during mitosis, which increases about three-fold during G_1 . In contrast to the lipid probes, however, the protein probe shows a gradual decrease in mobility during the S-phase and a return to the original mitotic value at the S- G_2 transition.

The modulation of the lateral mobilities of diI and GM1 during the cell cycle is correlated with the changes in rotational mobility of a lipid probe (DPH) as measured by fluorescence polarisation (see report for 1975, sect.V.k, report for 1976, sect.III.A.1b, and this report, ref.10). This suggests that both the rotational and lateral mobilities of membrane lipids are subject to the same constraint: the fluidity of the membrane lipid matrix. Shortly after mitosis possibly an excess insertion of phospholipids into the plasma membrane takes place, leading to an increase in membrane fluidity, an increase in the lateral mobility of membrane lipids and proteins, and a decrease in the density of intramembranous particles (IMPs), as seen in freeze-fracture electron microscopy (see previous report, sect.III.A.1d (ii)). In the same period the membrane potential undergoes a depolarisation associated with a decrease in the intracellular K^+ activity (see previous reports). The gradual decrease in the lateral mobility of proteins during S-phase is associated with an increase in the IMP density, a hyperpolarisation of the membrane potential, and an increase in the intracellular K^+ activity (see previous reports). It seems plausible that a relative increase in the amount of membrane proteins or the insertion of specific membrane proteins causes modulations of the lipid-protein and protein-protein interactions, which in their turn may be responsible for the observed changes in membrane properties during S-phase.

Morphological differentiation of Neuro-2A cells was induced by adding 1mM dibutyryl cAMP + 200 μ gr isobutyl-1-methylxanthine/ml to the culture medium. The lateral mobility of all three probes was measured 24 hr after the induction of differentiation. In the region of the perikaryon the diffusion coefficients of diI, GM1 and membrane protein are hardly affected by the process of neurite formation. However, neurites show an increased mobility for all probes used ($\sim 2x$). We have found earlier that the microviscosity of the plasma membrane decreases upon differentiation (see report for 1975, sect.V. 1c; submitted for publication). Taking this finding together with the results obtained by FPR, it can be concluded that outgrowing neurites acquire different dynamic membrane properties during differentiation. Evidently a topographical heterogeneity is established in the plasma membrane.

1d. Freeze-fracture electron microscopy of the plasma membrane

The study initiated last year (see previous report, sect.III.A.1d (ii)) is being continued. A method has been developed to obtain quantitative data on the size distribution and topographical distribution of intramembranous particles (IMPs). Using a Wang 2200 B-II minicomputer system equipped with a digitiser, digital plotter, and dual floppy disc memory, the sizes and relative coordinates of the IMPs are recorded from electron micrographs (magn. $\times 252,800$, area $0.5 \mu\text{m}^2$). From these data a frequency distribution of IMP classes based on their diameter is calculated. The minimal difference between the classes is 10 \AA diameter. For any range of IMP classes the recorded topographical distribution can be compared with a computer-generated random distribution. To this end the recorded area is subdivided into square subunits, each having a length of three times the maximum IMP diameter or a multiple thereof. The recorded distribution of IMPs per subunit is compared with the random distribution, using the Kolmogorov-Smirnov test. Significant differences between the recorded and the generated distribution for various sizes of subunits reveal different rates of IMP clustering or aggregation. This method is at present being used for a detailed analysis of the ultrastructural changes in the plasma membrane during the cell cycle of Neuro-2A cells.

1e. Environmental pH as a growth regulator

It has been known for a long time that the saturation density of both normal and transformed cells *in vitro* clearly depends on the pH of the medium. Furthermore, at high cell densities a metabolic acidification of the medium can generally be observed. This has led to the concept that the environmental pH is implicated in the control of growth, the cell membrane probably acting as a mediator. More recently it has been found that some neuroblastoma cell lines differentiate at low pH.

We have started an investigation of the role of environmental pH in the control of growth in Neuro-2A cells. Reversible changes in growth rate, as a

result of changes in external pH, appear to be accompanied by changes in membrane fluidity, membrane structure, cAMP and ion metabolism, and desoxyglucose transport. Lowering the external pH from 8.0 to 6.5 leads to a reversible increase in the cell doubling time from 8 to more than 30 hr. This increase in cell cycle duration is associated with (1) a decrease in the microviscosity as measured by fluorescence polarisation of the lipid probe DPH, (2) a decrease in the density of intramembranous particles as observed in freeze-etch electron micrographs, (3) an increase in cAMP content of the cell, and (4) a depolarisation of the membrane potential. Similar characteristics can be observed at high cell densities, when metabolic acidification of the medium takes place and growth is inhibited. It seems as if a low external pH induces a G_1/G_0 membrane state. In proliferating cells all of the effects of low pH mentioned are reversed. In high-density cultures, where cell division no longer takes place and the external pH is low, raising the pH to 7.6 will largely overcome the growth inhibition.

1f. The role of plasma membrane composition in the regulation of the cell cycle and differentiation

A start has been made with the isolation of plasma membranes for further biochemical analysis. As a first step the assays for a number of enzymes specific for different membrane fractions have been worked out. The following markers are used for the plasma membrane: 5'-nucleotidase, Na/K-ATPase, alkaline phosphatase, adenylate cyclase, and radioactive iodination of plasma membrane proteins. Microsomal markers are glucose-6-phosphatase and NADPH-cytochrome-C-reductase. Cytochrome-C-oxidase and acid phosphatase are used as markers for mitochondrial and lysosomal membranes, respectively. Two methods for plasma membrane isolation are being compared: the nitrogen cavitation method according to Wallach and the Zn^{++} -method according to Warren. The former method yields small membrane vesicles, the latter large membrane sheets; both types of membrane can be purified by differential and density centrifugation.

IV. OTHER RESEARCH PROJECTS

1. *A new method of local artificial insemination (Xenopus laevis)* (K.Hara, P.Tydeman, C.W.A.Gorlee)

The methods for artificial insemination of amphibian eggs in use so far do not allow control of the place where the sperm enters the egg. For detailed studies on the establishment of bilateral symmetry in the egg (see section I. A.i above) such control is an absolute requirement. To this end a simple but reliable method for local artificial insemination was developed. The unfertilised egg, stripped from the female and with its jelly layer intact, is placed in "dry" condition against a sperm localiser made of plastic plate,

which contains a sperm reservoir. In this way fertilisation occurs within a restricted area. The method has been submitted for publication.

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

2a. The regulation of reproduction and spawning (*Discoplossus pictus*, *Rana lessonae*, *Rana pipiens*, *Bombina orientalis*)

The research described in section IV.2b of the previous report is being continued.

As regards *Rana pipiens*, it was found that mammalian gonadotropins are ineffective for the induction of spawning; we are finding out whether a species-specific gonadotropin is commercially available. Attention has been paid to various environmental conditions for the hibernation of this species (day-length, water and air temperature, aeration).

2b. Permanent marking of adult amphibians (*Xenopus laevis*, *Ambystoma mexicanum*)

The tests of the method of freeze-branding in *Xenopus* (see previous report, sect.IV.2c) have been extended and the marks are now conspicuous and persist for more than a year. Changes in the marks are documented photographically. The method does not work for axolotls. We are trying to tag these by inserting tiny plastic strips into the tail musculature.

2c. Abnormalities of egg maturation (*Xenopus laevis*)

We are investigating whether occasional periods of bad egg quality could be due to faulty egg maturation. In some cases cytological abnormalities of maturation have been found.

2d. Mutants and tumours

At the request of a British colleague we have checked our axolotl colony for the presence of nuclear size mutants; all histological tests were negative.

Several amphibian tumours found at post-mortem examinations were sent to the National Museum of Natural History, Washington, D.C. for identification; two axolotl granulomas and a hematopoietic neoplasm of *Xenopus* were incorporated into the Museum's Registry of Tumours in Lower Animals.

V. MISCELLANEOUS

1. Dr.A.Sutasurya used the Library facilities for a period of ten weeks while writing a book with Prof.Nieuwkoop (see sect.I.A.iv.3).

2. Prof.R.Chandebois used the Library facilities for a period of two weeks.

Published

1. Bluemink, J.G. and S.W.de Laat — Plasma membrane assembly as related to cell division. In: The synthesis, assembly and turnover of cell surface components; Eds. G.Poste and G.L.Nicolson; Cell Surface Reviews, vol 4; Amsterdam, North Holland; p.403-461 (1977).
2. Boterenbrood, E.C. — Concise Catalogue of the Central Embryological Collection of the Hubrecht Laboratory; Utrecht, Hubrecht Laboratory; 95 pp. (1977).
3. Durston, A.J. — The control of morphogenesis in *Dictyostelium discoideum*. In: Eucaryotic microbes as model developmental systems; Eds. D.H.O'Day and P.A.Horgen; New York, Dekker; p.294-321 (1977).
4. Durston, A.J. and F.Vork — The control of morphogenesis and pattern in the *Dictyostelium discoideum* slug. In: Development and Differentiation in the Cellular Slime Moulds; Eds. P.Cappuccinelli and J.M.Ashworth; Amsterdam, Elsevier/North Holland; p.17-26 (1977).
5. Hara, K. — The cleavage pattern of the axolotl egg studied by cinematography and cell counting. Wilhelm Roux's Arch. Devl. Biol. 181, 73-87 (1977).
6. Hara, K. and E.C.Boterenbrood — Refinement of Harrison's normal table for the morula and blastula of the axolotl. Wilhelm Roux's Arch. Devl. Biol. 181, 89-93 (1977).
7. Hara, K., P.Tydemann and R.T.M.Henast — Cinematographic observation of "post-fertilization waves" (PFW) on the zygote of *Xenopus laevis*. Wilhelm Roux's Arch. Devl. Biol. 181, 189-192 (1977).
8. Hara, K. — Cinematographic observation of surface changes on the zygotes of *Xenopus laevis* and *Ambystoma mexicanum*. In: Programme and Abstracts; the Eighth International Congress of the International Society of Developmental Biologists, Tokyo, Aug.29 - Sept.2, 1977; p.67 (1977).
9. Hara, K. — Organizer in birds (in Japanese). In: Organizer (in Japanese); Eds. O.Nakamura and I.Kawakami; Tokyo, Misuzu Shobo; p.206-246 (1977).
10. Laat, S.W.de, P.T.van der Saag and M.Shinitzky — Microviscosity modulation during the cell cycle of neuroblastoma cells. Proc. Nat. Acad. Sci. USA 74, 4453-4461 (1977).
11. Nieuwkoop, P.D. — Origin and establishment of embryonic polar axes in amphibian development. Curr. Topics Devl. Biol. 11, 115-132 (1977).
12. Nieuwkoop, P.D. — Preface (in Japanese). In: Organizer (in Japanese); Eds. O.Nakamura and I.Kawakami; Tokyo, Misuzu Shobo; p.I-II (1977).
13. Verhoeff-de Fremery, R., Ch.A.W.Gorlee and P.Tydemann — A method for collecting naturally fertilized eggs of *Xenopus laevis* immediately after laying. Lab. Anim. 11, 263-264 (1977).
14. Weijer, C.J., P.D.Nieuwkoop and A.Lindenmayer — A diffusion model for

Accepted for publication

15. Bluemink, J.G. — Use of cytochalasins in the study of amphibian development. In: Biochemistry and Cell Biology of Cytochalasins; Frontiers of Biology Series; Amsterdam, North Holland.
16. Bluemink, J.G. and L.G.J.Tertoolen — Freeze-fracture electron microscopy of the plasma membrane of the *Xenopus* egg: evidence for particle-associated filaments. Cytobiologie 16, 353-366 (1978).
17. Bluemink, J.G. and L.G.J.Tertoolen — The plasma-membrane IMP pattern as related to animal/vegetal polarity in the amphibian egg. Devl. Biol. 62, 334-343 (1978).
18. Durston, A.J., F.Vork and C.Weinberger — The control of later morphogenesis by chemotactic signals in *Dictyostelium discoideum*. In: Biophysical and Biochemical Information Transfer in Recognition; Eds. J.G.Vassileva Popova and E.V.Jensen; New York, Plenum.
19. Hara, K. — Spemann's organizer in birds. In: Organizer — a Milestone of a Half Century from Spemann; Eds. O.Nakamura and S.Toivonen; Amsterdam, North Holland.
20. Hara, K. — Studies on "Spemann's organizer" in birds — A review. Quart. Bull. Dept. Biol. I.T.B. Bandung.
21. Hara, K. — Surface changes in amphibian zygotes, and the temporal pattern of cleavage (16mm films). Quart. Bull. Dept. Biol. I.T.B. Bandung.
22. Ikenishi, K. and P.D.Nieuwkoop — Location and ultrastructure of primordial germ cells (PGCs) in *Ambystoma mexicanum*. Devl. Growth Different.
23. Kubota, H.Y. and A.J.Durston — Cinematographical study of cell migration in the opened gastrula of *Ambystoma mexicanum*. J. Embryol. Exp. Morphol.
24. Laat, S.W.de, P.T.van der Saag and J.G.Bluemink — Regulation of the cell cycle and differentiation of neuroblastoma cells *in vitro* (in Russian). Ontogenez. 9, 110-111 (1978).
25. Laat, S.W.de, J.G.Bluemink and P.T.van der Saag — Membrane assembly during the cell cycle. Biol. Cellul.
26. Nieuwkoop, P.D. — Preface. In: Organizer — a Milestone of a Half Century from Spemann; Eds. O.Nakamura and S.Toivonen; Amsterdam, North Holland.
27. Paleček, J., G.A.Ubbels and K.Rzehak — Changes of the external and internal pigment pattern upon fertilisation in the egg of *Xenopus laevis*. J. Embryol. Exp. Morphol. 45.
28. Ubbels, G.A. and R.T.M.Hengst — A cytochemical study of the distribution of glycogen and mucosubstances in the early embryo of *Ambystoma mexicanum*. Differentiation 10, 109-121 (1978).
29. Ubbels, G.A. — Symmetrisation of the fertilised egg of *Xenopus laevis* (studied by cytological, cytochemical and ultrastructural methods). Mém. Soc. Zool. France, 41.

Limnological Institute

Progress Report 1977

Edited by C.L.M. Steenbergen and D. van der Mei

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INTRODUCTION

D. van der Mei

The Institute was founded in 1957 as a result of a 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. This effort resulted in the establishment of three Institutes: two involved in aquatic ecology, namely the "Hydrobiological Institute" and the "Division Delta-Research of the Hydrobiological Institute" and one - "The Institute for Ecological Investigation" - concentrating on problems of a terrestrial ecological character. The "Hydrobiological Institute" filled a gap that existed in biological and chemical research of the abundantly present fresh-water systems in The Netherlands. Due to the separate development of the two institutes for hydrobiological research their names were changed in 1968 in: "Limnological Institute" and "Delta Institute for Hydrobiological Research", respectively.

The Institute has two departments, one in Nieuwersluis, the original site of the Institute, and one in Oosterzee (Friesland) (see Fig. 1). The Institute is financed by the Ministry of Science and Education by means of funds allotted to the Academy of Arts and Sciences. It has nowadays a staff of 12 scientists. Additionally research fellows, guest workers, students and trainees take part in the research programme. The Institute has close ties with several Universities, especially those of Utrecht, Amsterdam and the Agricultural University at Wageningen.

The Research Programme

Hydrobiological research in fresh-water systems was in its earliest phase of development when the Institute started its work. The research in the first years had therefore a mainly inventory character. In the late sixties this was followed by a phase in which several processes in the fresh-water ecosystem under study were described more thoroughly.

In Nieuwersluis these two phases have now resulted in a major research effort in lake Vechten, aiming at a complete analysis of the ecosystem in this lake. At Oosterzee the investigations concentrate on algological problems and on foodchain and production studies.

The "Ecosystem study of lake Vechten" involves three working groups: "Primary and Secondary Production", "Ecophysiology of Water Plants" and "Mineralization of Organic Matter". The "Tjeukemeer Research" is carried out by the working groups "Algology" and "Foodchain and Production Studies" (see Table 1).

The Institute works along programmes for periods of five years, which are set up in consultation with a commission installed by the Academy of Arts and Sciences.

The Institute organizes courses and training programmes for students from the Universities. Training and research facilities are also offered to scientists from abroad. Apart from the main projects carried out by the working groups, a number of separate subjects are studied in close co-operation with departments and organizations that are involved in the management of fresh-water systems in The Netherlands.

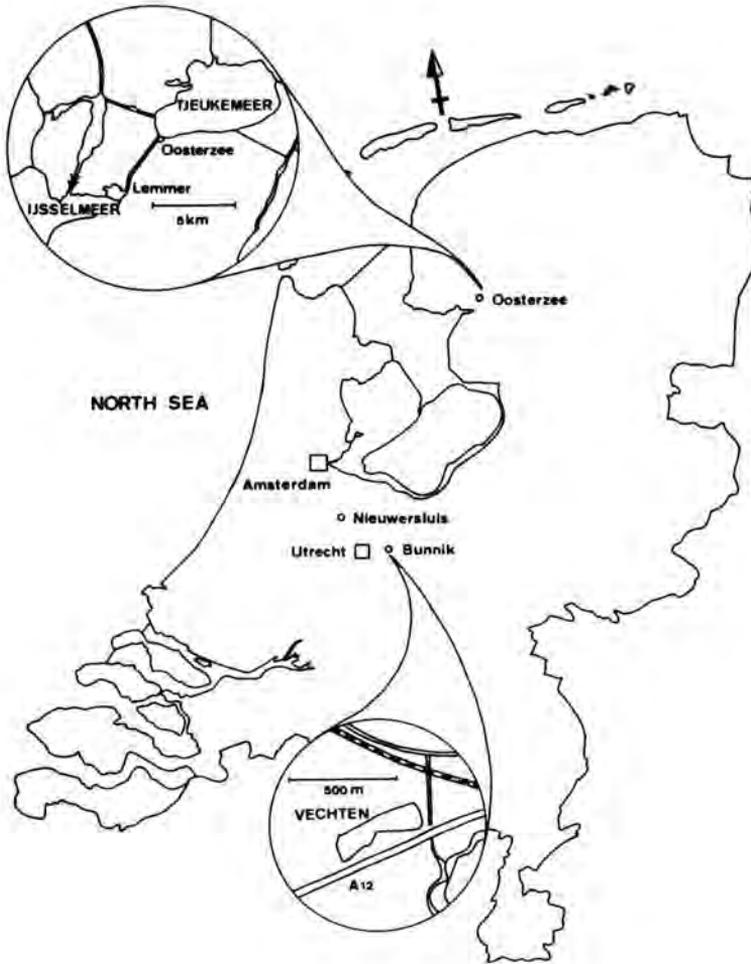


Fig. 1 Map of study areas of the Limnological Institute - Tjeukemeer research, Friesland and lake Vechten research, Utrecht.

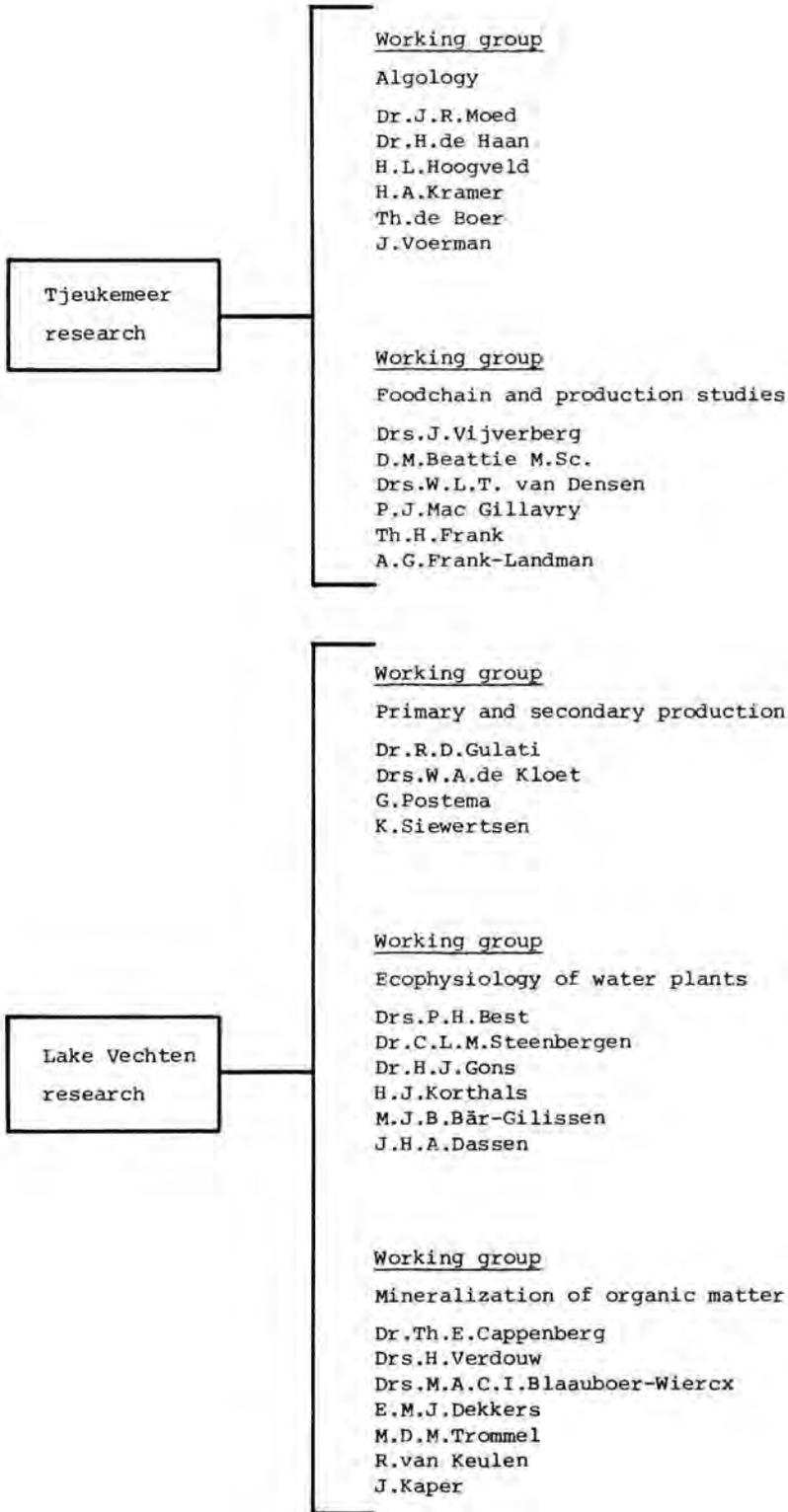


Table 1. Research scheme of the Limnological Institute.

WORKING GROUP "ALGOLOGY"

J.R.Moed, H.de Haan, H.L.Foogveld, H.A.Kramer, Th.de Boer & J.Voerman

Introduction

A study of the food-chain in Tjeukemeer within the framework of the International Biological Programme, also including the determinations of amounts of nutrients and numbers of algae was started about 1967 and finished in 1971.

In 1976 the working-group Algology was formed, in which the programmes of chemical and algological analysis and research on humic substances and on the ecology of diatoms were combined. The ultimate aim of the working-group is to explain the nature, magnitude and periodicity of algal populations in Tjeukemeer.

Comparison of several Friesian lakes with respect to their algal populations has shown Tjeukemeer to be representative for these peaty lakes. Tjeukemeer is about 20 km² in area with an average depth of about 1.5 m. It is part of the Friesian reservoir system, which in wet seasons collects water, rich in nutrients and humus, from the surrounding polders. In dry and warm seasons quite different water from IJsselmeer is allowed to enter, to maintain a particular level of groundwater.

It is difficult to determine the amounts of water (and nutrients) transported towards Tjeukemeer. Specific methods are needed to measure the amounts of nutrients released from the bottom of Tjeukemeer (including mineralization) in the presence of algal populations. Consequently, the actual investigations are not aimed at the explanation of the magnitude of algal populations in relation to the amounts of nutrients, but at the nature and periodicity of these populations in respect to a.o. physical-chemical factors in Tjeukemeer.

Therefore, chemical analytical methods are developed which will give more insight into the influence of the physical-chemical form of the nutrients (like Fe and P) on their availability for algae. In this respect one might think of inorganic ionic, inorganic and/or mixed colloidal, and complexed (chelated) forms of the nutrients.

The main reason for this study was the expectation that interactions between the abundantly present dissolved humic material in Tjeukemeer and nutrients occur.

To obtain the relevant compounds gel-filtration and ultra-filtration are applied, whereas the availability for algae will be tested in chemostats.

It has appeared that certain species of algae are annually dominant, whereas others are only incidentally dominant. Therefore, it is necessary to observe regularly the wax and wane of algal populations in Tjeukemeer. To represent the course of the algal populations both in numbers and bio-

mass, determination of cell numbers and length of threads are performed.

Particular attention is paid to the ecology of diatoms; nowadays the effects of depletion of nutrients and conditions of the bottom of Tjeukemeer on the shape and viability of diatoms are investigated. From such studies more knowledge may be gained about the mechanisms involved in the adaptation of algae to the changing conditions of Tjeukemeer. *Diatoma elongatum* was chosen as a study organism. This alga is one of the dominant diatoms in the Tjeukemeer. Besides its ability to grow both in the "free water" and attached to plants it shows a pronounced variability in cell-length in nature.

Unpublished results

Determination of chlorophyll-a

In co-operation with Dr. G. Hallegraef (Limnological Lab. Univ. Amsterdam) the acidification procedure to correct for phaeopigment in the determination of chlorophyll-a was improved. Acidification to pH= 2.6-2.8 appears to give reliable results. Relevant required amounts of acid are dependent on the nature of the solvent and its watercontent.

Determination of nitrate

It was found that nitrate using suspensions of zinc (powder) at alkaline pH is converted quantitatively to nitrite. Interfering substances present in Tjeukemeer and polderwater can be removed by passage through an Al_2O_3 column. The obtained values of nitrite agreed closely with those determined after the Devarda's method using Nessler's reagent.

Ultra-filtration experiments

Starting with filtered (0.2 μ m) summer Tjeukemeer- and polderwater and applying ultra-filtration it was found that:

- (a) most of the so-called dissolved P was present in inorganic colloidal particles (70%)
- (b) this also held for Fe (>90%)
- (c) in the relatively humus rich polderwater Fe was present (30%) in fractions, having a strong complexing capacity for Cu

Research on fulvic acids

A study of the possible interactions between fulvic acids, amino acids and carbohydrates from Tjeukemeer, based on gel filtration at pH 7.0

Sephadex G-25 gel filtration and dialysis at pH 3.0 and 7.0 of fulvic acids from Tjeukemeer indicated that more than half of the carbohydrates and amino acids, are probably not associated with the large molecular weight fulvic acids (M.W. \geq 5000) with which they were eluted from Sephadex at pH 7.0. If they are associated, this can only be by weak, acid-labile bonds (i.e. hydrogen bonds). Ion exchange experiments, using Dowex 2-X8, Dowex 50W-X8 and SP-Sephadex C-25, confirmed these results.

Curie point pyrolysis mass-spectrometry of the dissolved organic matter from Tjeukemeer.

In co-operation with Drs. G. Halma (Agricultural University, Wageningen) Curie point pyrolysis mass-spectrometry of the dissolved organic matter of Tjeukemeer was performed. The patterns obtained showed predominant peaks related to polysaccharides and amino sugars. Lower peaks were typical for proteinaceous material and phenols. These results resemble those of the pyrolysis of soil fulvic acids.

Algology

The survey of algae present in Tjeukemeer (1977) has shown that in February and March numbers of the diatoms *Melosira* spp, *Asterionella formosa*, unicellular centric and *Diatoma elongatum* strongly increased. In April

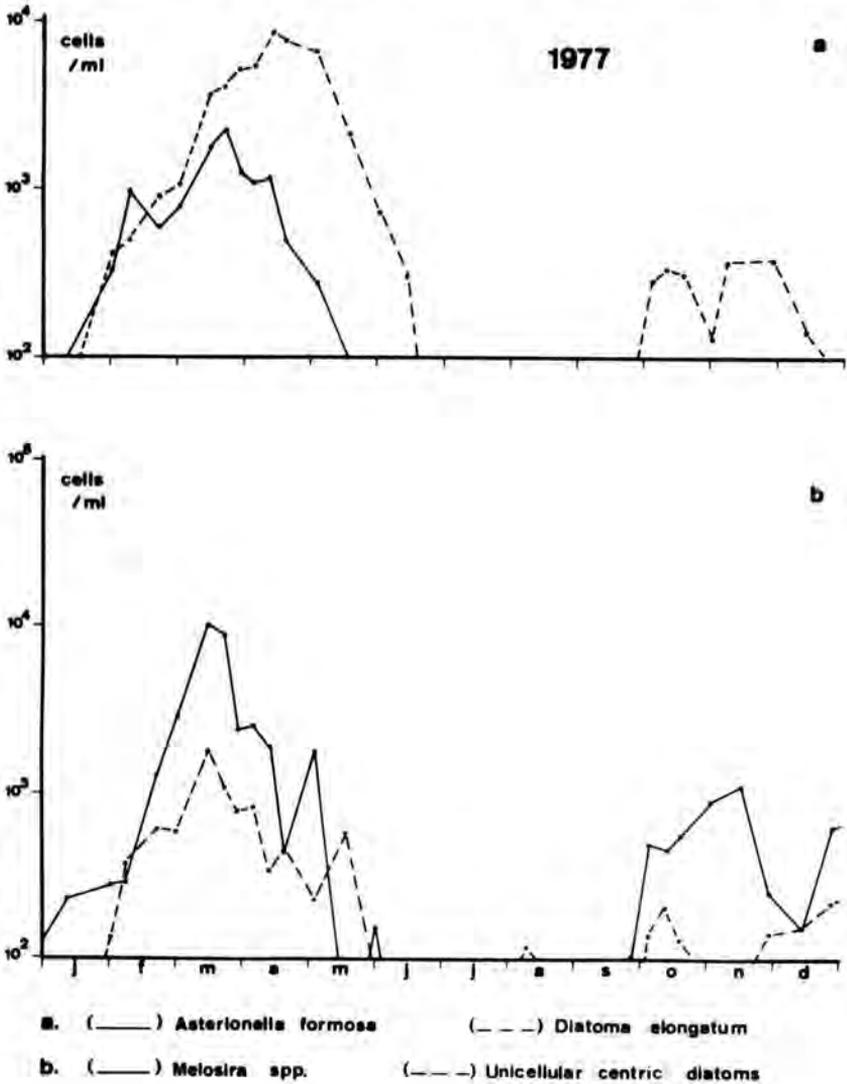


Fig. 2 Course of numbers of dominant diatoms in Tjeukemeer

numbers of *D.elongatum* increased as well. Furthermore in October-November a slight development of mainly *Melosira* spp. and *Diatoma elongatum* could be observed (Fig. 2a-b).

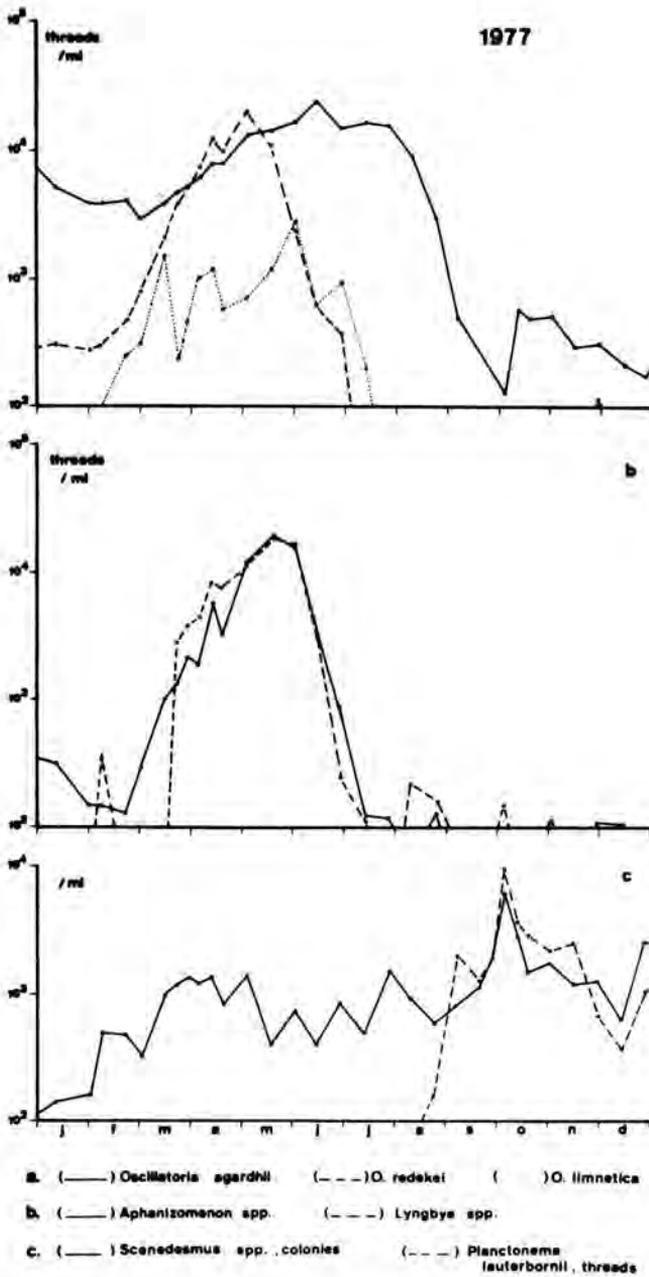


Fig. 3 Course of numbers of dominant blue-green algae (a-b) and dominant green algae (c) in Tjeukemeer.

From March on populations of the blue-green algae *Aphanizomenon flos aqua*, *Lyngbia* sp., *Oscillatoria limnetica*, *O. redekei* and *O. agardhii* started to develop. After the end of May numbers of the blue-green algae decreased except for *O. agardhii* which remained at a high level until the end of July (Fig. 3a-b).

As main green algae *Scenedesmus* spp., with maximal numbers in October, and *Planctonema lauterbornii* with a considerable development from the end of August till November were registered (see Fig. 3c). This considerable bloom of *Planctonema* can be considered as exceptional, since during the last 10 years only one time earlier, notably in 1970, a bloom of this alga has been observed.

Laboratory studies

Evidence was obtained that Si-depletion is (co-) operative in the formation of curved cells of *Diatoma elongatum*. Analogous forms have been observed in nature. It is likely that during dark incubation of cells of *D. elongatum* degradation of chlorophyll-a occurs, which is revealed by the appearance of green spots during thin-layer chromatography of pigment extracts and by changes in the absorption spectrum of intact cells.

Articles in press

Seasonal variations of fulvic acids, amino acids, and sugars in Tjeukemeer, The Netherlands.
H.de Haan & Th.de Boer. Arch. Hydrobiol.

The concentrations of dissolved amino acids in the humus-rich, eutrophic lake, Tjeukemeer, was recorded monthly during two years. The average content of dissolved amino acids is 2.9 mg l^{-1} which is about 6% of the total dissolved organic matter. Maximum amounts (13% of total dissolved organic matter) were observed just after diatom, green and blue-green algal blooms. The dissolved amino acids were fractionated by Sephadex gel filtration into three fractions differing in molecular size. The distribution of the amino acids over these three fractions was almost constant throughout the investigation. Most of the dissolved amino acids were in relatively high molecular weight fractions (M.W. 5000). Only 15% were free amino acids and/or simple peptides.

In summer, the pattern of variation for dissolved sugars was similar to the pattern observed for amino acids. However, the orcinol-sulfuric acid method used for determination of sugars did not give reliable measurements in the absolute sense - apparently owing to interference by undefined low molecular weight allochthonous substances - and so the investigation of dissolved sugars must be regarded as preliminary.

Introduction

In this working group the interrelation between the fish populations and their food organisms are studied (see Fig. 4).

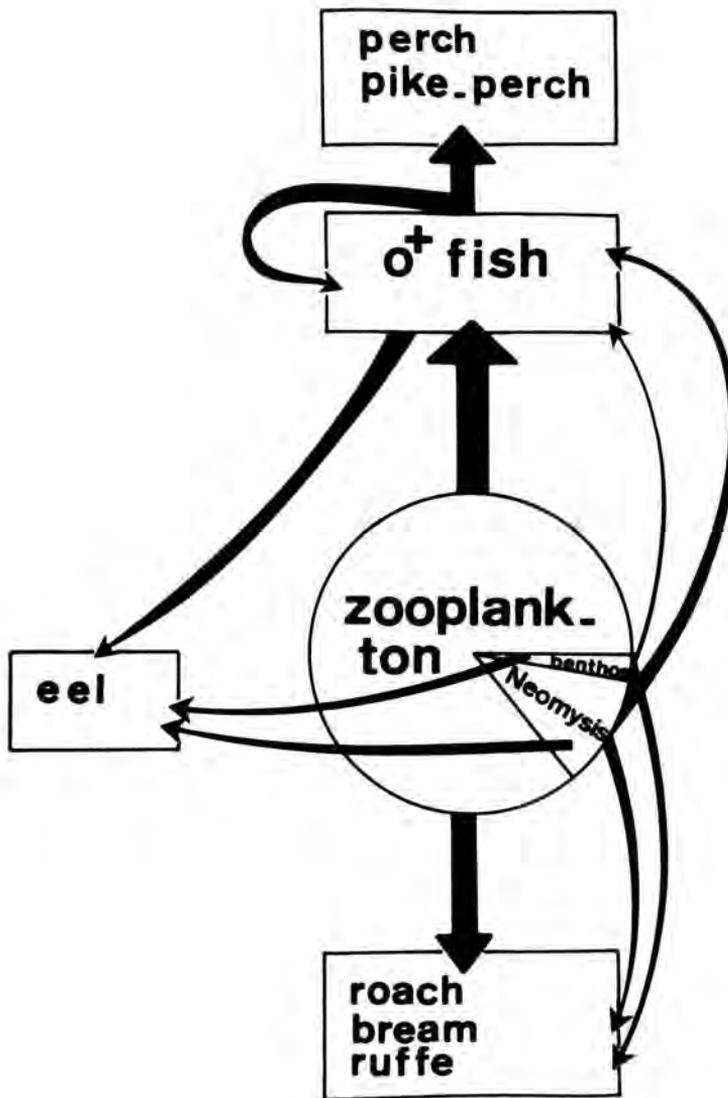


Fig. 4 Field of research covered by the working group "Foodchain and production in Tjeukemeer" (1974 - 1981).

Population structure, population density, fecundity, mortality, growth, standing crop biomass and production are estimated for both the fish and the most important fish-food species. The latter includes zooplankton (copepods and cladocerans) and macrofauna elements as chironomids, gammarids and the opossum shrimp *Neomysis integer*. Much work is directed to the ecology of fish-feeding under natural -as well as under experimental conditions in the aquarium. The results of these studies will be used in a simulation model to enable a description of this sub-system and to get more insight in its dynamics.

The work is carried out in phases. At the moment the interrelationship between O^+ fish and their food-organisms is investigated. The O^+ fish are a prominent part of the sub-system in study, since the production of this year class is relatively high in relation to that of the older year classes. Generally O^+ fish production represents 50-90% of the total production of a fish species.

Results

The programme carried out during 1977 was very similar to that of the previous year. The O^+ fish and its food and feeding was intensively studied (see student reports: Sijbesma 1977, Vogel 1977, Beyderwellen 1978, de Graaf 1978 and Lammens 1978).

The recruitment of all species, except for the smelt and the ruffe, was poor resulting in an overall-dominance of smelt in the open-water. Because of the good survival of smelt in June and July this species could build up a large stock with low mortality during the rest of the growing season. In this way the other O^+ fish species were indirectly protected against heavy predation by pike-perch. The poor survival of smelt during the comparable period in 1976 had led to a heavy predation-mortality on all other species and had resulted in a dramatic decrease of numbers and a very poor year-class 1976. Both years gave extreme examples of depensatory mortality.

Growth of the planktivorous bream and roach was poor because of low temperature during the growing season. Perch and ruffe grew very well on *Neomysis integer*, which food species was very abundant this year. The good growth of smelt was in accordance with the high abundance of *Daphnia hyalina*, while the preferred *Leptodora kindtii* was less important as a food source than during the warm summer of 1976. The retarded growth of O^+ pike-perch at the beginning of the growing season made it impossible for this species to become piscivorous and forced it to feed on *Neomysis integer* at the end of the season although large numbers of prey-fish (smelt) were present. At the end of 1976 pike-perch was also forced to feed on *Neomysis integer*. At that time this was not due to retarded growth but to the very low density of prey fish in the lake.

Total O^+ fish production during 1977 was comparable to that of 1976 and

amounts to 150-200 kg ha⁻¹. In contrast to 1976, when all species had a comparable contribution to the total O⁺ production, the production of 1977 was dominated by smelt.

To estimate the daily ration of O⁺ fish mean stomach-contents and gastric evacuation rates were determined for 24 hour periods. From observations on the feeding periodicity of smelt and bream they appeared to be typically day-time planktivores. Simultaneously performed observations on the evacuation rate in the field gave a negative bias. Further observations on the gastric evacuation rate for smelt, perch and pike-perch will be done in the laboratory under conditions of constant feeding.

In 1977 also the population structure, growth, fecundity, food and feeding periodicity of the ruffe in Tjeukemeer was studied (see student reports: Klein Breteler 1978 and Schouten 1978). This intensively predated benthophagic fish has a life-span of only 3 years and is already mature after the first year of life. The frequency distribution of the size of the eggs suggests that the younger females have a lower percentage of ripe eggs than the older ones.

During routine sampling of the older fish it appeared that in 1977 the bream population showed considerable increase by the recruitment of the very strong year-class 1975, which together with the year-class 1970 dominates the population.

The stock of pike-perch in the lake has an extremely homogeneous composition and is completely dominated by the strong year-class 1975, which is not followed by a recruitment of any size since then.

Published articles

A comparison of the population ecology of *Asellus aquaticus* (L) and *Asellus meridianus* Rac. in the reed beds of the Tjeukemeer. Chambers, M.R. *Hydrobiologia* 53; 147-154.

The population ecology of *Asellus aquaticus* and *Asellus meridianus* was studied in a Tjeukemeer reed bed from March to October. The densities of the two species are similar at the beginning and end of the reproductive season, although *A. meridianus* is up to 3 times more abundant inbetween. In both species egg production is positively correlated to body length and summer brood sizes are smaller than in the spring. *A. meridianus* begins to reproduce at smaller sizes than *A. aquaticus* and is also more fecund for overlapping size classes. Over the season as a whole both species have the same reproductive output. The life cycles of the two species are very similar with three main periods of reproduction and the populations being replaced twice during the year. Despite the differences between the species no factors are found which gives one species an obvious competitive advantage against the other.

The population ecology of *Gammarus tigrinus* (Sexton) in the reed beds of the Tjeukemeer.

Chambers, M.R. *Hydrobiologia* 53; 155-164.

The population ecology of *Gammarus tigrinus* (Sexton) was studied in the Tjeukemeer during 1969 and 1970. *G. tigrinus* reaches very high densities up to 24,000/m² in parts of the study area. In 1970, the summer densities were 2-2½ times greater than in 1969. Individuals do not grow to such large sizes in the summer as at other times of the year. Females begin to carry eggs in March or April and reproduction ceases in November. Large females have larger broods than smaller animals and the average size of the brood varies with the time of year. The egg incubation period and growth rate are dependent upon temperature. At summer temperatures females became sexually mature after about four weeks and the egg incubation period is about 10 days. The entire population is turned over about three times during the year. A combination of rapid growth rate, early onset of sexual maturity and high fecundity are probably responsible for the rapid spread of *G. tigrinus* throughout much of the Netherlands.

The return of marked roach (*Rutilus rutilus* L.) to spawning grounds in Tjeukemeer, The Netherlands.

Goldspink, C.R. *J.Fish Biol.* 11; 599-603.

A reciprocal transplant of marked roach between two spatially separated (3.3 km) spawning grounds is described. The recapture rates on the 'home grounds' were higher than those obtained elsewhere in the lake, which suggests that the roach exhibit a degree of fidelity to specific spawning grounds.

Population structure, life histories and abundance of copepods in Tjeukemeer.

Vijverberg, J. *Freshwat. Biol.* 7; 579-597.

The copepod zooplankton of Tjeukemeer was sampled at weekly intervals for 3 years (1969 - 1971) and ten species were recorded. Five species contributed more than 99% of all individuals collected. *Acanthocyclops robustus* is the most abundant species (66%), followed by *Mesocyclops leuckarti* (13%), *Cyclops vicinus* (11%), *Eurytemora affinis* (9%) and *Diacyclops bicuspidatus* (1%).

The ecology of the *A. robustus* population in Tjeukemeer differs from other *A. robustus* populations in that it dominates the open-water zone. This is an additional argument for a taxonomic revision of the *A. vernalis-robustus* complex.

Seasonal periodicity in abundance of each species was very similar in 3 successive years.

All cyclopoid species went into diapause. *A. robustus* and *M. leuckarti* during winter, *D. bicuspidatus* and *C. vicinus* during summer. Most species diapause in a particular copepodite stage.

The seasonal variation in the population structure, the fecundity and the sex ratio are described. Both population structure and sex ratio were influenced by diapause and size-dependent mortality. The fecundity was more influenced by the water temperature and the age structure of the female population than by food situation or size of the females.

Articles in preparation or in press

Life cycle and changes in carbohydrates, proteins and lipids of *Pentapedilum uncinatum* Goet. (Diptera, Chironomidae).
Beattie, D.M. Freshwat. Biol. 8; 109-113.

The chemical composition and total individual biomass is described for the different larval instars, as well for the pupae and adults. During the winter-diapause absolute weight and chemical composition remains the same. When larval growth is resumed the greatest percentage increases are in fat and sugar content.

Chironomid populations in the Tjeukemeer.
Beattie, D.M. Thesis Univ. Leiden Pp. 150.
Beattie, D.M. Verh.int. Verein theor. angew. Limnol. 20

Chironomid larvae usually form an important link in the food chain between phytoplankton and fish. The virtual absence of these larvae in the open-water of the Friesian lake ecosystems is an unusual feature and forms the subject of this thesis.

Population dynamics and biomass production of *Neomysis integer* (Leach) in the Bergumermeer.
Beattie, D.M. & H.N.Kruif. Verh. int. Verein theor. angew. Limnol. 20

The aim of this work was twofold. Firstly to develop an appropriate sampling method and sampling programme on bases of which the population dynamics of this species could be studied. Secondly to describe changes in the population structure, population densities and standing crop biomass, in order to estimate potential biomass production.

The effect of temperature on the development-and growth-rate of microcrustaceans from Tjeukemeer, the Netherlands.
Part I Cladocera. Part II Copepoda.
Vijverberg, J. (in prep.)

The effect of temperature on growth-rate and egg development time of the common cladoceran and copepod species from Tjeukemeer are described. Belehrádek's temperature functions have been fitted to the development times of eggs, combined naupliar instars and combined copepodite instars, as well as to the longevity of the adult copepods.

Distribution patterns of zooplankton in Tjeukemeer and its consequences for the accuracy of the population density estimates.
Nie, H.W., de & J. Vijverberg (in prep.)

As part of a study on the population dynamics and production of Copepoda and Cladocera in Tjeukemeer the accuracy of the absolute population density estimates were calculated and discussed.

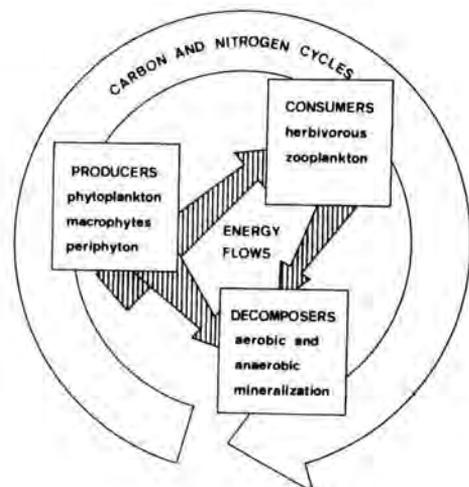
LAKE VECHTEN RESEARCH

Introduction

Lake Vechten, situated near Bunnik (see Fig. 1), was excavated during 1938-1939 to obtain sand for road building. It is isolated from other water bodies and can be characterized as a seepage lake. So it is free from external polluting influences, a factor which contributes the most to the suitability of the lake as an object of limnological studies, particularly for a fundamental ecosystem approach. Besides this the area of the lake (4.7 ha), its depth (maximum 10-12 m; average 6 m) and the consequent summer stratification and the physico-chemical and biochemical changes accompanying this are important factors contributing to the choice of this lake for long term research. In addition to this Vechten can be characterized as slightly eutrophic, has a well developed littoral region and an intensive anaerobic milieu in the bottom deposits.

Earlier studies of lake Vechten clearly indicated the complexities involved in interpreting the results. This was mainly due to the overlapping nature of the problems. Evidently none of the processes involving the three main properties of ecosystems, i.e. energy flow between producers and consumers, mineralization and nutrient recycling, and population self regulation, can proceed in isolation. In view of this there was a strong and unanimous desirability of both co-ordinated and integrated approach to limnological problems in lake Vechten. In this regard a three pronged attack for solution of problems was planned and during 1976 and 1977 three working groups were established. These groups concentrate on carbon and nitrogen recycling and aspects of the energy flow.

Scheme 1 Processes and relations between groups of organisms studied by the project group "Ecosystem study of Lake Vechten".



They are united in a project group called "Ecosystem study of lake Vechten". Scheme 1 represents their main activities, of which further details are given below.

WORKING GROUP "PRIMARY AND SECONDARY PRODUCTION"
R.D.Gulati, W.A.de Kloet, G.Postema & K.Siewertsen

Introduction

The production studies on lake Vechten are aimed at understanding the processes relating to: firstly, the photosynthetic fixation of sun energy in the limnetic region of the lake, i.e., the primary production of phytoplankton; and secondly, the energy flow from the first to second trophic level, i.e. transformation of the primary production by the herbivore zooplankton community.

In 1977, the seasonal and vertical changes in the chlorophyll and total pigments of phytoplankton and the *in situ* primary production rates of the two major fractions (seston size fraction below 33 μm and 33-105 μm , filter mesh size, the so-called nanoplankton and netplankton) were simultaneously measured. The grazing and assimilation rates of the crustacean zooplankton were studied, employing the ^{14}C labelled nanoplankton as tracer food. The data on temporal, and spatial distribution of the dominant Crustaceans were analysed further to examine the interactions among the species populations, e.g., the species niche dimensions, breadth and overlap.

A.Roostenberg-Sinnige (guest worker, State University, Utrecht) carried out a study entitled: The role of *Eudiaptomus gracilis* in the secondary production in lake Vechten during the winter period.

Results and comments

Primary production

The chlorophyll concentrations (7-10 mg m^{-3}) in the surface waters were relatively high during the winter and early spring. The nanoplankton (*Chlorella* sp., *Stephanodiscus astraea*) contributed almost exclusively to the phytoplankton. The late spring and the summer decrease of nanoplankton in the surface layers was accompanied by a significant increase of netplankton. The depth distribution of chlorophyll during late summer shows a marked heterogeneity, that is, accumulation (up to 50 mg m^{-3}) of sestonic particles, belonging to both the fractions, in the hypolimnion. This was particularly significant in the vicinity of and above the anaerobic zone. *Chlorella* sp., *Mallomonas caudata* and *Lyngbya* sp. contributed mainly to the increased concentrations of chlorophyll.

The primary production rates varied between 60 and 375 $\text{mg C m}^{-2}\text{d}^{-1}$, with

the summer mean of 222 mg. The rates are the lowest since 1969 when the production studies were first started. The causes for this sharp decrease in the production rates are not known since the chlorophyll and seston concentrations do not seem to have declined, nor are there any indications of decreased light penetration and increased nutrient limitation. Nevertheless, a sharp decrease in the gross photosynthetic efficiency this year (0.07%) in contrast to that in the preceding years (0.1-0.3%) may be due to changes in the algal composition. Secondly, increased excretion rates of the recently synthesized organic material may be the possible cause. However, comparative data in this regard are lacking. The excretion studies during 1977 have revealed that of the total photosynthetic fixation, from 15-60% of the carbon was excreted as extra-cellular products (see under Working group "Mineralization of organic matter" - C-fixation and excretion).

Zooplankton grazing

During the summer, the mean concentrations of crustacean filterfeeders, 0.9 g C m^{-2} , and their food (seston $< 33 \mu\text{m}$), 2.1 g C m^{-2} , were similar to those in 1976. This was also true for the ingestion and assimilation rates which were, respectively, 0.41 and $0.22 \text{ g C m}^{-2} \text{ d}^{-1}$. The fluctuations in the rates are illustrated in fig. 5. Though the grazing pressure of zooplankton in water strata below 3.6 m. was about 33% higher than in the strata above, the differences between the two zones were less remarkable than in 1976 (see Gulati, Verh.int.Verein theor.angew.Limnol., 20; 950-956).

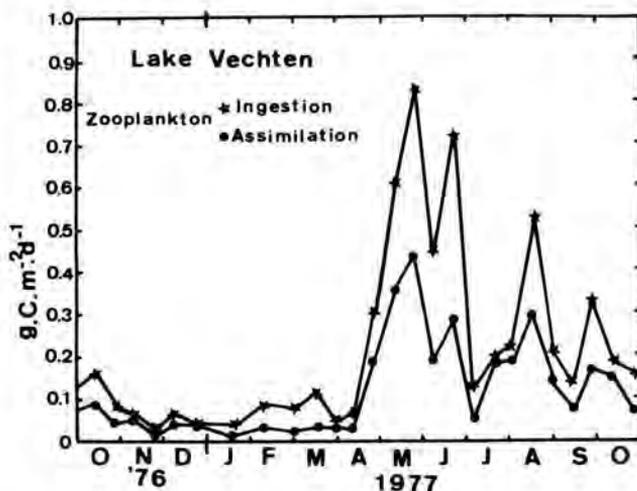


Fig. 5 The daily ingestion and assimilation rates of zooplankton in lake Vechten during 1977.

Table 2. A comparison of the summer means of the data relating to the grazing and assimilation activities of the filterfeeders community of zooplankton during the periods 1972, '73 and '74 (A) and 1975, '76 and '77 (B).

	<u>A</u>	<u>B</u>	B/A
1. Seston g C m ⁻²	2.84	2.15	0.76
2. Filterfeeders g C m ⁻¹	0.64	0.92	1.43
3. Ratio 1 : 2	0.22	0.43	1.95
4. Grazing % d ⁻¹	15.60	23.50	1.50
5. Assimilation % d ⁻¹	5.60	12.80	2.28
6. Consumption g C m ⁻² d ⁻¹	0.44	0.48	1.10
7. Assimilation g C m ⁻² d ⁻¹	0.15	0.25	1.70
8. Assimil. efficiency, %	34	51	1.50
9. Specific consumption (daily ration)%	70.3	52	0.74
10. Specific assimil.,% (ratio 7 : 2)	22.8	27.7	1.21

The recurrent phenomenon of sharp increase in the zooplankton grazing in late spring/early summer (Gulati 1978) appears to be related to time-lag reaction in the growth of *Daphnia* and *Bosmina* populations as a consequence of increase in their food supply in late winter and early spring.

A comparison of the summer data of the six years, 1972 - '77, indicates that the data of 1972 - '74 are remarkably different from those of 1975 - '77 (Table 2). In summary, the 25% decrease in the seston mean concentration during 1975 - '77 is inversely related to the 43% increase in that of the filterfeeders concentration. Consequently, the grazing and assimilation (% d⁻¹) increased significantly so that the grazing pressure became more significant than during 1972 - '74. It is interesting that despite the decreased food supply, the zooplankton consumption and assimilation rates rose, particularly the latter which increased by 74%. This could have only been achieved by more efficient utilization of the food as is reflected in the higher assimilation efficiencies in the latter period. An improvement in the quality of food, either through changes in the algal composition or through an increase in the bacterial component of colonized detritus, might contribute significantly to the changed situation.

Furthermore, since the mean phytoplankton primary production of 222 mg C m⁻² d⁻¹ in 1977 formed only 55% of the zooplankton daily consumption rate, their food demand must be partially met by the alternate sources of food i.e. detritus and bacteria. The linear regression between chlorophyll and sestonic carbon indicates that the mean detrital carbon in seston increases from 28% in the surface water to 67% in the deeper layers. Also, the bacterial studies (see under Working group "Mineralization of organic matter" - Course of bacterial numbers) in open water started during 1977 indicate

that the bacteria are encountered in significant numbers, 10 to $40 \times 10^{12} \text{ m}^{-2}$ from late July - October. The role of these alternate sources of food in zooplankton grazing will be investigated in the coming years.

The contribution of filter feeders to the community grazing was examined further. *Daphnia* spp., *Eudiaptomus gracilis* and *Bosmina longirostris*, as in 1976, were the dominant filterfeeders. All the major filterfeeders, except *Bosmina* sp., filtered the nanoplankton more efficiently than they filtered the netplankton. The assimilation efficiencies on netplankton were much lower than on nanoplankton.

The feeding rates of zooplankton at dusk are generally higher than during dawn and day. The results of several experiments reveal that the mean rates during the three periods were, respectively, 26.2, 16.3 and 20.6 $\text{mg C m}^{-2} \text{ hr}^{-1}$. An analysis of the data of 1976 reveals that nearly 78% of the consumption at dusk takes place in the water strata above 3.6 m; the respective figures for dawn and day are 65 and 48%. However, this is mainly related to the diurnal ascent and descent of zooplankton rather than to any diurnal changes in the specific grazing rates.

The diurnal data on zooplankton show significant differences in the total biomass (on area basis) at different periods. The biomass at night is invariably the highest and that during the day the lowest. This needs further investigation as to if the efficiency of zooplankton capture using the present Friedinger sampler is optimal.

Structure of filterfeeders community

The data on temporal and spatial and diurnal distribution of the dominant crustaceans were further analysed. The probability of co-occurrence of different populations, their niche breadths (B_1), niche dimension (D), niche overlap (a) and community niche breadth (B^*) and relative niche breadths (B_1/B^*) were calculated. Some remarks are here made.

The *Ceriodaphnia* population has the lowest niche breadth, the population being specialized in the deeper hypolimnetic water. *Eudiaptomus*, *Daphnia* and *Diaphanosoma* are all generalists since they occupy broader niche breadth and are encountered throughout the entire water column. *Bosmina* occupies an intermediate position between the two extreme categories.

The community niche seems to be strongly influenced by the populations of *Ceriodaphnia* sp and *Bosmina* sp. In other words, despite the three generalists, the community has a tendency to stratify with increasing depth, primarily due to increase in the number of the individuals of all populations with depth. The fact that most of the data pertain to day-time collections has certainly biased the results to an extent.

The probability of co-occurrence of different populations, i.e. niche overlap (a) was also calculated. The interaction among the pairs of gene-

ralists (those with larger niche breadths) is strong and that of a generalist with a specialist weak. For example, a values of *Eudiaptomus* against *Daphnia* and *Diaphanosoma* are 0.84 and 0.81, respectively, but against *Bosmina* and *Ceriodaphnia*, the a values are 0.62 and 0.37. Nevertheless, competitive exclusion of generalist by specialist would be prevented by the higher density of the former. It remains to be examined as to if the competitive existence is through habitat selection or through resource allocation.

Evaluation of biomass parameters

The simultaneous determination of phytoplankton (seston) and zooplankton biomass using carbon, nitrogen, calories and biochemical composition (protein, carbohydrates and fats) as parameters indicates, in general, a good correlation among the different parameters. The results when seen in the light of recommended conversion factors in the literature differ significantly in some cases.

The phytoplankton and zooplankton biomass values expressed as carbon derived from the COD data, are about 8 and 5.6 % higher than when calculated from the proteins, carbohydrates and fats using the standard conversion tables. The found ratios between organic matter and carbon data of phyto-and zooplankton are, respectively, 1.86 and 1.82 rather than the usual 2 cited in literature.

The mean percentages of protein, carbohydrate and fat were: phytoplankton, 48; 33 and 19%; and zooplankton, 65; 16 and 19%. The protein: N ratios were 5 and 4.94, indicating presence of significant amounts of non-proteinous nitrogen in plankton. The biochemical composition changed seasonally. The changes were related to temperature. The zooplankton proteins had a positive relation with temperature; on the contrary, phytoplankton protein level decreased in summer and the carbohydrate proportion increased. The fat content of zooplankton also increased in winter. The measured heat of combustion values (calories mg^{-1} org.d.w.) were significantly different in plankton: zooplankton, 5.78; and phytoplankton, 4.53.

The calorific values calculated from biochemical data were respectively 6.02 and 5.84 Cal mg^{-1} org.d.w. for phyto-and zooplankton. The conversion factor for carbon to energy units was found to be 10.95 Cal mg^{-1}C both for phytoplankton and zooplankton in contrast with the factor 10 usually employed. The data are being analysed to find the most suitable criterion for biomass, particularly with regard to the energy flow studies.

Published articles

Influence of temperature on animal life with special reference to food uptake and metabolism of zooplankton.
Gulati, R.D. In: Proc.World Conf. "Towards a plan of actions for mankind"; vol. 3; Biological balance and thermal modifications; ed. by M.Marois. Pp. 205-218.

The paper describes the physiological and ecological effects of elevated temperature on freshwater zooplankton. Both, from the literature cited and the author's own studies it appears that the filtering, food consumption and assimilation rates of *Daphnia* spp. significantly increases if the water temperature is raised. The assimilation: biomass ratios (%) of the mixed natural populations of filter feeders increased significantly when the temperatures rose from 4-10 to 10-20°C ; the ratios at the two temperature ranges in the lakes investigated were: Vechten, from 13.9 to 32.7% ; and Tjeukemeer, 28.1 to 41.8%. The effects of increased temperature were also recorded in the case of respiration rates, egg development time and growth and production rates.

In conclusion the alterations in the temperature regimes of lakes due to the thermal effluents from the power stations are considered to result in drastic modifications in the energyflow patterns in the food chain.

Articles in press

The ecology of common planktonic crustacea of the freshwaters in the Netherlands.

Gulati, R.D. *Hydrobiologia* 59(2); 101-112. (1978).

The paper discusses the freshwater crustacea of the Netherlands, specially the common planktonic forms, with notes on their distinguishing features, habits, habitats and modes of food capture. The filter feeders belonging to both cladocera and copepoda are discussed with comments on their densities, distribution in depth, feeding and assimilation rates.

Important among the limnetic filter feeders are *Daphnia cucullata*, *D. hyalina*, *Bosmina longirostris*, *B. coregoni*, *Diaphanosoma brachyurum*, *Ceriodaphnia pulchella*, *Chydorus sphaericus* and *Eudiaptomus gracilis*. *D. magna* and *D. pulex* are encountered invariably in ditches and ponds rich in detritus, bacteria and dissolved organic matter. The oxygen content of such waters is usually relatively very low.

A study of food relations of the herbivore crustacea in two limnologically different lakes revealed remarkable differences. Average food levels in lake Vechten - a deep stratified lake - in the summer periods of 1972 to 1976 varied between 0.4 and 0.6 g C m⁻³; corresponding figures for lake Tjeukemeer were almost an order of the magnitude higher. In lake Vechten almost hundred percent of the daily primary production of phytoplankton during summer (0.3 - 0.5 g C m⁻² d⁻¹) is consumed by herbivores; in Tjeukemeer only 30 to 50% of algal primary production (ca 3 g C m⁻² d⁻¹) is grazed down by the primary consumers.

In summary, the zooplankton production rates in summer of 0.06 to 0.075 and 0.090 - 0.150 g C m⁻² d⁻¹ in lakes Vechten and Tjeukemeer, respectively, constitute 15 to 20 percent and 3 to 5 percent of the daily primary production rates in the two lakes.

Vertical changes in the filtering, feeding and assimilation rates of dominant zooplankters in a stratified lake.

Gulati, R.D. Verh.int.Verein theor.angew.Limnol., 20; 950-956.

The paper deals mainly with a study of vertical and seasonal changes in the feeding rates of the dominant crustacean filter feeders carried out in Lake Vechten during 1976. The contribution of the different water strata and of the filter feeders, particularly in the period May - September, to herbivores' total ingestion and assimilation was examined in detail. The grazing pressure of zooplankton was significantly higher in the deeper waters during day-time throughout the summer period. In comparison with the mean grazing intensity of $24\% \text{ d}^{-1}$ during the study period, that in strata below 5.4 m up to the anoxic layers reached $100\% \text{ d}^{-1}$ in early August. About 70% of the annual grazing occurred in the summer months. Together with the spring period it consisted of 90% of the community grazing.

The mean filtering and feeding rates of *Eudiaptomus gracilis* were the highest of all the filter feeding crustaceans. Nevertheless, the *Daphnia* spp., because of their much higher densities particularly at the intermediate depths contributed the most to the community consumption and assimilation rates (37% each), followed by *Eudiaptomus* sp. and *Bosmina longirostris*, *Daphanosoma brachyurum* and *Ceriodaphnia pulchella* played a relatively insignificant role in the community's food intake.

Study of the phosphorus loading and retention in IJsselmeer.

Kloet, W.A., de. Verh.int.Verein theor.angew.Limnol. 20.

The investigation concerns the retention of phosphate in the IJsselmeer, with particular reference to the role of Ketelmeer as "P sink". The lake receives water from river IJssel - a Rhine tributary - which is the main source of phosphate to IJsselmeer via Ketelmeer. In 1969 the IJsselmeer received about 4 g Tot-P.m^{-2} ; about 75% of this was retained in the lake. A comparison of the decrease in the concentrations of $\text{PO}_4\text{-P}$ and Part-P shows that approximately 20 to 40% of the incoming phosphate is retained in the Ketelmeer. Decrease of $\text{PO}_4\text{-P}$ may be due to 1) physico-chemical processes and 2) to biological fixation. Further, it appears that sedimentation of Part-P plays a major role in the decrease of phosphate concentrations in this area.

WORKING GROUP "ECOPHYSIOLOGY OF WATER PLANTS"

P.H.Best, C.L.M.Steenbergen, H.J.Gons, H.J.Korthals, M.D.M.Trommel, M.J.B.Bär-Gilissen & J.H.A.Dassen

Introduction

The aim of this group is to study ecophysiological aspects of three different groups of primary producers, notably aquatic macrophytes, with macrophytes associated periphytic algae and phytoplankton. Special attention is paid to the influence of the abiotic factors light, temperature and

nutrients on colonization, growth and development of these organisms. Additional information to data obtained from field observations is gained under laboratory conditions by means of experiments in batch and continuous cultures.

Finally this working group studies the photosynthetic fixation of sun energy of macrophytes and periphyton and the role of these two groups of organism in the carbon and nitrogen cycles of the lake.

Photosynthetic carbon fixation in situ

Of the littoral region in Lake Vechten the zone of the submerged macrophytes is most important. Here the following three macrophytic species predominate; *Elodea canadensis*, *Ceratophyllum demersum* and *Myriophyllum verticillatum*. In order to quantify the role of these species and its periphyton in the carbon cycle of the lake, primary production was measured *in situ* using the ^{14}C method, from June 1977 on. It was observed that the carbon fixation rate, on dry weight basis, of *Elodea* and *Ceratophyllum* decreased at the end of June. In *Ceratophyllum* it remained almost constant from June till late October. In the case of *Elodea* the carbon fixation rate increased from the beginning of August and reached a maximum value late in October. The carbon fixation rate of *Myriophyllum* showed the same tendency as that of *Elodea*. In June and July the carbon fixation rate of *Elodea* and *Ceratophyllum* was similar. From the beginning of August the fixation rate of *Elodea* and *Myriophyllum* was much higher than of *Ceratophyllum*. Excretion was always lower than 1% of the fixation.

From these data, combined with data on cell counts of periphyton, it may be concluded that maximum fixation of the macrophytes occurs in May - June, as generally is the case in temperate lakes. In that period the photosynthetic tissues of the plants are well developed, the plants are growing fast and their surfaces are not yet densely occupied with periphyton. As the periphyton biomass increases greatly during summer and autumn almost all carbon fixed in the autumn must be attributed to the periphytic algae.

In 1978 the studies on primary production and the role of macrophytes and periphyton in the carbon cycle will be continued.

Laboratory studies on carbon fixation of macrophytes

In the laboratory several aspects of photosynthesis in *Ceratophyllum demersum* were studied. The carbon fixation and excretion of plant units, consisting of an apex and six whorls of leaves, in dormant, quiescent and fully grown state was compared by means of the ^{14}C method. Fixation was highest in fully grown plants and decreased in the process of ageing. Light and dark respiration of quiescent plant units was measured also. Light respiration was much higher than dark respiration, which indicated an efficient refixation of respired carbon dioxide. Excretion of the used plant units was always very low, but increased in the process of ageing.

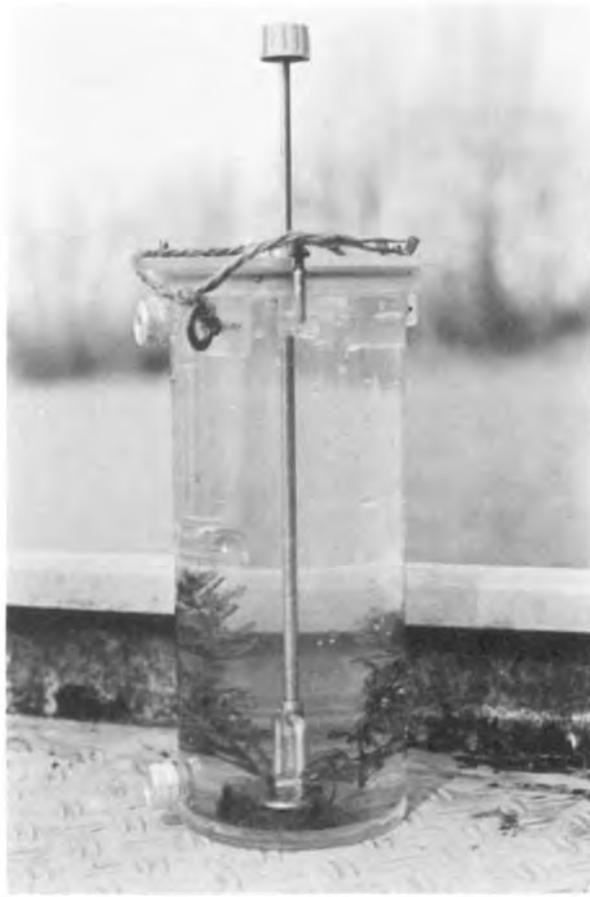


PLATE I. *Incubator for in situ measurements of primary production of macrophytes and periphyton.*

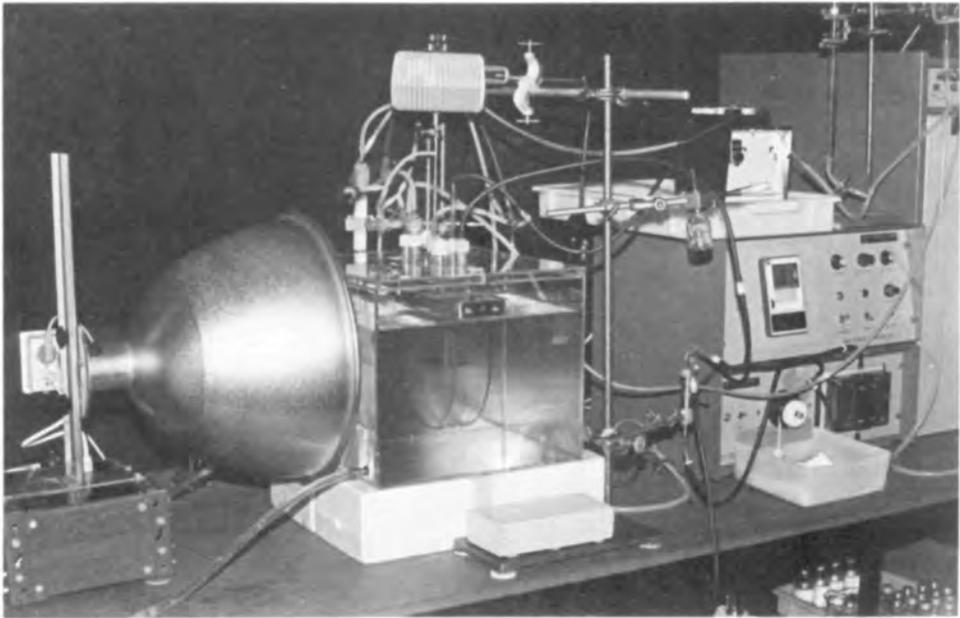


PLATE II. *Turbidostat for culturing diatoms.*

The presence of photorespiration in aquatic plants and the potential of reduced net photosynthetic carbon productivity under conditions which favour high rates of this process led to the question whether the C_4 photosynthetic system occurs in any aquatic plant.

To determine whether *Ceratophyllum* has a C_3 or a C_4 photosynthetic cycle analysis of the first products in photosynthesis was started by means of thin layer chromatography. C_3 as well as C_4 products were detected.

A method was developed to determine the gaseous composition of the gas lacunae of water plants.

Nitrogen uptake of macrophytes

In order to investigate the role of the dominant macrophytic species in the nitrogen cycle of the lake some preliminary experiments were carried out. The effects of nitrogen on growth and morphology of *Ceratophyllum* were studied. In long term experiments, nitrogen added as ammonium-N was lethal in high concentrations (45-105 mg l⁻¹). Nitrate-N in similar concentrations, however, did not affect growth. Ammonium-N was absorbed faster from the medium than nitrate as the plants are probably ammonium-adapted. The N-contents of all N-treated plants were higher than that of untreated plants. Plants treated with ammonium contained relatively more free amino acids than controls or plants treated with nitrate. These experiments will be continued. Moreover short term experiments will be carried out to investigate the uptake kinetics and turnover rates of nitrogen, using ¹⁵N techniques. Methodological studies for qualitative and quantitative analysis of amino acids are in progress.

Periphyton studies

In 1977 a regular investigation was started on the Lake Vechten periphyton, with emphasis on the role of the periphytic algae.

Most attention was directed towards the periphyton associated with the predominating submerged macrophytes (i.e. *Ceratophyllum*, *Elodea* and *Myriophyllum*), as most of the littoral zone is densely overgrown with these plants during the greater part of the year. Periphyton was sampled fortnightly by collecting tips, having a length of about 75 cm, of the macrophytes. This was done by SCUBA diving. The plant parts and their periphyton were separated using a Vibromixer (Chemap A.G., Zürich). In this way the more loosely associated algae can be easily removed quantitatively. Later in the year this method proved to be inadequate which was due to the formation of carbonate encrustations on the macrophytes. The macrophyte and all the associated periphyton could be separated effectively, however, by vibration in medium kept at pH 5 with pH-stat control. With this method the algae were not damaged and mixing of plant and algal material did not occur.

The numbers of periphytic algae were determined using an inverted coun-

ting microscope. Their pigment composition was studied using paper chromatography. The composition of the algal species did not differ significantly between the different macrophytes. However, much more periphytic algae, per macrophyte biomass, were counted in the *Ceratophyllum* and *Myriophyllum* samples than in the samples of *Elodea*. In general there was a substantial amount of metaphyton present, i.e. algae which may also be abundant in the phytoplankton. Thus, in early spring, the green algae *Chlorella* and *Scenedesmus* predominated in both the phytoplankton and periphyton; these algae and representatives of the planktonic diatoms (e.g. *Asterionella* and *Stephanodiscus*) and flagellates could also occur later in the year in great numbers in the periphyton. During May and June filamentous, mostly green, algae (e.g. *Spirogyra*, *Mougeotia*) predominated. In turn, epiphytic microalgae could be observed on the filamentous algae. From June up to the end of the macrophytic growing season pennate diatoms predominated, in particular representatives of the genera *Nitzschia*, *Navicula*, *Amphipleura*, *Epithemia* and *Fragilaria* (see Fig. 6).

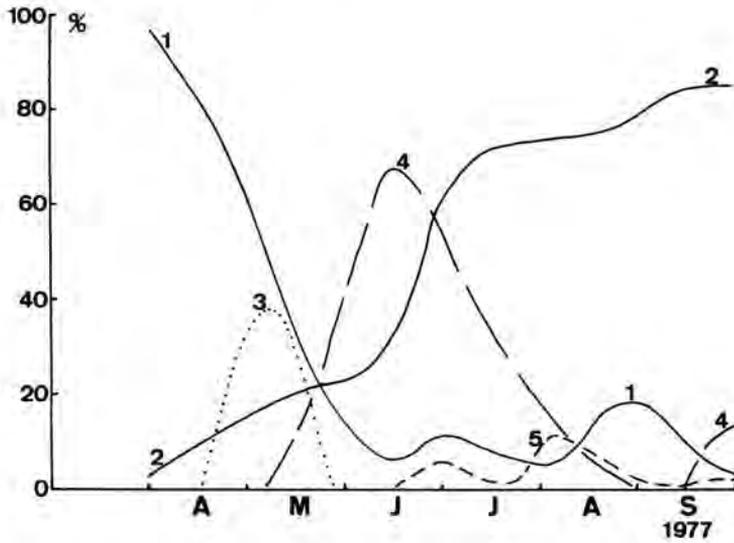


Fig. 6 Seasonal periodicity of the most important groups of periphytic algae on *Ceratophyllum* tips, on the basis of total cell counts; (1) chlorococcal green algae, (2) pennate diatoms, (3) flagellates, (4) filamentous green algae, (5) blue-green algae.

The species composition of the periphyton was completely different from that of the epilimnetic phytoplankton, where *Chlorella*, *Ceratium* and flagellates predominated during that time. That the pennate diatoms were also in quantitative respect by far the most important group in the periphyton was substantiated by the results of the paper chromatographic analysis of

the pigment composition.

The periphytic biomass may be very large in Lake Vechten. In September the periphyton dry weight, which consisted for the greater part of detritus and algae, appeared to be two to three times higher than that of the plant shoot (*Ceratophyllum* or *Myriophyllum*) from which it was collected. The ash-free dry weight of the periphyton could be as much as two times higher than that of the relative macrophyte tip. Whether the periphyton as a whole is important in the primary production in Lake Vechten will depend on the photosynthetic capacity of the algal biomass relative to the bacterial breakdown of the detritus. In 1978 this will be investigated by means of incubations of plant shoots and their associated periphyton, simultaneous measurements of carbon fixation and oxygen production in the light, and oxygen uptake in the dark.

Phytoplankton studies

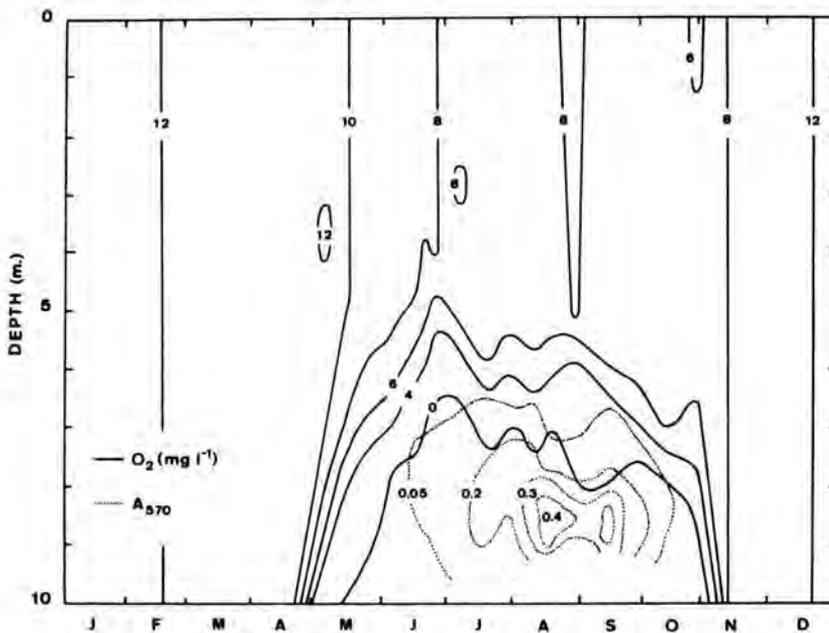


Fig. 7 Isopleths for the oxygen concentration and for the relative phycoerythrin content, the accessory photosynthetic pigment of blue green algae measured as absorbance at 570 nm in intact cells collected on glass fiber filters using an Unicam SP 1800 spectrophotometer.

Regarding the study of phytoplankton special attention was paid to the vertical distribution of blue-green algae. This year the blue-green algal population consisted mainly of *Lyngbya limnetica* and *Merismopedia* sp.: furthermore species of *Pseudoanabaena*, *Cylindrospermum*, *Anabaena* and

Oscillatoria were present. The maximum population density was found during summer in the anaerobic hypolimnion (see Fig. 7). These algae occur together with photosynthetic bacteria as was determined by means of paper chromatography of the photosynthetic pigments in the samples. In samples which contained the highest concentrations of accessory photosynthetic pigments of blue-green algae also high concentrations of bacteriochlorophyll were found.

Several species of blue-green algae were isolated from the hypolimnion and from the mud. These algae will be studied in continuous cultures under aerobic and anaerobic conditions. Furthermore the role of these algae in the carbon and nitrogen cycle and their relationships with the photosynthetic bacteria will be studied.

Published articles

Seasonal changes in mineral and organic components of *Ceratophyllum demersum* and *Elodea canadensis*.

Best, Elly P.H. *Aquat.Bot.*, 3; 337-348.

Seasonal changes of some organic and mineral components of the two dominant macrophytes in a sandpit were investigated. Both species - *Ceratophyllum demersum* L. and *Elodea canadensis* Michx. - occur in two distinct forms: the normal vegetative form in summer and the dormant form in winter. Samples were regularly obtained by means of SCUBA diving. Chemical analyses showed that carbon, nitrogen, protein and starch fluctuated with season. Preceding vegetative growth and during this growth in spring, a high percentage of the nitrogen is available in the form of protein, pointing to a high rate of enzyme synthesis. Starch is accumulated in winter and metabolized during growth in spring. The ATP content is higher in summer than in autumn and winter. The content of total carbohydrate is variable with a minimum in summer. The calcium and manganese contents are dependent on season, but iron and magnesium are not.

A preliminary study of the internal gas composition of *Lemna gibba* L.

Best, Elly P.H., A.H.Pietersen, R.Soekarjo & L.de Lange

Acta Bot.Neerl. 26(2); 109-113.

The internal atmosphere of gibbous as well as flat modifications of *Lemna gibba* G3 was determined by gas chromatography after cultivation in vitro under conditions of continuous light. A similar analysis was done on a gibbous strain of *L. gibba* collected in the field. The gaseous constituents of the air chambers were found to be N_2 , O_2 and CO_2 . The CO_2 concentration was approx. 1% in gibbous forms of strain G3 as well as the field strain and approx. 3% in flat forms of strain G3. The O_2 concentrations tended to be slightly higher in the gibbous forms than in the flat. The CO_2 to O_2 ratio of the flat modification was much higher than in the gibbous forms. The remaining part of the internal atmosphere consisted of

nitrogen. Methane and ethylene could not be detected.

Articles in preparation or in press

Primary productivity in the submerged aquatic macrophyte *Ceratophyllum demersum*.

Best, Elly P.H. & John T.Meulemans.

Verh.int.Verein theor.angew.Limnol. 20

Primary productivity, respiration and excretion in *Ceratophyllum demersum* were studied to investigate the role of this species in the carbon cycle of Lake Vechten.

Under experimental conditions, the effects of incubation during different periods in the light - combined with different pre-or postincubations - on photosynthesis were studied using the ^{14}C method. In the experiments apices were used from a preconditioned culture. These apices are photosynthetically the most active parts of the plants. Photosynthesis was highest during 2 - 4 hours after transfer to experimental conditions, ^{14}C fixation amounting to $0.002\% \text{ mg dry weight}^{-1} \text{ hr}^{-1}$ ($0.44 \text{ mg C g}^{-1} \text{ hr}^{-1}$). Excretion of non gaseous organic compounds was very low, excretion of $^{14}\text{CO}_2$ considerable. No correction was used for respiration as the ^{14}C assay is considered to be a method to measure approximate net photosynthesis in macrophytes, when short incubation periods are used. The data were low compared to those in the literature, probably caused by the late dormant state of the plant material. A seasonal effect in the rate of photosynthesis was detectable. The experiments will be followed by *in situ* measurements.

WORKING GROUP "MINERALIZATION OF ORGANIC MATTER"

Th.E.Cappenberg, H.Verdouw, M.A.C.I.Blaauboer-Wiercx, E.Jongejan, E.M.J.Dekkers, M.D.M.Trommel, R.van Keulen & J.Kaper

General introduction

This group investigates the dynamics of the carbon and nitrogen cycle in the open-water and in the sediments. Particular interest is given to aerobic and anaerobic mineralization processes of organic carbon (C) and nitrogen (N) fixed during the primary production. Ecological relationships between micro-organisms, which can be inferred from field observations, are tested in the laboratory by means of continuous cultivation techniques and studies of the kinetics of processes using labelled compounds.

Aerobic mineralization

Introduction

As part of the study on aerobic mineralization special attention is given to the relationship between extracellular products of phytoplankton and number and activity of heterotrophic bacteria. This year information was obtained by simultaneous measurements of photosynthetic carbon fixation, excretion of photosynthetic products, bacterial cell counts and of

chlorophyll a (as an index of phytoplankton biomass) at various depths in the lake.

C-fixation and excretion

Both the total carbon fixation (sum of carbon fixation and carbon excretion), as measured by the ^{14}C -method, and the concentration of chlorophyll a showed two main maxima in 1977 (see Fig. 8).

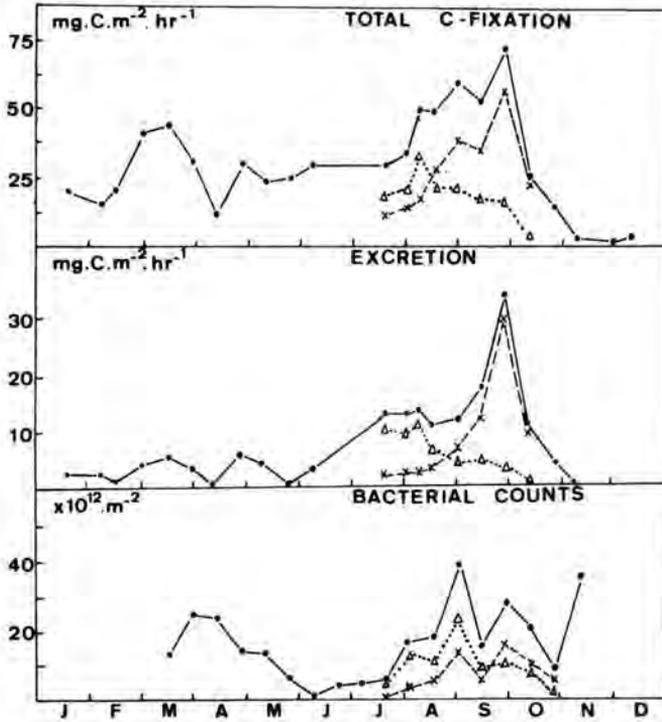


Fig. 8 Rate of total carbon fixation and carbon excretion of phytoplankton measured in situ and numbers of bacteria; the drawn, broken and dotted lines represent respectively the values for the water columns 0-8.5 m, 0-5 m and 5-8.5 m depth.

One occurring in March, when diatoms and *Chlorella* sp. were the dominant phytoplankton species. A percentage extracellular release (p.e.r.) of 10 - 15% of the total fixed carbon was observed during this period (Fig.8). Concomitantly with the growth of zooplankton (in May, cf. Fig. 5) the biomass of phytoplankton decreases, which may explain the simultaneously occurring decrease of the carbon fixation rate.

The highest maximum in C-fixation was demonstrated in August and September. In the beginning of this period a high phytoplankton biomass, consisting mainly of *Lyngbya limnetica*, *Mallomonas caudata* and *Chlorella* sp., and a high productivity in the metalimnion at a depth of 6 - 8 m was observed (cf. Fig. 11).

In spite of the reduced radiation at this depth (1 - 2% of the surface light intensity) 65% of the total carbon fixation took place in this layer. Furthermore excretion of assimilated carbon is notably high in this stratum and amounted to 40 - 60%. From the second half of August *Mallomonas* was distributed over the whole water column in lower numbers. The bluegreen algae remained at a depth of 7 - 8 m until the end of September, though p.e.r. fell to a low level (10 - 15%). At the same time numbers of *Ceratium hirundinella* and diatoms increased, which caused a high C-fixation rate predominantly in the epilimnion. The p.e.r. was low at the onset of the *Ceratium* bloom, but increased in September to about 40% (see Fig. 8).

During October the biomass of phytoplankton and the rate of C-fixation decreased gradually, in contrast to the p.e.r. which remained relatively high (30%). In the first week of November the overturn took place and in the last two months of the year amounts of C-fixation were very low.

In conclusion the p.e.r. appeared to be independent of depth; there was no increase in p.e.r. when C-fixation was inhibited by the high light intensities near the surface. No significant correlation was found between photosynthetic C-fixation and respectively chlorophyll a concentration, the amount of dissolved inorganic phosphorus and total light energy. Therefore total carbon fixation seems to be determined by the species composition of the phytoplankton community.

Course of bacterial numbers

In April the number of bacteria increased concomitantly with the decrease of diatom numbers. A second maximum in bacterial counts was demonstrated in August and September (see Fig. 8), when both C-fixation and excretion rates were very high. The highest bacterial numbers were observed at a depth where the amount of p.e.r. was relatively high i.e. during August in the metalimnion. Later (during September) the same correlation (relatively high amounts of p.e.r. coinciding with high numbers of bacteria) was found in the epilimnion. In October the bacterial numbers decreased simultaneously with the biomass and productivity of phytoplankton. After the overturn (mid November) a great number of bacteria (about $5.5 \times 10^9 \text{ l}^{-1}$) was present throughout the whole water column.

The following working hypothesis can be made on the basis of these results. During spring the detritus resulting from the spring-bloom will be the main energy and carbon source for the growth of the heterotrophic bacteria. Evidence is obtained that in summer the extracellular products of phytoplankton form an important substrate for bacterial growth. To test this hypothesis these events will be investigated in more detail during 1978. Also attempts will be made to analyse the excreted carbon compounds on their chemical structure.

Culture studies

In addition to the *in situ* work excretion of carbon compounds was studied in chemostat cultures under light-and phosphorus (P)-limited conditions. The organism used was *Chlorella* sp. isolated from Lake Vechten and present throughout the year. At all growth rates the content of dissolved organic carbon (DOC) in filtrates obtained from light-limited cultures was 4% on the basis of dry weight. The DOC content of filtrates obtained from P-limited cultures amounted to 8% on a dry weight basis. Increased light intensities during P-limited growth had no effect on the level of excretion. At present the effects of temperature and nitrogen (N)-limitation in this respect are studied, as well as N- and P-content, and molecular weight distribution of the excreted C-compounds. An inverse relationship between yield on phosphorus and growth rate was found in P-limited cultures i.e. at low growth rates (below $\mu = 0.01 \text{ hr}^{-1}$) the yield appeared to be 5-fold of the yield at near maximum growth rates ($\mu = 0.033 \text{ hr}^{-1}$). Some experiments will be done on the effect of temperature on this relationship.

Anaerobic mineralization

Introduction

Part of the carbon fixed during photosynthesis will settle and will reach the bottom of the lake. There the carbon compounds will be broken down partly by a number of different microbial processes, i.e. the anaerobic mineralization.

This mineralization plays an important role in the carbon cycle of the lake and consists of the formation of acids followed by the formation of gases. The first process i.e. the conversion of compounds as e.g. cellulose and chitin into mainly fatty acids and hydrogen gas is accomplished by a heterogeneous group of bacteria. The fatty acids are used by a unique group of bacteria, the methane producing bacteria. They produce CH_4 and CO_2 . Still another group of bacteria converts lower fatty acids and alcohols to CO_2 and H_2 . These two gases are used by H_2 / CO_2 -fermenting methanogenic bacteria, i.e. the phenomenon of interspecies hydrogen transfer (see Fig.9).

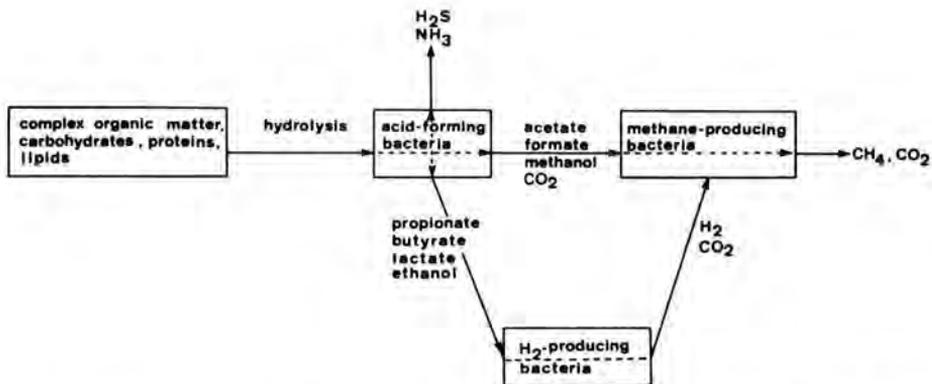


Fig. 9 Schematic representation of the anaerobic mineralization in the sediments of Lake Vechten.

Breakdown kinetics

This year the method for analysing the pool sizes of acetic acid in sediments was improved, using gas chromatography. In the mud core accurate values between $0.3 \pm 0.05 \text{ mg l}^{-1}$ and $1.5 \pm 0.05 \text{ mg l}^{-1}$ could be obtained. The rate of dissimilation of acetate was measured by the disappearance of ^{14}C -labelled acetate from mud samples using a gas chromatography-gas proportional counting system. The higher turnover of acetate ($0.07 \text{ mg l}^{-1}\text{hr}^{-1}$ versus $0.04 \text{ mg l}^{-1}\text{hr}^{-1}$) found deeper in the mud is in agreement with earlier observations in that at that depth maximum numbers of acetate-fermenting methanogenic bacteria were found.

The breakdown of acetate to CH_4 and CO_2 was followed by experiments with uniformly and non-uniformly ^{14}C -labelled acetic acid. The following conclusions concerning this breakdown, based on the distribution of the radioactivity, can be drawn: the splitting of one mole of acetate into one mole of CH_4 and one mole of CO_2 is more important in the lower layers. On the other hand in the upper layers of the mud the oxidation of one mole of acetate into two moles of CO_2 is more important. In addition, the label distribution between $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ in relation to the depth in the mud core was studied. From the results it was clear that oxidation of the methyl carbon of acetate to CO_2 is more important in the upper layers. By radio gas chromatography we found that incubation with CH_4 of mud samples or of pure cultures of *Desulfovibrio desulfuricans* does not result in formation of CO_2 . Therefore, it seems likely that part of the carbon of acetate is converted to CO_2 by anaerobic sulfur-reducing organisms, since by oxidation of FeS free sulfur is available in the upper layers of the mud in Lake Vechten.

Since the turnover rate constants, pool sizes and breakdown pathways of acetate are depth dependent, generalizations regarding the turnover and breakdown of acetate in mud of Lake Vechten are rather difficult. We found that about 70% of the methane produced, is derived from acetic acid. There is now evidence that the importance of acetic acid, the precursor of methanogenesis, is depth dependent.

Methodological work was done on the determination of non-volatile lower fatty acids, as lactate in sediments. Presently the method is based on the conversion of the acid into the benzyl esters via lipophilic tetra-n-butylammonium salts. The benzyl esters are detected and determined by gas-liquid chromatography and the labelled benzyl esters are assayed by radio gas chromatography. From the kinetic studies on the breakdown of lactate in mud samples this process could not be described according to the simple model of formation of only acetate and subsequent formation of CH_4 and CO_2 . The conclusions based on calculations employing first-order kinetic equations and on experiments with uniformly and non-uniformly ^{14}C -labelled lactate showed that lactate is broken down into propionic, butyric, acetic acid and

CO₂ simultaneously; with different turnover rate constant values. This is not only a kinetically complex system, but moreover the kinetic parameters are depth dependent in the mud. A further study to unravel this complex system is necessary.

Ecological relationships

Data on the ecological relationships between bacterial species involved in the anaerobic mineralization were obtained from mixed continuous culture studies. The influence of the H₂-producing, sulfate-limited *Desulfovibrio desulfuricans* on the fermentation of limiting amounts of H₂ by *Methanobacterium formicicum* was investigated. The metabolic coupling found, is an interspecies hydrogen transfer of electrons in the form of molecular hydrogen from *Desulfovibrio* to the H₂-utilizing terminal electron acceptor *Methanobacterium*. As lactate is always present in the mud of Lake Vechten, but sulfate is depleted during summer stratification by the activity of sulfate reducers, a seasonal variation in the products formed by *Desulfovibrio* can be expected, i.e. in summer more H₂ and in winter more acetate. Field observations have shown that the maximal abundance of sulfate reducers in the mud is in the 0 - 2 cm layer, and that of H₂ / CO₂-fermenting methanogenics is in the 2 - 3 cm layer. These latter bacteria may thus serve as an hydrogen sink and provide favourable growth conditions for the sulfate reducers. However, the chemostat studies are model systems and may not represent the *in situ* activities of the bacteria. A more natural approach, i.e. use of auto-radiographical and anti-body fluorescent techniques, is desirable.

Studies on the first stage in anaerobic mineralization - acid formation - involved the isolation, using the Hungate roll-tube technique, of various strains of bacteria, which are able to ferment cellulose or algal cell walls (*Chlorella* sp.). The major end products from these isolates, mainly *Clostridia*, were acetic, lactic, butyric, propionic and capronic acid, and the gases H₂ and CO₂. Preliminary experiments concerning the cellulolytic activity in mud by the use of U-¹⁴C- cellulose and U-¹⁴C-algal cell walls showed that breakdown of these organic compounds is probably the rate-limiting step in anaerobic mineralization. Field observations on the number of cellulose-splitting anaerobic bacteria in the mud of Lake Vechten indicated a maximal abundance of about 5.10⁶ ml⁻¹ of wet mud at a depth of 7 cm. Further investigations on breakdown of these organic material and the importance in carbon cycling will be carried out.

Nitrogen cycle

Introduction

This year studies on the nitrogen cycle in Lake Vechten were continued. The main purpose of this study is to follow the course of nitrogen minera-

lization in organic material together with that of carbon. In 1977 the changes in the amount of different nitrogen compounds (or groups of compounds) in the eastern part of lake Vechten were determined. At the deepest point of the eastern depression samples were taken from surface to bottom and the following determinations were carried out: ammonia ($\text{NH}_3\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON). Taking in account the volumes of the different water layers, the total amounts of the N-species at different depths were calculated.

Results

In Fig. 10 the changes during 1977 of the amounts of different N-compounds are depicted for the whole water column (0 - 10.5 m) and for the epilimnion (0 - 6 m), which contains about 80% of the total volume. Clearly nitrogen disappears completely from the epilimnion during spring and summer, but is recovered in the hypolimnion as ammonia and particulate nitrogen.

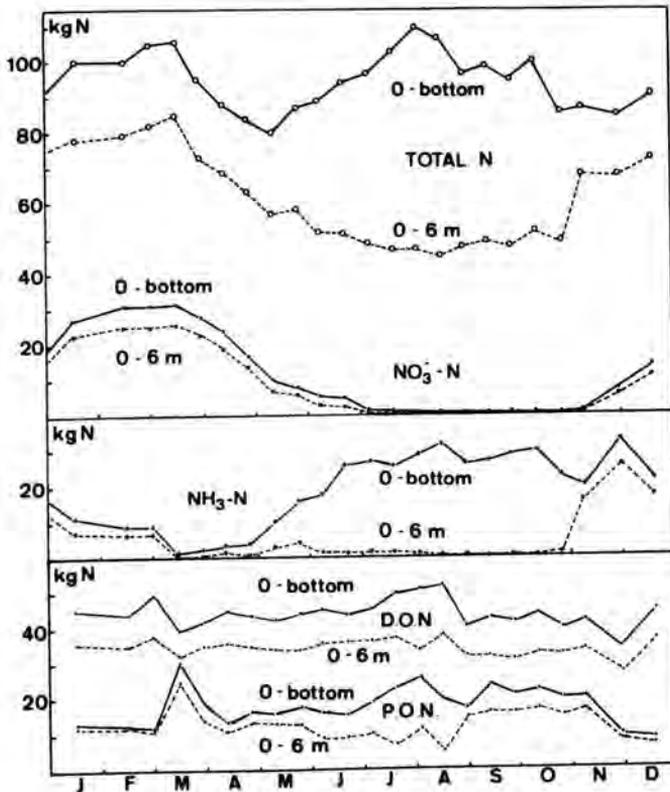


Fig. 10 Changes in the amount of different nitrogen compounds during 1977 .

No definite conclusions can be drawn as to where the ammonia has been formed: during the sedimentation or after reaching the bottom. Decrease of PON-concentrations close to the bottom (below 8.4 m) suggests a very active mineralization during sedimentation. Nitrogen is supplied to the epilimnion as ammonia when stratification is destroyed, followed by nitrification to nitrate. Tracer studies with ^{15}N concerning nitrogen uptake by phytoplankton and macrophytes, recycling of ammonia by zooplankton, turnover rates of the hypolimnetic- and sediment ammonia fractions, and of nitrification are in progress.

The downward flow of particulate organic matter was studied using sediment traps, which were suspended at three depths to entrap sedimenting organic matter. The traps were replaced with time intervals of 1 to 4 weeks. Total nitrogen and dissolved nitrogen fractions were determined in the traps. Particulate material was collected by filtration, dried at 105°C and analysed for carbon and nitrogen content.

Mineralization in the traps can be considerable, especially when the traps had been in the lake for extended periods of time: up to 30% of the nitrogen was present as ammonia in some cases.

From March till December the following ranges in sedimentation rates at different depths were found: $1.4 - 18.9 \text{ ug N cm}^{-2}\text{day}^{-1}$ at 9.7 m, $3.0 - 7.6$ at 7.8 m and $1.6 - 6.0$ at 4.7 m. Highest sedimentation rates occurred just before the overturn (end of October), lowest values were found in December.

Differences between traps at the various depths were most pronounced during stratification, the lower traps receiving more sedimenting material than the upper ones. During circulation periods of the lake the differences were relatively small. The well-known funnel effect in lakes, resembling Lake Vechten, makes calculations of total amounts of material reaching the bottom deposits difficult, so comparisons with the flow of nitrogen as indicated in Fig. 10 are not yet possible. No significant differences in C/N relations could be detected in traps from different depths of the lake. Further studies on the flow of particulate organic material to the bottom deposits are being carried out.

Articles in press

Microenvironments for sulfate reduction and methane production in freshwater sediments.

Cappenberg, Th.E. & E.Jongejan. In: Biogeochemistry of defined microenvironments in aquatic and terrestrial systems. Proceedings 3rd int.Symp.Environm.Biogeochem.; ed. by W.Krumbein, 3 vls Pp. 129-138. (1978)

Summarising the ecological relationships between sulfate-reducing and methane-producing bacteria in freshwater sediments, we find a depth dependence of several parameters. The abundance of the sulfate reducers is highest in the upper layers of the mud and is limited by the sulfate concentration. The products of these bacteria, hydrogen sulfide and acetate,

govern the abundance of acetate-fermenting methanogenic bacteria, which are found deeper in the mud. On the one hand acetate is an important substrate for methanogenic bacteria, on the other hand high concentrations of sulfide inhibit the production of methane. Sulfide is partly or completely precipitated depending on the concentrations of ferrous ions. Also the redox potential is an additional factor in this respect. Emphasis is laid on the kinetic aspects of these processes in sediments. Turnover rate constants (k-values) are given of important intermediates and the breakdown of these products towards CO₂ and CH₄ is followed. An accurate method is developed, using radio gas chromatography, to analyse these processes and the complexity of the mud system is stressed.

Anaerobic breakdown processes of organic matter in freshwater sediments. Cappenberg, Th.E., E.Jongejan & J.Kaper. In: Proceedings int.microb.ecol. Symp.; ed. by J.A.R.Miles and M.W.Loutit. Berlin etc., Springer Verlag. "Proceedings in Life Sciences".

The kinetics of anaerobic breakdown of organic matter in the sediments into methane and carbon dioxide was investigated. Rates of dissimilation of important intermediate metabolites, i.e., acetate and lactate, were measured by the disappearance from mud-samples of ¹⁴C-labelled substrates using a gas chromatography-gas proportional counting system. The turnover rate constants (k-values) were calculated and rates of disappearance given by the product of the turnover rate and its pool size. The breakdown of intermediates to methane and carbon dioxide were followed by experiments with uniformly and non-uniformly ¹⁴C-labelled substrates in which the labelled compounds are not broken down fully; the conclusions drawn are based on the distribution of the radioactivity.

Finally, mixed continuous cultures were used as a tool to study the complex ecological interrelations between important bacterial species involved in the anaerobic mineralization, i.e., *Desulfovibrio desulfuricans* and *Methanobacterium formicicum*. The findings found, gave evidence to an inter-species hydrogen transfer.

Ammonia determination based on indophenol formation with sodium salicylate. Verdouw, H., C.J.A. van Echteld & E.M.J.Dekkers. Water Res. 12.

An ammonia determination based on formation of a substituted indophenol with sodium salicylate as phenolic reagent has been developed and compared with other methods. Sensitivity and reproducibility are comparable with results obtained in a method where phenol was used, while a number of the disadvantages inherent to the use of phenol are avoided. The salicylate method is specific for NH₃-N and interferences are generally absent in samples from natural fresh waters. The method can be easily applied for seawater analysis.

Seasonal periodicity of phytoplankton

M.A.C.I. Blaauboer-Wiercx & M.D.M. Trommel

This year the dominant species were counted in composite samples collected at 7 different depths in order to get information about their vertical distribution.

It was found that in winter, spring and early summer the algae were evenly distributed, whereas during the summer stratification certain species occurred at definite depths.

The spring-bloom reached its maximum in March. Dominant species at that time were *Chlorella* sp., *Scenedesmus* sp. (both belonging to the Division of the Chlorophyta), the diatoms *Stephanodiscus astraea*, *Asterionella formosa* and *Centronella reicheltii* and to a lesser extent *Tetraedron minimum* (Chlorophyta). In April the cell numbers of *Chlorella* sp. and the cell numbers of the diatoms decreased. From the diatoms only *S. astraea* remained present in low numbers during the summer. During their disappearance high numbers of diatoms were found at greater depths, which was probably caused by rapid settling of the cells.

In early summer (April till June) *Gemellicystis* sp., *Oocystis* sp. and *Closteriopsis* sp., three species belonging to the Chlorophyta, and small flagellated algae together with *Rhodomonas minuta* (Pyrrhophyta) and *Dinobryon divergens* (Chrysophyta) predominated.

During July and August high numbers of *Mallomonas caudata* (Chrysophyta) were found at a depth of 5 to 7 meters. At the same time *Lyngbya limnetica* (Cyanophyta) occurred in the layer just below *M. caudata* (see Fig. 11).

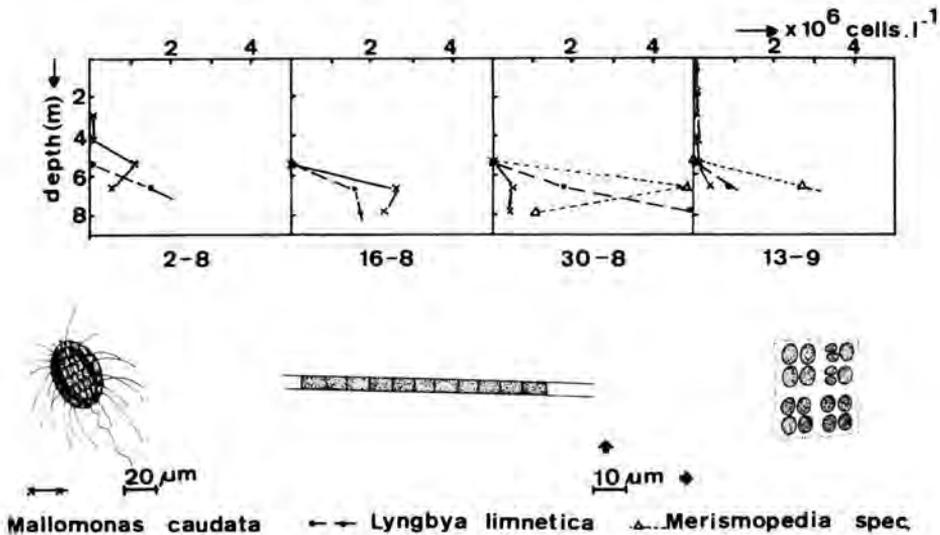


Fig. 11 Occurrence of phytoplankton species in the deeper water layers of Vechten in summer 1977.

In September the alga *Merismopedia* sp. (Cyanophyta) became apparent at a depth of 7 m (Fig. 11). Also in September the definite layer consisting of *M. caudata* cells disappeared, the cells becoming distributed over the whole water column. The Cyanophyta remained present till the end of September.

In early autumn the diatoms, which were abundant in the spring, again appeared, predominantly in the epilimnion, together with *Ceratium hirundinella* (Pyrrhophyta) and *Botryococcus braunii* (Chlorophyta). The latter species has not been found for 5 years.

Finally, after the overturn in November, *Rhodomonas minuta*, *Cryptomonas* sp. (Cryptophyta), *Mallomonas akrokomos* and *Chlorella* sp. were present. These algae can be regarded as winter species and they dominated, along with certain diatoms, the phytoplankton during December.

MISCELLANEOUS

PROJECT BERGUMERMEER

H.de Nie, J.B.W.Wanders & J.S.Swart

Introduction

Research has been carried out on the Bergumermeer since 1975. The Bergumermeer is the most northerly of the Frisian Lakes and supplies the water for direct cooling of a 640 MW steam electric power station. Because of the need for basic research on the effects of artificial heating of the ecosystem of a eutrophic lake, the influence of thermal discharges on the plankton community is being investigated. This research is financially supported by the Ministerie van Volksgezondheid & Milieuhygiëne (Ministry of Public Health and the Environment).

Results

In 1977 the mean difference in temperature between inlet and outlet water was 5.3° C. The temperature in the outlet harbour exceeded 20° C for 19 weeks, the same as in the hot summer of 1976. Near the inlet the temperature hardly exceeded 20° C in 1977, while in 1976 this occurred for a period of 6 weeks. Other temperature data and hydrological details are given in the Progress Report 1976.

Quantitative and qualitative differences in the phytoplankton community structure of the heated and non-heated areas were only temporary and of a local nature. They seemed to be caused more by the hydrological situation (see Progress Report 1976) than by heat disposal by the power station.

Passage of lake water through the power station has a considerable instantaneous influence on the physiology of the phytoplankton community as could be concluded from oxygen production and consumption experiments. Heating (4 - 7° C) of the lake water generally resulted in an increase of the oxygen production (photosynthesis) and oxygen consumption (respiration). However, the increase in photosynthesis of outlet water was about 10% lower

and the increase in respiration was about 30% higher than could be expected on account of temperature increase alone. When outlet water was cooled down to the temperature of the inlet water immediately after passage through the power station the apparent loss of photosynthetic activity was still noticeable for some hours. After 1 day recovery of photosynthesis and respiration was almost complete.

The growing and flowering season of a field of Fringed Waterlily (*Nymphoides peltata* (Gmel.) Kuntze) near the outlet of the power station, started about 2 weeks earlier and ended about 4 weeks later than that of a field near the inlet. The leaves of plants near the outlet were considerably larger (1.5 - 4.0 times) than the leaves from plants near the inlet.

The decrease in vitality of cladocerans after passage through the power station was quantified. There was an increase in mortality during the first 3.5 hours after passage, whereas the mortality in water from the inlet was 1.7 to 2.5%, the mortality in heated water after passage was 4.5 to 4.9%. The influences of heating and of passage were determined separately. The mortality of all species was most strongly influenced by passage.

In the heated water near the outlet zooplankton densities in the surface layer were lower than those near the inlet and other sampling points in the lake. This has been caused by vertical migration due to heating.

Work on a computer simulation model to evaluate the influence of temperature and heat disposal on the population dynamics of the most common cladoceran and copepod species has been started.

PLEOMORPHISM OF SCENEDESMUS QUADRICAUDA

C.L.M. Steenbergen, M.J.B. Bär-Glissen & H.J. Korthals

Introduction

One aspect of the morphological variability of spiny *Scenedesmus* species is their capacity to form unicellular as well as coenobial stages (see Fig. 12).

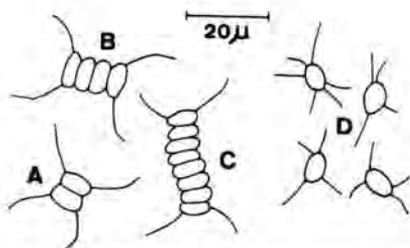


Fig. 12 A, B, C and D respectively 2, 4 and 8 celled coenobia and unicells of *S. quadricauda* (Camera lucida drawings).

This phenomenon has been found in natural environments and in cultures. The unicells are oval and possess usually two spines at each pole. Coenobia consist of 2, 4 or 8 elongated cells, which adhere to one another and are arranged in a plane, in such a way that their individual axes run parallel. The two outmost cells of a coenobium bear one spine at each pole.

The ecological significance of this pleomorphism is as yet unknown, although one may suppose that the ability of an organism to change its form in certain environmental conditions favours the potential for survival.

Results

In 1977 the study on the morphological variability of *S. quadricauda* was continued. Evidence was obtained that, besides nutrition, light has a distinct effect on the morphology. A relatively long photoperiod supports the production of unicells, whereas a relatively short photoperiod favours formation of coenobia. This light effect was further studied to ascertain whether this phenomenon can be explained on the basis of total light energy received or whether it is controlled by daylength. The evidence to postulate a genuine photoperiodic control of the morphology of our *S. quadricauda* strain is still inconclusive. At present the relationship between light intensity and morphology is investigated using continuous cultures. It is expected that these experiments will be finished in 1978.

Published

Nuclear divisions and cytokinesis during unicell and coenobia formation in synchronized cultures of *Scenedesmus quadricauda* (Turp.) Bréb. (Chlorophyceae). Steenbergen, C.L.M. & M.J.B. Bär-Gilissen. Acta Bot. Neerl., 26(2); 177-182.

Mitotic activity, cytokinesis and arrangement of daughter cells within the parental wall are studied in synchronized cultures of *Scenedesmus quadricauda*, which yield either unicells or coenobia. Irrespective of the formation of either unicells or coenobia, no essential differences occur in the nuclear patterns and the positions of the cleavage planes. Only the stages of daughter cell arrangement differ significantly in these two cycles. Daughter unicells are arranged within the parental wall more or less radially, whereas daughter coenobia become arranged with their axis parallel to the axis of the mother cell.

In press

Pleomorphism of *Scenedesmus quadricauda* (Turp.) Bréb. (Chlorophyceae) in synchronized cultures. Steenbergen, C.L.M. Mitt. Internat. Verein. Limnol. 21.

Cells of a pleomorphic *Scenedesmus quadricauda* strain are synchronized by 24 hour light-dark cycles (LD: 14, 10 regime). Synchronized cultures are diluted daily with fresh nutrient medium to the original cell density of ca. 0.4×10^6 cells per ml. Using different temperatures and different media morphologically stable synchronized culture systems are established which yield either coenobia or unicells. In synchronized cultures grown at a temperature of 30°C and with a medium containing 1800.5 mg per litre total inorganic salts only 4-celled coenobia are formed. When grown at a temperature of 20°C with a medium containing 130.5 mg per litre total inorganic salts partially synchronized cultures develop which consist of

2-celled, 4-celled and 8-celled coenobia. Attempts to change the latter coenobia-yielding system into a unicell-yielding system by increasing the concentration of P from 1.14 up to 127.4 mg per litre and by increasing the concentrations of both P (up to 127.4 mg per litre) and N (from 11.7 up to 105.4 mg per litre) failed.

A completely synchronized morphologically stable unicell-yielding culture system is achieved at a temperature of 20° C with high nutrient levels (i.e. 1800.5 mg per litre). This system becomes partly coenobial when subjected to short daylengths (e.g. LD: 3, 21 regime). Upon restoration of the original light-dark cycles (LD: 14, 10 regime) the coenobia disappear and the unicell-yielding system is re-established.

PARTICIPATION IN LAKE MAARSSEVEEN RESEARCH

Studies of the littoral zone

P.H. Best and H.L. Gons participated together with the Limnological and the Zoological laboratory of the University of Amsterdam, with the Botanical Department of the Free University (Amsterdam) and with the National Institute for Nature Management (RIN) in a project that was started in 1977. The aim of this project is to investigate several aspects of the littoral zone to come to a better understanding of relationships between different organisms, plants as well as animals.

An survey of the submerged macrophytes and macro-algae was made. The results were used to make a vegetation map of the lake. Dominant species were: *Elodea nuttallii*, *E. canadensis*, *Chara vulgaris*, *Zannichellia palustris*, *Potamogeton perfoliatus* and *P. lucens*. Frequently *Elodea*, *Zannichellia* and *P. perfoliatus* were sampled by means of SCUBA diving to study the periphyton of these species.

In 1978 more attention will be paid to changes in biomass of macrophytes and periphyton.

Methodological studies

Phytoplankton

W.A. de Kloet and B. Flik (Limnological Laboratory, University of Amsterdam) started a study, using the ¹⁴C-tracer technique, to compare the primary production *in situ* of lake Vechten water with the primary production of the water when incubated in a laboratory incubator.

Zooplankton

R.D. Gulati and J. Ringelberg (Limnological Laboratory, University of Amsterdam) collaborated in 1977 in a project in lake Maarsseveen. The purpose of this study was to compare the coulter counter technique with the ¹⁴C-method in grazing studies of herbivorous zooplankton. The study formed the basis for a joint research project to be financed by the Netherlands Orga-

nization for the Advancement of Pure Research (BION-ZWO) in 1978 and subsequent years.

LAKES IN THE VECHT RIVER AREA

R.D. Gulati and J. van Beuzekom (State University, Utrecht) studied the influence of river Vecht water on the limnological changes in lake Wijde Blik, particularly with regard to pollution *via* the Hilversum canal.

BORDER LAKES OF THE FLEVO POLDERS

H.L.Golterman & A.G.Wisselo

The work on the exchange processes of sediment phosphate of the border lakes of the Flevo Polders (a former part of the IJsselmeer) was continued. For this investigation the Board of Zuiderzee Works of Rijkswaterstaat has constructed 3 artificial ponds.

The work is carried out in collaboration with Rijkswaterstaat, i.e. results are exchanged and each year the research-programme is adjusted on the basis of the obtained results of all the participants.

ACKNOWLEDGEMENTS

The editors are indebted to Mrs. M.J.B.Bär-Gilissen, who designed and made Fig. 1 and Scheme 1, to Mr. E.M.Mariën for his contributions in layout and photographic work and to Mrs. R.Regensburg-Huiting for the care she has expended on the typing and lay-out of the manuscript.

Editors: Dr.C.L.M.Steenbergen

Dr.D.van der Mei (acting-director)

- Best, P.H. Seasonal changes in mineral and organic components of *Ceratophyllum demersum* and *Elodea canadensis*. *Aquat. Bot.*, 3; 337-348.
- Best, P.H., A.H.Pieterse, R.Soekarjo & L. de Lange. A preliminary study of the internal gas composition of *Lemma gibba* L. *Acta Bot. Neerl.*, 26; 109-113.
- Chambers, M.R. A comparison of the population ecology of *Asellus aquaticus* (L) and *Asellus meridianus* Rac. in the reed beds of the Tjeukemeer. *Hydrobiologia*, 53; 147-154.
- Chambers, M.R. The population ecology of *Gammarus tigrinus* (Sexton) in the reed beds of the Tjeukemeer. *Hydrobiologia*, 53; 155-164.
- Goldspink, C.R. The return of marked roach (*Rutilus rutilus* L.) to spawning grounds in Tjeukemeer, The Netherlands. *J.Fish Biol.*, 11; 599-603.
- Golterman, H.L. De eutrophiëring van het noordelijke gedeelte van de Adriatische Zee. *H₂O*, 10(7); 154.
- Golterman, H.L. Sediments as a source of phosphate for algal growth. In: Interactions between sediments and fresh water; Proceedings international Symposium, Amsterdam, 1976; ed. by H.L.Golterman. Blz. 286-293.
- Golterman, H.L. Waarom en hoe limnologisch onderzoek in Nederland. *H₂O*, 10(22); 514-516.
- Golterman, H.L. ed. Interactions between sediments and fresh water; Proceedings international Symposium, Amsterdam, September 6-10, 1976; ed. by H.L.Golterman. The Hague and Wageningen, W.Junk and Pudoc, 1977.
- Gons, H.J. On the light-limited growth of *Scenedesmus protuberans* Fritsch; ecological and physiological aspects of the energy requirements for growth and maintenance of a planktonic green alga. Is: Thesis, Univ. Amsterdam, 25 May 1977, Pp 120.
- Gulati, R.D. Influence of temperature on animal life with special reference to food uptake and metabolism of zooplankton. In: Proc. World Conf. "Towards a plan of actions for mankind"; vol. 3; Biological balance and thermal modifications; ed. by M.Marois. Pp 205-218.
- Haan, H.de Effect of benzoate on microbial decomposition of fulvic acids in Tjeukemeer (The Netherlands). *Limnol. Oceanogr.*, 22(1); 38-55.
- Steenbergen, C.L.M. & M.J.B.Bär-Gilissen. Nuclear division and cytokinesis during unicell and coenobia formation in synchronized cultures of *Scenedesmus quadricauda* (Turp.) Bréb. (Chlorophyceae). *Acta Bot. Neerl.*, 26; 177-182.
- Vijverberg, J. Population structure, life histories and abundance of copepods in Tjeukemeer. *Freshwat. Biol.*, 7; 579-597.
- Wanders, J.B.W. The role of benthic algae in the shallow reef of Curaçao. I: Primary productivity in the coral reef. *Aquat. Bot.*, 2; 235-270(1976).
- Wanders, J.B.W. The role of benthic algae in the shallow reef of Curaçao.

II: Primary productivity of the *Sargassum* beds on the north-east sub-marine plateau. *Aquat. Bot.*, 2; 327-335(1976).

Wanders, J.B.W. The role of benthic algae in the shallow reef of Curaçao.

III: The significance of grazing. *Aquat. Bot.*, 3.

ARTICLES IN PRESS

Beattie, D.M. Life cycle and changes in carbohydrates, proteins and lipids of *Pentapedilum uncinatum* Goet. (Diptera, Chironomidae). *Freshwat. Biol.* 8; 109-113(1978).

Beattie, D.M. Chironomid populations in the Tjeukemeer. Is: Thesis, Univ. Leiden, 19 June 1978, Pp 150.

Beattie, D.M. Chironomid populations in the Tjeukemeer. *Verh. int. Verein theor. angew. Limnol.*, 20.

Beattie, D.M., H.L.Golterman & J.Vijverberg. An introduction to the Limnology of the Friesian lakes. *Hydrobiologia* 58; 49-64(1978).

Beattie, D.M. & H.N.Kruif. Population dynamics and biomass production of *Neomysis integer* (Leach) in the Bergumermeer. *Verh. int. Verein theor. angew. Limnol.*, 20.

Best, P.H. & J.T.Meulemans. Primary productivity in the submerged aquatic macrophyte *Ceratophyllum demersum*. *Verh.int. Verein theor. angew. Limnol.*, 20.

Cappenberg, Th.E. Kinetics of breakdown processes of organic matter in freshwater sediments. *Beihefte Ergebnisse der Limnologie*.

Cappenberg, Th.E. & E.Jongejan. Microenvironments for sulfate reduction and methane production in freshwater sediments. In: *Biogeochemistry of defined microenvironments in aquatic and terrestrial systems. Proceedings 3rd int. Symp. Environm. Biogeochem.*; ed. by W.Krumbein, 3 vls. Pp 129-138(1978).

Cappenberg, Th.E., E.Jongejan & J.Kaper. Anaerobic breakdown processes of organic matter in freshwater sediments. In: *Proceedings int. microb. ecol. Symp.*; ed. by J.A.R.Miles and M.W.Loutit. Berlin etc., Springer Verlag. "Proceedings in Life Sciences".

Gons, H.J. Growth of *Scenedesmus protuberans* Fritsch in phosphorus-limited continuous cultures. *Verh. int. Verein theor. angew. Limnol.*, 20.

Gulati, R.D. Vertical changes in the filtering, feeding and assimilation rates of dominant zooplankters in a stratified lake. *Verh. int. Verein theor. angew. Limnol.*, 20.

Gulati, R.D. The ecology of common planktonic crustacea of the freshwaters in the Netherlands. *Hydrobiologia*, 59(2); 101-112(1978).

Haan, H.de & T.de Boer. Seasonal variation of fulvic acids, amino acids and sugars in Tjeukemeer, The Netherlands. *Arch. Hydrobiol.*

- Kloet, W.A.de Study of phosphorus loading and retention in IJsselmeer. Verh. int. Verein theor. angew. Limnol., 20.
- Verdouw, H., C.J.A. van Echteld & E.M.J.Dekkers. Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12.
- Steenbergen, C.L.M. Pleomorphism of *Scenedesmus quadricauda* (Turp.) Bréb. (Chlorophyceae) in synchronized cultures. Mitt. Internat. Verein Limnol., 21; 216-223.
- Wanders, J.B.W. The role of benthic algae in the shallow reef of Curaçao (Netherlands Antilles) . Is: Thesis, Univ. Groningen, 5 June 1978, Pp 84.

REPORTS DURING 1977

- Cappenberg, Th.E. Verslag studiereis Nieuw-Zeeland en Australië, waarbij inbegrepen het congresbezoek aan het International Symposium on "Microbial Ecology" (Dunedin, Nieuw-Zeeland) en het werkbezoek aan het Baas Becking Geobiological Laboratory (Canberra, Australië) van 18 augustus tot 6 oktober 1977.
- Cappenberg, Th.E. Verslag congresbezoek Third International Symposium on "Environmental Biogeochemistry", Wolfenbüttel (West-Duitsland) van 27 maart tot 3 april 1977.
- Densen, W.van De dynamiek en onderlinge afhankelijkheid van O⁺ vis en zijn voedselorganismen.
- Haan, H.de & J.Kattestaart. Enkele aspecten van de lozing van afvalwater in het Tjeukemeer te Oosterzee.
- Jongejan, E. Radio-gaschromatografisch onderzoek naar de omzetting van lagere vetzuren in sedimenten.
- Jongejan, E. De afbraak van lactaat: En nadere analyse.
- Jongejan, E. Gas-chromatografische bepaling van vetzuren als benzylesters.
- Jongejan, E. Bepaling van lagere vetzuren in modder.
- Limnologisch Instituut; afd. Oosterzee. Artikelen, verschenen in de "Zuid-Friesland".
- Steenbergen, C.L.M. Verslag van het "Annual European Symposium on Photomorphogenesis"; 19-25 maart 1977; gehouden te Bet HaPoel HaChaklai Bet Dagan, Israël.
- Werkplan 1977-1982 van het Limnologisch Instituut "Vijverhof", Nieuwersluis; Oecosysteem onderzoek "Vechten". Nieuwersluis, 1977. 50 blz.

STUDENT REPORTS 1977-1978

Working group "Algology"

- Kattestaart, J. (trainee analyst, Leeuwarden): Enkele aspecten van de lozing van afvalwater in het Tjeukemeer. 1977.
- Looy, J. (trainee Agricultural School, Groningen): Pigment analyse van *Diatoma elongatum*, 1977.

Nelissen, C. (Agricultural University, Wageningen): Levende algen in anaërobe slibafzettingen van het Tjeukemeer. 1977.

Working group "Foodchain and production studies in Tjeukemeer"

Beijderwellen, W. (State University, Utrecht): Voedselsamenstelling en voedselselektie bij O^+ snoekbaars in relatie tot groei en aantalsverloop. 1978.

Graaf, G.J.de (Agricultural University, Wageningen): De bouw van de kop van O^+ baars en snoekbaars in relatie tot hun voedselopname. 1978.

Klein Breteler, J. (Agricultural University, Wageningen): Voedsel, groei en fecunditeit van de pos, *Acerina cernua*. 1978.

Lammens, E. (Catholic University, Nijmegen): Produktie en voedselopname van O^+ brasem, *Abramis brama*. 1978.

Richter, B. (State University, Utrecht): Een computersimulatie van de crustaceeën populaties van het Tjeukemeer. 1977.

Schouten, R. (Agricultural University, Wageningen): Oecologie van de pos met speciale aandacht voor de produktie en voedselopname van O^+ pos. 1978.

Sijbesma, B. (trainee analyst, Utrecht): I. Voedselsamenstelling en voedselselektie bij O^+ spiering. II. De baars populatie in het Tjeukemeer. 1977.

Vogel, S. (Agricultural University, Wageningen): Voedsel en voedselselektie van de O^+ baars in het Tjeukemeer. 1977.

Working group "Primary and secondary production"

Beuzekom, J. van (State University, Utrecht): De invloed van Vecht-water op de limnologie van de Wijde Blik. 1977.

Working group "Ecophysiology of water plants"

Meulemans, J.T. (State University, Utrecht): Fotosynthese van *Ceratophyllum*. 1977.

Wittenboer, J.P. van den (State University Utrecht): De invloed van het herbicide paraquat op de fotosynthese en groei van *Ceratophyllum* en *Elodea*. 1977-1978.

Steketee, J.J. (Agricultural University, Wageningen): Pigmentsamenstelling van fytoplankton en perifyton gedurende de jaarcyclus. 1977-1978.

Working group "Mineralization of organic matter"

Duyf, F.van (State University, Utrecht): Anaërobe afbraak van cellulose en algencelwanden in het sediment. 1977-1978.

Goosjohan, B. (State University, Utrecht): Uitscheidingsprodukten van *Chlorella* in chemostaat-cultures onder fosfaat beperking. 1977-1978.

Institute for Ecological Research

Progress Report 1977



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1. FUNCTION AND ORGANIZATION OF THE INSTITUTE

The Institute for Ecological Research was founded in 1954 by the Division of Sciences of the Netherlands Academy of Arts and Sciences. Its function is to perform and to encourage terrestrial ecological research in a broad sense and to co-operate 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 Department for Dune Research has its seat at Oostvoorne. Recently, a new seat for experimental research was established at Heteren, near Arnhem. Field work is carried out in various parts of The Netherlands (see Fig. 1).

The main objects of study are the properties of plant and animals in relation to their specific occurrence. In this respect special attention is paid to plants of dune grassland and to birds, particularly the Great Tit.

The research projects are performed on a multidisciplinary basis. For parts of these projects the institute collaborates closely with some departments of the Dutch universities. In addition, the institute administers the ringing of birds in The Netherlands. It is also the site of the Euring Data Bank, in which all recoveries of birds ringed in Europe are to be assembled.

The institute is supervised by a committee appointed by the Division of Sciences of the Academy, and is financed by the Government.

The addresses of the institute are:

Headquarters: Kemperbergerweg 67, 6816 RM Arnhem. Tel. 085-432841.

Department for Dune Research: Weevers' Duin, Duinzoom 20a,
3233 EG Oostvoorne. Tel. 01885-2400.

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C. van Dijk (Plant-microorganism relationships)

P.A.I. Oremus (Plant-microorganism relationships)

D. van der Laan (Synecology)

S.R. Troelstra (Soil science)

M.J. Adriani (Guest worker)



FIG. 1. Location of the Institute for Ecological Research and its field work sites

1. Headquarters in Arnhem
2. National Park De Hoge Veluwe, where most of the field work on the Great Tit is done
3. Heteren (experimental research)
4. Vlieland (additional field work)
5. Westeinder Plassen, where most of the field work on the Coot is done
6. Oosterhout (additional field work on the Great Tit)
7. Liesbosch (additional field work on the Great Tit)
- 8-9. Zuidelijk and Oostelijk Flevoland, newly reclaimed IJsselmeer polders, where the field work of the Department of Distributional Ecology is done
10. Dune system on the island of Voorne, with the Department for Dune Research Weevers' Duin
11. Dunes of Goeree (additional field work of the Department for Dune Research)

3. POPULATION ECOLOGY

3.1. POPULATION DYNAMICS OF THE GREAT TIT, *PARUS MAJOR*

3.1.1. Population fluctuations, foraging behaviour and winter food

(J.H. VAN BALEN)

The study on the importance of winter feeding conditions for the annual survival of yearling and older Great Tits (*cf.* VAN BALEN 1976) was continued. Multiple regression calculations showed that both the size of the beechmast crop and the amount of additional food (seeds) provided by us, affected the annual survival of the tits. The survival of the yearlings in particular was promoted by the size of the beechmast crop, whereas the survival of the older birds was mainly affected by the additional supply of seed food. The influence of weather factors (frost, snow) is of doubtful importance. A more sophisticated analysis, performed by canonical correlation, confirmed these conclusions.

The rather wide distribution of beechmast, both inside and outside the study area, seems to account for the effect on the survival of the yearlings, many of which do not have a strong attachment to a restricted area (territory). On the other hand, the dominance of the older birds over most of the yearlings could to a large extent prevent the latter from profiting from the additional food supplied.

The canonical correlation calculation referred to above showed additionally that the number of yearlings in the breeding population of a given year was positively correlated with the number of fledglings in the preceding year and negatively correlated with the size of last year's breeding population. This result might reflect the importance of the number of "available sites" in the population for the yearlings' chances of establishing a territory.

The alternation of winters with a good and a bad crop of beechmast continued. The rich crop in the autumn of 1976 which was followed by an extremely high breeding density of Great Tits (in all areas studied by us; see Table 1), was

TABLE 1. *Numbers of breeding pairs of the Great Tit in the last five years*

Area	1973	1974	1975	1976	1977
Hoge Veluwe	118	124	169	133	201
Warnsborn	30	26	41	15	53
Vlieland	66	56	61	56	109
Oosterhout	26	25	22	16	30
Liesbosch	44	27	31	27	51

succeeded by a very poor crop in the autumn of 1977. Most of the beeches in the Hoge Veluwe study area bore no nuts and there were very few on the other trees. Most of the tits foraged in oak, birch, and Scots Pine during the autumn and winter. Within this group of tree species the foraging stations

changed from the twigs (in the autumn) to the boles and the soil under the trees (in the winter). In February and March of 1978 some samples of stomach contents were collected by the use of an emetic. The majority of the samples contained animal food. This was even the case during a period of severe cold with a snow cover in February.

The very high breeding density in 1977, combined with a high breeding success, resulted in a large summer population. This was reduced by various causes to an extremely low population in February 1978. The usual dispersal of yearlings in the summer was followed by another period of emigration in October-November during which many of the non-territorial birds left the study area. Furthermore, predation by Sparrowhawks and Tawny Owls was responsible for a continuous removal of tits throughout the autumn, winter, and spring. The cold spell in February resulted in a sudden decline in numbers, probably due partially to death and partially to emigration. Later on, the numbers were increased by immigration of newcomers and of birds that had left the area previously.

Reference

BALEN, J.H. VAN - Factors affecting the size of the breeding population. In: Progress Report 1975, Institute for Ecological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 67, 3-5 (1976).

3.1.2. Territory-occupancy and reproduction (P.J. DRENT)

From 1974 onward, the composition of the Great Tit population during the breeding season -in terms of territorial and non-territorial males or pairs- is well known for part of the main study area in Hoge Veluwe region.

In each breeding season the majority of the males present occupied a territory in which they attempted to reproduce (local- territorial males). The number and percentage of males that were present but did not possess a territory in this part of the study area (guest males), differed markedly from year to year (Fig. 2). During the breeding seasons of 1975 and 1977, both of which followed a winter with a good beechmast crop, the number of territorial males and the number and percentage of guest males were high. This might be related to the good local survival of non-territorial males observed during such winters. In the spring the surviving males succeeded territorial males which had disappeared, stayed in the study area as non-territorial males (local non-territorial males; see Fig. 3), or emigrated to the surrounding

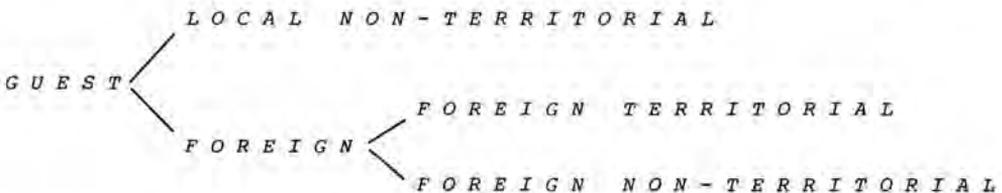


FIG. 3. Classification of guest males or pairs

N total	47*	83	50	79
N loc. territ.	45	64	47	65

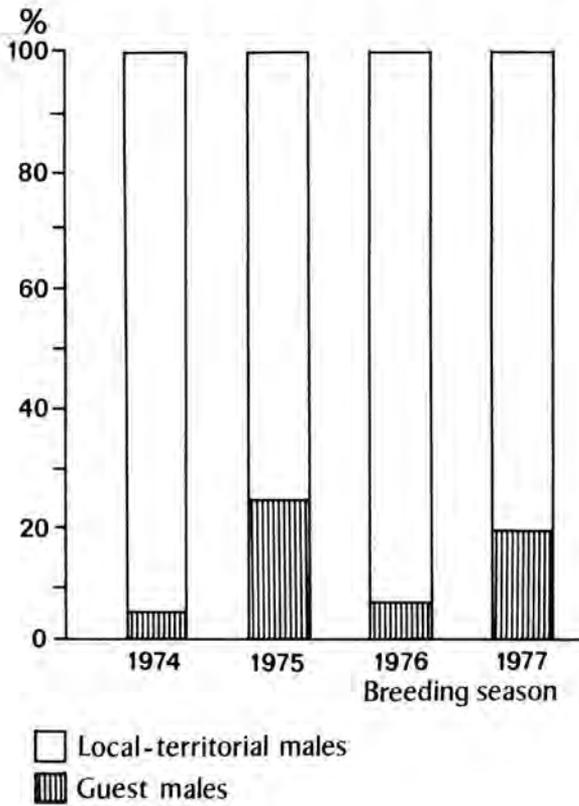


FIG. 2. The percentages of local-territorial and guest males in the breeding season.

* (Calculated only from recapture data before, during, and after the breeding season)

areas. Since there is a shortage of suitable nest-holes (nest-boxes) in some of the surrounding areas, some territorial pairs, but particularly non-territorial pairs, were unable to reproduce. Such pairs, i.e., residents and recently immigrated, visited our study-area, where there is a surplus of nest-boxes, to find a nest-site. These pairs, called foreign guest pairs, can be divided into foreign territorial and non-territorial guest pairs (see Fig.3).

Unlike the local-territorial males, all foreign guest pairs disappeared from the study area after their breeding effort. Since the percentage of foreign guest pairs is high in years with a high breeding density, this could have an effect on the local survival of the total breeding population.

TABLE 2. *The survival (in %) of various categories of male tits between breeding season n and breeding season n + 1. The number of birds in breeding season n is given between parentheses*

years	all	local-territorials	foreign guests
1974/1975	57(47)	60(45)	0(2)
1975/1976	46(83)	59(64)	0(13)
1976/1977	56(50)	60(47)	0(2)

Table 2 shows that in 1975/1976 the local survival of the breeding males was relatively low, but that this was due solely to the low survival of the guest males and their large contribution to the 1975 breeding population. The local survival of the local-territorial males did not vary in the three years of the present study.

The presence of these different categories of males (or i.e., pairs) makes it possible to study proximate effects of territory occupancy on reproduction. Although territory occupancy is not necessary for mating, females joined territorial males preferentially. Moreover, females mated with non-territorial males left them if they had the opportunity to join an unmated territorial male. Consequently, in 1975, when there was a shortage of females in the area, especially the non-territorial males remained unmated. The observations showed clearly that all of them tried to reproduce, regardless of their territorial status. Guest pairs nested in free holes or nest-boxes in the territory of another pair, the host pair. These guest pairs always had the second choice of holes. They defended their nesthole and its direct surroundings against other birds, but remained subordinate to the host pair.

Although the guest pairs were already observed in the general area before the egg-laying period, they started egg-laying much later than the local-territorial pairs (Table 3).

TABLE 3. *Mean laying date of first egg of pairs of different status. The number of observations is given between parentheses*

	local-territorial pairs	guest pairs	host pairs
1975	31.6(60)	37.5(14)	25.3(13)
1977	31.3(65)	36.7(12)	27.6(11)

The difference became more pronounced when only the guest pairs and their host pairs were compared. These data indicate that the guest pairs started their breeding effort in territories where the female started to lay early. Observations had shown that agonistic behaviour of especially the male of the

host pair is at least partly responsible for this phenomenon. Although the guest pairs showed some nest-building activities at the same time as local territorial pairs, they were prevented from completing the nest by the agonistic behaviour of the host pairs. Only when (with the progression of his reproductive cycle) the host male decreased his agonistic behaviour and restricted it to the direct surroundings of his own nest did the guest pair succeed in building a nest. Consequently, guest pairs could start their reproductive effort earliest in the territories of pairs which had started relatively early. This is important, because the data of the first egg determines the fledging date. Early-fledged young are known to be more successful than late-fledged young in establishing a territory in the autumn. Having a territory in the autumn improves the chances for surviving the winter and for having a territory in the same location in the next breeding season.

The different categories of pairs did not differ in the number of broods from which at least one young-fledged; in the numbers of eggs, hatched young, and fledged young per brood; and in the weight of the young just before fledging. In contrast to the local-territorial pairs, the guest pairs did not normally produce a second clutch. The late start of the first clutch might be important in this respect. Important differences occurred, however, in the number of young recaptured after fledging in the study area. Many more young of local-territorial pairs than of guest pairs were recaptured (see Fig. 4).

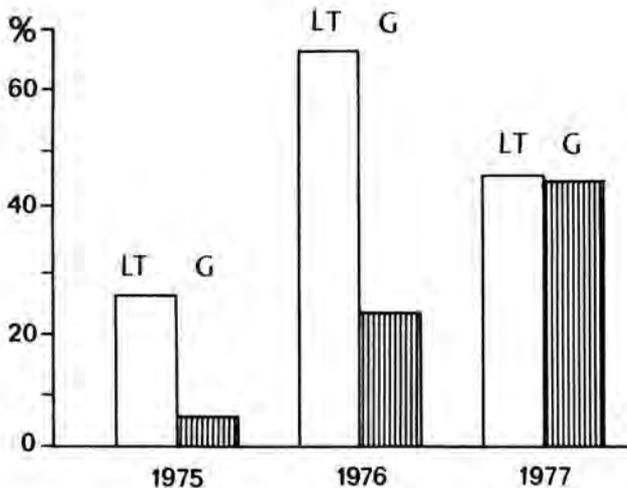


FIG. 4. Percentage of recaptured fledged young of local-territorial pairs (LT) and of guest pairs (G)

Of the different categories of guest pairs (see Fig. 3), the young of local non-territorial guest pairs were recaptured more often than those of foreign guest pairs. More precise observations showed that these differences arose from the influence of the parents on the movements of the brood during the period after fledging when the young are still dependent on them for food. After fledging, the broods either stayed in the territory or returned to the parents' territory (foreign territorial guest pairs), or, in the case of non-territorial pairs, returned to the parents' former daily-activity range. In contrast to the broods of local-territorial pairs and of local-non-territorial pairs, which stayed in the immediate neighbourhood of the nest site during the first few days after fledging, the broods of foreign guest pairs started within an hour after fledging to return to the former activity range of the parents. The movements were carried out with such rapidity that some of the young could not keep up with the parents. These young run a high risk of starving or being predated.

After some days the parents started to move with their young daily (or sometimes even for several days) from the territory to other localities which were rich in food. Observations made during recent years had shown that at this stage the parents often returned to those localities where they had foraged during their first summer. As a consequence, some broods of local pairs left the study area at this stage, whereas some of the broods that had emigrated shortly after fledging now returned.

The above-mentioned movements led to differences in local survival due to selective mortality, emigration, or immigration. These findings are important, because the number of young that establish a territory in the autumn depends to some extent on the number of young males present in the area at that time. Thus, since the proportion of guest pairs (and therefore also the proportion of broods which emigrated) is high in years with a high breeding density, it is not correct to take the number of fledged young as a measure of the young present in the autumn in seeking for correlations between this factor and parameters typical for processes occurring in these periods of the year.

3.1.3. The breeding of Great Tits in natural sites (J.H. VAN BALEN, J.A. VAN FRANEKER & E. OSIECK)

The regular inspection of tree holes in an area north of Arnhem was continued. The results obtained in 1975-1977 were similar in many respects. In each year the Starling (*Sturnus vulgaris*) was the main occupant of the holes, with the Great Tit taking second place. The percentage of occupied holes varied considerably, decreasing from 78 in 1975 to 61 in 1976 and increasing again to 87 in 1977. Only a small proportion of the territory-holding pairs of Great Tits succeeded in obtaining a hole for nesting. The remaining pairs either stayed in the area without nesting or moved to nearby nestbox areas.

When the entrances of most of the holes with wide entrances in part of the

study area were narrowed to 30 mm, the pattern of occupation in the experimental plot changed completely. Whereas in 1975 and 1976 the Great Tit occupied only 21 and 14% of the suitable holes, respectively, in 1977 the occupancy rose to 47%. In this situation about 70% of the territory-holding pairs succeeded in obtaining a nest site.

The breeding success of the Great Tit was very low. Due to interspecific competition and to predation, only 56% of the nests that were started resulted in at least one fledged young. The number of fledged young in successful broods in tree holes was similar to that in nest boxes.

The comparison of several breeding parameters with characteristics of the nest holes gave results differing from those reported earlier (VAN BALEN & BOOIJ 1976). The calculation of partial correlation coefficients for the 1977 data showed that the size of the entrance hole is the only hole characteristic that is significantly related to the clutch size and the number of fledged young. In holes with a small entrance the clutches and the number of fledglings are larger, and the effects of competition and predation are smaller than in holes with wide entrances.

Reference

BALEN, J.H. VAN & K. BOOIJ - The breeding of Great Tits in natural nest sites. In: Progress Report 1975, Institute for Ecological Research, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 67, 11-12 (1976).

3.1.4. Population genetics of the Great Tit: heritability of clutch size

(A.J. VAN NOORDWIJK)

The presence of genetic variability is a necessary condition for the process of evolution, and is implied in many hypotheses in evolutionary ecology about characters with ecological significance, e.g. date of laying and clutch size.

Direct demonstration of genetic variability in natural populations is rare. This is not surprising, because it concerns quantitative characters, which in general are rather sensitive to environmental variation. Environmental and genetic differences are mixed up in the total phenotypic variance of the character concerned. The phenotypic variance can be separated into its environmental and genetic components by the statistical methods of biometrical genetics. The Great Tit is one of the very few organisms besides man for which sufficient data with a known family structure are available in natural populations to make such an analysis feasible.

A maximum value of the genetic variance can be obtained by determining the repeatability of the character, i.e., the ratio of the variance caused by differences between measurements in the same individual and the total phenotypic variance. More accurate methods used to determine the share of genetic variation in the phenotypic variation are based on comparisons of individuals sharing a known part of their genes as a consequence of family relationship. The reduction in variance within families and the correlation between parents

TABLE 4. Results of correlation and regression calculations on clutch-size data. (Hoge Veluwe 1955-1972, first clutches. No transformations or corrections applied)

Category	Number of clutches	Number of individuals or pairs	Correlation or regression coefficient + S.E.	Degree of kinship	Heritability
1. Repeatability for females with different partners	323	140	0.46	1.0	0.46
2. Repeatability for males with different partners	308	135	-0.00	0.0	-
3. Repeatability for females/males with same partners	155	73	0.34	1.0	0.34
4. Regression daughter-mother; clutch by clutch	630	187	0.18+0.04	0.5	0.46
5. Regression son-father; clutch by clutch	669	253	-0.05+0.04	0.0	
6. Regression daughter-mother; mean/individual	-	187	0.25+0.08	0.5	0.54
7. Regression son-father; mean/individual	-	253	-0.02+0.07	0.0	
8. Regression daughter-mother; clutches from same year	-	95	0.24+0.10	0.5	0.52
9. Regression son-father; clutches from same year	-	102	-0.02+0.11	0.0	
10. Correlation between full sisters from same nest					
mean/individual	-	21	0.41	0.5	(0.82)
clutches from same year	-	19	0.30	0.5	(0.60)

and progeny makes it possible to calculate the ratio of genetic variance to the phenotypic variance. This ratio is called the heritability. Because it is a relative measure, it is dependent on the amount of variation originating from other sources represented in the data, e.g., the range of environments included and possible systematic age effects. In this analysis of the heritability of clutch size, use was made of data on first clutches from the Hoge Veluwe collected in 1955-1972 and the only attempt made to eliminate other sources of variation was the exclusion of very small (≤ 6) and very large clutches (≥ 16), where the probability of artefacts is higher.

With respect to the data in Table 4, it must be kept in mind that the identity of genes is not the only property shared by some combinations. For example, in both the comparison of full sisters and the repeatability measurements an influence of common conditions in the nest might also be a source of similarity, and systematic age effects introduce an unwanted source of dissimilarity in the repeatability.

If there had been an influence of the male on the clutch size of his partner (possibly through heterogeneity of the environment or via an autocorrelation resulting from uneven spreading of good and bad years), we would have obtained a positive correlation between the different partners of the same male. The absence of this correlation leads to the conclusion that environmental heterogeneity within the study area is unlikely to be important as a source of variation in clutch size. This is in agreement with the fact that no consistent differences in average clutch size were found between the various parts of the area.

On the whole, we may conclude that almost half of the observed variance in the size of first clutches is caused by genetic heterogeneity. A first attempt to establish selection did not yield results.

These investigations are being continued in the expectation that the accumulation of more data will permit a more detailed splitting into different groups. Other characters under investigation are the date of laying, body size, and egg characteristics. The initial results on the date of laying indicate a high degree of heritability after deletion of the variation between years.

3.1.5. Eco-physiological research (J.A.L. MERTENS)

Since beechmast seems to be a major food resource when snow covers the ground in periods of cold weather, the digestibility of this type of food was determined. This was done by placing an adult Great Tit of known weight in a metabolic chamber with a weighed amount of peeled beech nuts of known composition, estimating the bird's energy expenditure over a ten-day period by respiration-gas analysis, and collecting the faeces (F), urine (U), and wasted food every other day. The food and water supply was replenished at the same intervals.

The composition and energy content of the wasted food were determined, and the latter was subtracted from the amount of energy supplied in the food to obtain the amount of energy ingested (C). The amount of energy excreted in the faeces (F) and urine (U) was also determined. The difference between C and F is the amount of energy present in the digested amount of food (D), and the difference between D and U is the assimilated amount of energy (A) which theoretically should equal the sum of the amount of energy expended by the bird and the amount of energy lost or gained in changes of its body weight. This equality is never attained, due to incomplete collection of the wasted food, faeces, and urine. The amount of energy expended by the bird (as heat) was measured to estimate the size of the error introduced via the collection of the wasted food, etc.

The digestibility coefficient of peeled beech nuts ($\frac{D}{C}$) digested by Great Tits was 0.442 and the assimilation/consumption efficiency ($\frac{A}{C}$) was 0.403. These values are very low compared with data found in the literature. Since such a low digestibility coefficient might be due to the unsuitability of beech nuts as food or to the fact that the Great Tit is not a true granivorous species, the digestibility of certain seeds for Great Tits and one or more granivorous species was studied. Because beech nuts were no longer available, we compared the digestibility of sunflower seeds for Great Tits and Greenfinches (*Chloris chloris*). Although for Great Tits sunflower seeds proved to be much more digestible than beech nuts (0.700), the Greenfinches digested the sunflower seeds even better (0.830). The corresponding assimilation/consumption efficiencies are 0.672 and 0.801. These studies are to be repeated next year with beech nuts. In view of the low digestibility coefficient of beech nuts, this type of food must be regarded as an emergency food.

During the breeding season of 1977 the growth and temperature-regulation development of nestling Stock Doves (*Columba oenas*) was investigated. These investigations form the first stage of a comparative study on energy requirements and the development of temperature-regulation and heat-transport mechanisms in contrasting bird species during the reproductive period.

These initial investigations showed that unlike Great Tits, nestling Stock Doves already have the ability to increase their heat production in response to decreasing ambient temperatures when they are three days old. The basal energy expenditure per unit body weight of newly hatched Stock Doves lies below that of adult Stock Doves, increases during the nestling period, and reaches the adult level when the nestling are only four days old. The basal energy expenditure per unit body weight of nestling Great Tits also increases during the nestling period, but here it reaches the adult level only by fledging time. The basal energy expenditure per unit body weight of nestling Stock Doves surpasses the adult level after the fourth day of development and closely approaches the level of passerine species when the nestling Stock Doves have reached 1/3 of their fledging weight. After that, the energy

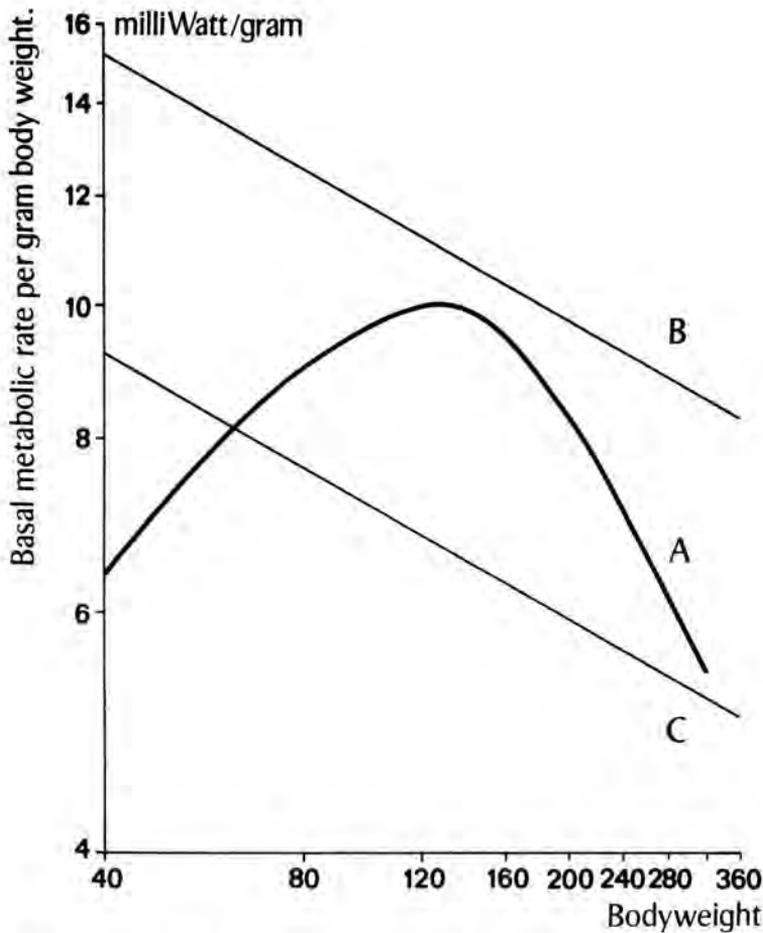


FIG. 5. A. Basal methabolic rate (BMR) of nestling Stock Doves per unit body weight
 B. Level of BMR of adult passerine species
 C. Level of BMR of adult non-passerine species

requirements per unit body weight decrease and reach the level of adult Stock Doves (non-passerines) at the time of fledging again (Fig. 5).

3.2. ECOLOGY OF THE COOT, *FULICA ATRA* (J. VISSER)

The differences in clutch size between age classes and between populations led us to study the weight and composition of the eggs (VISSER 1976). Differences in egg weight proved to occur between different populations and between yearling and older females. Furthermore, there are indications that the egg weight is highest early in the season. In some cases there is a marked variation in egg weight within clutches. Due to the variation in egg weight, variation could also be expected in the quantities of the components: fat,

protein, water, and undetermined components (remainder), some of these components may be important for the survival of young chicks.

From 1975 through 1977, 43 fresh and 26 pipped eggs were sampled and analysed. The relationship between egg weight and the weight of the components is given in Table 5 for both fresh and pipped eggs. There is no significant

TABLE 5. *Correlation between egg weight and the weight of fat, protein, remainder, water, and the shell of fresh and pipped eggs*

component		percentages		absolute value	
		correl. coeff.	P value	correl. coeff.	P value
fat	fresh	r = -0.034	>0.05	r = 0.581	<0.01
	pippe	r = -0.024	>0.05	r = 0.623	<0.01
protein	fresh	r = 0.153	>0.05	r = 0.848	<0.01
	pippe	r = -0.363	>0.05	r = 0.742	<0.01
remainder	fresh	r = -0.213	>0.05	r = 0.256	>0.05
	pippe	r = -0.255	>0.05	r = 0.237	>0.05
water	fresh	r = -0.073	>0.05	r = 0.976	<0.01
	pippe	r = 0.256	>0.05	r = 0.897	<0.01
shell	fresh	r = 0.066	>0.05	r = 0.630	<0.01
	pippe	r = -0.138	>0.05	r = 0.928	<0.01

correlation between the weight of the eggs and the percentage weight of the components; in other words, the composition of the eggs is not related to the total weight. Therefore, an increase could be expected in the absolute amount of the components with increasing egg weight. This is confirmed by the significant correlation coefficient for fat, protein, and water in Table 5. The correlation between egg weight and the weight of the undetermined component is not significant. It is clear that the egg-shell increases in weight with increasing egg size.

In 1976 and 1977, 18 pipped eggs were sampled from which the fresh weight was known. Table 6 shows the composition of the eggs at pipping in relation to

TABLE 6. *Correlation between the weight of fat, protein, remainder, and water of pipped eggs and the fresh weight of the same eggs*

component	correlation coefficient	P value
fat	0.706	<0.01
protein	0.934	<0.01
remainder	0.235	>0.05
water	0.889	<0.01

that of the fresh egg. The amount of fat, protein, and water in the chick just before hatching proved to be positively correlated with the weight of the egg at the time of laying. Again, no significant correlation was found between the undetermined component of the chick and the weight of the egg.

It is known that there is a large variation not only in individual chick weight within a brood but also in the weight of different broods of the same age (VISSER 1974). The variation in weight within a brood can be partially ascribed to differences in sex and age (asynchronous hatching). The analyses of fresh and piped eggs indicate that the variation in egg weight within a clutch can also contribute to the variation in chick weight at the time of hatching. Differences in the mean weight of broods of the same age can also be partially ascribed to differences in the mean weight of clutches.

To investigate the seasonal trend in egg composition, fresh eggs from 43 clutches for which the laying date was known, were analysed in 1975 and 1976. The laying dates ranged from March 12th to June 16th. The correlation between the weight of the egg components and the time of laying is shown in Table 7.

TABLE 7. *Correlation between the absolute weight of components of fresh eggs and time of egg laying*

component	correlation coefficient	P value
fat	-0.378	<0.05
protein	-0.212	>0.05
remainder	0.094	>0.05
water	-0.165	>0.05
shell	0.205	>0.05
total weight	-0.160	>0.05

The seasonal decline in total weight is not significant. Nevertheless, there is a significant decrease in the absolute fat weight in the course of the season. Protein and water also show a seasonal decline in weight, but here the correlation is not significant. The weight of the shell shows an increase; the undetermined component shows no correlation at all. These results support the conclusions drawn from earlier investigations (VISSER 1976). We intend to investigate the consequences of the variation in egg composition and the seasonal decline in fat weight for the survival of newly-hatched chicks.

References

- VISSER, J. - The post-embryonic development of the Coot *Fulica atra*. *Ardea* 62, 172-189 (1974).
 VISSER, J. - Ecology of the Coot, *Fulica atra*. In: Progress Report 1975, Institute for Ecological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 67, 12-14 (1976).

4. BIRD MIGRATION

THE ANALYSIS OF MIGRATION PATTERNS FROM DATA OF RINGED BIRDS (A.C. PERDECK)

Introduction

The purpose of this analysis is to distinguish patterns of movement or dispersion of bird populations on the basis of recoveries of ringed individuals. In the absolute sense this is only possible if these recoveries give a true picture of the distribution of the birds. As mentioned in the preceding progress report (PERDECK 1977a) and discussed in greater detail elsewhere (PERDECK 1977b) this is generally not the case. It can be argued, however, that relative differences between distributions can be extracted from such data, provided we study related species or populations of one species. This means that the comparative approach is the most promising.

If we interpret the distribution in space and time as the result of movements, the following features of the movements are of particular interest: 1) extent; 2) orientation; 3) scatter (including such features as skewness of the distribution, e.g. in partial migration); 4) annual periodicity of 1), 2), and 3); 5) age dependency, 6) calendar-year dependency; 7) detailed pattern (e.g., location in space and time of breeding, wintering and other resting areas, routes, etc.).

Periodic regression analysis

A method to describe a distribution changing with time is to be found in a special kind of regression analysis based on Fourier analysis (PERDECK 1977a, b). When the data are distributed according to the basic assumptions underlying multiple regression analysis, a description of the pattern covering most of the features mentioned above can be given. But if these basic assumptions not fulfilled, the method is liable to give misleading results. Such assumptions are: (1) that the independent variate (the time of finding) is the one that is controlled, without measurement inaccuracies, (2) that the dependent variates (the geographical co-ordinates of the recovery site, converted into space co-ordinates) have, for each value of the independent variate, the same expected variance, and (3) that they are distributed normally.

That the first condition is met could be questioned, but the other two are certainly not (see below). Trials to modify the co-ordinates such that they fitted the assumptions better, were not successful. Moreover, variance inequalities and skew distributions might be the result of phenomena of interest from a biological viewpoint, such as partial migration or concentration at certain places for breeding or feeding. It therefore seems incorrect to delete such features just to obtain nice curve fitting.

Nevertheless, regression analysis might be useful for a general description of the route. With lower numbers the periodic regression gives rise to simple cosine curves. Combination into a two-dimensional picture (a map) gives elliptical patterns yielding little information. When there are few observations during a certain period only, higher harmonics (the shorter wavelengths) that

are functional outside this period oscillated within it too. This gives rise to strange curls in the two-dimensional representation. From the above considerations it is clear that the periodic regression method cannot solve all of the problems encountered in the analysis of migration patterns. For detailed analysis, other methods are needed. This holds especially for the scatter of the observations at different times of the year.

Principal component analysis

One complication of recovery sites is that they are in fact distributed in three dimensions, since they lie on the globe. This is a rather academic point for small areas, which can be considered flat, but one would like to evaluate the influence of this flattening. This holds especially when long-distance migrants are considered. A method for this evaluation is to convert the two (polar) geographical co-ordinates of the recovery sites into three rectangular space co-ordinates or axes (PERDECK 1977b, Fig. 2). A principal component analysis is then carried out on the converted co-ordinates of the recoveries, neglecting the time of finding. As a result of this procedure the original axes are rotated such that one of them (the first component) becomes the best-fitting straight line through the points concerned, while the second axis, remaining rectangular to the first, is the best fitting one if all points are projected on the first axis, and so on. This analysis yields three eigenvalues representing the variance of the projections of each point on the three axes, which makes it possible to estimate how much of the variation is due to each component. If one or two of the eigenvalues are very small, the three-dimensional distribution can be reduced to two dimensions or one dimension, respectively.

Another interesting aspect of the principal component analysis is the fact that the components are not correlated with each other. To visualize this, imagine a bird flying to the southwest. If its course is expressed in latitude and longitude, it is clear that these co-ordinates are strongly correlated for each point of the route. However, if the bird's direction is due south -i.e., the north-south axis (longitude) is the best-fitting line through its respective positions- then latitude and longitude are uncorrelated (longitude has become a constant). From the statistical point of view this has advantages. For instance, in periodic regression analysis the three original equations of the space co-ordinates should be solved in a multivariate way, by multivariate multiple-regression analysis (there are three dependent variates, all correlated to each other). But if the co-ordinates are converted into principal components, three univariate multiple regressions can be carried out, which simplifies the computation.

Results

To make the material as homogeneous as possible, only the recoveries of dead

birds were used and these were divided into two groups "found" and "shot" (for definitions and the reason for this division, see PERDECK 1977a, b). Within one analysis only one type of recovery was used. For this report, the species ringed as nestlings were analysed from the recovery category "found". This concerns the following species: Long-eared Owl (*Asio otus*), Oystercatcher (*Haematopus ostralegus*), Kestrel (*Falco tinnunculus*), Grey Heron (*Ardea cinerea*), and Lapwing (*Vanellus vanellus*). The category "shot" was used for the Barnacle Goose (*Branta leucopsis*), and the Bean Goose (*Anser fabalis*).

The birds had been ringed at different places in The Netherlands, but no differentiation was made in this respect. However, they may move over distances much shorter than the distance between the ringing localities, and this will give rise to extra scatter of the recovery distribution that is not due to movements. Therefore, the mean geographical co-ordinates of the centre of gravity of the ringing places were calculated (method 3 in PERDECK 1977b). The co-ordinates of the recovery site were adjusted to the shift of the original ringing place. This shift kept the orthodromic (great circle) distance correct, but therefore gave slight deviations in the (loxodromic) directions.

Next, a principal component analysis was carried out on the space co-ordinates of the corrected recovery sites. The variation due to each component was calculated by means of the eigenvalues. From column 1 in Table 8 it can be seen that 80% or more is covered by the first axis, and that the third axis can be neglected.

TABLE 8. Results of principal component and regression analysis

species	1			2	3		number of recoveries	
	share of components in total variation along axes (%)				relative standard dev. of comp. 1.	significance of terms (axis 1)		
	1	2	3			cos t		sin t
Long-eared Owl	84.1	15.5	0.4	1	ns	ns	303	
Oystercatcher	88.8	11.1	0.1	2	*	ns	315	
Kestrel	80.2	18.8	1.0	2	ns	**	594	
Grey Heron	92.8	5.8	1.4	3	ns	***	740	
Lapwing	90.6	8.9	0.5	5	***	***	395	
Barnacle Goose	96.0	3.6	0.4	9	***	*	106	
Bean Goose	84.0	12.7	3.3	11	***	***	751	

ns = not significant at 5% level
 * = significant, P < 0.05
 ** = significant, P < 0.01
 *** = significant, P < 0.001

This means that a two-dimensional analysis is valid here (implying the choice of a certain map projection), and even that reduction to one dimension (the first axis) does not violate the data seriously. An application of the latter is to use the eigenvalue of the first axis as a measure of the extent of the movements. For this purpose the eigenvalues, being variances, were transformed into relative standard deviations by taking the square root and considering as unit the value of the species with the least deviation. This is of course only legitimate when the relative share of the first component is roughly the same in all of the species under study (otherwise, the second axis should also be taken into account). The result is shown in column 2 of Table 8.

Each of the uncorrelated components was subjected to a Fourier or periodic regression analysis (PERDECK 1977b). The start was made with 5 harmonics, but the sine and cosine terms were kept separate in the analysis of variance.

These 10-termed regression equations were reduced to the significant terms by the backward elimination procedure. This procedure not only reduces the regression to a more simple one with a higher overall significance, but also tests the significance of the separate terms. This might be not very interesting for the second and higher harmonics, which are intended for better curve fitting, but for the first harmonic it serves as a kind of test for annual periodicity in the movements. The significance of the first harmonic terms ($\cos t$ and $\sin t$) from the partial F-test in the reduced equation is given in column 3 of Table 8. Clear differences between the species can be detected, and for the Long-eared Owl the conclusion is that there is no annual periodicity.

From this regression analysis a rough picture of the migration routes is obtained by converting, for each day of the year, the rectangular space coordinates into polar geographical co-ordinates and plotting them on a map (see PERDECK 1977b, for examples). Limitations of this method have already been pointed out in section Periodic regression analysis.

To obtain more insight in the scatter of the movements the data were, after the principal component analysis, divided into months. For both the first and the second axis the measurements for skewness and kurtosis were calculated and tested. Months with significant normal distributions were rare. Therefore, median, 20th and 80th percentiles were calculated and plotted against time (months), which gave a picture of both the movement and the scatter and skewness of the distribution in time. This is illustrated in Fig. 6 for the first component (the main axis) of some of the species in question. Sixty per cent of the observations can be found between the 20th and 80th percentile lines. It must be kept in mind of course, that these pictures concern only the movement of the birds along the main axis, the best-fitting straight line through all recovery sites. For more information the second axis should also be taken into account, but this axis covers only a small part of the variation. Such pictures could, in principle, be obtained for each age class and also for calendar years separately.

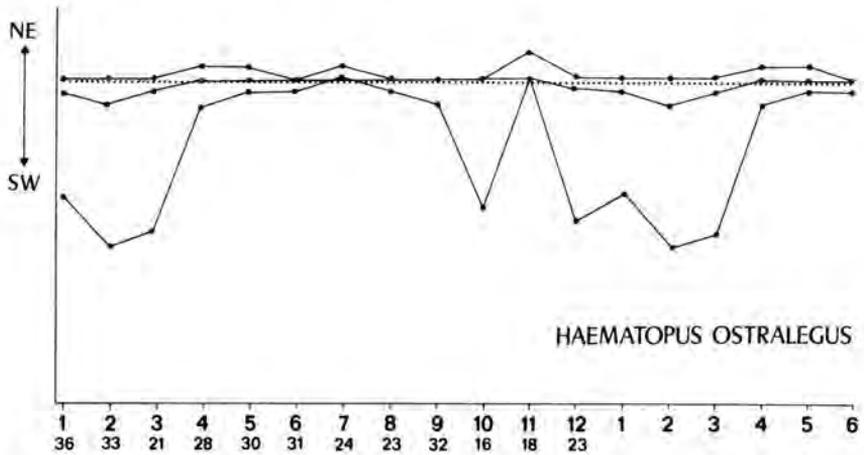
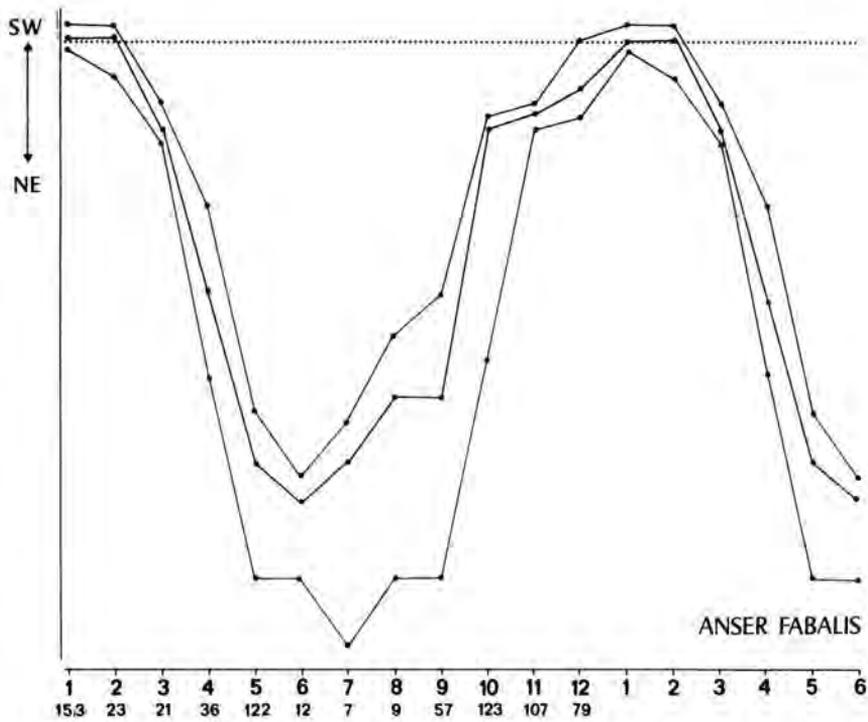
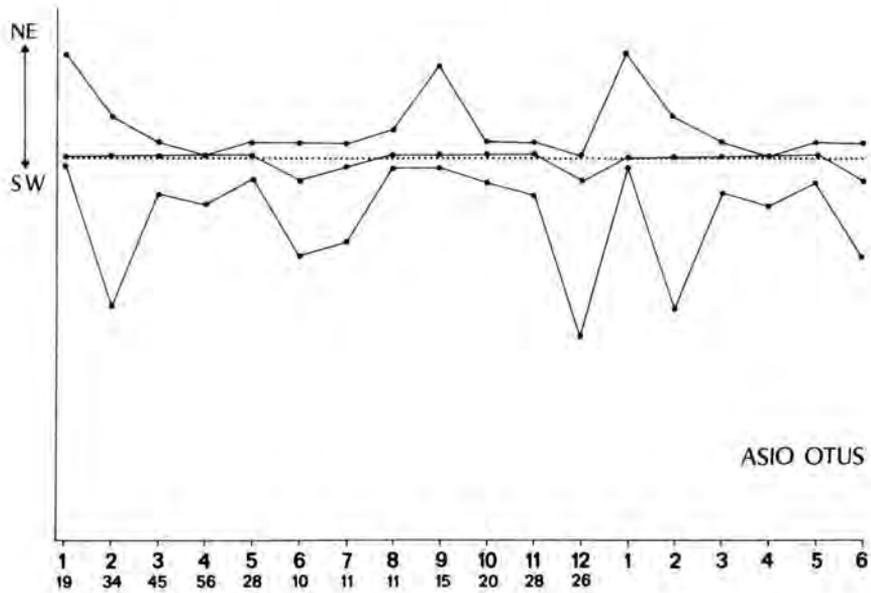
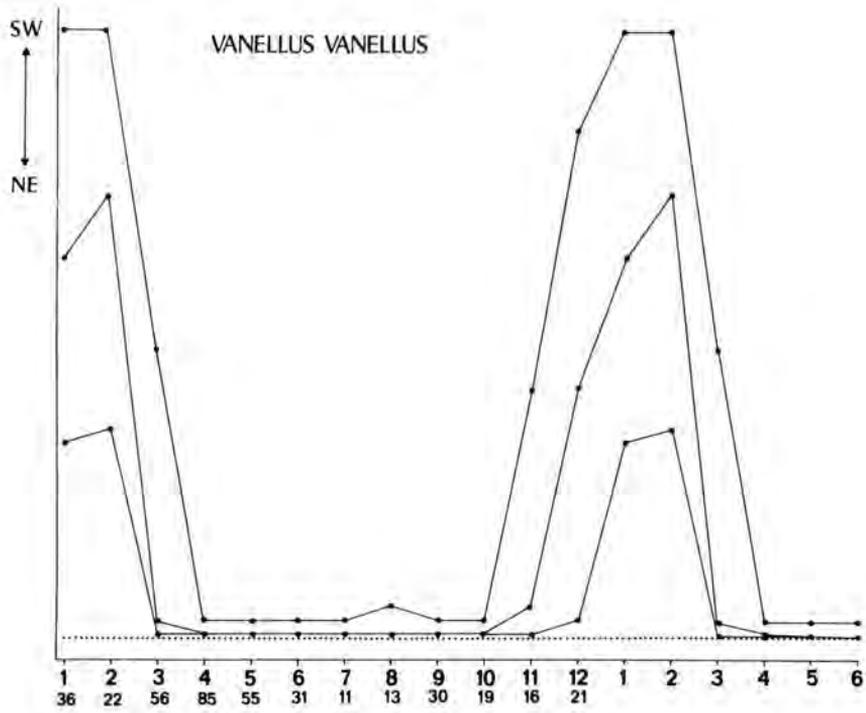


FIG. 6. Monthly position on main axis.
 Dotted line: position of mean ringling place
 Solid lines from top to bottom: 80th, 50th, 20th percentile.
 The scale of the ordinate is arbitrary, but the orientation of the axis is indicated.
 The figures on the absciss refer to the months (top) and the number of recoveries per month (bottom). For convenience half of the cycle is repeated.



Conclusion

Although a periodic regression model meets all the requirements for an analysis of the changing distributions of recoveries of ringed birds, practical application proves it to be inadequate, mainly because of the statistically abnormal distribution and the varying number of observations per time unit. For each separate feature to be described, the appropriate method has to be found. In most cases it seems that the material should be divided into groups of time units (e.g. months) and that the analysis should be carried out on the group means or medians. The loss of information is then compensated for by the deletion of spurious information. Furthermore, combined with dimension reduction by principal component analysis, the handling of the data becomes easier and the main features are less likely to be blurred by insignificant details.

References

- PERDECK, A.C. - The analysis of migration patterns. In: Progress Report 1976, Institute for Ecological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk. 2e Reeks 69, 117-119 (1977a).
- PERDECK, A.C. - The analysis of ringing data: pitfalls and prospects. Die Vogelwarte 29, Sonderheft, 33-44 (1977b).

5. DISTRIBUTIONAL ECOLOGY

5.1. EXPERIMENTS ON THE ESTABLISHMENT OF PLANT SPECIES IN A REED FIELD

(J. VAN DER TOORN & K. REININK)

In an earlier report the vegetation development of differently treated reedbeds in the Zuid-Flevoland area -an IJsselmeer polder reclaimed in 1968- was described (REININK & VAN DER TOORN 1976). Reed-accompanying plant species only became established in the dry, undisturbed parcels. This establishment was more or less restricted to sites where aggregates of *Epilobium hirsutum* had been killed by a heavy infestation of *Haltica lythri* (Coleoptera, Chrysomelidae). One of the plant species that became dominant was the perennial *Cirsium arvense*, which formed aggregates extending by rhizomes from the old *Epilobium* patches.

The extent to which seed propagation (of *Cirsium* and other species) played a role in this process is not certain. To answer this question, the experiments described below were performed.

In the establishment of species from seed, two factors are thought to be important, the presence of reed litter and interference with reed shoots.

In the dry, undisturbed reedbeds (drained by ditches about 60 cm deep) a thick layer (about 10 cm) of reed litter is present. This layer is loose and dry at the top, but more compressed and humid at the bottom.

A field experiment was set up in which reed-litter was removed from 1 m² plots, which were compared with untreated plots (treatments "without litter"

and "with litter", respectively). In half of both kinds of plot the reed was clipped several times during the growing season; in the other half the reed shoots were allowed to develop normally (treatments "without reed" and "with reed", respectively).

To investigate how many seeds already present in the litter could germinate, reed litter was collected during May and placed in sowing-pans which were kept outdoors and moistened frequently to give favourable germination conditions. The results, shown in Table 9, indicate that a considerable number of seeds

TABLE 9. Number of germinated plants in reed-litter collected from three plots measuring 1 m²

Species	Plot no.		
	1	2	3
<i>Cirsium arvense</i>	31	31	16
<i>Cirsium vulgare</i>	28	23	12
<i>Atriplex hastata</i>	159		
<i>Polygonum lapathifolium</i>	2		43
<i>Sonchus asper</i>	6	7	17
<i>Ranunculus sceleratus</i>		9	1
<i>Epilobium spec.</i>	2	3	
<i>Stellaria media</i>		2	
<i>Chenopodium rubrum</i>	1		
<i>Senecio vulgaris</i>	1		
<i>Taraxacum spec.</i>	1	2	
<i>Phragmites australis</i>	3	21	8
<i>Senecio congestus</i>		2	
Total	234	100	97

are present in the litter. Among the most numerous are the seeds of two species (*Atriplex hastata* and *Polygonum lapathifolium*) showing large local differences in numbers. These species unlike the others, do not have airborne seeds. Two marshland species (*Phragmites australis* and *Senecio congestus*) do not occur in the field as seedling, probably because of the relatively dry conditions prevailing there.

Because it was not known how many seeds were present in the litter and soil, some plots were sown with *Cirsium arvense* or *Atriplex hastata* (200 seeds per m²), but this did not lead to a significant increase in the numbers of germinated plants. Therefore, for the further calculations sown plots were combined with the other plots. The results, which are given in Tables 10 and 11, are based on measurements in 0.25 m² quadrats (one quadrat per plot).

When litter is not removed (Table 10; "with litter") germination in the

TABLE 10. Density and cover of plant species in field experiment

	density (m ²)						cover (%)					
	without reed			with reed			without reed			with reed		
	j	a	s	j	a	s	j	a	s	j	a	s
<u>with litter:</u>												
<i>Cirsium arvense</i>		1	3	1	1		0.1	0.1	0.1	0.1	0.1	
<i>Cirsium vulgare</i>		1					0.1					
<i>Atriplex hastata</i>	1	1	<u>1</u>				0.1	0.1	<u>0.1</u>			
<i>Polygonum lapathifolium</i>		1					0.1					
<i>Sonchus asper</i>				1						0.1		
Other species		1					0.1					
Total	1	5	4	2	1		0.1	0.5	0.2	0.2	0.1	
=====												
<u>without litter:</u>												
<i>Cirsium arvense</i>	61	45	17	64	24	0.4	2	9	2	2	3	<0.1
<i>Cirsium vulgare</i>	23	26	18	19	12		1	7	3	1	1	
<i>Atriplex hastata</i>	10	10	<u>4</u>	29	26	1	0.4	2	<u>0.3</u>	1	1	0.1
<i>Polygonum lapathifolium</i>	83	46	<u>20</u>	67	20	0.4	3	7	<u>3</u>	3	2	<0.1
<i>Sonchus asper</i>	7	<u>9</u>		8	3		1	<u>3</u>		1	0.4	
<i>Ranunculus sceleratus</i>	82	80	37	74	60	4	1	4	1	1	1	0.3
<i>Epilobium spec.</i>	5	10	17		1		<0.1	1	1			<0.1
<i>Stellaria media</i>			1		0.4				<0.1			<0.1
<i>Chenopodium rubrum</i>	2	2	<u>3</u>	1	1	0.4	0.3	0.3	<u>1</u>	0.1	0.1	<0.1
<i>Rumex maritimus</i>	5	11	<u>9</u>	25	20		0.3	1	<u>0.4</u>	1	1	
Other species	37	2	1	38	2		1	<0.1	<0.1	<u>0.4</u>	0.2	
Total	315	241	127	325	169	6	10	34	12	11	10	0.4

j = 28/6; a = 1/8; s = 12/9. Underlined values refer to fertile stages. Number of quadrats (0.25 m²) per treatment = 9 (without reed) and 6 (with reed)

TABLE 11. Dry weight (gram per m²) of above-ground parts in field experiment (September, 1977)

	without reed	with reed
<u>with litter:</u>		
Reed	0	1068
Other species	<0.01	0
=====		
<u>without litter:</u>		
Reed	0	972
Other species	52	<0.01

field is very poor compared to the results of the germination experiment shown in Table 9, and also in comparison to the treatments without litter shown in Table 10 (in the latter case the germinating seeds lie in the mineral soil and may -at least partially- originate from earlier succession phases before the reed became dominant, since the pioneer species *Ranunculus sceleratus* and *Rumex maritimus* are more abundant here than in the litter; see Table 9). Thus, the germination of seeds is hampered by the litter layer. Several causes are conceivable, e.g.:

- (1) seeds in the litter-layer cannot germinate because the humidity is too low;
- (2) seeds under the litter cannot germinate because of light shortage; and
- (3) seeds do germinate under the litter but the seedlings cannot penetrate the compact mass of litter.

The influence of the reed itself is clearly expressed in the treatments without litter (Table 10). Clipping of reed promotes the growth of other species, as can be seen from:

- (1) the density in August and September;
- (2) the cover in August (cover in September decreases in both treatments due to natural death);
- (3) the occurrence of fertile plants (only annuals); and
- (4) the biomass in September (Table 11).

Cirsium arvense developed rhizomes in the clipped plots but not in the non-clipped treatments.

From the foregoing it may be concluded that establishment was only successful on the clipped plots. The diminishing plant growth on the other plots is probably to be ascribed to the dense shading by the reed. The reed biomass was relatively high in 1977 (Table 11) compared to preceding years; it had reached a minimum (552 gr/m^2) in 1973 (REININK & VAN DER TOORN 1976). In those years the shading effect may have been much smaller.

When litter is not removed there is little or no effect of reed-clipping (Table 10 and 11). Not only was germination very poor but the dry weight of the plants present in September was also low. Therefore, litter seems to have had a negative influence not only on germination but also on the further development of the plants.

In conclusion, it can be said that in the reedbeds under study establishment from seed can only occur on sites without litter or reed. This was more or less the case for the places where *Epilobium* died in 1973. In the other places establishment from seed also occurred on disturbed sites, such as paths made by man as well as by hares, pheasants, and roe deer.

Establishment from seed can be very efficient after incidental burning. A year after this treatment (by which the litter is destroyed) the reed may be heavily infested by the moth larva *Rhizedra lutosa*, which results in a very low shoot biomass, as in one of the dry treatments in 1976. This lead to mass development of several plant species, especially *Cirsium vulgare* and *C. arvense*.

Reference

REININK, K. & J. VAN DER TOORN - Effect of changes in water table on the vegetation development of reedbeds in the Zuid-Flevoland area. In: Progress Report 1975, Institute for Ecological Research. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 67, 120-126 (1976).

5.2. DETERMINATION OF THE HEAT TRANSFER COEFFICIENT (P. STOUTJESDIJK)

In the energy budget of the earth's surface, the heat given off by convection appears as a closing item; all other components of the energy balance can be obtained by measurement. This convection term is the product of the temperature difference (Δt) between surface and air times the heat transfer coefficient (α). In the technical literature relations are found between wind velocity, Δt , and α , for both moving air (forced convection) and still air (free convection).

In the field, application of these relations encounters several difficulties because the wind velocity changes very rapidly and over a wide range, especially near the surface. For this reason alone it would be hard to use simple measurements to obtain a characteristic value suitable as a reference value in heat transfer calculations. Furthermore, heat transfer relations discussed in the technical literature refer to laminar flow or completely still air. In the field, air movement is usually turbulent and conditions are such that there is an intermediate stage between free and forced convection. Therefore, it is not surprising that for freely exposed leaves, calculated values of α were often up to 2.5 times higher than would be expected from theoretical considerations.

For the estimation of the heat transfer coefficient at the surface of the earth, the literature contains virtually no indications. Because this quantity is not only decisive for the temperature of the soil surface and low vegetation but also directly related to evaporation processes, it seemed worth while to attempt to measure it directly or at least to find a quantity that is related to it in a simple way. We therefore attempted to design a device to measure α under conditions approaching those at the earth's surface as closely as possible. The result is shown in Fig. 7. Two heat flux plates with surfaces of polished copper are placed side by side. When one of the two is heated from below, the following relation holds:

$$H_1 - H_2 = \alpha (T_1 - T_2)$$

If we assume that such small amount of solar radiation as are absorbed by the polished copper are the same for both surfaces, H_1 and H_2 are the heat fluxes through the heated and the unheated surface, respectively, and T_1 and T_2 are their surface temperatures.

H_1 and H_2 can be measured, because the surfaces that exchange heat with the air are part of what are called print plates in electronics. These plates consist of a layer of isolating material sandwiched between two thin copper plates, one of the plates is heated evenly via a copper plate which in turn is

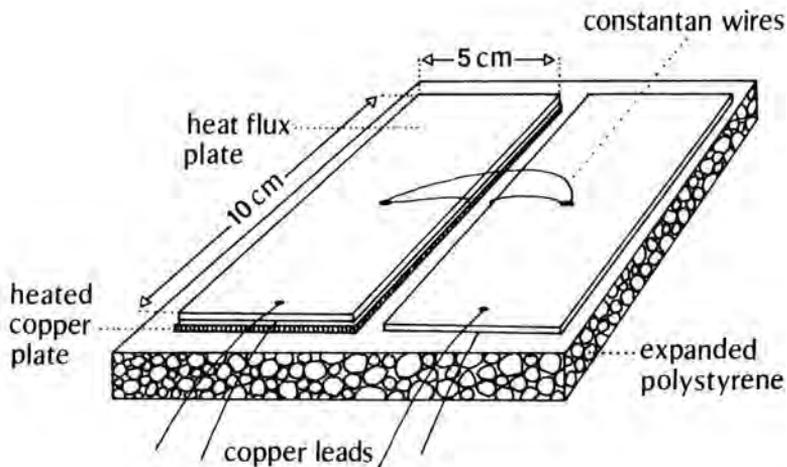


FIG. 7. Design of the device for the determination of the heat transfer coefficient. The constantan wires form part of the circuits that measure the temperature differences between the surface of the heat flux plates

heated on the lower side by a zigzag wire of manganine. Since the heat flux through the print plates is proportional to the temperature difference between their upper and lower sides, it can be measured after calibration. The temperature differences are measured thermoelectrically.

In Fig. 8 measured values of α are shown in relation to wind velocity. The values of α are mean values of a short series of measurements; the range of the wind velocities measured in this series with a hot-wire anemometer at a height of 10 cm is represented by a vertical line. It is clear from this Figure 8 that the relation between α and the wind velocity is not clear-cut, which was to be expected in view of the enormous fluctuations of the latter. It is quite possible that refined recording and analysis of wind velocity and structure would provide a good estimate of heat transfer conditions at the soil surface, but for general field work this will be hard to realize.

Very low values of α (0.005-0.007 cal/cm² min.) are found on still, bright evenings when the temperature profile of the air is stable. Under these conditions the values of α lie close to those calculated for still air. Many observations are contained in the interval $\alpha = 0.008-0.015$ cal/cm² min °C characterized by strong variation of the maximum wind velocities and low minima. In the interval $\alpha = 0.015-0.020$ cal/cm² min °C, the minima are higher the maxima are not. Values of $\alpha = 0.020$ cal/cm² min °C are hardly exceeded in sheltered conditions, even with strong winds. In exposed situations values of α up to 0.068 cal/cm² min °C were observed.

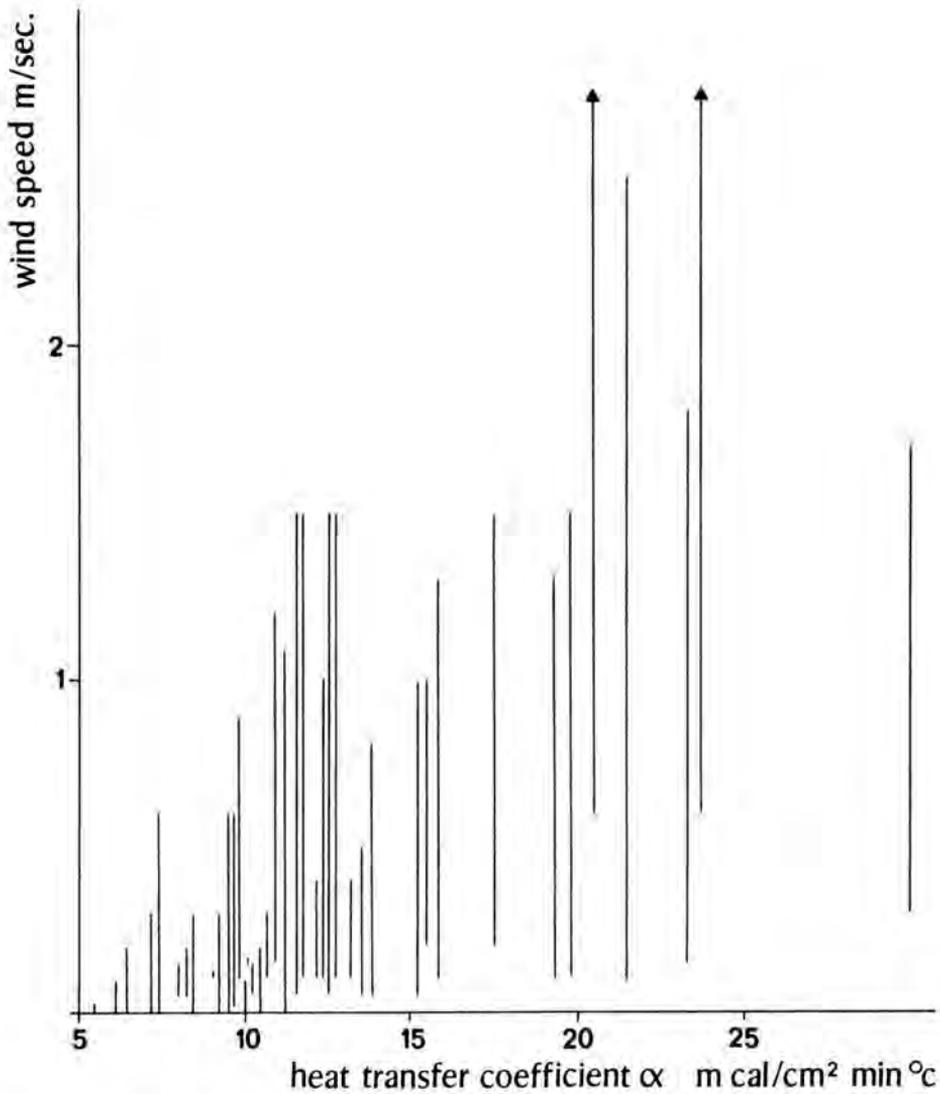


FIG. 8. Heat transfer coefficient (α) in relation to wind velocities at a height of 10 cm. The values of α are means of short series of measurements. The vertical lines indicate the range of wind velocities prevailing during this series.

Of course, it is not certain that the heat transfer coefficients obtained in this way are exactly the same as those at the surface of the earth. Yet in the small number of cases in which α could be determined from the energy budget of the surface, the values are in good agreement with the above data. The same holds for a large number of estimates based on surface temperatures, radiation data, and plausible values for the smaller items of the energy budget.

In conclusion it may be said that the method described here gives a better indication of the heat transfer conditions at the surface of the soil than wind measurements can give.

6. ECOLOGICAL DUNE RESEARCH WEEVERS' DUIN

6.1. SEPARATE AND COMBINED EFFECTS OF TRAMPLING AND SOIL COMPACTION ON THE GROWTH OF FOUR *PLANTAGO* SPECIES ON EXPERIMENTAL PLOTS (C.W.P.M. BLOM)

Introduction and methods

The research on the effects of trampling and soil compaction on the growth and development of four *Plantago* species was continued in trampling experiments performed on artificial plots. The aim was to separate the effects of trampling and soil compaction; the influence of soil compaction being studied separately and the effects of trampling and compaction on the behaviour of the plants in combination. In analogy with greenhouse experiments (BLOM 1976, 1977), three series of plots were prepared; the dune-sand soil of series A was not compacted, that of series B was moderately compacted, and the substrate of series C was strongly compacted. Each series comprised three plots. In April of 1976 four-week-old seedlings of *P. lanceolata*, *P. coronopus*, *P. major* ssp. *major*, and *P. media* were planted in rows; the distance between the plants was 15 cm. The plants growing on half of each plot were trampled by means of a trampling machine (see photograph Progress Report 1974); in the other half the effects of soil compaction on growth, flowering capacity, and seedling production were studied. The following trampling regimes were applied: each plant in series A was trampled once a day (lightly trampled), in series B the trampling frequency was three times a day (moderately trampled), and in series C each plant was trampled six times a day (heavily trampled). The force applied to the plants was 0.25 kg/cm^2 which roughly equals that applied by a man of medium weight (cf. LIDDLE 1975; CANAWAY 1975). The duration of each trampling treatment was 5 seconds, and the trampling regimes were started a month after planting.

Preliminary results and discussion

The data of the first three plots (A_1 , B_1 , and C_1 ; each treatment 12 plants per species) have been analysed and the diameters of the rosettes give a good idea of differences in reaction between the four species. With reference to the factor soil compaction, the largest differences in reaction between the three treatments were found for *P. lanceolata* and *P. coronopus*. Both species showed larger plants on the untrampled loose soils than on the moderately or strongly compacted substrates (Fig. 9a,c); the highest numbers of leaves per plant were also found on the loose soils. The differences in the size of *P. major* and *P. media* between the untrampled treatments were much smaller (Fig. 9b,d). Compacted soils provided even better conditions for the growth of these species than did loose sandy soils, which can be ascribed to the relatively high water availability in compacted sandy soils (cf. LIDDLE & GREIG-SMITH 1975). In the field, *P. major*, and to a lower degree *P. media*, occur mainly on relatively moist sites. It is probable that on the plots with the loose soil the growth of both species was inhibited by the low moisture content. As described below, also root competition caused a reduction in growth, especially in the case of *P. major*.

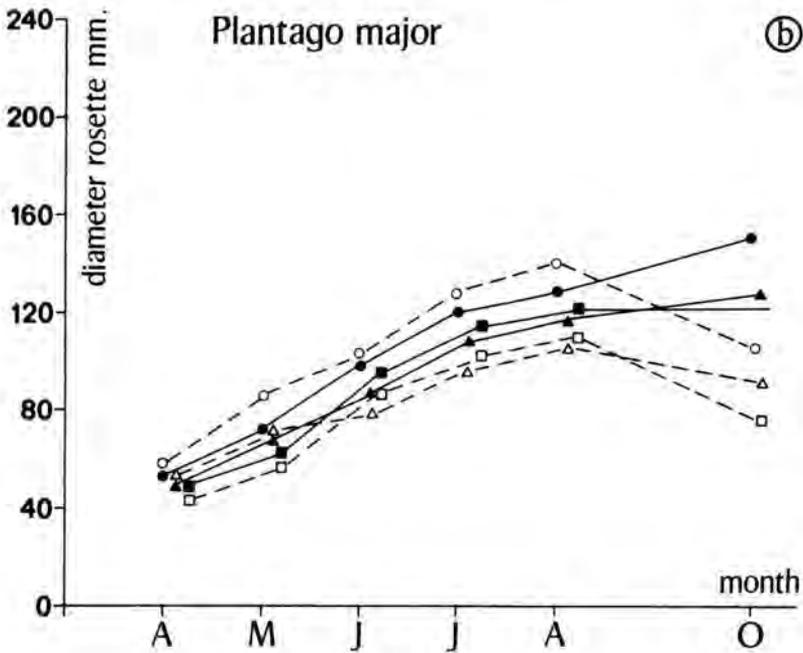
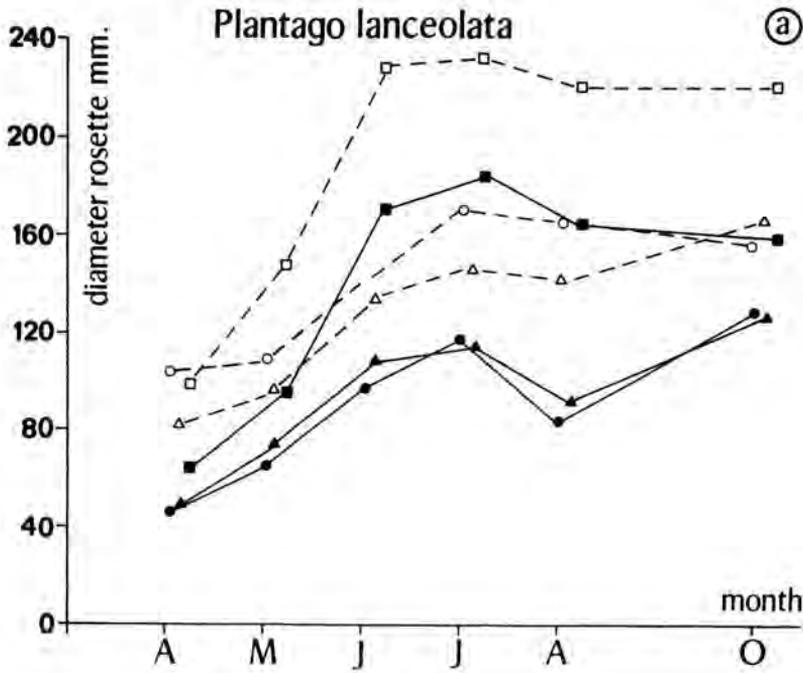
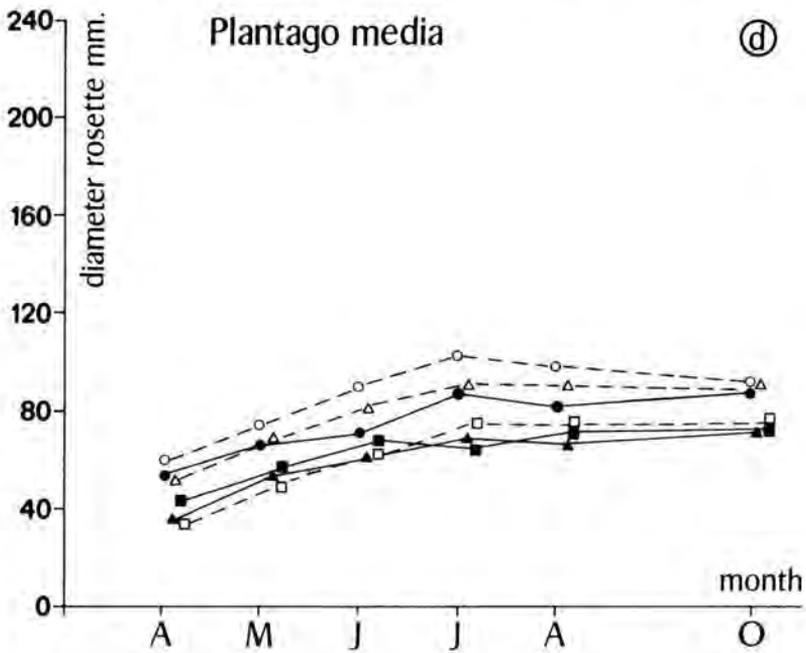
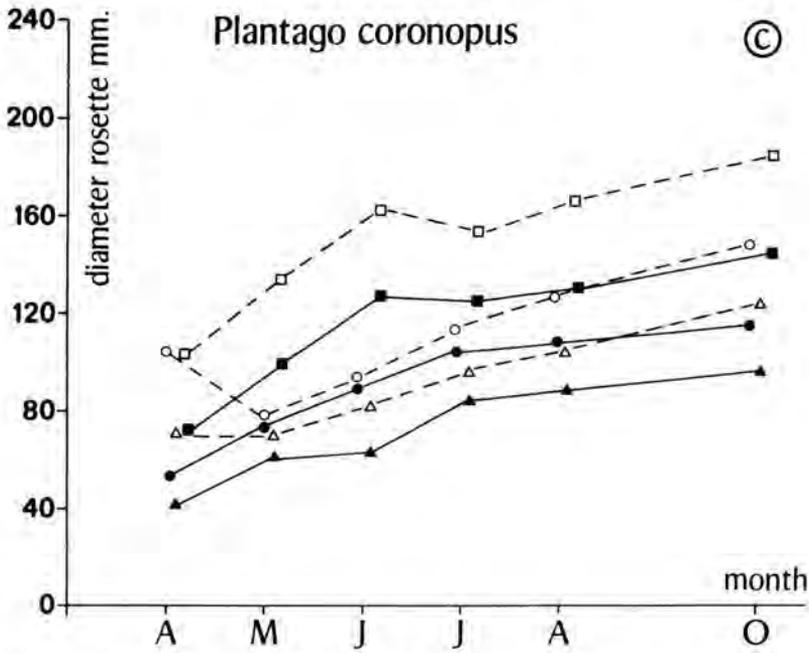


FIG. 9. The effects of soil compaction (open symbols) and of compaction in combination with various trampling regimes (closed symbols) on the rosette diameter of four *Plantago* species growing in experimental plots. The diameters measured during 1977 are given



○—○ *strongly compacted*
 △—△ *moderately compacted*
 □—□ *loose soil*

●—● *heavily trampled*
 ▲—▲ *moderately trampled*
 ■—■ *lightly trampled*

The combined effects of trampling and soil compaction are also given in Fig. 9. For *P. lanceolata* and *P. coronopus*, an increase in trampling resulted in a considerable decrease of rosette diameter (Fig. 9a,c). *P. major* and *P. media* were more resistant to trampling (Fig. 9b,d), which was also the case during earlier stages in their life-cycle (BLOM 1977). Comparison of the effects of compaction alone and trampling combined with compaction shows larger differences in diameter for *P. lanceolata* and *P. coronopus* than for *P. major* and *P. media* (Fig. 9). This striking contrast demonstrates that under these conditions reduced growth of *P. major* and *P. media* plants must be attributed more to limited factors due to physical soil conditions than to vulnerability to trampling.

Besides the rosette diameter, the number and size of leaves and spikes as well as the number of seeds produced in 1977 were determined. The first results show clearly for *P. lanceolata* and *P. coronopus* the mean values of all these characteristics decrease with increasing compaction and trampling. For these species the values for the trampled plots are in general much lower than those found for the untrampled sites; only the numbers of spikes of *P. coronopus* were more than two times lower on the untrampled than on the comparable trampled plots, as was also found for *P. lanceolata* on the loose and lightly trampled plots. The differences in the vegetative characteristics of *P. media* and *P. major* between untrampled and trampled series were much smaller; especially for *P. major*, the fewest and the smallest leaves were found in the untrampled series.

The data on produced seeds indicate that the seed production of *P. lanceolata* and *P. media* increased strongly with decreasing trampling and compaction; for the other species this increase seems to be smaller. *P. major*, and to a lesser degree, *P. coronopus*, were the only species able to produce a reasonable number of seeds in the heavily trampled series.

As shown in Table 12, the density of the soil of all trodden plots was much

TABLE 12. *The compaction of the dune-sand soil in the open plots of the trampling experiment. The values were obtained by measurement with a penetrometer (conus A, see BLOM 1977) 5 and 10 cm below the soil surface. Mean values of 10 measurements are given in kg/cm²*

	loose soil		moderately compacted soil				strongly compacted soil					
	untrampled		lightly trampled		untrampled		moderately trampled		untrampled		heavily trampled	
	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm
1974												
\bar{X}	5.8	8.8	-	-	10.0	21.2	-	-	10.2	24.2	-	-
S.D	1.2	1.4	-	-	1.4	1.8	-	-	1.2	2.6	-	-
1977												
\bar{X}	4.8	8.0	11.0	16.0	10.8	20.4	15.8	29.8	13.6	29.6	19.9	35.4
S.D	1.2	3.0	0.8	2.2	0.6	1.4	1.2	1.8	2.2	0.7	2.0	0.6

higher at the end of 1977 than in 1974. Probably due to relatively intense vertical soil-water movements, the density of the untrampled, strongly compacted soil had also increased. Compared with the 1974 values, the density of the soil in the other untrampled plots had slightly decreased in 1977, which can be ascribed to the mechanical activity of the roots in these plots. In September of 1977, the root systems in plots A₃, B₃, and C₃ were studied with the "pinboard" method (see SCHUURMAN & GOEDEWAAGEN 1965). All species showed a marked increase in length, width, and weight of the root systems with decreasing compaction. The smallest root systems were observed in the heavily trampled plots. Between the treatments, the largest differences in size and shape were found for *P. lanceolata* followed by *P. media* and *P. coronopus*; *P. major* showed relatively small differences. It was remarkable that the large root systems of *P. lanceolata* seemed to inhibit the growth of *P. major* roots.

References

- BLOM, C.W.P.M. - Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. I. Soil compaction, soil moisture and seedling emergence. *Oecol. Plant.*, 11, 225-241 (1976).
- BLOM, C.W.P.M. - Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. II. Trampling and seedling establishment. *Oecol. Plant.*, 12, 363-381 (1977).
- CANAWAY, P.M. - Fundamental techniques in the study of turfgrass wear: an advance report on research. *J. Sports Turf Res. Inst.* 51, 104-115 (1975).
- LIDDLE, M.J. - A selective review of the ecological effects of human trampling on natural ecosystems. *Biol. Conserv.*, 7, 17-36 (1975).
- LIDDLE, M.J. & P. GREIG-SMITH - A survey of tracks and paths in a sand dune ecosystem. I. *Soils. J. Appl. Ecol.*, 12, 893-908 (1975).
- SCHUURMAN, J.J. & M.A.J. GOEDEWAAGEN - Methods for the examination of root systems and roots. Centre for Agricultural Publications and Documentation, Wageningen (1965).

6.2. THE AVAILABILITY OF NITRATE AS A NITROGEN SOURCE IN NATURAL GRASSLANDS (A.J. SMIT & J.W. WOLDENDORP)

As part of a study on the specific adaptations of grassland plants to their environment, special attention is being paid to nitrogen nutrition. In this respect it was necessary to determine the contribution of nitrate and ammonium to this nutrition in the various grasslands under study. To develop the appropriate techniques for this purpose, attention was focussed on *Plantago* species occurring in an old established dune grassland on the island of Goeree.

It has been claimed in the literature that nitrate plays a minor role in permanent grassland soils due to a suppression of nitrification by allelopathic compounds excreted by plant roots (THERON 1951). Such suppression should occur particularly in climax vegetations (RICE 1974). However, other investigators (e.g. PURCHASE 1974) have ascribed the low nitrification rate to the absence of sufficient ammonium ions thought to be used preferentially in nitrogen immobilization and uptake by plant roots.

In the studies cited above, numbers of nitrifying bacteria were used as an indication of nitrate formation. However, in these studies the data were not correlated with parameters of the nitrogen status of the plant, such as accumulation of nitrate and the level of nitrate reductase in the various parts of the plant. The latter should be determined to obtain an impression of the extent to which the nitrate formed by the nitrifying bacteria is indeed taken up by the plants, as has been discussed by WOLDENDORP (1978). This combined approach was adopted for the present study.

To establish the level of nitrate reductase (NaR), synthesis of which in plants is induced by nitrate, *Plantago lanceolata*, *P. coronopus*, *P. major* ssp. *major*, and *P. media* were pre-cultivated in the glasshouse on a modified Hoagland solution with ammonium as the nitrogen source kept at pH 6.5 by automatic titration. A week before the NaR determinations, the plants were transferred to a nitrogen-free nutrient solution before exposure for 20 hours to ammonium, ammoniumnitrate, or nitrate, after which the NaR activity was measured; a control without nitrogen was also included. Enzyme activity was measured in the leaves at 2.00 p.m. (Dutch summer time) according to a modification of the method described by JAWORSKI (1971). The results of the experiment are given in Table 13.

TABLE 13. Levels of nitrate reductase (expressed as $\mu\text{M NO}_2^- \cdot \text{g dr.w.}^{-1} \cdot \text{h}^{-1}$) in the shoots of four *Plantago* species pre-cultivated with ammonium as the nitrogen source before exposure to ammonium, ammonium nitrate, nitrate, or no nitrogen

Treatment	<i>P. lanceolata</i>	<i>P. coronopus</i>	<i>P. major</i>	<i>P. media</i>
No nitrogen	0.05+0.02	0.03+0.01	0.55+0.13	0.42+0.11
Ammonium	0.04+0.02	0.02+0.02	0.29+0.05	0.20+0.04
Nitrate	2.22+0.29	3.96+0.08	4.67+0.24	4.36+0.31
Ammonium nitrate	1.59+0.35	0.97+0.12	3.60+0.14	3.01+0.38

As can be seen from this Table, in the absence of nitrogen the NaR levels are considerably higher for *P. major* and *P. media* than for *P. lanceolata* and *P. coronopus*. This points to a specific adaptation of the former species to a nitrogen-rich environment, since the maintenance of superfluous levels of NaR in the absence of nitrate can be considered a waste of energy. The results shown in Table 13 are in agreement with those of KRUYNE *et al.* (1967) who analysed 1,500 Dutch grasslands and found *P. major* and *P. media* to be present in a nutrient-rich environment and *P. lanceolata* at low levels of nutrients. As compared with the control, ammonium suppressed NaR activity to some extent in all species, whereas the enzyme was induced more or less strongly by nitrate. When both nitrogenous compounds were present, levels of the enzyme were lower than with nitrate alone. Particularly in the case of *P. coronopus* there was a considerable suppression by ammonium.

The NaR levels found in the laboratory were compared with those obtained in the field and analysed for correlation with the pH, the quantities of ammonium and nitrate, and the numbers of nitrifying bacteria in the rhizosphere of individual plants. Data obtained for a number of *P. lanceolata* plants are shown in Table 14. For these data the correlations in Table 15 were calculated with Spearman's non-parametric rank-correlation test (SIEGEL 1956).

TABLE 14. Relationship between the NaR activity in the shoot and some rhizosphere parameters of a number of *Plantago lanceolata* plants collected in August in an old natural dune grassland

pH H ₂ O	NH ₄ ⁺ (ppm)	NO ₃ ⁻ (ppm)	Numbers* of nitrifiers (per g soil)		NaR level ($\mu\text{M NO}_2^- \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$)	NO ₃ ⁻ (ppm.g ⁻¹)
			NH ₄ ⁺ -oxidizers	NO ₃ ⁻ -oxidizers		
4.75	7.7	0.9	14,400	14,400	1.15 \pm 0.50	<10
5.73	9.2	1.1	7,200	7,200	0.49 \pm 0.19	<10
5.53	4.5	0.3	7,200	3,600	0.26 \pm 0.07	<10
4.79	3.5	0.3	7,200	7,200	0.17 \pm 0.04	<10
5.05	7.7	0.5	3,600	7,200	0.62 \pm 0.14	<10
5.40	5.4	0.4	1,800	900	0.06 \pm 0.05	<10
4.92	5.7	0.5	3,600	900	0.27 \pm 0.09	<10
5.01	4.9	0.4	7,200	7,200	0.40 \pm 0.17	<10
4.99	8.4	0.1	14,400	7,200	0.88 \pm 0.37	<10
4.84	6.7	0.5	900	900	0.16 \pm 0.05	<10
4.87	8.6	0.6	1,800	1,800	0.24 \pm 0.08	<10
5.45	8.8	0.6	1,800	900	0.20 \pm 0.06	<10
5.12	9.6	0.3	900	450	0.10 \pm 0.04	<10

* Numbers per gram roots + adhering soil

TABLE 15. Correlation** between the NaR levels in *Plantago lanceolata* and some rhizosphere parameters

correlation (n=13)	r _s
NH ₄ ⁺ in the soil: numbers of NH ₄ ⁺ -oxidizers	-0.283
NH ₄ ⁻ in the soil: numbers of NO ₂ ⁻ -oxidizers	-0.210
Numbers of NH ₄ ⁺ -oxidizers: NaR in shoots	0.807
Numbers of NO ₂ ⁻ -oxidizers: NaR in shoots	0.794

** The levels of significance with n=13 for $\alpha = 0.05$ and $\alpha = 0.01$ are 0.553 and 0.684, respectively

From the results presented in Tables 14 and 15 the following conclusions can be drawn. The presence of considerable numbers of propagules of both groups of nitrifying bacteria in the rhizosphere of *P. lanceolata* plant offers unequivocal

evidence of the formation of nitrate in this established grassland soil, because these bacteria can only generate energy for growth and maintenance by the oxidation of ammonium and nitrite. The NaR levels in the shoots were found to be significantly higher than those shown in Table 13 for plants grown in the absence of nitrogen or with ammonium as the nitrogen source. Evidently, at least part of the nitrogen taken up under field conditions by these *P. lanceolata* plants was in the nitrate form. The close correlation found between the numbers of nitrifying bacteria in the rhizosphere and the NaR levels in the plants suggests that the nitrate taken up derives at least partly from formation in the rhizosphere. Preliminary calculations concerning the amounts of nitrate formed in the rhizosphere indicate that nitrate formation is considerably lower than the uptake capacity of the root system as determined by FREIJSEN (unpublished results). The latter conclusion is in accordance with the insignificantly low levels of nitrate in the rhizosphere and with the absence of free nitrate in the shoots of *P. lanceolata*. No relationship was found between the amount of extractable ammonium in the rhizosphere and the numbers of nitrifying bacteria. Apparently, the steady-state concentrations of ammonium are not a good measure of nitrate formation in the soil.

Preliminary experiments with *P. coronopus* and *P. major* likewise showed a correlation between the numbers of nitrifying bacteria and the NaR levels in the shoots. For the former species the values were somewhat higher than those found with *P. lanceolata* and those of *P. major* were considerably higher. The latter result once more demonstrates that *P. major* is bound to comparatively more nutrient-rich situations than the other *Plantago* species.

In conclusion it can be stated that the combined approach described above made it possible to demonstrate the formation of nitrate in the rhizosphere and its utilization by plants in an old well-established grassland. No indications were obtained that nitrification is suppressed under such conditions, as has been suggested by some authors (RICE 1974). Experiments concerning the quantification of nitrate formation and plant uptake are in progress.

References

- JAWORSKI, E.G. - Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Comm.* 43, 1274-1279 (1971).
- KRUYNE, A.A., D.H. DE VRIES & H. MOOI - Bijdrage tot de oecologie van de Nederlandse graslandplanten. Pudoc, Wageningen, 65pp (1967).
- PURCHASE, B.S. - Evaluation of the claim that root exudates inhibit nitrification. *Plant Soil* 41, 527-539 (1974).
- RICE, E.L. - Allelopathy. *Acad. Press., New York.* 353 pp (1974).
- SIEGEL, S. - Nonparametric statistics for the behavioral sciences. *Mc Graw-Hill, New York.* 312 pp (1956).
- THERON, J.J. - The influence of plants on the mineralization of nitrogen and maintenance of organic matter in the soil. *J. agric. Sci.* 41, 289-296 (1951).

Introduction

As already reported (VAN DIJK 1975), inoculation of *Alnus glutinosa* seedlings with soil suspensions led in some cases to the formation of root nodules resulting from fungus infection instead of from the actinomycete *Frankia alni*. Myco-nodules can only be distinguished from young actinomycete nodules by microscopical investigation of the nodule content. We were able to isolate the fungus on Czapek-Dox agar. The fungus was found to be *Penicillium nigricans*, which differs slightly from the species described by POMMER (1956), who found root-nodule formation on *Alnus* caused by *Penicillium album*. In a series of nodulation experiments the infectivity of both actinomycete and fungus as well as possible interactions between them were studied.

Sensitivity to nitrate

It is known that actinomycete nodulation in *Alnus* reaches an optimum at low nitrate concentrations. Comparison of both myco-nodulation and actinomycete nodulation at different nitrate concentrations in the nutrient solution showed that both types of nodulation had an optimum at 0.2 meq $\text{NO}_3/1$ Hoagland nutrient solution and that nodulation declined steadily at higher nitrate concentrations until zero was reached at 2.5 meq NO_3 . The uniform response of both types of nodulation to nitrate shows clearly that the low nitrate tolerance of the actinomycete nodulation is not necessarily to be considered a "useful" feedback system developed by evolutionary forces in connection with the nitrogen-fixing ability of the symbiosis. Therefore, it can also be expected that the much higher sensitivity of root nodule formation to nitrate in *Hippophae rhamnoides* is due to host characteristics rather than to one special host-endophyte relationship.

Maximum nodulation

For both types of nodulation the quantity of nodules produced per plant depends on the concentration of the inoculum. Maximum numbers of nodules per plant appeared to be equal for both inoculum species.

Interactions between *Frankia alni* and *Penicillium* sp.

Interactions between myco- and actinomycete nodulation as compared with single-species nodulation were studied in a series of nodulation experiments with test plants of *Alnus glutinosa* on Hoagland nutrient solution containing 0.25 meq NO_3/litre .

Combined inoculation showed that low concentrations of actinomycete inoculum (nodulation level: \pm 3% of max. nodulation) did not reduce myco-nodulation significantly and, conversely, actinomycete nodulation was only slightly reduced by low levels of myco-inoculum. High concentrations of actinomycete inoculum reduced myco-nodulation proportionally and *vice versa*. Reduction of myco-nodulation was considerably lower when the actinomycete inoculum was

added 14 days after the myco-inoculum. In all cases of combined inoculi there was a slight reduction of the maximum numbers of nodules per plant as compared with single-species nodulation. It is concluded that *Frankia alni* and *Penicillium nigricans* compete for the same infection sites on the root system. The ratios of myco- and actinomycete nodules from mixed inoculation indicate that competition is mainly determined by the ratio of infective particles of both species, but additional experiments are needed to determine whether some qualitative predominance of the *Penicillium* species exists.

Host plant genotype and nodulation

Nodulation characteristics may be influenced by genetic variation among the test plants. An impression of such variation was obtained from nodulation experiments with plants raised from five different seed samples. Four of the samples had been collected from different trees in the dune area (4 half-sib families). The origin and genetic uniformity of the fifth seed sample (commercial) were unknown. Plants from all seed samples were inoculated separately with either myco- or actinomycete inoculum at the maximum nodulation level (25 plants per combination). Comparison of the maximum nodulation levels thus obtained showed that within 4 seed samples both types of nodulation reached the same level. One seed sample from the dune area showed a myco-nodulation level which was significantly higher than that of the actinomycete nodulation. The validity of this observation is under study. Different levels of maximum nodulation were found between the seed samples. These differences were not correlated with the biomass of stem and leaves. The results obtained so far also indicate that both infectants compete for the same infection sites on the root. Plants of different genotype may have different numbers of infection sites on the root system. This difference could be determined either by slight differences in growth rate between plants of different seed batches or by a difference in susceptibility to infection.

Effects of pymaricine on myco- and actinomycete nodulation

Pymaricine (Pymafucin susp. 2.5%, Mycofarm, Delft) belongs to the polyene antibiotics with a wide-spectrum fungitoxic action. It does not act on bacteria and actinomycetes, and phytotoxicity should be insignificant or absent at fungitoxic concentrations. Because spontaneous nodulation by *Penicillium* sp. from soil suspensions may disturb nodulation tests performed for the estimation of the number of infective actinomycete particles in soil, the effect of pymaricine as selective inhibitor of myco-nodulation was studied in a series of nodulation experiments. Alder seedling were inoculated with either an actinomycete or a myco inoculum. Prior to inoculation, the test plants were provided with a concentration series of pymaricine ranging from 0-100 ppm in the Hoagland nutrient solution. The nutrient solutions were kept in the dark and the pymaricine concentrations were refreshed every 5 days. Four weeks after inoculation, plant habitus and

nodulation were determined. Concentrations of 0-2.5 ppm pymaricine did not influence either of the type of nodulation or plant habitus. A concentration of 5 ppm pymaricine strongly reduced the number of both types of nodules, but test plants were macroscopically unaltered. Pymaricine concentrations of 10-100 ppm completely inhibited both types of nodulation, and an increasing reduction of root length which resulted in abnormal root growth was observed. In all probability pymaricine inhibited the infection process by altering the root organization rather than via reactions with the inoculum species. The observed phytotoxicity of pymaricine at levels close to the expected inhibitory levels for *Penicillium* makes this compound unsuitable for use in nodulation experiments.

References

- COSTA, A. & C. RODRIGUEZ-BARRUECO - The effect of antibiotics on the infective viability of an alder (*Alnus glutinosa* L. Gaertn.) crushed nodule inoculum. *Ann. Microbiol. (Inst. Pasteur)* 128A, 217-227 (1977).
- DIJK, C. VAN - Ecology of the root-nodule endophyte of *Alnus glutinosa* L. (Vill.). In: Progress Report 1975, Institute for Ecological Research. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks*, 67, 48-53 (1976).
- POMMER, E.H. - Beiträge zur Anatomie und Biologie der Würzelknöllchen von *Alnus glutinosa* Gaertn. *Flora* 143, 603-634 (1956).

6.4. SYNECOLOGICAL RESEARCH ON THE DUNE SLACKS ON VOORNE: A STUDY ON THE TEMPORAL VARIATION IN VEGETATION (D. VAN DER LAAN)

Introduction

The analysis of the vegetational and environmental data collected in the wet dune slacks on the island of Voorne is still the main project. A considerable part of these data concerns permanent plots. In these permanent plots the changes in the vegetational composition are being recorded over a series of years. The data provide information on the behaviour and ecology of phytocoenoses and the individual species as well as on the processes associated with environmental changes.

Vegetational changes

In studies on permanent plots any difference between two successive observations made in the same plot may be defined as a change. An example of such change would be the invasion or disappearance of species. Another would be an alteration in the percentage of cover, or in the number of individuals, or a shift in the proportion of flowering and vegetative shoots of a given species. Both long-term and short-term changes may occur. Changes of short duration which sometimes recur are generally called fluctuation. The long-term unidirectional shifts in the vegetation are usually called succession. It appeared to be useful to differentiate the short-term from the long-term changes.

TABLE 16. *Various values characterizing the vegetation of the permanent plots, the percentage organic matter of the upper soil layer (10 cm) and the average ground-water level during the vegetation period between 1966 and 1976*

site	1	3	4	6	8	16	17	19	4	45	46	57	70	72	73	75	100	101	111	133	140	141
number of records	10	13	10	13	11	11	11	11	12	9	11	11	10	11	11	8	6	6	6	6	8	8
number of species	35	82	64	58	66	70	50	57	64	54	46	64	62	59	59	61	66	66	72	51	39	55
% "permanent" species	36	46	53	24	30	43	36	36	25	33	30	30	33	26	19	22	52	49	55	45	41	33
% "successional" species	12	26	19	37	27	26	24	33	35	33	28	31	27	14	23	27	16	20	14	22	19	6
% "fluctuating" species	52	28	27	39	44	30	40	31	40	33	42	39	40	60	58	52	32	31	31	33	41	62
number of "unidentified" species	2	0	2	7	2	1	8	2	1	0	3	3	7	2	7	1	4	1	14	2	2	3
average ground-water level (in cm below soil surface)	12	34	26	8	10	30	18	30	19	29	19	56	33	31	37	5	48	47	108	60	21	14
% organic matter	18.6	9.3	12.0	13.6	15.3	13.4	17.0	18.6	10.8	2.4	2.4	7.4	5.8	0.9	1.8	3.2	8.9	11.5	10.0	1.9	2.0	2.0

For a preliminary analysis of the dune-slack data, a restriction was made in the number of permanent plots and also in the number of vegetational characteristics used to characterize a change between two successive observations. The selection of the permanent plots had to represent the variation in two important environmental factors, i.e., the content of organic matter in the upper soil-layer and the height relative to the ground-water table. On this basis 22 plots were selected. For a characterization of the changes in the vegetation only the presence or absence of the species was taken into account in this preliminary study.

Succession and fluctuation

To establish whether observed changes were the result of succession or could be considered fluctuations, the species were classified as follows:

- a. Species present since the first observation and persisting throughout the whole observation period. These species were considered "permanent".
- b. Species present at the beginning which disappeared after three or more years and species appearing in a given year which remained for at least the next three years, were considered "successional" species.
- c. Species irregularly present or absent were considered "fluctuating" species.

Results

Once the species were classified according to these criteria, the contribution of these three types of species to the composition of a given permanent plot could be established (Table 16). Twenty of the 22 plots were easy to classify and included 8 plots with a distinctly permanent character and 12 plots with a distinctly fluctuating character. The 8 "permanent" plots proved to be rather rich in humus and to lie moderately high above the ground-water level. The 12 "fluctuating" plots either had a low organic matter content and were wet to moderately wet or were rich in humus with a high ground-water level. Seven plots deviated considerably from the mean rate of succession; these plots showed both high and low succession rates.

Succession and fluctuation of species

Finally, an attempt was made to characterize the individual species on the basis of their behaviour in the 22 permanent plots over a period ranging from 6 to 13 years. For this purpose, the percentage frequency of each species in each of the three groups (permanent, succession, and fluctuation) was calculated.

The results of this analysis show that an appreciable proportion of the species belong completely or mainly to one of the three groups, whereas other species can be determined by their absence in one of the three groups. Only a small proportion of the species are equally well represented in all three groups.

This analysis was presented at the third symposium of the Working Group on Succession Research on Permanent Plots of the International Society of

Vegetation Science, which was held in Bialowieza, Poland, in September, 1977. The text of this paper, which reports the results of this study in more detail, will be published in a coming issue of PHYTOCOENOSIS, the phytosociological bulletin of the University of Warsaw.

The results obtained with the methods described here give an impression of the nature of the changes occurring in permanent plots and also provide information on the behaviour of phytocoenoses and of individual species. These results are based only on the presence or absence of a species. It is evident that more knowledge about the ecology of a phytocoenosis and of the individual species can be obtained if additional characteristics of the vegetation are used, such as abundance, percentage cover, and phenological stage. Analyses using these data are in progress.

6.5. ON THE DETERMINATION OF TOTAL ORGANIC PHOSPHORUS IN SANDY DUNE SOILS

(S.R. TROELSTRA & M.A. VAN DER MEULEN)

Introduction

In recent decades various methods and modified procedures for the determination of the organic fraction of soil phosphorus have been developed, and a rough division into two essentially different categories can be made, i.e., extraction methods and ignition methods.

Extraction methods involve a series of successive extractions, and the difference between the amounts of total phosphorus and inorganic phosphorus present in the combined extracts is taken as the total organic phosphorus content of the soil. In ignition methods the organic phosphorus is converted to inorganic forms by ignition and the difference between the amounts of inorganic phosphorus present in the extracts of ignited and non-ignited soil samples is taken as the total organic phosphorus content. Thus, in both types of method the amount of organic phosphorus is determined indirectly. As yet, reliable direct methods or satisfactory absolute standards for comparison are not available.

Potential sources of error are:

(a) extraction methods

- (1) hydrolysis of organic P;
- (2) incomplete extraction of organic P;

(b) ignition methods

- (1) increase in extractability of inorganic P due to ignition;
- (2) hydrolysis of organic P during the extraction of non-ignited samples;
- (3) incomplete extraction of oxidized organic P;
- (4) incomplete oxidation of organic P;
- (5) volatilization of P during ignition.

If the determinations of total and inorganic phosphorus in the extracts are accurate, all the points mentioned except b1 will cause the organic phosphorus values to be too low.

During the last twenty years, particularly the extraction method of MEHTA *et al.* (1954) and the ignition procedures of LEGG & BLACK (1955) and SAUNDERS & WILLIAMS (1955) have been extensively used, and several modifications of these methods have been developed and recommended (e.g. ANDERSON 1960; BORNEMISZA *et al.* 1967; DORMAAR & WEBSTER 1963b; KAILA 1962; KAILA & VIRTANEN 1955). There have also been many comparative studies of existing methods (e.g. BORNEMISZA & IGUE 1967; VAN DIEST & BLACK 1958; DORMAAR 1964, 1968; DORMAAR & WEBSTER 1963a, 1964; ENWEZOR & MOORE 1966; HANCE & ANDERSON 1962; IPINMIDUN 1973; MCKERCHER & ANDERSON 1968; SEN GUPTA & CORNFIELD 1962; WILLIAMS & WALKER 1967; WILLIAMS *et al.* 1970). The results are often contradictory, and the soil type is likely to be an important factor in the choice of the most suitable technique for determination of the total organic phosphorus content in soils. The method yielding the highest value for organic phosphorus can only be assumed to be the most efficient when the above-indicated potential sources of error are not operative and the determinations of total and inorganic phosphorus in the extracts are accurately performed.

In general, extraction methods and ignition methods using relatively high temperatures are considered to produce organic P values which are too low and too high, respectively, the real value lying somewhere in between. LEGG & BLACK (1955) suggest the use of a relatively low ignition temperature of 240 °C. At this temperature a positive error due to an increase in solubility of inorganic phosphorus would be compensated for by a negative error due to incomplete decomposition of organic phosphorus. Using an ignition temperature of 550 °C, SAUNDERS & WILLIAMS (1955) obtained ignition values that were very similar to and only slightly higher than the extraction values.

However, since soils comparable with sandy dune soils had not been investigated yet, the extraction method of MEHTA *et al.* (1954) and the ignition method of LEGG & BLACK (1955) applied at various ignition temperatures were tested for a first orientation, using samples of sandy dune soils from several locations in The Netherlands.

Materials and methods

Soil samples from three coastal dune areas in The Netherlands were used: Bergen, Voorne, and Goeree (Westduinen). All of these samples have a fine sandy texture; the major properties are summarized in Table 17. Except for Westduinen C, all soils are surface soils of varying sampling depth. In general air-dried, sieved (2 mm), and ground soil samples were used, and in two cases the results obtained with ground and unground samples were compared. In order to get reproducible results with these relatively coarse-textured samples in which the organic matter is rather loosely distributed between the mineral particles, it is recommendable to grind a representative part of the sample for determinations such as carbonates, percentage loss-on-ignition, total phosphorus, total organic phosphorus, total nitrogen, etc.

TABLE 17. Some characteristics of the soils under study

soil sample		pH-H ₂ O*	pH-KCl*	% CaCO ₃	% org. matter**	total P (mg/100 g)
Voorne	h	7.3	7.0	3.2	7.8	34.4
Voorne	2	8.3	8.0	5.3	1.4	21.5
Voorne	8998	7.5	7.3	1.1	14.1	42.2
Voorne	9086	8.1	7.7	5.1	3.4	21.0
Bergen	1b	8.8	8.5	0.5	0.6	5.7
Westduinen	1A2	5.7	5.2	0	12.2	30.5
Westduinen	A	4.2	3.4	0	2.0	11.6
Westduinen	C	8.5	8.2	1.5	0.3	11.2

* 1:2½ (w/v) suspension; ** loss-on-ignition, 430 °C for 24 h

Total phosphorus was determined by digesting 2.5 g soil with 20 ml Fleischmann's acid (mixture of equal volumes of conc. H₂SO₄ and of conc. HNO₃) according to HOUBA *et al.* (1974).

Inorganic phosphorus fractions were obtained with a modified Chang and Jackson procedure (HOUBA *et al.* 1974; see also under 6.6.).

The two analytical procedures employed for the organic phosphorus determinations are summarized below.

Extraction method (MEHTA *et al.* 1954)

- (1) add 10 ml conc. HCl to soil samples (1-2 g) in a 100 ml centrifuge tube and place in a waterbath at about 70 °C for 10 min;
- (2) add an additional 10 ml conc. HCl and allow to stand at room temperature for 1 h;
- (3) add about 50 ml H₂O, centrifuge the suspension, and pour the HCl extract into a 250 ml volumetric flask containing about 50 ml H₂O;
- (4) add 30 ml 0.5 N NaOH to the soil residue and allow to stand at room temperature for 1 h;
- (5) centrifuge the suspension and pour the (first) NaOH extract into the volumetric flask with the preceding extract;
- (6) add 60 ml 0.5 N NaOH to the soil residue and heat in an oven at 90 °C for 8 h;
- (7) cool, centrifuge the suspension, and pour the (second) NaOH extract into the volumetric flask containing the previous extracts;
- (8) dilute to volume, determine total P (after digestion of an aliquot combined extract with flocculated organic matter suspended) and inorganic P (in supernatant of the combined extract after settling of the suspension) and take the difference as organic P.

For step 6, a slightly different and more convenient procedure was adopted by placing the samples, at the end of the afternoon when step 5 is completed, in an oven at room temperature with a time-switch pre-set to heat the oven at 90 °C from midnight until 8.00 a.m. In other words, step 6 is preceded by a period of about 8 hours at room temperature.

Ignition method (LEGG & BLACK 1955)

- (1) ignite soil samples (1-2 g) in a muffle furnace at 240 °C (LEGG & BLACK 1955) and at higher temperatures up to about 600 °C for 1 h;
- (2) add 10 ml conc. HCl to ignited and comparable non-ignited samples in 100 ml centrifuge tubes and place in a waterbath at about 70 °C for 10 min;
- (3) add an additional 10 ml conc. HCl and allow to stand at room temperature for 1 h;
- (4) add about 50 ml H₂O, centrifuge the suspensions, and pour the extracts into 250 ml volumetric flasks;
- (5) dilute to volume, determine inorganic P in the extracts, and take the difference (ignited-nonignited) as organic P.

In both procedures centrifuging and decanting the suspensions presented some quantitative problems, because a solid soil cake was not formed with these relatively coarse-textured samples when an angle head was used at >10,000 rpm; a swing out head was not available, but might give some improvement.

With the ignition method, filtration instead of centrifugation of the extracts was adopted and 200 ml Erlenmeyer flasks instead of 100 ml centrifuge tubes were used. With the extraction method centrifuging and decanting was performed as quantitatively as possible; the possibility of a little loss of soil during the extractions cannot be excluded completely, however.

Results and discussion

From the data shown in Table 18, the following conclusions can be drawn.

- (1) An ignition temperature of 240 °C (LEGG & BLACK 1955) is too low for samples of sandy dune soils. This is consistent with findings of e.g. DORMAAR (1964) and BORNEMISZA & IGUE (1967) for other soil types.
- (2) The results of the extraction method (MEHTA *et al.* 1954) and the ignition values at temperatures >400 °C show a fairly good agreement. When the LEGG & BLACK ignition procedure is used, selection of an ignition temperature between 500 and 600 °C will be necessary. With this modification the ignition and extraction procedures give almost identical estimates of organic phosphorus.
- (3) For all soil samples in Table 18, the average extraction value of 8.4 mg org. P/100 g compares favourably with the average (500 °C) ignition value of 8.3 mg org. P/100 g. The ignition method of LEGG & BLACK (1955), but using a temperature of 500 °C instead of 240 °C, was selected for further routine determinations in our laboratory.
- (4) Grinding of the soil samples does not affect the average value. Since subsampling is more reproducible, however, more consistent values are obtained for ground samples.

In spite of the fact that the extraction and ignition values are very similar, the question remains whether these values really represent a measure of total organic phosphorus, since no absolute standard method is available for comparison. A tentative answer to this question can nevertheless be obtained from three separate and independent determinations on the soil samples, viz. total phosphorus, sum of

TABLE 18. *Organic phosphorus content determined by different methods*

soil sample*	organic P (mg/100 g)		
	extraction value** (MEHTA <i>et al.</i> 1954)	ignition temperature (°C)	ignition value*** (LEGG & BLACK 1955)
Voorne h, unground	11.6+0.86	240	2.9
		250	1.8+0.74
		320	9.0+1.22
		430	11.5+0.51
		500	11.8+0.62
		575	11.6+1.31
Voorne h	11.6+1.13	240	2.3+0.14
		310	9.2+0.28
		360	12.3+0.99
		400	12.2+0.14
		500	12.4+0.00
		610	12.4+0.28
Voorne 2, unground	2.9+1.13	240	-0.1+0.21
		310	1.2
		370	1.3+1.13
		420	2.5+0.28
		500	2.6+0.07
		600	2.2+0.00
Voorne 8998	21.3	500	19.2
Voorne 9086	10.2+0.28	500	10.1
Bergen 1b, unground	1.8+0.61	240	0.3+0.57
		310	1.0+0.28
		370	1.9+0.07
		420	1.8+0.07
		500	1.6+0.00
		600	1.6+0.28
Bergen 1b	1.8+2.24	240	0.1+0.28
		310	0.9+0.14
		360	1.0+0.00
		400	1.5+0.07
		500	1.8+0.42
		610	1.7+0.35
Westduinen 1A2	16.0+0.64	310	11.7+0.92
		410	16.2+0.42
		500	16.0+0.81
		600	16.5+0.64
Westduinen A	6.2+0.00	310	5.5+0.92
		410	6.4+0.21
		500	6.4+0.58
		600	6.7+0.14
Westduinen C	1.0+0.07	310	0.9+0.49
		410	0.6+0.14
		500	1.0+0.10
		600	1.1+0.07
mean for all samples	8.4	500	8.3

* ground samples unless indicated otherwise

** mean + standard deviation (1-9 determinations)

*** mean + standard deviation (1-4 determinations)

inorganic phosphorus fractions (fractionation procedure), and total organic phosphorus.

If these values balance according to:

$$\text{total P} = \text{organic P} + \Sigma \text{ inorganic P fractions,}$$

it will be even more justified to consider the obtained organic P values to be valid estimates.

The results in Table 19 indicate a rather good balance between the different phosphorus quantities, and the positive balance deficits can be at least in part explained by methodical difficulties, especially for the inorganic phosphorus fractionation procedure. Undoubtedly, some loss of soil occurred during the many successive extractions involved. Since Ca-P is quantitatively the most important inorganic P fraction in these soils, and is determined at the end of the fractionation scheme after several extractions and intermediate washings (see under 6.6.), this "loss of soil" effect may account almost entirely for the observed deficit.

If total P and inorganic P data are available for this type of samples, the organic P content can be approximately estimated as:

$$\text{total P} - \text{sum inorganic P fractions} = 2.7,$$

where 2.7 is the mean of the last column in Table 19.

TABLE 19. Total, organic, and inorganic phosphorus analyses of 13 samples

soil sample*	mg/100 g			
	total P	organic P (ignition method, 500 °C)	inorganic P** (sum of fractions)	a-(b+c)
	(a)	(b)	(c)	
Voorne h	34.4	12.4	19.3	2.7
1400	32.6	21.2	5.1	6.3
1404	22.5	12.8	5.7	4.0
1408	17.3	12.5	3.3	1.5
1405	14.5	6.1	5.4	3.0
1406	14.1	3.8	7.3	3.0
1407	12.5	2.1	7.7	2.7
1409	10.8	3.5	4.9	2.4
1410	10.5	1.0	7.1	2.4
1411	10.4	0.5	7.2	2.7
1402	9.6	2.1	5.9	1.6
1401	8.6	2.1	4.3	2.2
1403	7.9	1.2	6.5	0.2

* Voorne h, 1400, 1404, 1408: surface soils; other samples: subsoils (10-40 cm below the surface); 1400/1411: three soil profiles of four consecutive 10 cm layers from Goeree (Westduinen)

** saloid P + Al-bound P + Fe-bound P + Ca-bound P (the presence of reductant-soluble and occluded P forms is assumed to be zero for this type of samples)

References

- ANDERSON, G. - Factors affecting the estimation of phosphate esters in soils. *J. Sci. Food Agric.* 11, 497-503 (1960).
- BORNEMISZA, E. & K. IGUE - Comparison of three methods for determining organic phosphorus in Costa Rican soils. *Soil Sci.* 103, 347-353 (1967).
- BORNEMISZA, E., L.S. VIEIRA & K. IGUE - The method of Mehta *et al.* modified for determination of organic phosphorus in soils high in extractable iron. *Soil Sci. Soc. Amer. Proc.* 31, 576-577 (1967).
- DIEST, A. VAN & C.A. BLACK - Determination of organic phosphorus in soils. III. Comparison of methods. *Soil Sci. Soc. Amer. Proc.* 22, 286-287 (1958).
- DORMAAR, J.F. - Evaluation of methods for determination of total organic phosphorus in chernozemic soils of southern Alberta. *Can. J. Soil Sci.* 44, 265-271 (1964).
- DORMAAR, J.F. - Extraction of organic phosphorus in chernozemic soils. *Plant and Soil* 28, 268-279 (1968).
- DORMAAR, J.F. & G.R. WEBSTER - Status of organic phosphorus in some Alberta soils. *Can. J. Soil Sci.* 43, 27-34 (1963a).
- DORMAAR, J.F. & G.R. WEBSTER - Determination of total organic phosphorus in soils by extraction methods. *Can. J. Soil Sci.* 43, 35-43 (1963b).
- DORMAAR, J.F. & G.R. WEBSTER - Losses inherent in ignition procedures for determining total organic phosphorus. *Can. J. Soil Sci.* 44, 1-6 (1964).
- ENWEZOR, W.O. & A.W. MOORE - Comparison of two methods for determining organic phosphorus in some Nigerian soils. *Soil Sci.* 102, 284-285 (1966).
- HANCE, R.J. & G. ANDERSON - A comparative study of methods of estimating soil organic phosphate. *J. Soil Sci.* 13, 225-230 (1962).
- HOUBA, V.J.G., J.Ch. VAN SCHOUWENBURG & I. WALINGA - Soil analysis. II. Methods of analysis for soils. M. Sc. Course, Wageningen (1974).
- IPINMIDUN, W.B. - Comparison of some methods for determining organic phosphorus in some Nigerian soils. *Soil Sci.* 115, 324-325 (1973).
- KAILA, A. - Determination of total organic phosphorus in samples of mineral soils. *Maataloust. Aikakausk.* 34, 187-196 (1962).
- KAILA, A. & O. VIRTANEN - Determination of organic phosphorus in samples of peat soils. *Maataloust. Aikakausk.* 27, 104-115 (1955).
- LEGG, J.O. & C.A. BLACK - Determination of organic phosphorus in soils. II. Ignition method. *Soil Sci. Soc. Amer. Proc.* 19, 139-143 (1955).
- MCKERCHER, R.B. & G. ANDERSON - Observations on the accuracy of an ignition and an extraction method for measuring organic phosphate in some Canadian soils. *Soil Sci.* 105, 198-200 (1968).
- MEHTA, N.C., J.O. LEGG, C.A.I. GORING & C.A. BLACK - Determination of organic phosphorus in soils. I. Extraction method. *Soil Sci. Soc. Amer. Proc.* 18, 443-449 (1954).
- SAUNDERS, W.M.H. & E.G. WILLIAMS - Observations on the determination of total organic phosphorus in soils. *J. Soil Sci.* 6, 254-267 (1955).
- SEN GUPTA, M.B. & A.H. CORNFIELD - Phosphorus in calcareous soils. II. Determination of the organic phosphorus content of calcareous soils and its relation to soil calcium carbonate content. *J. Sci. Food Agric.* 13, 655-658 (1962).
- WILLIAMS, J.D.H. & T.W. WALKER - Comparison of ignition and extraction methods for the determination of organic phosphate in rocks and soils. *Plant and Soil* 27, 457-459 (1967).
- WILLIAMS, J.D.H., J.K. SYERS, T.W. WALKER & R.W. REX - A comparison of methods for the determination of soil organic phosphorus. *Soil Sci.* 110, 13-18 (1970).

6.6. INORGANIC SOIL PHOSPHORUS FRACTIONS IN AN OLDER DUNE AREA (WESTDUINEN)
OF GOEREE (S.R. TROELSTRA)

Introduction

In the analyses of soils for P, a good understanding of the occurrence and nature of the phosphorus present (organic P versus inorganic P; inorganic P forms) is necessary for the interpretation of soil P availability indices obtained by isotopic dilution techniques, anion resin procedures, and chemical extraction methods, and may also be useful in mycorrhiza studies.

Particularly in more natural and non-agricultural situations, a quantitative knowledge of the forms of soil P that might contribute to plant nutrition will no doubt be of additional value in autecological greenhouse work in which an attempt is made to find correlations between values of available P obtained in various extraction procedures and plant-uptake values. Therefore, a study was performed to determine the inorganic P forms occurring in a relatively old dune area (Westduinen) on the island of Goeree, one of the Institute's research sites.

Materials and methods

Soil profiles with depths varying from 40 to 100 cm and successive soil layers of 10 or 20 cm were sampled, giving a total of about 70 soil samples.

The samples were air-dried, sieved (1 mm), ground, and analyzed for carbonates, loss-on-ignition (430 °C for 24 h), total P, and inorganic P fractions; the pH (1:2½ w/v suspension) was determined on unground samples. Use was made of a modified CHANG & JACKSON (1957) fractionation scheme as described by PETERSEN & COREY (1966) and HOUBA *et al.* (1974): 1 g soil samples in 100 ml centrifuge tubes are successively extracted with 50 ml 1 N NH_4Cl (saloid P: water-soluble and highly available P forms); 50 ml 0.5 N NH_4F pH 8.2 (Al-bound P); 50 ml 0.1 N NaOH (Fe-bound P); and 50 ml 0.5 N H_2SO_4 (Ca-bound P).

Since the presence of reductant-soluble and occluded P forms is not very likely in this type of samples, treatments with Na dithionite-Na citrate 0.3 M and 0.1 N NaOH after the Ca-P extraction were omitted. This assumption was verified by analyzing 5 samples for reductant-soluble and occluded P forms. Very low values of 0.3 to 1.3 mg P/100 g were found for the sum of both fractions, which can be ascribed at least in part to methodical difficulties such as incomplete removal of the residual solution in the tubes.

Centrifugation of these rather coarse-textured samples at >10,000 rpm did not result in very good sedimentation or a compact soil cake (for 100 ml tubes, only an angle head was available). After pipetting a 25 ml subsample for further analysis, the remainder of the solution was discarded carefully and as quantitatively as possible, using a glass capillary connected with a vacuum. Some loss of sample may have occurred, however.

TABLE 20. Results of inorganic phosphorus analyses

soil sample	soil layer (cm)	pH-H ₂ O	pH-KCl	% CaCO ₃	% loss-on-ignition	P (mg/100 g)					total P
						sal.P	Al-P	Fe-P	Ca-P	Σ fractions	
568	0- 20	5.0	4.1	-	1.7	0.1	0.3	0.4	2.9	3.7	10.3
569	20- 40	7.3	7.1	0.4	0.4	-	0.4	0.4	4.6	5.4	9.2
570	40- 60	8.1	8.0	2.1	0.2	0.1	0.3	0.2	6.1	6.7	10.4
571	60- 80	8.1	8.1	2.7	0.3	-	0.4	0.1	6.2	6.7	10.3
572	80-100	8.6	8.5	2.6	0.2	-	0.5	0.2	6.7	7.4	10.1
573	0- 20	5.3	4.7	-	3.1	-	0.4	0.5	3.7	4.6	12.1
574	20- 40	7.9	7.6	0.5	0.5	-	0.4	0.3	5.1	5.8	10.0
575	40- 60	8.6	8.5	2.5	0.3	0.1	0.5	0.1	6.4	7.1	10.0
576	60- 80	8.6	8.5	3.1	0.3	0.1	0.5	0.2	7.7	8.5	10.7
586	0- 10	4.8	4.1	-	5.6	1.1	0.9	1.8	2.9	6.7	18.7
587	10- 20	5.3	4.7	-	1.8	0.1	1.3	0.9	4.7	7.0	10.8
588	20- 30	5.7	5.0	-	0.6	0.9	0.7	0.6	5.4	7.6	9.6
589	30- 40	7.6	7.5	0.4	0.6	0.1	0.7	0.3	6.3	7.4	9.9
590	40- 50	8.2	8.2	1.6	0.4	0.3	1.1	0.4	7.0	8.8	10.4
591	50- 60	8.2	8.2	2.8	0.4	-	0.5	0.2	8.8	9.5	11.3
592	60- 80	8.6	8.4	4.3	0.3	0.4	0.8	0.2	8.6	10.0	10.9
593	80-100	8.6	8.5	4.6	0.3	0.6	0.7	0.2	7.7	9.2	11.0
601	0- 20	5.0	4.2	-	2.1	0.6	0.7	0.7	2.9	4.9	13.7
602	20- 40	5.4	4.4	-	0.5	0.5	0.8	0.6	4.0	5.9	9.8
603	40- 60	6.3	6.0	-	0.6	0.6	0.9	0.7	4.2	6.4	11.2
604	60- 80	7.3	7.2	0.2	0.5	0.2	0.7	0.4	5.1	6.4	10.5
605	80-100	8.1	8.1	1.4	0.4	0.1	0.5	0.3	7.1	8.0	11.3
1400	0- 10	4.5	3.7	-	15.0	0.3	0.5	2.3	2.0	5.1	32.6
1401	10- 20	5.4	4.2	-	1.4	0.1	0.5	0.4	3.4	4.4	8.6
1402	20- 30	5.8	4.6	-	1.0	0.1	0.5	0.3	5.0	5.9	9.6
1403	30- 40	6.2	4.9	-	0.4	0.1	0.5	0.4	5.4	6.4	7.9
1404	0- 10	5.6	4.6	-	4.7	0.5	0.7	1.0	3.5	5.7	22.5
1405	10- 20	5.8	4.6	-	1.4	0.2	0.4	0.9	3.8	5.3	14.5
1406	20- 30	6.9	6.2	0.2	0.8	0.1	0.7	0.8	5.6	7.2	14.1
1407	30- 40	7.9	7.6	0.7	0.5	0.1	0.5	0.7	6.4	7.7	12.5
1408	0- 10	5.4	4.4	-	6.6	0.1	0.3	0.7	2.2	3.3	17.3
1409	10- 20	5.8	4.6	-	1.6	-	0.4	0.6	3.9	4.9	10.8
1410	20- 30	6.4	5.2	-	0.5	-	0.8	0.7	5.6	7.1	10.5
1411	30- 40	8.7	8.2	0.6	0.3	-	0.5	0.4	6.3	7.2	10.4

Results

Some of the results are given in Table 20. Although not entirely quantitative, the fractionation results do give a clear insight into the relative occurrence of the different mineral phosphorus forms. Initially present carbonates have been leached completely from the surface layers (30 cm or deeper), but Ca is still the most abundant cation in the base exchange capacity (see under 6.7.), even at relatively low pH values, in this 1350-year-old dune area (younger age with respect to dune stabilization).

This pattern is confirmed by the inorganic P analyses. In general, Ca-bound P is the most important mineral phosphorus form and increases with depth along with pH, as expected (Table 20). The decrease in Ca-P toward the soil surface is more compensated for by an increase in organic phosphorus than by an increase in Fe-bound P. The parent material appears to be relatively low in Fe.

Because of the thermodynamic instability of Ca-P at low pH values, Ca-P may be a valuable source of P for plants growing on these soils.

References

- CHANG, S.C. & M.L. JACKSON - Fractionation of soil phosphorus. *Soil Sci.* 84, 133-144 (1957).
- HOUBA, V.J.G., J.Ch. VAN SCHOUWENBURG & I. WALINGA - Soil analysis. II. Methods of analysis for soils. M. Sc. Course, Wageningen (1974).
- PETERSEN, G.W. & R.B. COREY - A modified Chang and Jackson procedure for routine fractionation of inorganic soil phosphates. *Soil Sci. Soc. Amer. Proc.* 30, 563-565 (1966).

6.7. SPATIAL VARIABILITY OF SOIL CHEMICAL PROPERTIES IN AN OLDER DUNE AREA (WESTDUINEN) OF GOEREE (S.R. TROELSTRA)

Introduction

The dune complex of which the Westduinen area originally formed a part was deposited at the end of the post-Roman or Duinkerke II transgressional phase of the sea (A.D. 250-600). Since then erosion processes and leveling by man have made considerable changes. In its present and largely stabilized state the Westduinen site, which is one of the few remaining relatively undisturbed inner dune areas, shows a rolling relief with maximal differences in height of about 2-4 m and a natural grassland vegetation.

Of the soil-forming factors (parent material, topography, climate, vegetation and fauna, and time), the topography exerts a major influence on the soil profile development via its impact on microclimatic and hydrological regimes (slope aspects; due to rising of the ground-water, some depressions are flooded during the winter and early spring).

AC profiles have been developed to a varying degree (maximal organic matter accumulation in depressions) and carbonates have been leached completely from the upper 30 cm or deeper. Aside from soil texture, the depth of leaching of

carbonates is determined at least in part by the net water movement through the soil profile, and in some depressions carbonates may be present within 30 cm. Podzolization processes (chemical migration of aluminium and iron and/or organic matter) are not important yet. In some places, leaching of organic matter may have occurred to some extent directly underneath the A horizon.

Other characteristics of the area are: extensive stock grazing during the summer period (dung and urine patches), a low rate of fertilizer application on the flatter parts, burrowing activities by rabbits on the upper slopes and summits, and some digging by man (aerials of a marine transmitting station; drinking places). The latter points may enable carbonate-rich material to reach the surface again. The present investigation was designed to study the spatial variability of soil chemical properties within the Westduinen area. The work was done as an integral part of an extensive plant ecological program carried out in this area. In addition to mean values, ecologists are also interested in range values for a given area (BALL & WILLIAMS 1971).

Materials and methods

Soil variability within the Westduinen area has two main features, i.e., a small-scale variability within a topographically uniform unit (no slope effects) and a topographic variation.

To investigate the small-scale variability, a grid 60 cm square subdivided into sixteen units of 15 cmsquare was applied to 19 rather different locations in the area (Fig. 10). Eight units of the grid were sampled separately (replicate samples) and the other eight (shaded in the Figure) were combined and treated as a bulk sample; 4 soil cores per sampling unit were taken; sampling depths were 0-10 cm or 0-15 cm and 10-20 cm or 15-25 cm (in some places deeper soil layers as well).

After drying and sieving (1 mm), bulk samples were mechanically subdivided (Retsch subsampler type PTZ) to obtain 8 subsamples, and from this point on replicate samples and bulk subsamples were treated in the same way: subdivision, grinding of part of the sample in a mortar mill (Retsch type RMO), and analysis.

Comparison of means and standard deviations makes it possible to evaluate the efficiency of the two sampling procedures. It has been tacitly assumed that, on average, bulk samples and replicate samples have the same composition. Separate treatment of pairs of grid-subunits (drying, sieving, subdivision into two parts, one of them for bulk sample) would have been more correct but also more laborious.

The topographic variation is being studied on the basis of two transects, each about 50 m long, and including several depressions and summits of moderate dimensions. Maximal differences in height are 2.5 m for the first and 1.5 m for the second transect. Sampling points were taken 50 cm or 1 m apart and sampling depths up to 30 cm. Each sampling point represents an area of about 15 cm square, and 4 to 5 soil cores per sampling point were taken. After drying

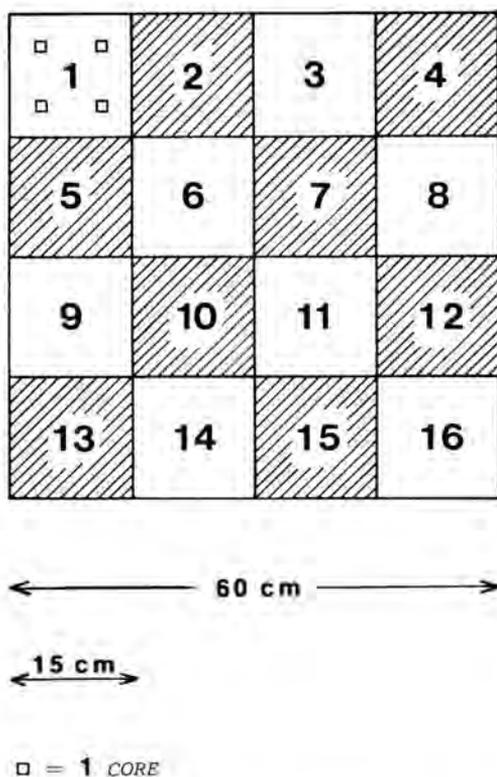


FIG. 10. Grid applied in variability study

and sieving (1 mm) samples were treated as indicated above for replicate samples and bulk subsamples.

The pH of the soil was measured potentiometrically in 1:2½ (w/v) suspensions of unground soil in H₂O or 1 N KCl (pH-H₂O and pH-KCl). Carbonates were measured gas-volumetrically 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 the ignition method using ground soil samples (see under 6.5.). Olsen P (bicarbonate P) was determined after shaking unground soil samples with 0.5 M NaHCO₃ (OLSEN *et al.* 1954; WATANABE & OLSEN 1965). Exchangeable cations were determined by atomic absorption spectrophotometry after shaking unground soil with neutral ammonium acetate. Chloride and electrical-conductivity analyses were carried out on 1:5 water extracts of unground soil.

TABLE 21. Spatial variability of soil chemical factors in the Westduinen area (Goeree)

	Small-scale variability (area of 0.36 m ²)						Topographic variation					
	0-10 cm (0-15 cm)			10-20 cm (15-25 cm)			transect I	transect II	"whole area"***	transect I	transect II	
	average range	maximum range	minimum range	average range	maximum range	minimum range				0-10 cm	10-20 cm or 10-30 cm	10-20 cm
pH-H ₂ O	0.3 pH	4.7-5.5	4.6-4.8	0.3 pH	7.7-8.2	6.0-6.1	4.2-5.4	4.6-5.7	4.2-6.4	5.0-8.1	4.9-7.7	5.2-8.5
pH-KCl	0.3 pH	5.1-5.6 3.9-4.4	3.3-3.4	0.2 pH (0.3 pH?)	4.5-5.0 (6.7-7.9?)	3.7-3.8	3.3-4.5	3.6-5.2	3.3-5.9	4.0-8.0	3.9-6.9	4.2-8.1
% CaCO ₃	-	-	-	-	-	-	0	0	0-0.9	0	0	0-1.5
% org. matter (loss-on-ignition)	1.8	10.9-15.9	2.7-3.4	0.3	0.5-1.2	0.3-0.4	2.0-8.6	3.3-7.7	0.9-33.5	0.4-2.7	0.6-2.8	0.2-1.1
total P (mg/100 g)	4.6	12.6-21.6	9.8-11.1	1.6	10.5-16.2	8.5-8.9	10.8-21.2	14.9-24.4	7.4-50.9	6.2-14.3	9.3-15.6	7.8-12.4
organic P* (mg/100 g)	-	16.0-20.2	4.8-8.0	-	1.4-2.6	0.9-1.7	4.2-13.9	8.7-15.5	4.2-ca.36	0.7-6.8	3.1-9.2	1.2-6.4
NaHCO ₃ -P (mg/100 g)	-	-	-	-	-	-	0.30-0.92	0.28-0.94	-	0.02-0.65	0.07-0.47	0.07-0.35
Ca* (me/100 g)	-	5.43-6.38	0.12-0.33	-	0.39-3.31	0.35-0.46	0.28-4.59	1.14-6.88	0.12-11.51	0.00-2.18	0.36-2.81	0.36-1.89
Mg* (me/100 g)	-	0.84-1.19	0.09-0.12	-	0.08-0.20	0.08-0.09	0.15-0.65	0.35-0.95	0.08-2.47	0.02-0.20	0.10-0.40	0.10-0.30
K* (me/100 g)	-	0.22-0.32	0.04-0.06	-	0.02-0.03	0.00-0.01	0.05-0.17	0.04-0.20	0.03-0.67	0.00-0.10	0.01-0.14	0.01-0.07
Na* (me/100 g)	-	0.45-0.63	0.03-0.06	-	0.00-0.03	0.00-0.01	0.04-0.25	0.05-0.18	0.03->1.30	0.00-0.04	0.01-0.06	0.01-0.05
Cl** (me/100 g)	0.06	0.33-0.46	0.04-0.07	0.02	0.01-0.04	0.01-0.02	0.03-0.19	0.03-0.16	0.01-1.07	0.01-0.07	0.00-0.08	0.00-0.05
EC** 1:5 extr. (μS cm ⁻¹)	30	190-265	27-39	12	32-88	12-16	25-115	35-110	23-585	12-74	12-75	14-70

* grid analyses of 2 or 3 extreme locations

** grid analyses of 10 locations

*** combined results of plot, transect, and other analyses

TABLE 22. *Coefficients of variation (per cent)*

	0-10 cm (0-15 cm)		10-20 cm (15-25 cm)		deeper layers	
	replicate	samples bulk subsamples	replicate	samples bulk subsamples	replicate	samples bulk subsamples
pH-H ₂ O	1.4- 6.1 (2.4)*	0.2- 1.1(0.5)	0.8- 2.9(1.8)	0.2- 1.4(0.6)	0.9	0.3- 1.4(0.8)
pH-KCl	1.0- 5.0(2.5)	0.2- 0.9(0.5)	0.8- 5.3(2.0)	0.2- 1.4(0.5)	1.3	0.1- 0.7(0.4)
% org. matter (loss-on-ignition)	3.4-18 (10)	1.0- 9.4(2.7)	7.0-35 (15)	1.7- 9.0(4.7)	5.5	1.9-20 (6.7)
total P	3.4-18 (8.2)	0.8- 4.0(2.0)	2.0-13 (5.8)	1.6- 5.2(3.2)	11	0.1- 5.1(2.8)
organic P	9.2; 14	1.2; 8.1	4.7; 18	5.7; 19		
Cl	12 -40 (22)	2.8-17 (9.2)	10 -45 (30)	9.3-27 (17)		5.1-34 (17)
EC 1:5 extract	6.8-16 (13)	3.2-12 (6.0)	4.7-39 (15)	2.1-10 (6.5)		4.1-12 (8.4)

* mean

Results and discussion

The results are summarized very briefly in Tables 21 and 22. Unless indicated otherwise, small-scale variability values in Table 21 refer to the results of replicate sampling of a total of 19 plots.

As expected, mean values for replicate and bulk sampling procedures (not shown in the Tables), in general do not differ significantly and also reflect possible variations in pretreatment, analytical variations, etc. Where significant differences do occur, they are of no quantitative importance.

Standard deviations and coefficients of variation (Table 22) of the replicate samples are of considerable magnitude in some instances. In other words, the determination of only a mean value of a bulk sample may involve the loss of ecologically important information related to the small-scale variability of the plot. Whether the extra effort required to obtain this knowledge is justified, will depend on the actual order of magnitude in combination with the type of research.

The effect is more pronounced in the upper soil layers, because here most properties reach their highest value and the "noise" level of the analytical methods does not have a marked influence on the results. Chloride and conductivity values are very low, and these determinations were carried out for orientation rather than statistical comparison.

The coefficients of variation can be compared with values given by e.g. BALL & WILLIAMS (1968, 1971) and BECKETT & WEBSTER (1971).

For most of the properties the range of values varies considerably for the topographic variation, especially for the area as a whole (column 9 in Table 21). Nevertheless, because locations with the higher values are not very widely distributed within the area, the results of both transects give a fairly good idea of the average situation. The pH variation in the upper 10 cm is relatively small.

For most factors the observed variation is directly correlated with the organic matter content variation: organic phosphorus, total phosphorus via organic phosphorus, exchangeable cations (organic matter as component of the clay-humus exchange complex), chlorides (coefficients of correlation with organic matter + 0.93, + 0.84, and + 0.58 for surface samples of 10 plots and 2 transects, respectively) and electrical conductivity. Since the formation of AC profiles is the most important soil development within the area, this conclusion seems rather logical.

Organic matter values are high and certainly not to be taken for percentages of "active" humus. The surface samples may contain an appreciable amount of undecomposed or partly decomposed material.

In the upper 10 cm, 50 to 70 per cent of the total P is present in organic forms. The subsoil (10-30 cm) shows a lower percentage and more variation: between 10 and 60 per cent. Total nitrogen was determined in a small set of samples only and is not shown in the Tables, partly because this value is closely correlated with organic matter content and has limited value for the

prediction of N availability; the nitrogen content of the organic matter amounts to 3 to 4 per cent.

C/N/P ratios were determined in a limited set of samples too. For surface samples and subsoils the ratios were $(118-190)/10/(0.30-1.04)$ and $(100-142)/10/(0.58-1.65)$, respectively, which is in very good agreement with values reported in the literature (BARROW 1961; N is set equal to 10).

It should be noted that the analytical results are expressed on a soil-weight basis, although from an ecological point of view a soil-volume basis (soil layer thickness constant) would be preferable. Therefore, to determine correlation with plant performance the bulk density variation must also be taken into consideration, since soil with a higher organic matter content in depressions will have a lower bulk density value. Expressed on a soil-volume basis instead of a soil-weight basis, the topographic variation will be somewhat less extreme.

References

- BALL, D.F. & W.M. WILLIAMS - Variability of soil chemical properties in two uncultivated Brown Earths. *J. Soil Sci.* 19, 379-391 (1968).
- BALL, D.F. & W.M. WILLIAMS - Further studies on variability of soil chemical properties: efficiency of sampling programmes on an uncultivated Brown Earth. *J. Soil Sci.* 22, 60-68 (1971).
- BARROW, N.J. - Phosphorus in soil organic matter. *Soils and Fert.* 24, 169-173 (1961).
- BECKETT, P.H.T. & R. WEBSTER - Soil variability: a review. *Soils and Fert.* 34, 1-15 (1971).
- DAVIES, B.E. - Loss-on-ignition as an estimate of soil organic matter. *Soil Sci. Soc. Amer. Proc.* 38, 150-151 (1974).
- HOUBA, V.J.G., J.Ch. VAN SCHOUWENBURG & I. WALINGA - Soil analysis. II. Methods of analysis for soils. *M. Sci. Course, Wageningen* (1974).
- OLSEN, S.R., C.V. COLE, F.S. WATANABE & L.A. DEAN - Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circ.* 939 (1954).
- WATANABE, F.S. & S.R. OLSEN - Test of an ascorbic acid method for determining phosphorus in water and NaHCO_3 extracts from soil. *Soil Sci. Soc. Amer. Proc.* 29, 677-678 (1965).

7. PUBLICATIONS IN 1977

- BLOM, C.W.P.M. - Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. II. Trampling and seedling establishment. *Oecologia Plantarum* 12(4), 363-381 (1977).
- CAVÉ, A.J. - Pitfalls in the estimation of age-dependent survival rates from ringing and recovery data. *Die Vogelwarte* 29, Sonderheft, 160-171 (1977).
- KLUYVER, H.N., J.H. VAN BALEN & A.J. CAVÉ - The occurrence of time-saving mechanisms in the breeding biology of the Great Tit, *Parus major*. In: *Evolutionary Ecology*, B.Stonehouse & C.M. Perrins (editors), 153-169 (1977).
- MERTENS, J.A.L. - Thermal conditions for successful breeding in Great Tits, *Parus major* L. I. Relation of growth and development of temperature regulation in nestling Great Tits. *Oecologia* 28, 1-29 (1977);
II. Thermal properties of nests and nestboxes, and their implications for the range of temperature tolerance of Great Tit broods. *Oecologia* 28, 31-56 (1977).
- MERTENS, J.A.L. - Temperatuurregulatie in de nestholte van de Koolmees, *Parus major* L. Proefschrift, R.U., Groningen (1977).
- PERDECK, A.C. - The analysis of ringing data: pitfalls and prospects. *Die Vogelwarte* 29, Sonderheft, 33-34 (1977).
- SPAANS, A.L. - Are Starlings faithful to their individual winter quarters? *Ardea* 65, 83-87 (1977).
- SPAANS, A.L. - Molt of flight and tail feathers of the Least Sandpiper in Surinam, South America. *Bird Banding* 47, 359-364 (1977).
- STOUTJESDIJK, Ph. - On the range of micrometeorological differentiation in the vegetation. In: *Vegetation und Klima*, H. Dierschke (editor), 21-34, Vaduz (1977).
- STOUTJESDIJK, Ph. - High surface temperatures in the winter and their biological significance. *Int. J. Biometeorology* 21, 325-331 (1977).
- TOORN, J. VAN DER & K. REININK - Rietproefveld, Zuidelijk Flevoland en Moerasvegetatie Oostvaardersdiep-gebied, Zuidelijk Flevoland. In: *Onderzoek naar de toepassingsmogelijkheden van multispectrale scanning*. NIWARS publ. 44, 294-313 (1977).
- TURIN, H., J. HAECK & R. HENGEVELD - Atlas of the Carabid beetles of The Netherlands. K.N.A.W. Verh. Afd. Natuurk., 2e Reeks, 68, 1-228 (1977).
- PROGRESS REPORT 1976. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2e Reeks, 69, 108-147 (1977).
- Contents:
- BALEN, J.H. VAN & P.J. DRENT - Population fluctuations, site tenacity and winter food. p. 111-112 (4-5).
- BALEN, J.H. VAN & R. DE JONG - The timing of the moult in adult Great Tits. p. 112-113 (5-6).
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- CAVÉ, A.J. - The analysis of survival rate. p. 119-122 (12-15).
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BLOM, C.W.P.M. & J. VAN HEESIJK - Some remarks on the germination of six *Plantago* species. p. 137-142 (30-35).

LAAN, D. VAN DER - Synecological research on the dune slacks on Voorne; analysis of vegetational data. p. 142-146 (35-39).

N.B. Page numbers between parentheses refer to the reprint of the Progress Report issued before the appearance of the journal.

