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HUBRECHT LABORATORY

International Embryological Institute – Utrecht

PROGRESS REPORT 1968 [1])

Composition of Scientific Staff

P. D. Nieuwkoop, Ph.D. – Director, Prof. of Experimental Embryology, University of Utrecht
J. A. Leussink, M.Sc. – Administrator
B. Z. Salomé, M.Sc. – Chief Librarian
Elze C. Boterenbrood, Ph.D. – Head, research unit of amphibian experimental morphology
K. Hara, Ph.D. – Head, research unit of photo- and cinematography, and avian experimental morphology
J. G. Bluemink, Ph.D. – Head, research unit of ultrastructural research
Geertje A. Ubbels, Ph.D. – Head, research unit of histo- and cytochemistry
P. Th. M. van der Saag, M.Sc. – Head, research unit of biochemistry
J. Faber, Ph.D. – Head, research unit of developmental genetics
Th. M. Konijn, Ph.D. – Head, research unit of developmental physiology
Kirstie A. Lawson, Ph.D. – Head, research unit of tissue and organ culture

Sixth International Team in Embryology

The Sixth International Team met from February 1st till July 31st, 1968. Its central topic was: "Cell contacts and their role in morphogenesis and cytodifferentiation". Prof. L. Weiss of the Roswell Park Memorial Institute, Buffalo, N.Y. acted as co-leader to the Team.

The Team consisted of the following members:
Dr. R. Adler * (Buenos Aires, Argentine)
Dr. D. Beig (Rio Claro, Brasil)
Dr. Mary Beth Burnside * (Austin, Tex., U.S.A.)
Dr. R. Hauser (Bern, Switzerland)
S. Kochav (Jerusalem, Israel)
Dr. Louise Luckenbill * (Boston, Mass., U.S.A.)
Dr. J. Marthy (Basel, Switzerland)
Dr. Teresa Rogulska (Warsaw, Poland)
Aysel Şeftalioglu (Ankara, Turkey)
Dr. A. Scisławski (Krakow, Poland)

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[1] This report was edited by Dr. J. Faber; the final draft was approved by the Director and by all Staff Members. Parts of the report may be quoted under the title "Hubrecht Laboratory, Royal Netherlands Academy of Sciences and Letters, Progress Report 1968". It should however be realized that much of the subject matter in the report is of a preliminary nature, and is consequently subject to extensions and alterations at a later date.

Dr. R. Singh (Jaipur, India)
Sri Sudarwati * (Bandung, Indonesia)
Dr. A. Švajger (Zagreb, Yugoslavia)

The Team Members carried out a variety of research projects planned so as to fit into the research programmes of the Laboratory's various research units. In most cases only preliminary results were obtained. Research projects of Team Members are mentioned very briefly or by title only, unless they yielded more or less definite results. The Team Members whose names are marked with an asterisk stayed at the Laboratory as Visiting Investigators after the end of the Team.

Visiting Investigators other than Team Members

Dr. Rosine Chandebois (Marseille, France)
Judith Chegwidden (London, England)
Renata Czołowska (Warsaw, Poland)
Dr. R. W. Glade (Burlington, Vt., U.S.A.)
Dr. J. G. Hollyfield (Austin, Tex., U.S.A.)
Dr. M. Klíma (Brno, Czechoslovakia)
Dr. W. P. Luckett (Madison, Wis., U.S.A.)
Dr. N. Nikitin (Leningrad, U.S.S.R.)
Dr. I. Slabý (Plzeň, Czechoslovakia)
Dr. K. Ulrich (Kgs. Lyngby, Denmark)
Dr. L. Weiss (Buffalo, N.Y., U.S.A.)

Ph.D. students (University of Utrecht)

N. J. de Both
W. J. Ouweneel

Graduate students (University of Utrecht)

A. M. A. ten Berge
J. H. H. Eussen
C. E. Hulstaert
S. W. de Laat
Marie-Thérèse van Oldenborgh
J. J. Zeegers

The Progress Report 1968 consists of the following sections:

I. EARLY AMPHIBIAN DEVELOPMENT

In 1967 a number of research units within the Laboratory embarked on a joint project, viz. a multidisciplinary approach to the problems of early amphibian development (mainly concentrating on the developmental period extending from the egg up to and including gastrulation). The progress made so far is described below, arranged according to the methods used. Most of the work was on the axolotl *(Ambystoma mexicanum)*.

a. *Experimental morphology*

1. Recombination experiments using various animal-vegetative zones of the blastula (P. D. Nieuwkoop)

It had been found in 1967 that the entire mesoderm arises from the animal, ectodermal "half" of the blastula as a result of an inductive action emanating from the vegetative, endodermal "half". In an extension of these experiments it was found that recombinates of the animal cap of the blastula with the vegetative nutritive yolk material (i.e. after removal of the intervening marginal zone material) could develop into virtually normal larvae, provided healing of the wound (closure of the "surface coat") had been completed prior to the onset of gastrulation. In such recombinates the nutritive yolk material forms the entire range of endodermal organs. Further analysis by means of transposition of the recombinant parts showed that the dorso-ventrality of the induced mesoderm is determined entirely by the dorso-ventral polarity of the endoderm, and that the portion of the grey crescent present in the animal cap plays no causal role whatsoever in the formation of the axial mesodermal structures. Finally indications were found that the first phenomena of

gastrulation (formation of flask cells) are induced by the animal "half" of the blastula.

2. The regional inductive capacity of the blastula endoderm (E. C. Boterenbrood)

In connection with the findings mentioned in the preceding section, experiments are being carried out to analyse in more detail the way in which the dorso-ventral polarity apparently present in the endoderm leads to the appearance of dorso-ventral organisation in the induced mesoderm.

3. The induction of the mesoderm in the anuran blastula *(Xenopus laevis)* (S. Sudarwati – thesis subject, Bandung Institute of Technology)

The preliminary results of this investigation show that the induction of the future mesoderm in anurans is not essentially different from that in urodeles. The possible differences in mesodermal competence between the inner and outer layer of the two-layered anuran ectoderm are being investigated.

b. *Electron microscopy*

1. Electron microscopic studies of cleavage and of the development of cell contacts during early embryogenesis (J. G. Bluemink)

The earliest indication of the first cleavage division of the axolotl egg is the appearance of a shallow groove at the surface of the animal hemisphere. In many eggs transverse folds radiate out from this groove. In the ridge of such a fold the egg cortex is characterized, among other things, by accumulations of osmiophilic material. In the part of the fold bordering on the cleavage groove this material shows a periodic structure reminiscent of myelin, which suggests the presence of unsaturated lipids. It seems plausible to assume that this material will be used to build up the new cell surface.

In a later stage of the first cleavage division the bottom of the cleavage groove is underlain by a layer of filaments lying immediately below the cell membrane and running parallel to the cleavage plane. During the further progress of cleavage the space between the future blastomeres is bridged by extensions arising from the cell surfaces facing each other. In those places where the cleavage cleft is completely obliterated, attachments of the "zonula occludens" type develop between the apposed trilaminar cell membranes.

2. Electron microscopic study of the so-called "surface coat" (L. Luckenbill – continued Team research project)

Since the "surface coat" is held to be characterized by contractility upon damage inflicted to the egg surface, the results of such damage were examined with the electron microscope. The results have so far failed to yield any evidence supporting Holtfreter's conception of the

existence of a "surface coat" as a separate, possibly extracellular entity. The possibility is being investigated that the elastic properties of the egg surface might be attributable to the structure of the cortical layers of the egg.

c. *Histo- and cytochemistry*

1. The origin and further development of the "grey crescent" in *Ambystoma mexicanum* and *Xenopus laevis* (R. W. Glade)

This cytological and cytochemical study was initiated by examining the distribution in the egg of yolk platelets of different size classes. It will later be extended to later stages and experimentally treated eggs.

2. Early chemical differentiation in various regions of the embryo of *Ambystoma mexicanum* and *Xenopus laevis* (G. A. Ubbels)

The preliminary results of this study show that the ectoderm and mesoderm of the early axolotl gastrula are not histochemically homogeneous: individual cells show marked differences in polysaccharide content. This holds also for the animal blastomeres of the morula of *Xenopus*. These differences are at present being traced back to earlier stages, while at the same time the nature of the polysaccharide component is being studied.

3. The distribution of cytoplasmic RNA in the gastrula and neurula (A. Scisławski – Team research project)

d. *Biochemistry*

1. Aspects of the molecular biology of early amphibian development (P. Th. M. van der Saag and co-workers)

The research unit of biochemistry was established during 1967, and research did not start fully until 1968. As a basis for further work, ribosomes, ribosomal RNA, and transfer-RNA of embryos of *Ambystoma mexicanum* are being purified and characterized by means of sucrose density gradient centrifugation. Material of *Xenopus laevis*, a species more fully studied biochemically, is used for comparative purposes. In axolotl material purification meets with difficulties on account of the large number of yolk platelets and glycogen granules present in the cytoplasm.

1a. DNA, RNA and protein synthesis in dissociated embryonic cells (P. Th. M. van der Saag and J. H. H. Eussen)

Because the outer surface of amphibian embryos is relatively impermeable even to small molecules, the embryos are dissociated into single cell suspensions. The synthetic capacities of these cells are being analysed by means of incorporation of radio-active precursors. The following specific inhibitors of DNA, RNA and protein synthesis are being used: hydroxyurea, puromycin aminonucleoside, puromycin, and cycloheximide.

1b. Patterns of protein synthesis in the embryo (P. Th. M. van der Saag and J. J. Zeegers)

A very sensitive method for the separation of proteins, viz. disc-electrophoresis in polyacrylamide gel, is being applied to amphibian material. First the patterns of protein synthesis in whole embryos (fertilized egg up to and including the tail bud stage) are being analysed. Later the method will be combined with radio-active labeling of whole embryos and dissociated cells.

1c. Elaboration of a method for obtaining large numbers of ecto-, meso-, and endodermal cells of the early embryo (I. Slabý)

In direct connection with the preceding project a method is being developed to obtain the three main cell types of the early embryo in a viable state and in large numbers, in order to use them for further biochemical work. The method is based on the size and density differences between the cell types; the embryos are dissociated, and the cell suspension is centrifuged in a Ficoll density gradient, which yields fractions containing cells of approximately the same size and density.

e. *Biophysics*

The equipment for the new research unit of biophysics is being installed (S. W. de Laat). The actual research has not yet begun; it will be concentrated on measurements of the membrane potential and membrane resistance of the amphibian egg surface, and on the role of membrane permeability in the exchange of information between the blastomeres in amphibians.

II. AMPHIBIAN DEVELOPMENT (general)

In this section investigations will be reported which fall outside the scope of "early amphibian development" because they deal with very special problems or with later stages of development, and also because they are in part continuations of research projects started earlier. Unless stated otherwise, the experimental animal was the axolotl.

1. A cytochemical investigation of the origin of the so-called "germinal cytoplasm" in the oocyte of *Xenopus laevis* (R. Czołowska – thesis subject, Univ. of Warsaw)

The main conclusions from this study are the following: the germinal cytoplasm, which is localized near the vegetative pole of the egg and later forms part of the cytoplasm of the primordial germ cells, is probably of multiple origin. Some of its components, such as the mitochondria, probably already accumulate in the basal part of the oocyte during an early phase of oogenesis. This holds also for part of the RNA, but there are indications that during egg maturation also RNA originating from the germinal vesicle reaches the germinal cytoplasm.

2. The spatial distribution of inductive capacities in the archenteron roof and the overlying neural plate of *Triturus alpestris* (J. A. Leussink)

Median, paramedian, and lateral parts of the archenteron roof and the neural plate were isolated from four cranio-caudal levels at the open neural plate stage. Their inductive capacities were tested by combining them into "sandwich" explants with competent ectoderm from early gastrulae of another species, *Ambystoma mexicanum*. The results were analysed quantitatively by determining the volumes of the induced neural masses. It was shown that the inductive capacity of isolated pieces of the archenteron roof decreases strongly in medio-lateral direction. The inductive capacity of paramedian and lateral pieces is negligible, and is not enhanced when they are combined with median pieces; the inductive factors they contain apparently originate from the median area, i.e. the prospective notochord. For the neural plate a similar, though somewhat less pronounced spatial distribution was found. In view of the close adhesion between the prospective notochord and the neural plate it seems likely that an intimate contact with the prospective notochord is a prerequisite for the development of the neural plate. Apparently both in the archenteron roof and in the neural plate the induction field expands from the median line laterally and cranially with decreasing intensity, the whole field being maintained by the prospective notochord.

3. The influence of excess vitamin A on neural competence (S. Sudarwati – Team research project)

This investigation was carried out because it is known that vitamin A in excess leads to an increase in the autolytic activities of cells by altering the permeability of membranes such as the lysosomal membrane, and that autolytic processes probably play a role in neural induction and artificial neuralization. Only a slight increase of the neural competence of gastrula ectoderm was found.

4. Cell movements in artificially activated gastrula ectoderm (J. Marthy – Team research project)

For this microcinematographic investigation the ectoderm was attached with its inner surface to a fully transparent collodion membrane, so that it could be filmed from both sides. The membrane had been perforated in one place; as a result of the slightly harmful influence of the culture solution, neuralisation of the ectoderm took place there where it was not protected by the "surface coat" (along its periphery and over the perforation). The ectoderm first shows marked epiboly as a prelude to epidermal differentiation. In those areas where the cells are switched into the neural pathway of differentiation, this epiboly is succeeded by a pronounced convergence and surface contraction of cellular material.

5. Pattern formation in the presumptive prosencephalon at the open neural plate stage (E. C. Boterenbrood)

The regional distribution of differentiation tendencies in the future prosencephalic area of the neural plate was studied by transplanting small fragments of this area to a "neutral" region of host embryos. The fragments were taken from a median strip encompassing the whole cranio-caudal extent of the presumptive prosencephalon. The results were analysed quantitatively by determining the volumes of the various tissue components formed.

Fragments taken from the most anterior zone but one, and from the most caudal zone yielded identical results, forming eye tissue as well as telencephalic and diencephalic structures. Fragments from the intervening zones formed almost exclusively eye tissue, while the most anterior zone produced telencephalic structures only. It may be concluded from these results that the fixation of the prosencephalic pattern begins both in the centre and at the cranial border of the presumptive prosencephalon with an enhancement of specific differentiation tendencies for the eye and the telencephalon respectively.

6. The possible chemotactic role of pharyngeal endoderm during the migration of neural crest cells in vitro (R. Hauser – Team research project)

During the initial formation of the splanchnocranium neural crest cells migrate towards the pharyngeal endoderm. A chemotactic influence of the latter could not be shown in *in vitro* experiments. However, the investigation led to the development of an improved culture method for embryonic amphibian tissues based on the use of a culture chamber. At the same time an improved technique for the aspiration of blastocoelic fluid and for the injection of solutions into the blastocoel was developed, which has additional importance for the radio-active labeling of blastulae.

7. The role of cell contacts in tissue displacements during the closure of the dorsal wall of the gut in the neurula (A. Şeftalioglu – Team research project)

8. Cell production and cell migration in the various layers of the neural retina in metamorphosing anuran larvae (J. G. Hollyfield)

It had been shown previously (see bibliography, section IX) that cell production by mitosis in *Rana pipiens* is restricted to the periphery of the retina, and is followed by centripetal migration of the newly formed cells through certain ganglionic layers. In an extension of this study to *Xenopus laevis* it appeared that in this species the zone of production of new cells is much less sharply localized, while at the same time the migrating new cells still frequently undergo mitosis, which in *Rana pipiens* only occurs sporadically.

III. AVIAN DEVELOPMENT (chick embryo)

1. Germ layer formation in the early blastoderm (S. Kochav – Team research project)

The problem of the delamination of the hypoblast from the epiblast was attacked by means of dis- and reaggregation of early blastoderm cells *in vitro*, using the vitelline membrane as a substrate for the further development of the reaggregates. Very early blastoderms were obtained by removing eggs from the uterus.

2. The origin of primordial germ cells in the early blastoderm (T. Rogulska – Team research project)

Parts of the early blastoderm were transplanted into the coelom of $2\frac{1}{2}$-day host embryos, whose own primordial germ cells had been previously eliminated by means of UV-irradiation.

3. The origin of the regional pattern of neural differentiation (K. Hara)

The results were evaluated of experiments carried out in 1966 and 1967, in which parts of the neuro-ectoderm situated in front of Hensen's node were isolated at various stages, and recombined either with the originally underlying mesoderm, or with mesoderm from more cranial or more caudal levels. The recombinates (so-called "open sandwiches") were cultured *in vivo* by means of transplantation into the coelom of older host embryos. Special attention was paid to the differentiation of dorsal brain parts arising from lateral areas of the neural plate (such as the pineal body, telencephalic cortex, optic lobe, and choroid plexus), since these provide information with regard to the medio-lateral organisation of the nervous system.

The results may be summarized as follows. Neural organisation starts prior to the definitive primitive-streak stage. The cranio-caudal pattern is more or less fixed at the early head-process stage: ectoderm developing in prosencephalic direction upon "activation" can no longer be "transformed" by a rhombencephalic inductor; conversely, once the ectoderm has been transformed, it can no longer be forced into the prosencephalic pathway of differentiation by a prosencephalic inductor. Consequently, in normal development activation and transformation must succeed each other rapidly.

Medio-lateral organisation, on the other hand, is still labile at the early head-process stage; it becomes fixed, at least in the prospective prosencephalic area, at the late head-process stage.

4. The time required for neural induction (K. Hara)

This problem is being studied in the following manner. "Open sandwiches" consisting of competent ectoderm and mesoderm are transplanted into the coelom of older host embryos. After 1–5 h the transplants are

dissected from the hosts, the mesodermal inductor is removed, and the ectoderm transplanted into a new host.

5. The nature of the contact between the inductor and the reaction system (D. Beig – Team research project)

For this investigation "open sandwiches" of competent ectoderm and mesoderm were made as described in the preceding sections. Prospective notochordal mesoderm adheres firmly to the ectoderm within 5 min, while 15–20 min are required for the firm attachment of prechordal mesoderm. In intracoelomically transplanted "open sandwiches" the formation of the neural plate begins after about 8 h. Whether during this period changes occur in the tissue contacts between the inductor and the reaction system cannot be ascertained in histological sections. This will have to be analysed further with the help of the electron microscope.

IV. AMPHIBIAN LIMB REGENERATION (*Ambystoma mexicanum*)

1. Epidermal-mesenchymal interactions in limb regeneration (J. Faber)

Blastemal mesenchyme transplanted to the muscles of the back, and covered by wound epidermis migrating across it from the adjacent back skin, can grow out and give rise to limb structures. In the present experiments blastemal mesenchyme was transplanted beneath the wound epidermis of wounds previously made in the skin of the back. The wound epidermis varied in age from three to 12 days after wounding. Young wound epidermis (3–5 days) always thickens, which is sometimes followed by outgrowth of the mesenchyme. Wound epidermis older than seven days fails to thicken, and outgrowth is never observed. Preliminary histological checks have revealed that 6–7 days after wounding the first signs of a new dermis are observed beneath the wound epidermis; probably the dermal fibres block epidermal-mesenchymal interactions.

2. Autonomous regional organisation of transplanted forelimb blastemas (N. J. de Both)

This section is a summary of a study carried out during the years 1965–1968 with financial support of the Netherlands Organisation for the Advancement of Pure Research. The transplantation sites used were the back and the orbit (after previous removal of the eye).

In order to counteract the usual regression of the transplanted blastemas two different procedures were adopted. Either a limb nerve was deviated to the transplantation site on the back, or mesenchyme from several blastemas was combined into one transplant, which developed into a single limb. Under these conditions mesenchyme of the early conical blastema, obtained by amputation through the upper arm, is able to form skeletal elements of the forearm and upper-arm in addition to elements of the hand (if transplanted singly without additional innervation

such a blastema forms hand structures only). Mesenchyme of the distal portion (hand plate) of an older regenerate from the same amputation level, transplanted under the same conditions, can also form forearm and upper-arm elements in addition to the hand. Finally, even mesenchyme from blastemas obtained by amputation through the base of the carpus, which normally would never form anything but hand structures, can form forearm elements in addition to the hand, provided mesenchyme from several blastemas is combined into one transplant in the orbit.

Thus, the conclusion is justified that in all these cases the transplanted blastemal mesenchyme contains the information needed for the development of the entire limb. This information expresses itself only if the initial mass of undifferentiated mesenchyme is sufficiently large; its expression is autonomous with regard to the limb stump, leading to the establishment of an integrated limb pattern in the absence of continued stump influences. However, it is very likely that interactions between the mesenchyme and the wound epidermis play an essential role in the establishment of the limb pattern.

Apart from the main study, experiments were carried out with regard to the immunological aspects of blastemal transplantation in the axolotl. It was found that the axolotl does not tolerate xenografts, but that homografts are tolerated fully or to a considerable extent, depending on the genetic relatedness of the populations providing the donor and host animals. In addition, it was found that homografted larval heads can show prolonged survival and development, remaining intact both structurally and functionally (see bibliography, section IX).

V. DEVELOPMENTAL GENETICS IN INSECTS *(Drosophila melanogaster)*

1. A genetic and developmental analysis of homoeotic differentiation in the *loboid-ophthalmoptera* strain (W. J. Ouweneel)

This section is a summary of research carried out during the years 1967 and 1968 with financial support of the Netherlands Organisation for the Advancement of Pure Research. A homoeotic strain was studied which is characterized by outgrowths arising from the hypodermis of the eye area, and consisting of wing tissue. It could be shown that the "key gene" in this strain is *loboid* (*ld*, III-102); this causes reduction of the facetted area of the eye, as a result of which extra hypodermal tissue is formed, which undergoes a homoeotic change into wing tissue. This change is mainly controlled by the modifier gene *ophthalmoptera (opht)*, which could be localized on the X-chromosome (I-5 ±). This modifier can produce the same homoeotic effect in combination with other eye-reducing key genes (e.g. Dfd^{r-L}).

Homoeotic wing outgrowths could also be obtained by selection in several strains which did not contain the ophthalmoptera factor. This and other data led to the conclusion that a polygenic system of enhancer

genes is also involved in the homoeotic effect. This system is of rather wide-spread occurrence, and is able to produce the effect independent of *opht*, provided an eye-reducing key gene is present.

The gene *multiple wing hairs (mwh)*, if present in the *ld-opht* strain, has the same effect on the hairs of the homoeotic outgrowths as it has on the hairs of the normal wing.

A morphological analysis of the heads of *ld-opht* flies, in conjunction with a histological analysis of the optic imaginal discs in *ld-opht* larvae, led to the conclusion that the homoeotic effect is probably due to local hyperplasia of the folded border of the optic disc (i.e., the future hypodermal tissue of the edge of the eye area). The homoeotic effect could therefore be comparable to the phenomenon of transdetermination discovered by Hadorn and co-workers, which is also related to enhanced proliferation.

A variety of external agents were studied as to their effect on the development of the *ld-opht* strain. Most of the agents were chosen because their effect could be expected to have a bearing on the above tentative interpretation of the homoeotic effect (eye reduction combined with local hyperplasia in the optic disc). To be mentioned are only temperature, maternal age, starvation, UV-irradiation, and chemicals such as acetamide, uracil, and borax. All of them have larger or smaller effects on the penetrance of the homoeotic effect. As regards the effect of UV-irradiation there are two sensitive periods, both very early in larval development; the first is characterized by a decrease, the second by an increase in penetrance.

Finally a beginning was made with transplantations of head complexes of very young *ld-opht* larvae into the abdomens of adult wild-type females, to see how far the homoeotic effect depends upon the internal milieu of the *ld-opht* larva.

VI. DEVELOPMENT OF THE ACRASIEAE OR CELLULAR SLIME MOLDS

1. The identification of 3′, 5′-cyclic AMP as the chemotactically active substance in the aggregation of *Dictyostelium discoideum (Acrasieae)* (Th. M. Konijn)

During a stay at the Department of Biology, Princeton University in the second half of 1967 and the first months of 1968, a study was carried out together with Dr. J. T. Bonner and some of his co-workers, in which 3′, 5′-cyclic AMP could be identified as the (main) chemotactic agent acting during cell aggregation in *D. discoideum* (see bibliography, section IX). The substance was initially isolated from bacteria (the natural food of myxamoebae), which are also chemotactically active. It was later shown to be produced by the myxamoebae themselves, particularly shortly before and during the aggregation phase, during which period the myxamoebae are also most sensitive to its effect. Cyclic AMP is

inactivated by the enzyme phosphodiesterase (Chang), which is produced continuously by myxamoebae, leading to the establishment or enhancement of concentration gradients of this substance. Cyclic AMP also enhances the mutual adhesiveness of myxamoebae.

Subsequently several compounds that are chemically related to 3′, 5′-cyclic AMP were tested as to their chemotactic activity. Most of them were inactive; 3′, 5′-cyclic tubercidine, 3′, 5′-cyclic UMP, 3′, 5′-cyclic GMP and 3′, 5′-cyclic CMP attracted myxamoebae, but were less active than 3′, 5′-cyclic AMP.

2. Analysis of specific and generic differences in sensitivity to the effect of cyclic AMP (Th. M. Konijn and M. Th. van Oldenborgh)

At present aggregation is being studied in a species of *Dictyostelium* that does not react to cyclic AMP, and in the genus *Polysphondylium*, the myxamoebae of which are insensitive to cyclic AMP although they produce the substance themselves.

3. Isolation of possible other chemotactically active substances (Th. M. Konijn)

Since species which are insensitive to cyclic AMP nevertheless react chemotactically to products secreted by bacteria and myxamoebae, the purification of these products is being continued to see whether they contain chemotactically active components other than cyclic AMP.

4. Chemotaxis in myxamoebae of the true slime molds *(Myxomycetae)* (N. Nikitin)

This investigation was started in October 1968.

5. Development of a bio-assay for the quantitative determination of 3′, 5′-cyclic AMP in higher organisms (Th. M. Konijn)

Since 3′, 5′-cyclic AMP plays an important role in higher organisms as a "second messenger" in hormonal effects, the question is being investigated whether the myxamoebae test for the chemotactic activity of extracts developed previously is applicable to extracts of tissues of higher organisms.

6. The effect of 3′, 5′-cyclic AMP on the permeability of the cell membrane of myxamoebae (R. Singh – Team research project)

Possible changes of cell volume in suspensions of myxamoebae were investigated by means of a photoelectric densitometer. No effect of cyclic AMP was found.

7. Polysaccharides in the cell surface of myxamoebae of *Dictyostelium* and *Polysphondylium* in the vegetative phase (M. B. Burnside – continued Team research project)

During the Team period an investigation was started on the role of

polysaccharides in the reaggregation of dissociated neural retina cells of the chick embryo. Since these cells proved to be unsuitable, the investigation was continued using myxamoebae in the vegetative growth phase preceding aggregation. By means of incorporation of ^{3}H-glucosamine and subsequent autoradiographic and electron-microscopic analysis, an answer is sought to the question whether, and if so, which polysaccharides occur in the cell surface of myxamoebae.

VII. ORGANOGENESIS IN VITRO (birds and mammals)

1. The effect of the thyroid hormone triiodothyronine on the growth of embryonic chick limb bones (K. A. Lawson and co-workers)

It had been found previously that the metatarsus and the radius of the $7\frac{1}{2}$ day old chick embryo, which are comparable in size and stage of development, nevertheless respond differently when exposed to the same concentration of triiodothyronine (T_3) in culture; overall growth of the metatarsus is retarded, whereas that of the radius is stimulated.

1a. Biochemical study of DNA synthesis (A. M. A. ten Berge)

The effect of 10^{-7}M T_3 on the rate of DNA synthesis in the metatarsus and radius during four days culture on cock plasma and chick embryo extract, equilibrated with 5% CO_2 in air, has been studied by measuring the incorporation of ^{3}H-thymidine into the hot TCA extractable material of the rudiments and expressing the incorporated radioactivity in terms of the total DNA in the extract. The rate of DNA synthesis is stimulated in both bones during the first 48 h in culture with a maximum increase of about 20% over the controls. The rate of stimulation is faster in the metatarsus than in the radius during the first 24 h. After 4 days in culture the rate of DNA synthesis in the T_3-treated radius has dropped to the control level, whereas that of the T_3-treated metatarsus has been reduced to about 40% of the control rate. This reduction in the rate of DNA synthesis is thought to be the result of precocious hypertrophy in the shaft with concomitant loss of part of the population of dividing cells.

That the initial temporary stimulation of DNA synthesis is not due to the transition of the rudiments from *in vivo* to *in vitro* conditions was shown by rudiments in which DNA synthesis was stimulated by the addition of T_3 after 1 or 3 days in culture. That it is not a phenomenon restricted to a very narrow period of development was shown by exposing to T_3 freshly isolated rudiments from $6\frac{1}{2}$ and 9 day old embryos. DNA synthesis was stimulated in both during the first 24 h of treatment.

1b. Autoradiographic study of DNA synthesis (C. E. Hulstaert)

Since a limb bone rudiment is a heterogeneous system in which not all the cells are dividing, autoradiographic examination of ^{3}H-thymidine incorporated into the different areas of the rudiment should reveal whether

the effects of T_3 on DNA synthesis are due to changes in the size of the population of dividing cells or to differences in the rate of DNA synthesis per cell.

1c. Chondroitin sulphate synthesis (K. A. Lawson)

Similar experiments to those described under 1a have been carried out using $^{35}SO_4$ and measuring its incorporation into the whole rudiments. The rate of incorporation of ^{35}S per μg DNA is substantially lower in the radius growing on control medium than in the metatarsus. Treatment with T_3 results in a stimulation of the rate of incorporation into both bones, which is noticeable within 6 h and rises to a maximum between 24 and 48 h, maintaining this level throughout the 4 day culture period. The relative increase on a whole bone basis, but not per μg DNA, is greater in the radius than in the metatarsus. These results suggest that differences between rudiments in their rate of synthesis of intercellular material may be important in determining the overall growth rate of the rudiment and its response to T_3.

2. Cartilage development in the external ear of the rat (A. Svajger – Team research project)

During normal development and also in homografts transplanted to the anterior chamber of the eye, the following components of the elastic cartilage of the external ear appear in chronological order: reticulin fibres, elastic fibres, amorphous acid mucopolysaccharide of the matrix, and finally fat droplets. In the present study the question was approached whether this set of four biosynthetic properties is intrinsic to the mesenchyme, or whether their individual expression is dependent on environmental conditions or on the previous metabolic history of the cells.

The chondrogenic mesenchyme from the embryonic pinna of 14, 15, or 19 day rat embryos, or the mesenchyme from the first gill arch of 13 day embryos was separated from the epidermis by treatment at 4 °C with 1% trypsin. Explants were supported by rayon cloth coated with a film of 0.5% agar on a medium of cock plasma and chick embryo extract. The atmosphere was equilibrated with 5% CO_2 in air.

The preliminary results indicate that the culture conditions used in these experiments are more favourable to the development of hyaline than elastic cartilage and that, under these conditions, presumptive elastic cartilage acquires a more hyaline character.

3. Specificity of cell contacts during neural tube development in the chick (R. Adler – continued Team research project)

Segments of the neural tube (optic lobe or telencephalon) from 4–6 day embryos were dissociated into single cell suspensions in Ca and Mg-free Tyrode's solution containing 1% trypsin. The suspensions were cultured in a gyratory shaker for periods of time ranging from 1.5 to 24 h. The

aggregates formed were either fixed or grafted onto the chorio-allantoic membrane (CAM) of 8-day host embryos.

Aggregates formed by cells from 4-day embryos are characterized by the presence of a large number of "rosettes", consisting of epithelial-like cells arranged around a central lumen, as in a miniature neural tube; the rosettes show many cell divisions, the mitotic spindles being nearly always oriented parallel to the lumen. Aggregates formed by cells from older embryos contain no rosettes, their main characteristic being the presence of a conspicuous fibrillar layer at the margin of the aggregate. The aggregates grafted to the CAM show differentiation of neuroblasts in the absence of typical organogenesis; in the "rosettes" mitoses disappear almost completely.

In subsequent experiments the donor embryos were first labeled with ^{3}H-thymidine. The autoradiographic analysis of the reaggregates suggests that a change in the capacity of cells to recognize each other occurs at the time of cessation of DNA synthesis, which is assumed to be the time when cellular differentiation starts. The autoradiographic method is also being used to study the pattern of reaggregation in mixtures of cells from the optic lobe and the telencephalon.

VIII. MISCELLANEOUS

1. Dr. L. Weiss, who was co-leader of the Sixth International Team (see p. 3), in addition carried out some preliminary experiments in association with Dr. P. F. Elbers and Prof. J. Th. G. Overbeek of the University of Utrecht. The problem to be investigated is the distribution of electric charges at the cell surface of dissociated chick embryo retina cells, studied by means of gold sol adsorption.

2. Dr. K. Ulrich stayed at the Laboratory during the beginning of the Team period to become acquainted with the technique of cell electrophoresis, and to take part in the introductory discussions of the Team.

3. Dr. R. Chandebois and Miss J. M. Chegwidden both stayed at the Laboratory during several weeks to learn embryological techniques.

4. Dr. W. P. Luckett and Dr. M. Klíma both for some weeks studied material present in the Central Embryological Collection, in the case of the former pertaining to the comparative development of the primate placenta and fetal membranes, and in the case of the latter to the development of the sternum and shoulder girdle in *Monotremata* and *Marsupialia*.

P. D. NIEUWKOOP

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CENTRAALBUREAU VOOR SCHIMMELCULTURES, BAARN

PROGRESS REPORT 1968

As a guest worker Dr. M. B. Schol-Schwarz carried on her investigation of the genus *Phialophora*. It appeared that this genus comprises various groups of related species. Those with light, often pink, colonies cannot be distinguished from one another with certainty by the shape of their conidia. After a long period of experimenting, ascigerous states belonging to *Coniochaeta* were obtained in some strains. The work on a revision of the accepted species was continued.

Dr. G. A. de Vries published the results of his study on *Aphanoascus* and *Anixiopsis*. Both genera were distinguished on the basis of differences in colour, size and wall structure of the ascomata. *Anixiopsis stercoraria* and *A. reticulispora* were considered to be varieties of *A. fulvescens*. A manuscript about an investigation into aspergillosis was completed. No correlation was found between certain morphological and physiological features of *Aspergillus fumigatus* and its presence in lung tissue. Important data were obtained, e.g. about the sensitivity of the strains examined to antimycotics, about the fluorescence and the anaerobic growth. Two Trichophyton strains in the collection, which probably belong to an undescribed species, are being examined at the moment. Dr. A. H. Klokke (Utrecht) investigated their pathogenicity for guinea pigs.

Drs. A. C. Stolk in cooperation with Dr. G. L. Hennebert prepared a manuscript describing two monoverticillate *Thysanophora* species. In addition a new genus, *Custingophora*, was described; this was based on *C. olivacea*, a fungus isolated from compost by Dr. A. von Klopotek (Giessen). The new genus has a superficial resemblance with *Aspergillus* but is in fact more closely related to *Thysanophora* and *Phialocephala*. The examination of the perfect *Penicillia* belonging to *Eupenicillium* was extended by the study of strains sent by Dr. S. Udagawa (Tokyo) and Dr. J. W. Paden (Victoria), some of them turned out to be new species. A manuscript with the descriptions of four new *Penicillium* species was submitted for publication.

Drs. M. A. A. Schipper went on with her study of the fungi of the genus *Mucor*. The influence of environmental factors on the development of various strains was investigated but the emphasis was placed on mating tests, during the course of which zygospores were found in species in which these were either not or only uncertainly known until now. Strains that could be mated with each other were considered to belong to one

species even where small morphological differences were found. The mating experiments and the morphological studies carried out at the same time lead to the conclusion that the generally current division of the genus *Mucor* into sections, as established by Zycha in 1935, is not tenable.

A *Zygorhynchus* isolated by Dr. V. Hintakka (Helsinki) was described with his cooperation as a new species. This fungus grew only at low temperatures, the best temperatures being 0–5 °C.

Drs. E. J. Hermanides-Nijhof continued her study of the species of the genus *Fusarium*. The two strains of *Fusarium javanicum* Koorders (=*Nectria haematococca* Berk. & Br.) in the collection which form perithecia proved to be homothallic. Two clearly macroscopically different groups could be distinguished in the single-ascospore cultures from both strains. One group readily formed perithecia, the other remained sterile and even the initiation of perithecial formation was absent. The cultures of the sterile group were extensively mated with each other and with other strains but none of the matings resulted in the formation of perithecia.

In addition all the strains belonging to the *Martiella* group were compared, e.g. in their reactions to light and temperature. U.V. light affected the formation of perithecia but not conidia formation.

Drs. A. J. van der Plaats-Niterink continued her work on the *Pythium* species occurring in the Netherlands. The heterothallic *Pythium sylvaticum* appeared to be one of the most common *Pythium* species in the Netherlands. Besides this species and *Pythium heterothallicum* also *Pythium intermedium* turned out to be heterothallic. Oogonia and oospores were formed after mating.

Many matings between *Pythium* species with filamentous sporangia, in which no sexual form is known, remained without results. The tests will be continued.

Drs. H. A. van der Aa continued the study of the type and secondary collections of *Phyllosticta* species. Many herbarium specimens were examined and, where possible, isolations were made of fungi that are comparable in vivo. Isolates of many leaf moulds of other genera were also obtained as a by-product of this work and many of them were incorporated in the collection after identification. Many isolates, especially those belonging to the genus *Coniothyrium*, could not yet be identified and are kept for further study.

Dr. K. W. Gams completed the examination of herbarium specimens for his study "Monographie Cephalosporium-artiger Hyphomyceten". He examined many type collections and some 300 preparations made from

material present at the Commonwealth Mycological Institute, Kew. Many new strains were isolated for the C.B.S. collection. An ascigerous state belonging to *Niesslia* was found by mating of *Monocillium*-like isolates. Many strains, some of them freshly isolated, were examined during a study of the genus *Mortierella*. A manuscript about the subdivision of the genus into sections was prepared for publication. Other publications are being prepared, in part with the cooperation of Prof. K. H. Domsch, i.a. an illustrated flora of soil fungi "Pilze aus Agrarböden".

Dr. G. W. van Eijk continued his work concerning the purification and identification of pigments and other components of fungi. Besides a green pigment, a very small quantity of a substance producing a strong blue fluorescence in U.V. light was also obtained in a pure state from a *Roesleria* species. The study of the ultraviolet, infrared, nuclear magnetic resonance and mass spectra of the latter one have given considerable data on its structure. Experiments are going on which are expected to elucidate the complete structure of both substances.

Several metabolites could be isolated and identified from the culture filtrate of a *Penicillium* species cultivated in a synthetic medium.

Dr. J. A. von Arx continued his studies for the publication "The genera of fungi in pure culture". He also worked on some ascomycetes isolated from wood pulp and other substrates. Together with Drs. G. de Bruin-Brink and other colleagues he spent much time on the publication of the 27th edition of the "List of Cultures" which appeared in June 1968.

In the yeast division in Delft Drs. W. Ch. Slooff completed her monograph of the genus *Lipomyces* for the new edition of "The Yeasts". As techniques with which she was unfamiliar were necessary for this investigation she enlisted the cooperation of Ir. P. Bos and Drs. P. J. Nieuwdorp of the Department of Electron Microscopy, Laboratory for Microbiology of the Technological University. The results of this cooperative effort were recorded for publication. She also continued the study of the genus *Pityrosporum*. *P. orbiculare*, especially, proved to be a difficult species to cultivate on laboratory media.

Drs. L. Rodrigues de Miranda continued his investigation on a possible relationship of some asporogenous yeasts with basidiomycetes. He tried to follow the life cycle of *Candida scottii* by using haploid mating types.

D. Yarrow identified a variety of yeasts. Two strains received from Prof. C. E. Sonck (Turku) were found to represent unknown species and were described as *Sterigmatomyces elviae* and *Trichosporon fennicum*. The work on lyophilized yeasts which was begun in 1966 was carried on.

The viability of strains that had been dried earlier, using 10% milk powder as the protective medium, was checked. It was found that some yeasts, e.g. *Candida albicans* and many *Saccharomyces* strains, were still viable after 15 to 20 years whereas the majority of the *Rhodotorula* strains were not.

J. A. VON ARX

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INSTITUTE OF ECOLOGICAL RESEARCH

PROGRESS REPORT 1968

DEPARTMENT OF POPULATION ECOLOGY

The study of the population dynamics of the Great Tit, Parus major, was continued. Much time was devoted to a study of the fate of fledglings in the Hoge Veluwe area, by frequent captures and recaptures during summer, autumn and winter. These captures provided data on the mortality, dispersal, moult and body weight of tits of different age classes. Preliminary calculations gave results similar to those from the 1967/1968 season, which are discussed below.

The age class, born in 1967, was sampled by catching at feeding stations until March 1968, and finally by catching the breeding population in May 1968. This enabled us to compute the changes in numbers from one summer to the next. This was done for several classes of tits: young tits born in the study area (termed first-year locals), or born outside the study area (first-year immigrants), and birds breeding in the area already in 1967 (breeding birds).

During the first three weeks after fledging the number of first-year locals in the study area decreased with circa 65%. This large decline is due to both mortality and emigration. The size of the decline was equal for young fledged from first and second broods. After this decline the numbers remained more or less constant, until a second decline occurred in September-October. As before, the share of mortality and emigration in causing the decline in numbers, is unknown. From October until March the numbers of first-year birds did not change appreciably.

In other classes of Great Tits we also find the phenomenon, that the decline in numbers is not uniform over the non-reproductive season. Among the older birds, which have spent one or more breeding seasons in the study area, a sharp decline in numbers between the end of August and the end of September was found, presumably connected with territorial activity in autumn. From September until March the number of breeding birds was remarkably constant.

During the whole catching season (July-March) immigrants entered the study area. Their numbers were maximal in July and March. A characteristic feature of all groups of immigrants was the sharp decline in numbers in the first 1–2 months after the arrival in the area. The decline was largest for the immigrants arriving in September-October, presumably as a result of mortality, and among the birds arriving in March, presumably as a result of migration through the area. Of all immigrants those arriving in November-January gave the largest contribution to the breeding population of 1968.

Movements of tits within the area may give indications on the tendency to migrate or disperse. From the extent of such movements it appeared that the first-year birds, remaining in the area, disperse especially during two periods, one immediately after fledging, the other in February-March. A decline in numbers in the study area during these periods may therefore be partly attributed to dispersal by birds crossing the artificial boundaries of the area.

The effect of the parasitic fly Protocalliphora sp. on young of the Great Tit was studied. The number of parasite larvae, which suck blood on the tit nestlings did not affect the mortality of the nestlings. Yet the parasite is important for the condition of young tits, as appears from the survival after fledging. The number of parasite larvae per nest is negatively correlated with the percentage of the fledglings recaptured within a year after fledging.

Additional evidence for this view comes from the unequal distribution of mortality over different broods. From some broods a large proportion of the fledglings survive, from other broods none at all. This phenomenon of a non-random distribution of recaptures occurs in all study areas, except on Vlieland, where Protocalliphora has yet to be found. This makes us suppose that the parasitic fly plays an important role in the disappearance of whole broods.

Measurements of heat production and water loss in young Great Tits have indicated the possibility of hyperthermia at normal outdoor temperatures. Following this line of research, some experiments were done on the occurrence of hyperthermia in broods of different size and at temperatures from 19° to 35 °C. At 35° broods of 13 and more young suffered acutely from hyperthermia, resulting in the death of a part of the brood. Small broods succeed in maintaining their body temperature by evaporation. At temperatures between 30° and 35° the weight loss of the nestlings (presumably caused by evaporation) was negatively correlated with the brood size. Probably high summer temperatures set a limit to the brood size in the Great Tit.

Within the framework of the study of the energy balance the chemical composition and the caloric value of a large number of nestling Great Tits from a marginal biotope were determined.

The water content of nestling tits decreases during their period of development (18 days) from 85% to 68%. The ash content increases more sharply during the first 5 days than in the subsequent period. The protein content increases more sharply after the first 5 days. This indicates the succession of the growth of the skeleton by that of muscle tissue.

There is a great variability in the caloric value per gram ash-free, dried tissue early in the breeding season, decreasing as the breeding

season advances. This variability is definitely correlated with the fat content. The large variability in the first part of the breeding season is presumably due to local food shortage.

During 1967 a project on the ecology of the Collared Dove, Streptopelia decaocto, was started. The ultimate aim of this study, which is supported by the International Biological Program, is to measure the energy flow through the population.

In doves, caught on Vlieland, the criteria for age and sex were studied. The age criteria, as reported in the literature, are valid for only a part of the year, depending on the moulting season.

The literature on the moulting season is not unanimous. Probably the timing of the moult is affected by environmental factors, such as climate and food supply. In first-year doves the date of hatching, which varies enormously, determines the start of the moult. We found that the moult of the primaries commences between early June and late September, and ends between late January and late May. The adult doves moult their primaries between April and January, largely coinciding with the breeding season. Doves collected near Harderwijk end their moult earlier, mostly late autumn.

From October 1968 Collared Doves were collected near Harderwijk to study the crop and stomach contents. These birds feed mainly on food provided on large duck farms. The total amount of food in the crops of the Harderwijk birds varied strongly, in connection with the time of the day when the birds were shot. Field observations showed that large numbers of birds fed on the duck farms during the morning hours, whereas during the rest of the day little feeding took place. This feeding pattern is reflected in the variable size of the crop content in the morning, when many crops are still empty, and other contain up to 44 grams of food. In the afternoon very few crops are empty, and the average crop contains 11 grams of food.

Judging from the crop and stomach contents, the main food in this area is the food offered to the ducks, mainly maize and other cereals. Moreover in many stomachs and in some crops stones of Prunus serotina were found, whole or in small pieces, presumably functioning as grinding stones. In some birds other natural food was found, for instance seeds of Sambucus nigra, Solanum nigrum and several Polygonum species. The weight of the natural food is seldom more than 5% of the total crop and stomach content.

A start was made with feeding trials with captive doves, to study the preference for certain seeds, and the amount of food consumed during different parts of the year.

The field station on the isle of Vlieland made another experimental contribution to the study of the population dynamics of the Great Tit.

After two years of normal reproduction (1965 and 1966) two years followed in which the reproduction was artificially reduced to 3 fledglings per breeding pair. The number of breeding pairs increased from 1964 to 1965, and remained at a higher level, probably due to amelioration of the biotope. It seemed useful to study once more the effect of reduced reproduction, this time at a higher breeding density. The results point in the same direction as those from the years 1960–1964, viz. that the number of breeding tits in the next year is not affected, due to the increased survival of fledglings and adult birds.

The survival of tits from one year to the next is calculated from the numbers of identified breeding birds. In some years difficulties are encountered, due to a surplus of unpaired males, which cannot be captured and identified. While in 1966–1967 a rather large surplus of males, mainly first-year birds, was found, in 1968 this was not the case.

A large number of nests of hole-breeding birds were collected and examined, to study the effect of the parasite Protocalliphora sp. This parasite appeared to be absent, at least in 1968, from the island.

The breeding population of the Blue Tit has steadily increased since 1965, presumably due to the increasing growth of deciduous trees in the pinewoods. This gives the opportunity to study the nesting requirements of this species and the competition for nesting sites between Great and Blue Tit. Preliminary results indicate that the Blue Tit, at least under Vlieland conditions, mainly nests in nestboxes which are, by the small size of the entrance, inaccessible to Great Tits.

A contribution was given to the study of Chaffinch migration, by catching and ringing more than 660 Chaffinches in spring and autumn. The number of recoveries is yet too small for valid conclusions about the migration routes of birds migrating through Vlieland.

DEPARTMENT OF BIRD MIGRATION (VOGELTREKSTATION)

This year a new method was tried to catch Starlings in the spring for a displacement experiment (the spring counterpart of the earlier autumn experiment).

On a pasture bordering on a large roost of starlings, a floodlight trap was built. It consisted of a funnel with a mouth of 9 m high, 28 m wide, and 15 m deep.

The funnel ended in a small opening near which a number of floodlights were placed. Behind the opening, which could be closed off, a tunnel of 8 m deep was built. In dark nights the roost was disturbed and the birds were driven in the direction of the trap. Then the lamps were switched on and a part of the birds were caught by the light and flew in thick crowds into the funnel. The method was successful. On 4 March 1500–2000 birds were caught and on 24 March more than 1000.

780 juveniles and 200 adults were used for the displacement experiment

to Switzerland. The number of recoveries is at the moment too low to allow any conclusions.

The homing experiments with Blackheaded Gulls were continued with a new type of orientation cage. The bird walks in this cage on a white sheet of paper on which radially placed strips are mounted. The surface of these strips is soaked with ink. The prints of the dirtened feet of the bird on the paper give a measure for the direction in which the bird is striving (the movements of the bird must fulfil certain conditions).

In 1968 126 birds of two colonies were used. The displacement was 30 km N, 30 km S and 150 km S. An indication of a homing tendency was only found in the 150 S displacement, and then only when both sun and landscape were visible. Next year a displacement 150 km N under the same conditions will be carried out.

The orientation of Chaffinches was studied in the Kramer cage on top of the laboratory and in self-registrating cages of the type described above. Although the individual scatter in the directions was large, both in autumn and spring directions were found corresponding more or less with those known from the field observations.

In the past several attempts have been made to assess the standard direction of autumn Chaffinch migration in the Northern part of The Netherlands. The observations near Norg in Drente resulted in directions not significantly different from those of the Veluwe. Nevertheless there are indications that, especially in the Wadden Area, more westerly directions prevail.

To give these indications a firmer base in 1968 observations were made in NW Friesland. Caused by the continuous bad weather, only few data could be obtained. Inland hardly any migration was seen at all. On the south pier of the Harlingen harbour we had three good days. The mean direction of 60 flocks was WSW. Only high flying flocks taking into the sea with a constant direction are included.

This direction again is not different from those in the middle of the country. To what degree the birds were influenced by the southern coast or the Afsluitdijk will be studied next year.

During the winter 1100 Fieldfares and 85 Redwings were caught with cannon nets on dump heaps of apples in three days.

At the ringing centre a start was made to change all old punch cards into new code. The new programme for calculating elapsed time, direction, distance, speed and year class was explained in a mimeographed report (in English) and sent to all European ringing centres (in the framework of *Euring*).

An important event was the appearance of the booklet "Handkenmerken". The ringers now have a reliable guide for sexing and aging and the identification of difficult species.

DEPARTMENT OF DISTRIBUTIONAL ECOLOGY

The fieldwork was concentrated in Zuidelijk Flevoland, a new polder in the IJsselmeer. The polder (48,000 ha) became gradually dry in the spring of 1968. At first the heavy clay soil was almost impassable. From the end of May onwards it was possible to visit parts some km from the surrounding dike and from the end of July the centre of the polder could be reached.

In the course of the summer a vegetation developed in the polder. In most places it consisted of about 15 plant species. Alongside the dike nearest to the "old" land the vegetation was richer in species and 86 plant species were found. Most of these species – with either anemochorous or hydrochorous dissemination – reached the new land before it came dry. For agricultural reasons *Phragmites communis* and (locally on artificial sand deposits) *Lolium perenne* were sown by aeroplane in May 1968.

During 1968 *Senecio congestus* was one of the most dominant species of the new land. This extreme pioneer plant migrated before and after reclamation by its anemochorous dissemination, which resulted in a density of about 10,000 per ha at the end of the first growing season. Relatively few of these plants did flower, the others flowered in the spring of 1969.

Another important pioneer species is *Ranunculus sceleratus*. Although its density, compared with *Senecio congestus*, was low in the beginning, it increased considerably and resulted, at the end of 1968, in a density of approx. 28,000 per ha. Obviously this is reached by:

(1) its short life cycle (2½ months),
(2) its very high seed output,
(3) its effective hydrochorous dissemination (it appeared that the first generation, which migrated from the old land before reclamation, had a very regular dissemination pattern in the whole polder, contrary to another hydrochorous species, *Scirpus maritimus*, which established itself in a zone of 7–10 km wide along the old coast only. The following generations of *Ranunculus sceleratus* were disseminated by rainwater pools),
(4) its ability to germinate, at least of part of the seeds, immediately after seed fall.

The pioneer grass *Catabrosa aquatica* proved to have a great power of vegetation extension. In 6 months after germination clones were built up with a diameter of up to 7 m. These large clones had a ring-shaped structure, because the centre died off.

Plant succession was studied by describing and photographing permanent vegetation plots. A method was developed to photograph plots vertically from a height of 2½ m.

The zoological fieldwork in Zuidelijk Flevoland concentrated on carabid beetles and phytophagous insects living on Common Reed and Willows. During the first visits to the new polder it became clear that already a few species of Carabids had reached it before. Pitfall traps were placed along a SW-NE transsect through the centre of the polder and in some places on the dikes and the old land. In one place, about 1 km away from the dike window traps were placed to catch flying beetles. In 1968 only monomorphous winged species and macropterous forms of dimorphous species were caught in the polder. It is evident, that the winged species have better possibilities for spreading than the unwinged ones and in the dimorphous species the winged form can be regarded as the dispersive form. In the course of the years the percentage of winged species and individuals will shift; the question is how and how fast.

As an example the dimorphous (woodland) species *Trechus obtusus* can be taken. During the summer of 1968 we caught more than 100 specimens in the window traps and hundreds of individuals in the pitfall traps in the polder, all of them macropterous. In the adjoining polder Oostelijk Flevoland (11 years old) the macropterous specimen amounted to 59% and on the old land in Drenthe to 4% (Den Boer, 1968, Meded. Bot. tuin, Belmonte Arb., *11*) of all individuals of this species.

The catch of 30 individuals of *Amara majuscula* is also remarkable. This species originates from Asia and spreads westwards from the 1920's onwards. In our country only a few specimen were known from Emmen (Drenthe) in 1961.

The dispersal of a number of species of insects living on reed and willows was also studied along the same SW-NE transsect. Here it also appeared that many flying species are able to disperse over great distances. It was remarkable that the aphid *Hyalopteris pruni* (sampled in September) showed a sharp decrease with distance from the old land on reed plants standing on dry soil, whereas on reed standing in wet places no decrease was found. Because the species hibernates only on *Prunus*, which is not present in the polder, the colonization by this aphid can be followed repeatedly during the next years.

In the laboratory the observations on the acoustical behaviour of Chloropid flies living on reed were continued. The signals (substrate vibrations) of males of 6 species were recorded and found to differ considerably, whereas the answering signals of the females of 4 species, which could be recorded so far, show much resemblance. Although differences are known in the occurrence on different types of reed shoots and in the time of emergence of the adults, there is much ecological overlap between the species, so that it is reasonable to suppose that the differences in the acoustical signals of the males play a role in reproductive isolation. Male signals of 5 species were played back to females of the three largest species *(Lipara lucens* and the two sibling

species known as *Lipara rufitarsis)*. The females responded almost exclusively to the signals of their own species.

BIOLOGICAL FIELD STATION "WEEVERS' DUIN", OOSTVOORNE

The annual description of the permanent sampling plots was carried out as usual following the Braun-Blanquet method. Soil samples from these plots were analysed for pH, N, P, $K^{\cdot}$, $Na^{\cdot}$, Cl' and water content.

Much attention was given to the relation between vegetation and phreatic level. Many transsects were made for this purpose over the whole area. Soil samples taken along these transsects were analysed as specified above. By means of these measurements we hope to arrive at a better typology of the dune vegetation.

A detailed chart of a permanent square of Tortulo-Phleetum vegetation was made, as was done since 1961. Gradually a clear picture is obtained of the migration of the different species into the sample plot. The increase of the rare Teucrium scordium since 1964 and 1965 was shown by a detailed distribution chart of this species in a small dune valley.

In close co-operation with "Rijkswaterstaat" (Ministry of Water Management) the research project on the influence of fertilizers and surface stabilizers on dune formation and dune forming plants on a newly built sanddike was continued. The growth of Elytrigia juncea, Ammophila arenaria, Elymus arenarius and Ammocalamagrostis baltica were stimulated significantly by a gift of nitrate. Surface stabilizers had no effect and application of potassium or phosphate hardly any. An extensive report will be published in 1969.

Fertilizing experiments were also done in natural dune vegetation on soils with a low mineral content. When the mineral content of the soil is increased the number of plant species is reduced and one or two grass species become dominant. These changes take place within one growth season. A low level of mineral nutrients is apparently essential for the development of a dune vegetation which is rich in herb species.

The micrometeorological research was concentrated on the heat- and water-economy of single leaves. A method was devised to calculate the resistance against water loss of a leaf from its temperature and the temperature and humidity of the ambient air. The water loss resistance of a leaf is rarely less than four times that of a free water surface under the same conditions. In this way it could be shown that the relatively very low temperatures of some desert plants are due to the dry air and not to special properties of the leaves.

Succulents and xerophytes can have a low water loss resistance and swamp-plants a very high one but general rules cannot be given at the moment.

W. H. VAN DOBBEN

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STAFF OF THE INSTITUTE

	W. H. van Dobben (director)
Departments:	
Population ecology	J. H. van Balen (head) J. A. L. Mertens (fysiology) H. N. Kluyver (guest worker)
Bird migration	A. C. Perdeck (head) A. J. Cavé
Distributional ecology	J. H. Mook (head) J. Haeck (zoology) J. van der Toorn (botany)
Botanical ecology. Biological Fieldstation "Weevers' Duin", Oostvoorne	M. J. Adriani (head) Ph. Stoutjesdijk A. H. J. Freysen P. A. I. Oremus Miss E. Heeres.

LIMNOLOGICAL INSTITUTE NIEUWERSLUIS, THE NETHERLANDS

PROGRESS REPORT 1968

Project Tjeukemeer

A more or less complete survey was obtained of the changes in the chemical composition of the water throughout this year. When the water of the surrounding polders enters the lake in the autumn, the pH and the concentrations of Cl^-, HCO_3^- and Ca^{2+} decrease, while the concentration of phosphate, ammonium, silicate and iron increase, as is shown in the following table:

meq/l							mg/l	
	w	s		w	s		w	s
Ca^{2+}	1.6	2.3	HCO_3^-	1.3	2.0	NH_3-N	2	0
Mg^{2+}	0.7	0.8	Cl^-	1.2	2.4	PO_4-P	0.3	0.04
Na^+	1.0	1.1	SO_4^{2-}	0.6	0.5	Tot-P	0.75	0.2
K^+	0.2	0.2				SiO_2-Si	4	0.1
Fe	(0.1)					pH	7½	8–9

The decrease of the Cl^--concentration in the autumn (inlet polderwater) and the increase in the spring (inlet of IJselmeer water) are so far the only data for hydrographical studies (e.g. renewal time). But these data are invalid when the chloride concentration in the lake is the same as in the incoming water. Unfortunately, water current meters – although made from stainless steel – were so heavily corroded by the aggressive Tjeukemeer water, that their use was a complete failure.

Apparatus was ordered to record the water level, but had not been delivered by the end of 1968. The "Provinciale Waterstaat" provided the data of water inlet and outlet of the whole Frisian poldersystem near Lemmer and Stavoren. It is proposed to calculate the correlation between these data and the water renewal time in Tjeukemeer calculated from the changes of the Cl^- concentration and to use this correlation for the periods that the Cl^- concentration remains constant.

The increase of pH and of the Ca^{2+} and HCO_3^- concentrations in the spring cannot be explained. The same hold true for the difference between changes of Na^+ and Cl^- concentrations, which must further be studied. The decrease of the phosphate may be explained by a precipitation of hydroxyapatite. The product of the ionic concentrations was larger than the experimentally found value in saturated solutions. More work is necessary about the relations between Ca^{2+}, CO_3^{2-} and PO_4^{3-} concentrations.

Miss de Bruyn continued the inventarisation of the phytoplankton,

by taking samples each fortnight. With a grant of the Beyerinck Popping fund she was also able to investigate the polders channels.

In the following table are comprised the most important data:

organism	period	max. cell numbers/ml
Melosira div. spec.	14/2–24/4	1000
	11/9–15/10	400–900
Oscillatoria agardhii	10/4– 3/7	max. 80% of total
Scenedesmus div. spec.	29/4– 5/6	3000 (22/5)
	1/8– 9/10	12000
Centric diatoms	27/3– 1/5	800 (20/4)
(*Actinocyclus normannii*)	24/9	400 (24/9)
Stephanodiscus astrea		
Cyclotella spec.)		

As the light penetration in the lake water is poor ($Z_{o.d.} = 10$ à 20 cm) and the weather often rough the determination of the primary production with suspended bottles (either for the O_2 – or for the ^{14}C method) is unreliable. As a preliminary estimate from the O_2 bottles, values may be mentioned of 200 ± 100 and 2000 ± 1000 mg C.m^{-2}. day^{-1} for the winter- and summer periods respectively. During the summer period oxygen production may be equal to or even less than the oxygen consumption per day. It is not yet certain, whether this is due mainly to respiration or to oxydation of dissolved organic material.

Since October progress has been made with the ^{14}C method using long glass tubes in order to achieve integration in situ.

The ratio of O_2 produced to CO_2 fixed is not constant, but changes between 0.3 and 4. As the Winkler method may be influenced by organic material a Clark recording O_2 electrode was made operative by Mr. P. Frederik. ^{14}C uptake and O_2 production can now be measured in the same bottle. Mr. Frederik found furthermore that part of the organically bound phosphate is indeed not taken up during photosynthesis. As this compound is extractable by butanol it is likely that humic – iron – phosphate complexes are involved.

Drs. H. de Haan started his investigations on the biochemistry and bacteriology of the humic compounds. His studies will try to establish the origin of the humic material in the water and to determine, whether biodegradation is significant.

Dr. Moed, who studied the pigments of the phytoplankton, found large differences in the chlorophyll concentrations throughout the year and a relative large concentration of pigments with extinction maxima near 417 and 435 mμ. With thin layer chromatography he found two green spots, as yet unidentified, although they may be quantitatively important.

The Crustacean zooplankton was studied by Miss H. H. Bromley and Mr. L. Slooten. Paired samples were taken, from 10 stations, regularly

from March – November and analysed individually for numbers of the different cladoceran species, total number of copepods and large rotifera. The population growth was estimated, both of cladocera and of individual species and their egg production.

Water temperature, food and predation are thought to be significantly influential in regulating the population increase (especially in June) and its sudden decrease (July). Special note was taken of the possible influence of water movements, both in the channels and in the lake itself. The effect of wind on the variation in mean cladoceran numbers was studied. It was concluded, that swarming or clumped distribution is often in evidence when there is little wind. The most important animals numerically are *Chydorus sphaericus* (max. $\pm$ 450 per litre; 3 July), *Bosmina longirostris* (max. 450 per litre; 9 July). *Bosmina coregoni* (max. 300 per litre; 9 July). In July the minimum value for *Cladocera* was 2×10^6 per sq.m.

The standing crop of copepodids and adults of *Copepod* species occurring in the open water was measured mainly by taking day samples.

The main species are in order of abundance *Acanthocyclops robustus*, *Mesocyclops leuckarti* and *Eurytemora affinis*. The numbers per litre decreased slowly after 16th July. *E. affinis* occurred in springtime in considerable numbers. There are some indications that this species was brought in with IJselmeer water.

Mr. Chambers studies the role and importance of the littoral fauna of the Tjeukemeer. As the Tjeukemeer is a very shallow lake, the littoral region is defined as comprising only those areas that are covered by beds of macrophitic vegetation.

Gammarus tigrinus and *Chironomid* larvae are the most abundant animal species on Potamogeton. A correlation has been established between the weight of Potamogeton and the number of *Gammarus* and chironomids on them. The ratio, plant weight to number of animals varies with the coating of the leaves with epiphytic algae and obviously with the natural fluctuations due to reproduction, predation etc.

A maximum number of *Gammarus* has been found in September ($\pm$ 400/m^2 of *Potamogeton* beds), and of *Chironomids* in July ($\pm$ 200/m^2) of *Potamogeton* beds. In the emergent vegetation (*Phragmites* and *Typha*) much higher densities are found – *Gammarus* up to 10.000/m^2 and chironomids up to 5.000/m^2. As the emergent vegetation provides a permanent habitat its contribution of food to the fish is potentially greater than that of the *Potamogeton*.

Preliminary examinations have shown that *Gammarus* forms the staple diet of the pope (*Acerina cernua*).

Mr. Beattie studied the non-littoral fauna (mainly *Chironomids*) and its role in the food-chain.

The general distribution for the bottom fauna is as follows (range of numbers/m^2):

	chironomids	oligochaetes	mites
Peat . . .	0–50	100–400	250–700
Sand . . .	50–250	250–1000	50–200
Mud . . .	50–500	500–1250	200–500

The *Oligochaetes* do not offer much prospect of being an useful food source. The *Chironomids* have by virtue of their particular life-cycle a greater potential for investigating the bottom production, although they do probably not form a major food source.

Chironomus anthracinus and *Polypedilum nebeculosam* have more than one generation per year and might thus yield more food than expected from their low densities. From these densities a standing-crop of 1 g/m^2 may be calculated. This is low compared with the required value for the annual increase of the substantial population of bream.

The main problem in the study of the bottom fauna is to explain why the densities of the animals are so low. In adjacent conditions of similar environment the densities are much higher.

Mr. Goldspink estimated the population of roach (*Rutilus rutilus*) and its growth and mortality rates.

Monthly samples of fish were taken to determine the increase in length and weight of each age class throughout the year. The age of the raoch was determined from their scales.

Population numbers was estimated by marking 20.543 roach by fin clipping between 18th March and 2nd April. Each fish marked was measured, to the fork of the tail fin, and allocated to a 0.5 cm length class. After four weeks the ratio of marked to unmarked fish at four different points were 0.88×10^{-2}, 0.75×10^{-2}, 0.84×10^{-2} and 0.32×10^{-2} which apart from the latter suggest that the fish are randomly distributed over the area where the fish are being caught. The total weight of roach is from the figures obtained 1.7×10^5 kg and the production per year 9×10^4 kg. The production per hectare may be estimated 40 kg/hectare, (for fish between 2 and 8 years). 78% is for females and 22% for males. Gonad production was about 30% of the total production.

IJselmeer Project

Drs. de Kloet continued his study of the primary production and the input of nutrients. Like last year each month a trip was made with one of the boats of the "Rijksdienst der Zuiderzeewerken". 26 sampling stations were first chosen, but two of these no longer exist, as they are now part of the newest polder. At each station the following determinations are carried out: O_2 content, temperature, Secchi disk, light penetration, pH, total-phosphorus and primary production.

The main water body is divided into North, Middle and South parts by the lines Enkhuizen–Staveren and Hoorn–Lelystad. Furthermore

special areas may be distinguished near *Amsterdam*, the *Randmeren* (lakes between the polders and the old land) and the *Ketelmeer*.

O_2 varies in the open parts between 150 and 70%, in the Randmeren between 142 and 58%. In the North part one 24 hours-cycle could be measured, the highest value of the oxygen content was 160% with a 40% decrease overnight. The Secchi disks readings varied in the open water between 100 cm (North; October) and 15 cm (South; March). In the open parts the primary production reached a value of 0.8 (M-part), 1.2 (N-part) and 1.5 mg/l.hr.$^{-1}$ (S-part) of CO_2 in August. In September and October lower values were found. As during the trip there is no time for in situ measurements the ^{14}C-uptake was followed in Winkler flasks in tanks under standard conditions. The border-areas followed the same pattern, with a greater variety and higher maximum values.

Total phosphorus varied for the North- and Middle-part between 50 and 260 μg/l, and for the South-part 70 and 540 μg/l. In the Border-areas the concentrations were 100–500 μg/l higher.

Drs. Boesewinkel began a study of the phytoplankton population composition.

'Vechten' Project

The routine chemical research in Vechten was continued again this year, whereby Drs. Hogendijk extended his work on manganese analysis in order to see if this element played any role in the electron transfer from ferrous iron to oxygen. CO_2, Ca and Mg exchange between the upper and lower layers was determined every week, by measuring the pH, bicarbonate, Ca and Mg concentration in vertical sections.

Drs. Hogendijk made a beginning with in vitro experiments using mud and water containing sulphate, to see if there is any connection between the amount of ferrous iron formed under anaerobic conditions and the turnover of sulphate. In connection with this the sulphate determination was subjected to further research. Further attention was given to the influence of the organic material on the reaction of the ferric phenanthrolin complex.

Experiments on the reduction of $Fe(OH)_3$ by H_2S were continued. By the very slow addition of H_2S a maximum of 50% of the iron in the $Fe(OH)_3$ could be brought into solution as ferrous iron at pH = 5.9.

Drs. Cappenberg began his research with the isolation of single species of bacteria. Attention was given to sulphur and methane bacteria.

A pure culture of Desulfovibrio desulfuricans was obtained, with which the carbon sources utilized by this strain shall be traced. Special attention shall be paid to CO_2 and CH_4. Connected with this work an attempt was made to gain an insight into the quantitative composition of the methane forming bacteria in the mud.

A beginning was made with the determination of methane by gas-chromatography. Estimates of methane oxydizing bacteria were made.

The greatest concentration was below the thermocline during the summer-stratification. Small peaks in concentration were also found in the epilimnion at the place of the oxygen maximum.

Dr. Moed carried out a number of pigment measurements at different depths in January and February. Whenever the deepest sample was taken *without* bottom material little difference was found in the composition of pigments. In a sample *with* bottom material a strong increase of yellow pigments was found; as in bottom material the breakdown of chlorophyll is more rapid than carotenoids.

The study of periodicity of phytoplankton was re-instigated by Drs. Boesewinkel.

Drs. Parma continued sampling the bottom of lake Vechten in four places. The population density of the larvae of *Chaoborus flavicans* (*Diptera; Chaoboridae*) showed that during the pupation period there was a minimum in summer. The increase of larvae in autumn is not synchronized for all depths but occurs earlier in shallower areas; here the third instars can form the largest part of the benthic population. It is clear that certain factors in the hypolimnion inhibit the third and early fourth instars from burrowing. The nature of this factor is completely unknown.

It appeared that during the vertical migration phase the larvae could be concentrated in certain parts of the lake under the influence of weather conditions. This fact is important for a better understanding of horizontal distribution.

With short days the fourth instar larvae development is much slower than with long days and has a very high mortality. When there is a possibility to burrow the development is slowed down and the mortality decreases strongly.

The research into the life-cycle of *Ch. crystallinus* was continued in the Institute pond, but came to a stop when mass-mortality destroyed the population.

Wijde Blik

Dr. Gulati, holding an I.T.H. fellowship, has been working at the Institute for a year and has made an inventory of Wijde Blik. His research includes the physical and chemical characteristics, and also the phyto- and zooplankton. This research of which the first report appeared, is important in giving an insight into the future development of excavated waters, which are occurring on a large scale nowadays.

Algology

Drs. Steenbergen continued his experiments with the synchronous cultures of *Scenedesmus obliquus*.

These experiments have given information about the course of photosynthesis during the life-cycle of this green alga. Both parts of the reaction

of photosynthesis, namely the photochemical and biochemical parts, show under certain circumstances a development that is so separate, that it is likely that they each possess a separate regulation. The colour and intensity of the light play a role in this regulation. This work emphasized the importance of further investigation of the influential light factor on the growth of phytoplankton.

Dr. Moed began laboratory experiments on the breakdown of chloroplast pigments, alongside his work to form an inventory of these pigments in the natural environment.

Using heat or chloroform treatment (2 hr.), *Scenedesmus* cells could have their pigments broken down. With $CHCl_3$ a breakdown could be obtained in two hours of pheophytine a from chlorophyll a; this breakdown could be prevented by previous heating of the cells (5 mins. at 75 °C) or by the use of a Trisbuffer. Further research about the release of H^+ ions during the autolysis is necessary. Chlorophyll b turned out to be broken down to a lesser extent and the extraction of this pigment was more difficult after autolysis.

Scientific contacts

In April the author took part in the I.B.P. meeting and "Intercol" symposium in Varna. Support was obtained from the I.B.P. PF committee for the founding of the International Calibration Centre. From 13–17 May the author was in England and Scotland. During the first days he visited the I.B.P. project at Loch Leven and took part in its annual meeting, where he gave a talk over the Tjeukemeer. On 16–17 May and in November he visited the I.B.P. office in London for talks about the founding of the International Chemical Calibration Centre, and with Dr. Clymo for the correction of the prints of the I.B.P. chemical manual.

On February 18th Drs. Parma gave a lecture in Rotterdam, entitled "De verspreiding van *Chaoborus* larven in Nederland", to the Nederlandse Entomologische Vereniging.

Dr. Maitland from the Loch Leven project payed a visit to the Tjeukemeer in September.

In the spring the Landbouwkundige Kring Utrecht and the Ecologische Studiekring Wageningen payed a visit to the Institute. Drs. Hogendijk, Steenbergen and the author talked about their work.

On May 21st the Utrechtse Chemische Club was received and different chemical techniques demonstrated.

On Friday June 28th Dr. J. Rzoska gave the attic lecture in the "Vijverhof" entitled "The ecology of papyrus swamps in Africa".

Drs. Hogendijk, Steenbergen, Parma and Dr. Moed and the author took part in the S.I.L. congress in Israel. Drs. Parma and the author gave lectures.

Drs. Hogendijk and the author gave a talk on April 26th to the Geoche-

mische Kring about the equilibriums between calcium phosphate- and carbonate ions.

Drs. de Kloet and the author addressed the Hydrobiologische Vereniging, about the eutrophication of the IJsselmeer, on May 3rd.

H. L. GOLTERMAN

DELTA INSTITUTE FOR HYDROBIOLOGICAL RESEARCH

PROGRESS REPORT 1968 *)

I. Introduction

Although this year's schedule did not provide for a definite closure of an estuary, much progress was made in many sectors. In the Haringvliet the temporary island, where the sluices had been built, was broken down and pillars for the cable to be used in the final closure, were constructed. In the Brouwershavense Gat the construction of culvert-caissons and preparation of the bottom where these caissons are to be placed, are in progress. In the Biesbosch preliminary works were started on such a large scale that in many places the biological environment was already drastically changed.

Towards the end of the year the decision about the place where the dam through the Oosterschelde is to be constructed, became known. Among many others our Institute had also been approached in order to advise the Department of Roads and Waterways in this matter. As will be elaborated in later chapters, our collaboration with this Department was very satisfactory, and with other agencies as well, as witnessed by the fact that our Institute was repeatedly requested to advise on various hydrobiological subjects such as eutrophication, desalinisation, heated effluents of power plants, nature conservancy and management of protected areas. Staff members of our Institute are on the board of various commissions dealing with these matters.

Our plans for enlargement of the buildings could not be realized this year, as some changes had to be made in the plans for central heating and electricity. We expect to start building in the spring of next year. The name of our Institute was changed into "Delta Institute for Hydrobiological Research" (Delta Instituut voor Hydrobiologisch Onderzoek) as per November.

II. Hydrography and soil structure

A. Oosterschelde

Owing to the, much esteemed, collaboration of the Hydrometry Section of the Department of Roads and Waterways at Zierikzee, Mr. F. Vegter was able to collect water samples in the mouth of the Oosterschelde beyond the operational limits of our own researchvessel.

River discharge was fairly high in summer but the annual maxima and minima did not fluctuate as much as in the year before, the chlorinity

* Communication no. 81 of the Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke, The Netherlands.

of Yerseke fell to 11.2‰ Cl′ only, whereas a value of 9.6‰ Cl′ was reached in 1967. During the period of maximal discharge the same division into three compartments was observed in the Oosterschelde as in previous years.

The three nitrogen components nitrate, nitrite and ammonia were added to the sampling plan and the results are being integrated with those of a study of plankton samples collected simultaneously.

B. Veerse Meer.

In this lake the deep pit near the sluices, known as "Dijkhuis", was selected as the object of a preliminary study of the nitrogen cycle. This 20 m deep depression is permanently stratified owing to the constant influx of saline water from the Oosterschelde through the sluices, and this stratification reduces the oxygen contents, very often until complete anaerobic conditions prevail. Nitrate proved to fluctuate from values around 1 mg/l $N\text{-}NO_3$ in winter to 0.1 in summer, and was seen to rise again towards September. Nitrite and ammonia fluctuate on a much lower level, but apparently follow a similar trend. In this case as well, the results will be correlated with data on the plankton.

C. Measurements of primary productivity by means of radioactive carbon.

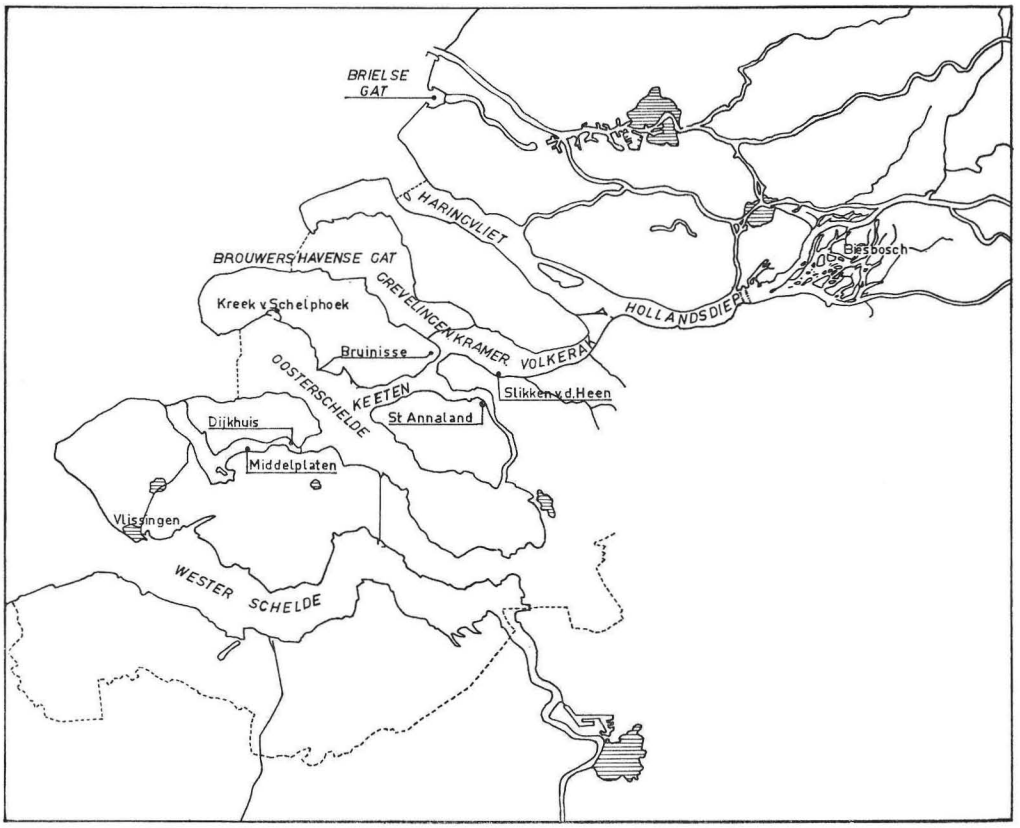
This year the Volkerak-area was studied four times, because the secundary dam through this water body will be finished by April 1969 and after that date no river water can flow south. This will produce a sudden and drastic rise in salinity in the Volkerak.

It was found this year that the usual minimum in production, found in the brackish area of mixing, can be obliterated when a good deal of freshwater plankton is flushed in by a strong river discharge. The plankton samples, taken along with the measurements on production, are being studied.

D. Hydrology

Mr. M. C. Daane, soil scientist in Dr. Beeftink's Department, studied the problem of penetration of rain and flooding with seawater into the ridges and depressions of the salt marshes in the Slikken van de Heen, with the aid of perforated tubes placed at different levels. It followed from a study of the salinity of the ground water in those tubes, that a vertical movement of the water takes place down into the anaerobic soil layers.

The mud flats and salt marshes beyond the dyke of the Stroodorpepolder showed a fluctuation in chlorinity of 10‰ NaCl in the upper layers between 0 and 1 cm and a fluctuation of about 4.5‰ in the layers between 1 and 5 cm. In the salt marsh near St. Annaland a special investigation was carried out, in order to study local variation in chlorinity of the soil moisture within different types of homogeneous vegetation.



In a layer of 0–5 cm under a low lying vegetation belonging to the *Puccinellietum maritimae*, a salinity was found varying from 22.3 to 25.6‰ NaCl, in the layer 5 to 20 cm: 20.0–28.2‰ and in the layer 20–40 cm: 22.0–28.2‰ NaCl. The corresponding values under a neighbouring vegetation belonging to the *Plantagini–Limonietum* type were resp. 16.7–22.9‰, 16.5–22.4‰ and 21.2–26‰ NaCl.

III. Zoological investigations

A. Littoral–Benthic fauna

Intensive sampling was carried out along the shores of Haringvliet, Hollands Diep, Volkerak, Krammer and northern part of Keeten in order to study the macrofauna. The sampling stations were chosen with an interspace of 1 km. The data have already been worked out and will form a base to study the changes bound to occur after the closure of the Volkerak by the secundary dam in April 1969.

Mr. A. J. J. Sandee started a survey of the polychaetes and archiannelids of the interstitial macrofauna as a continuation of earlier work

carried out by Den Hartog, Bilio and Boaden. An interesting finding was the polychaete *Microphthalmus listensis*.

Mr. G. R. Heerebout, a student from Leyden University, studied the ecology and distribution of bryozoa from brackish and saline water. In total 27 marine, 4 typically brackish and 6 species living in the euhalinicum but penetrating far into the brackish region, were found. *Electra crustulenta*, the most frequent species, was cultured in the aquarium in combinations of various temperatures and salinities. The results were in accordance with the observations on its distribution in the field. In the future this species is bound to flourish in the temporarily brackish lakes during the period the chlorinity is still over $2^0/_{00}$ Cl'.

Mr. L. Braber, a student from Utrecht University, studied ecology and distribution of *Caprellidae* and –mainly– *Anthozoa* for a period of three months, to be repeated next year. The horizontal distribution of 7 species of *Anthozoa* and of *Caprella linearis* could be established as a result of a large collection of live specimina.

Mr. L. de Wolf continued his measurements on the growth of *Hydrobia ulvae*, *H. stagnorum* and *Ovatella myosotis*. Sufficient measurements on *Assiminea grayana* are available to date and an analysis of the life-cycle and growth is now in progress.

Amateur divers discovered living specimina of *Nassarius reticulatus* along the north side of the Oosterschelde.

B. Sublittoral–Benthic fauna

After the collection of some grab-samples from the Haringvliet, the inventory of the area is almost completed. A total of 1800 samples is available to date and a few systematic groups only are not yet worked out.

Together with the Pisidium-specialist Mr. J. G. J. Kuiper (Paris), Mr. W. J. Wolff wrote an article on dispersion and ecology of this genus, followed by a prognosis on its future distribution during the change to fresh water. Other articles on the genus *Sphaerium* and on *Decapoda Reptantia* are in preparation.

Mr. Wolff spent most of his time on a study of polychaetes and has identified 90 species thus far. He wrote two short notes on the subject, one of which describes the discovery of the tropical polychaete *Mercierella enigmatica*, at the moment very frequent in the Veerse Meer, in the canal through Walcheren and in the docks at Vlissingen. At the Congress at Arcachon he lectured on the distribution of four species of *Nephtys* in our area.

In bottom samples taken off the coast Dr. W. Vervoort (State Museum of Nat. Hist., Leyden) identified the hydroid *Corymorpha nutans*, previously once encountered near Den Helder but unknown in the deltaic area.

Benthic-pelagic *Mysidacea* formed the better part of Mrs. C. H. Borg-

houts' programme this year. The Grevelingen was sampled every month and *Neomysis integer* and *Schistomysis kervillei* were found to dominate in this sea-arm. At the moment all relevant water-bodies have been surveyed. The species *Siriella armata*, previously only once found in the Grevelingen, was also sampled from the Krabbekreek.

C. Secundary production in the Grevelingen

From three biocoenoses in the Grevelingen, distinguished in 1967, representative samples were taken and the biomass measured by means of the ash-free dry weights.

The data found are in accordance with values stated in the litterature on similar estuaries abroad. This work will be intensivated next year.

D. Inland waters

In the canal through Walcheren Mrs. Borghouts found *Balanus amphitrite amphitrite*, previously found by her in the heated canal of the electricity plant at Vlissingen.

Mr. L. Blommers, a student from Amsterdam University, investigated the distribution of chironomids in water bodies of different salinity ranging from 0.2–15‰ Cl′. The larvae are sampled quantitatively and the adults are reared in the laboratory at Amsterdam. His results are in preparation.

During Heerebout's survey (see p. 44) an interesting phenomenon was encountered in the Kreek van Schelphoek on the island of Schouwen. This brackish inland pond is permanently stratified. In summer a positive temperature- and salinity stratification is found, and in winter a negative temperature stratification is maintained by means of a salinity stratification, as is sometimes the case in the Veerse Meer. But here the deeper strata differ in still another aspect from the upper ones, by their high concentrations of ions of iron and –possibly– tin, as a good deal of scrap iron was used to close the gap in the dyke in 1953.

E. Birds

The bi-weekly bird census along the coasts of Schouwen were stopped and the results are being worked out. From now on the avifauna is surveyed every month. Mr. Wolff took the initiative to establish a club of bird watchers in our area, which group produced a manuscript on the avifauna of Walcheren, written by Messrs. A. Joose and B. J. Smulders.

F. Fish

Dr. K. F. Vaas collected the gonads of plaice from the Veerse Meer just prior to the last stage of ripening. The eggs were sampled and counted. The plaice proved to have more eggs than fish of similar length from the Southern Bight in the North Sea and less than plaice from the Baltic. The production of sexual products could be incorporated into the cal-

culations on growth and respiration, carried out previously. The manuscript on population dynamics of the isolated population of plaice in the Veerse Meer was sent for consultation to the fishery biologists of the Institute of Fisheries at IJmuiden who collaborated so much to this study.

The fish fauna and dominant evertebrata of the Grevelingen, Westerschelde and Oosterschelde were repeatedly sampled. Our former collaborator W. J. M. Vader (Espegrend, Norway) published an article on the distribution of *Maurolicus mülleri* in the North Sea as a result of his analysis of finds along the Dutch coast (see Ann. Rep. 1964).

G. Physiological investigations

Dr. A. G. Vlasblom summarized his studies on *Marinogammarus marinus*. Investigations on the life-cycle of *Orchestia gammarella* are still in progress, similar studies on *O. cavimana* are being analysed at the moment and work on *Neomysis integer*, supervised by Mrs. Borghouts, is in progress.

The technicians, Ch. Almekinders and J. Verbrugge, constructed a microcryoscope. This apparatus, to be used for the investigation of osmoregulation of various organisms, is now being tested with the aid of chloride solutions of known concentrations. An accuracy of 0.030 °C, corresponding with 0.3‰ Cl′, was found. Survival-experiments with *Marinogammarus* and *Orchestia* spp. showed that usually ripe females are more susceptable to decreasing salinity than unripe ones. The animals begin to suffer below 8‰ Cl′ and their oxygen-consumption is also affected below this limit. In order to use statistical methods large numbers of experiments must be used in this work, as the factor temperature must also be dealt with.

IV. Plankton investigations

A. Veerse Meer

In 1967 the chlorinity of the lake fluctuated with a range of 4‰, but this year Mr. C. Bakker found a fluctuation between 9 and 10.5‰ Cl′.

As the water temperature did not rise to high values in summer –the maximal value being 19 °C– the oxygen consumption was not large. Supersaturation up till 120% was found during a bloom of *Sceletonema costatum* (220.10^6 cells/l). During this heavy bloom, the silico-flagellate *Ebria tripartita*, living on this diatom, also showed a very strong development.

As the average temperature did not exceed 18 °C between the end of June and the beginning of September, the summer copepode *Acartia tonsa* did not so well this year.

The epiphytic diatom *Melosira moniliformis*, a tychoplanktonic element in this lake, bloomed in early spring and showed additional maxima

in early and late summer, with a marked disruption in its development at the moment the diatom *Coscinodiscus centralis* came to the fore in September, with 2000 cells per litre at a chlorinity of approximately $10^0/_{00}$. It follows that the last mentioned species, an euhalobe, slightly euryhaline diatom, can also flourish in brackish water, provided the fluctuations are not too large. In its normal, marine environment the diameter of the cells of *Coscinodiscus centralis* is between 100 and 300μ, but the cells from the lake were found to be much smaller, measuring between 50 and 150μ. During the period the Zuiderzee changed into a fresh water lake it was also observed that the dimensions of various organisms were greatly reduced.

The larvae of the polychaete *Polydora* were very numerous this year, probably owing to the relative high salinity during spring.

B. Oosterschelde

The development of phytoplankton was similar to that of 1967 in spring but less intensive in summer.

For the first time plankton samples from the mouth of the Oosterschelde came available from regions beyond the radius of our own vessel, and these samples showed a luxurious development of polyhaline/euhaline diatoms and copepods, living in the contact zone between the water of the central Oosterschelde and the coastal North Sea.

Further inland in the mouth, in the region where the dam will be constructed, many plankton samples could be collected throughout the whole depth of the water. During a complete tidal cycle on August 27th a rich diatom association was encountered, dominated by *Schroederella schroederii*. An analysis on the spatial distribution of the diatom will be carried out, when data on current velocities –measured by the Department of Roads and Waterways– have become available.

C. Grevelingen

In this sea-arm, separated from the rest of the deltaic area by the dam at Bruinisse, Mr. R. Peelen found a smaller plankton development than in previous year, owing to lower summer temperatures and smaller insolation. The copepode *Acartia clausi* bloomed in August with 4000 animals per litre. Previous to the construction of the dam, this animal was never numeruos in the Grevelingen. *Rhizosolenia imbricata shrubsolei* was the principal diatom of the spring bloom.

D. Keeten-Krammer-Volkerak-Haringvliet-Hollands Diep

An interesting phenomenon was observed in the Keeten-Krammer-Volkerak-area. As summer temperature and insolation had been under normal and river discharge of the Rhine fairly high –$1\frac{1}{2}$–$2\frac{1}{2}$ times the annual average– the number of phytoplankton cells was expected to be low. But the reverse proved to be the case. Numbers of *Biddulphia*

aurita and *Melosira sulcata* in spring, as well as those of *Rhizosolenia imbricata* in summer and those of various autumn species, such as *Biddulphia regia* and *B. sinensis*, were significantly higher than in the year before. Zooplankton showed the same relation (*Eurytemora affinis*, *Polydora*- and *Balanus* larvae). In the Hollands Diep the number of *Asterionella formosa* and *Brachionus calyciflorus var. amphiceros* –to quote a member of each group– were 3 to 5 times higher than in 1967. Having recently established this phenomenon it is obvious that we cannot yet give a definite explanation. Dilution of noxious waste products might be a possibility.

As chlorinity fluctuated in the Haringvliet between 0.5 and 4‰ Cl′, the brackish diatom *Nitzschia closterium* did not reach high numbers, but freshwater organisms developed well.

The distribution of *Eurytemora affinis* in the delta area was studied during a number of sampling tours. A vertical migration from the surface towards the deeper strata at sunrise and a reverse movement in the evening was observed. In stagnant water with a chlorinity between 0.3 and 15‰ *Eurytemora* can subsist, but in estuaries between 1 and 3‰ Cl′ only. That this copepode can stand a good deal of pollution was proved by its strong development in the harbours of Rotterdam.

E. Biesbosch

The rhythm of cell-partition of the diatom *Actinocyclus normannii* was the subject of a special study carried out by Mr. Peelen. He found partition to start at a fixed number of hours after sunset, thus at different times of the night in different seasons. The formation of micro- and macrospores as well as auxospores could be observed.

F. Brielse Meer and Brielse Gat

Owing to the low salinity of 220 mg/l Cl′, *Microcystis aeruginosa* dominated over *Aphanizomenon flos-aquae*, reaching higher numbers than in 1967. In the Brielse Gat *Sceletonema costatum* reached numbers of about 2.5×10^6 cells per litre at a chlorinity of about 10‰.

G. Westerschelde

The investigations of Mr. N. de Pauw, from the State University Centre at Antwerpen, Belgium, carried out under supervision of Prof. Dr. F. Evens, were continued for another year. Again our Institute received a grant from the Beyerinck–Popping Fund in order to support this work. Cyclic development of various plankton organisms was studied in relation to fluctuations in the chemical and physical environment. A connection with Mr. Peelen's work is the frequent occurrence of *Eurytemora affinis* in the estuary of the Schelde. Here too this copepode did not show a clear-cut correlation with a special salinity range. As water pollution

is a prominent problem in the Westerschelde the bacterial flora was studied thoroughly. Sampling will be continued through 1969.

V. Botanical investigations

A. Veerse Neer

According to Dr. W. G. Beeftink's observations the development of the vegetation of the Middelplaten was similar to that of previous year. A few halophytes hold on, others deteriorate and are restricted more and more to the humid borders of the flats. Gras vegetation increased, possibly as a result of grazing.

The *Phragmites* vegetation was followed on the eastern island only, and only a few older plants were seen. One-year old plants were not observed, as had been the case in 1967.

B. Halophytic vegetation elsewhere in the Delta Area

The inventory of the flora growing outside the dykes was continued this year mainly in the northern part of the area, along the rivers in Brabant and along the eastern banks of the Schelde in Belgium. Ubiquists excluded, about eight different types of distribution patterns could be distinguished in combinations of soil-types and salinity. *Sonchus palustris* represents a pattern on its own, being limited to an area between the isohalines of 2 and 0.3‰ Cl′ at high water.

Prof. Dr. J. L. van Soest –Leyden– identified the collection of *Taraxacum* forms and distinguished 21 species with different distribution.

Long term changes in the vegetation of 225 permanent squares are being analysed.

The investigation of Mr. P. Dieleman, a student from Utrecht University, was prolonged for 6 months, in order to study the level where the various components of natural hedges grow. As a result of the study of numerous soil samples it was found that *Crataegus monogyna* and *Rosa* spp. grow on sandy clay, deposited by former creeks.

Mr. J. C. H. Peeters, a student from Leyden University, carried out similar research on hedges in the polder "De Breede Watering". His results are being worked out.

A third student, Mr. G. C. Redeker from Utrecht, investigated the flora of drinking pools in the vicinity of Goes and was able to classify them according to vegetation and environment.

The student C. van der Kraan, Free University of Amsterdam, following up the work of Miss J. G. Roeleveld, investigated the vegetation of the slopes of dykes boardering the sea, and studied the influence of exposition, use by men and cattle and salinity gradients.

C. Benthic Algae

This year Mr. P. H. Nienhuis carried out an intensive survey of the

algal vegetation of mud flats and salt marshes in the Grevelingen and constructed an eco-sociological classification. On the flats and marshes a number of distinct sociations can be identified in the sublittoral and in the lower part of the eulittoral zone. On fine sands an *Enteromorpha prolifera – E. linza* sociation can be distinguished, on musselbanks an *Ulva rigida/lactuca – Fucus vesiculosus f. mytili* sociation next to a mixed sociation, in which mainly elements from the *Enteromorpha*-sociation and some elements from the *Fucus*-sociation can be distinguished growing among the *Zostera* fields. On the mud flats and salt marshes in the upper eulittoral and supralittoral zones a more diversified vegetation is growing in a, likewise, more diversified environment. Eight vegetation units could be identified here. In some of them brue-green algae dominate, in others *Chlorophyceae*, such as *Rhizoclonium*, *Enteromorpha* and *Vaucheria*. The pattern of most important habitats occupied by algae was depicted on a map of the Grevelingen. These investigations form the base of a manuscript on the subject, finished in the second part of the year.

Results of the study of epilithic algae in the Grevelingen were used for a lecture on the importance of the substrate on algal distribution, held at the 6th International Symposium on Marine algae at Santiago de Compostela, Spain. This lecture will be published.

Periodicity and quantitative composition of the algal flora of the Springersgors was studied with the aid of permanent squares. In late winter *Ulothrix* spp. are prominent, in spring a bloom of *Enteromorpha* was observed. However, these species disappear gradually as the stand of phanerogams becomes more dense in early summer. Then blue-greens grow out with numerous species. A few vegetation-units, such as the *Fucus vesiculosus f. volubilis* and the *Bostrychia scorpioides*-sociation, are perennial.

Correlation of data on algal vegetation with data on salinity and humidity of the soil, shows that the blue-greens are most resistant against fluctuation in salinity.

The autecological study of *Rhizoclonium* and *Chaetomorpha* species yielded many interesting points of view. *Rhizoclonium* proved to be a genus with a large ecological amplitude, growing in many different biotops, preferably in stress situations: on places alternately wet and dry, salt and fresh. Culturing *Rhizoclonium* species, it was found that threads from salt marshes did well in fresh water and those from fresh soils in saline water. Many alleged taxonomical differences could be reduced to ecological differences induced by the environment.

K. F. VAAS

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