4. Malacology in the Netherlands: Experimental malacology

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4a. INTRODUCTION

In the past many experimental investigations of various kinds of molluscs have been carried out in the Netherlands. The names of H.J. Jordan (comparative physiology), Chr. P. Raven (embryology), J. Verwey (marine biology) and P. Korringa (biology of oysters) should be mentioned. For detailed information, see the supplement of Basteria (Vol. 23, 1-174, 1959) issued at the occasion of the 25th anniversary of the Netherlands Malacological Society. The present chapter deals with the current research in this field.

The help of Prof. N.H. Verdonk, Dr. A. de Zwaan and Dr. P.A.W.J. de Wilde, who wrote paragraphs 4b, 4c and 4e, respectively, is acknowledged.

4b. ZOOLOGICAL LABORATORY, STATE UNIVERSITY, UTRECHT

Research on the development of molluscs in the Netherlands dates back to 1938, when Chr.P. Raven was appointed professor of zoology at the State University of Utrecht. Trained as an embryologist in Woerdeman's laboratory in Amsterdam, Raven had made special studies of the development of amphibians. In Utrecht he introduced embryology as a new field of study. Convinced that in the Netherlands adequate attention was paid already to the development of amphibians, he focussed his attention to the study of molluscan development; not for reasons of special liking for these animals, but for the simple reason that molluscan eggs are suitable for analyzing factors that play a part in animal development. The snail *Lymnaea stagnalis*, which abounds in the Dutch ditches, provided the egg material.

Starting from the principle that an experimental approach must be preceded by a detailed study of normal development, the embryonic development of *Lymnaea* was first thoroughly investigated. As a result the embryology of this snail is the best known of all molluscs. From the beginning, however, attention was paid also to experimental research, e.g., by centrifugation and treatment with various mono- and divalent cations. Especially lithium chloride causes characteristic disturbances of development, primarily in the head region; the eyes and tentacles, normally situated at the periphery, approach each other towards the middle and eventually may fuse into one central eye and tentacle (cyclocephalic series). From an analysis of these malformations it was concluded that developmental factors, located in the cortex of the egg, form a gradient field with a maximum at the animal pole and gradually decreasing intensities on either side. Lithium treatment caused a weakening of the field. A detailed study of the cell lineage in normal and lithium treatment embryos by Verdonk revealed, however, that the primary pattern of the head region is radially symmetrical, whereas secondarily this pattern becomes bilaterally symmetrical and dorsoventral. Lithium suppresses the bilateral symmetry and this leads to the formation of cyclocephalic head malformations.

On the basis of these results the activities of the Utrecht research group were mainly concentrated on factors that determine the very early development of molluscs. In this group of animals the blastomeres are restricted to a specific developmental pathway already at an early cleavage stage. Local accumulations of cytoplasmic substances, which during cleavage are distributed over the daughter cells according to a fixed pattern, play an important role in the specification of the blastomeres.

Studying the ooplasmic segregation in the egg of *Lymnaea stagnalis*, Raven observed special cytoplasmic differentiations, situated immediately below the cortex in the equatorial region of the egg. These subcortical accumulations are arranged according to a dorsoventral and bilaterally symmetrical pattern, which may be related to the future organization of the embryo. Some molluscan species, however, produce eggs which so to speak are made for studying cytoplasmic localizations. In these eggs a so-called polar lobe is formed at the early cleavages. Through this protrusion at the vegetal pole of the egg, after cleavage fused with one of the blastomeres, specific cytoplasmic components are shunted to special blastomeres. In an electron-microscopic study of the polar lobes of the snails *Bithynia* and *Crepidula*, Dohmen observed that the contents consist of one or more aggregates of small vesicles with an electrondense substance, probably RNA. The polar lobes of several species (e.g. *Dentalium*, *Nassarius*, *Littorina*) are being studied with electron-microscopical and cytochemical techniques. The morphogenetic significance of cytoplasmic localizations is investigated with microchirurgical methods, in which the polar lobes or blastomeres with and without polar lobe material are removed. In this way Cather (University of Michigan, Ann Arbor, U.S.A.)during a stay in Utrecht proved that in *Bithynia* adult structures are not formed after removal of the polar lobe. Equal distribution of the polar lobe material over both the blastomeres at first cleavage in the scaphopod *Dentalium* produces a double embryo, whereas removal of the lobe causes a very abnormal embryo. In a detailed study of the development of normal and lobeless eggs of *Dentalium* Van Dongen found that of the four quadrants (A, B, C and D), constituting the embryo, the D-quadrant, which receives the material of the lobe, is essential for the organization of the embryo. This D-quadrant, which forms the dorsal side of the embryo, influences the A- and C-quadrants, whereas the ventral quadrant (B) follows its own pathway. In the lobeless embryos indications of bilateral symmetry and dorsoventrality are missing, and the embryo remains radially symmetrical.

The question arises how bilateral symmetry is determined in molluscan eggs, which have an equal cleavage pattern without formation of a polar lobe. These eggs remain radially symmetrical until the fifth cleavage. Then one of the macromeres becomes centrally positioned and intrudes far into the interior of the egg. This central blastomere determines the dorsal side of the embryo and forms the mesentoblast, which plays a major role in development as it gives rise to the entire mesoderm. Deletion experiments of Van den Biggelaar with eggs of *Patella* indicate that in principle each macromere may attain a dorsal position and form a mesentoblast. The differentiation between the four macromeres takes place under influence of the micromeres, which interact with only one of the macromeres. Recently, a new approach to this problem was made by Arnolds, who isolated two lethal mutants of *Lymnaea stagnalis*, in which as an innate defect, the development of the egg remains radially symmetrical.

In the development of molluscs epigenetical factors as well as cell-internal determinants play a role in establishing the cell lines of which the embryo is built up. The cell lineage studies prove that cleavage follows a predetermined pattern resulting in a regular distribution of the developmental factors. The study of the cell cycle and the cleavage rhythm is of great importance for a better understanding of this programmed cleavage. For this purpose the successive phases of the cell cycle are investigated in relation to their significance for later development. Geilenkirchen investigates the significance of c-AMP and c-GMP in the cell cycle of *Mactra* and *Lymnaea* eggs; Labordus irradiates eggs of *Lymnaea stagnalis* during certain phases of the cell cycle and traces the effects on development; Van der Wal investigates the transformation of the yolk in relation to the cell cycle (disturbances of the cleavage rhythm by heat shock or short term chemical treatment may seriously affect development). The effect of cleavage asynchronies on development are being studied by Mrs. Boon-Niermeijer.

In summary it may be stated that the Utrecht group – with nine scientists the largest group of molluscan embryologists in the world – is primarily interested in mechanisms controlling the early development. The eggs of molluscs have proved to be excellent material for solving problems related to animal development.

4c. LABORATORY OF CHEMICAL ANIMAL PHYSIOLOGY, STATE UNIVERSITY, UTRECHT

The laboratory has three main groups studying the interrelation between metabolism and development, the regulation of energy metabolism in insects and in molluscs, respectively. The group studying molluscs includes four staff-members: Holwerda, Kluytmans, Zandee and De Zwaan. The research concentrates on alterations in energy metabolism, especially in intertidal bivalves and gastropods, which are due to variations in oxygen availability. Four projects are studied at present: 1, alterations in metabolism related to the aerobic-anaerobic transition (regulation of alterations, interaction between tissues and organs, location within the cell); 2, metabolic consequences of switches from anaerobic to aerobic ways of life (fate of end-products, accumulated in the organism, upon returning to aerobic conditions); 3, metabolic adaptations during the development of aerobic living larvae into facultative anaerobic adults; 4, differences between facultative anaerobic and obligate aerobic organisms on the one hand and obligate anaerobic organisms on the other.

4d. DIVISION OF GENERAL AND EXPERIMENTAL ZOOLOGY, SUBFACULTY OF BIOLOGY, FREE UNIVERSITY, AMSTERDAM

The Subfaculty of Biology of the Free University was founded in 1950. During the initial period research in zoology was mainly restricted to histological and biochemical studies of the thyroid gland. It was soon deemed necessary to find a new, less specialized, theme with ramifications in many zoological directions, in order to cope with the interests of the increasing number of students.

At that time the phenomenon of neurosecretion (the production of hormones by nerve cells) was known to be important for the physiology of vertebrates and arthropods and it could be expected to be a fundamental process in all Metazoa. In 1956 it was decided to switch over to the study of neurosecretion in invertebrates and after some preliminary studies of representatives of several phyla, promising results were obtained with molluscs, especially with freshwater basommatophoran snails (ancylids, planorbids, lymnaeids). It was found that in the central ganglia of these animals many neurosecretory cells of different types are present. Moreover, upon the cerebral ganglia peculiar epithelial structures occur, the so-called dorsal bodies, while in the small lateral lobe of these ganglia several characteristic neurosecretory cells and a glandular follicle are present (Lever, 1957, 1958; Lever et al., 1958-1961).

Soon nearly all investigations dealt with Basommatophora, but also some thorough studies were made of neurosecretion in the freshwater bivalve *Dreissena polymorpha* (see Antheunisse, 1963) and in the stylommatophoran snail *Succinea putris* (see Cook, 1966), and on growth and reproduction in the marine bivalve *Macoma balthica* (see Lammens, 1967).

From the orientating work mentioned above, the question arose as to what might be the function of the various neurosecretory cells and of the dorsal bodies and the complex lateral lobes in the snails. In order to tackle these problems anaesthetization and operation techniques had to be developed (Joosse & Lever, 1959). As main experimental animal the largest basommatophoran species, *Lymnaea stagnalis*, was selected. It appeared that these snails can survive rather severe operations, such as extirpations of ganglia and cutting of commissures and connectives.

From such and other experiments (injections of ganglia-homogenates) it was before long concluded that the pleural ganglia influence the water balance (Hekstra & Lever, 1960; Lever et al., 1961). Joosse (1964) found that the neurosecretory products of the large groups of Gomori-positive medio-dorsal and latero-dorsal cells in the cerebral ganglia are transported to the bulb-shaped endings of their axons which are located in the periphery of the median lip nerves, while the neuro-haemal area of the phloxinophilic caudo-dorsal cells lies in the periphery of the cerebral commissure. Moreover, on the basis of synchronous activities in the dorsal bodies and in the ovotestis during the annual cycle, and of the effects of extirpations of these bodies, he showed that the latter are involved in reproduction. Boer (1965) made the first detailed histological and histochemical study of the types of neurosecretory cells mentioned above. He demonstrated that these are characteristically different in many respects and that the cells are active synthetizers of glandular products, which are released in an endocrine way.

Gradually the impression was gained that the various types of neurosecretory cells in the ganglia, the dorsal bodies and the structures in the lateral lobe must have important regulating effects upon many processes in the snails. This led to the conclusion that an extensive research programme, implying, in principle, studies of all structures and functions of these animals, was needed as a basis for the understanding of this complex endocrine system. During the last ten years large parts of this programme have been carried out.

Several descriptive, and in part also experimental, morphological studies (anatomy, histology, ultrastructure) were made of various tissues: skin (Zylstra, 1972), circulatory system (Bekius, 1972), blood cells and connective tissue (including haemocyanin-producing cells and immunological reactions; Sminia, 1975), digestive system (salivary glands: Boer et al., 1967; sorting mechanism in the pylorus: Veldhuyzen, 1974; digestive gland – of *Biomphalaria pfeifferi* –: Meuleman, 1972), kidney (Wendelaar Bonga & Boer, 1969; Boer & Sminia, 1976), ovotestis (Joosse, 1964) and reproductive tract (of *Biomphalaria glabrata*: De Jong, 1973), and musculature (Plesch, 1977).

With regard to sensory and neurophysiological functions, studies were made of the statocysts (Geuze, 1968), the tactile sence (De Vlieger, 1968; Janse, 1974), chemosensitivity (Jager, 1971), and photoreception (Stoll, 1973). In this respect also investigations of the neuronal regulation of the buccal mass (Goldschmeding, 1977) and of the tentacle retraction reflex (A.J. Lever, 1977) should be mentioned.

Other aspects which were studied experimentally are the water-balance (Van Aardt, 1968), the effects of external factors upon oviposition (Van der Steen, 1968), carbohydrate metabolism (Veldhuyzen, 1975), regulation of specific ions in the haemolymph (De With, 1977), calcium metabolism (Spronk, de With & Sminia, in progress), and food assimilation (Scheerboom, in progress).

In the mean time the endocrinological work was continued. The most important results were as follows. Wendelaar Bonga (1971) made an extensive study of the neurosecretory cells in all central ganglia, using especially a modified alcian blue-alcian yellow technique and quantitative electron microscopy. It appeared that at least ten different types of neurosecretory cells are present, all lying at characteristic locations. The neuro-haemal areas of these cells are very extensive and include the peripheral parts of many nerves and of all connectives of the visceral ring. With regard to the functions of the endocrine centres it was found that the dorsal bodies control vitellogenesis, growth and cell differentiation in the female accessory sex organs (Geraerts & Joosse, 1976; Geraerts & Algera, 1976) whereas the caudo-dorsal cells regulate ovulation (Geraerts & Bohlken, 1976) and the medio-dorsal and latero-dorsal cells (light green cells) are involved in growth (Geraerts, 1976). Furthermore, it could be concluded from extirpation experiments that the lateral lobes influence growth and reproduction, tissue and haemolymph carbohydrate and haemolymph protein content, and have effects upon aerobic, anaerobic and gluconeogenic metabolism (Geraerts, Mohamed & De Zwaan, 1976). Roubos (1976) found that the activity of the ovulation hormone producing caudo-dorsal cells is under stimulating and inhibiting nervous control, as shown in implantation and in in vitro experiments. Moreover, these cells show a circadian rhythmicity which is synchronized by light via the eyes (after blinding: desynchronization). The medio-dorsal and latero-dorsal cells also show a daily rhythm, which, however, is not modulated via the eyes. Moreover, it appeared that the so-called dark green cells of the pleural and parietal ganglia (which are involved in osmoregulation; see above) react upon the osmotic value of the medium (and of the blood). Neuronal pathways from other parts of the central nervous system have no effect upon this process (cells in implanted ganglia react quite normal).

In 1976, based upon the results obtained in recent years, an integrated programme started on the regulation of the activity of neurosecretory cells. This is subsidized in a generous way by the Netherlands biological research council BION-ZWO. The neurosecretory system of *L. stagnalis* is an excellent model for the study of this general problem, because in this animal the neurosecretory cells are exceptionally large, while many of the various types are localized in distinct groups or do even occur as single cells. Furthermore, the cells are easily visible and can be individually manipulated (cauterized or studied with microelectrodes) in the living animal.

This programme is carried out as a joint effort of the three experimental groups of the present *Division of General and Experimental Zoology* (chairman: Prof. Lever): 1, *Endocrinology and Chemical Zoophysiology* (head: Prof. Joosse): Bohlken, Mrs. Dogterom, Dogterom, Dr. Geraerts, Scheerboom, Dr. Spronk, Dr. Wijsman; 2, *Histology, Histochemistry and Electron Microscopy* (head: Dr. Boer): Dr. de Jong, Van Minnen, Dr. Roubos, Dr. Sminia; 3. *Neurophysiology* (head: Dr. de Vlieger): Dr. Goldschmeding, Dr. Janse, A.J. Lever, Dr. Stoll, Van Swigchem, Ter Maat.

In the past some of the research dealt with bilharzia vector snails. During recent years this occurs often in close co-operation with the Parasitology Group (Dr. Meuleman) of the Medical Faculty of the F.U., which has the parasite-host relationship as its central problem.

The animals needed for the experiments are bred under standard conditions in specially devised large tanks (Van der Steen et al., 1969). The annual production of the snail factory (head: Mrs. Mooy) is at present 25-30,000 specimens of *L. stagnalis*.

4e. NETHERLANDS INSTITUTE FOR SEA RESEARCH (N.I.O. TEXEL

Due to stressful ecological conditions the malacological diversity of the vast and rather monotonous tidal mud-flats of the Dutch Wadden Sea is low. Perhaps with the exception of the fossil material from the Eem period and with the remnants of the former Zuiderzee fauna, now limited to some land-locked brackish waters, the area is from a taxonomical point of view of little interest. In contrast the numerical abundance of the small number of gastropod and bivalve species over extensive areas make them predominantly suitable for ecological studies. During the last decade one of the main interests of the Netherlands Institute for Sea Research has been focussed on the structure and functioning of the ecosystem of the Wadden Sea and adjacent North Sea.

Benthic invertebrates are more numerous in this area as compared to most other marine habitats in the world. With a total percentage of 65, bivalves rank first when biomass is concerned. Measurements on density and growth rates of the most important species *Mytilus edulis, Cerastoderma edule, Mya arenaria* and *Macoma balthica* are carried out by Beukema. These investigations also include patterns of dispersal, age distribution, recruitment, mortality, biomass, calorific value and production of soft parts and shell-lime. From observations over extended periods the detection of long-term trends in growth and numerical abundance come within reach, giving insight into the causal relations between certain parameters of the populations and the abiotic and biotic environmental factors. *Macoma* perhaps is now the best investigated species. Mentioned here are the large variations in recruitment: the high initial mortality, the limited growing season in spring and early summer, the large seasonal changes in biomass and the gradual decline in numbers when they have passed their first year. Growth probably directly depends on the availability of primary produced food, which again is related to feeding behaviour and immersion times. Of special interest is the phenomenon of redispersal of the first year spat on the flats during their first winter.

Energy transfer in the food chains takes place by eating whole prey as well as by consumption of certain regenerating parts of the body of the prey. It was found that juvenile plaice *(Platessa platessa)* heavily predate on the syphons of *Macoma*. De Vlas investigates the importance of this kind of predation. By measuring syphon regeneration this normally overlooked part of secundary production is estimated.

De Wilde's contribution to budget studies shows a more ecophysiological approach. In *Macoma*, kept in micro-ecosystems in the laboratory, the influence of temperature on behaviour, energy metabolism and growth was studied. When measured under natural circumstances in a continuous flow respirometer, the oxygen uptake of *Macoma* shows remarkable patterns of rhythmicity, which could be correlated with certain behavioural activities. By offering temperature shocks it proved to be possible to induce spawning at will in *Macoma*. In this way the loss of energy occurring with spawning was estimated. The sexual products were collected and the calorific value measured.

Other lines of research in molluscs include the filtering mechanism of the edible mussel *(Mytilus edulis)*. Dral studied the behaviour of the latero-frontal cilia and the mechanisms used to manage high concentrations of food particles.

Swennen is interested in molluscs as hosts for numerous parasites. When the host is eaten by a tertiary producer (fish or bird) the life-cycle of the parasite can be closed.

Another project, carried out in the southern part of the North Sea, is partially dealing with molluscan life. Winkelman's investigations aim to describe the detailed patterns of dispersal, biomass and growth of the infauna in relation to water depth, bottom structure and chemical properties of the overlying water.

In the framework of the project Biological Research Ems-Dollard Estuary, Van Arkel studies the macrobenthic fauna of the Ems-Dollard Estuary, following in general the same programme as Beukema (described above).

Cadeé has contributed to the palaeontological-sedimentological knowledge of the Wadden Sea by a study on the transport of shell material. After death the empty shells from the tidal flats are transported and concentrated in the gullies. This wide-spread post-mortem transport of shells is in contrast to the near absence of a similar transport found in other embayments and in open shelf areas. Special attention is paid to the size-frequency distribution of shells in these transported assemblages. Juvenile shells may dominate here, which was always thought to be characteristic for in-situ accumulations. This indicates that size frequency (alone) cannot be used in palaeo-ecology to differentiate between transported and non-transported molluscan assemblages.

Finally as part of a research in the sedimentological history of the northern North Sea Eisma, in cooperation with Spaink (Geological Survey), studies changes in the molluscan fauna in cores.

Due to space limitations no references are given; interested persons should contact the author.