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Notes on the spawning, egg cleavage and early development of the bivalve *Macoma balthica*

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Larvae of *Macoma balthica* (L.) developed by chance in the laboratory. Experiments were made to spawn clams in the laboratory and to rear the eggs into larvae. The egg cell cleavage proceeded in an ordinary way typical of bivalves. Trocophore larvae developed at 4°C within 2–3 days. The velum began to form in 5-day-old larvae. Some larvae lived for one week, by which time they had a small membranous larval shell. The results of these experiments were promising; a better yield of larvae and perhaps also larvae in later stages might be obtained if more sophisticated culturing methods were to be used.

A note is given on intraovarian egg cleavage in some (diseased?) *M. balthica* females.

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1. Introduction

The early larvae of bivalves are much alike and thus difficult to distinguish (Loosanoff & Davis 1950). Larvae reared from eggs spawned and fertilized in the laboratory may help in the identification of wild larvae. Lebour (1938) reported the breeding seasons of about sixty species from Plymouth (GB). Over twenty of these could be bred in the laboratory to the gastrula, free-swimming larva, or young shelled, stages. Commercially important species have received most attention in the development of cultivation methods and in the studies of physiological and ecological requirements of the larvae. In spite of many earlier difficulties in the cultivation methods, larvae of about twenty species had been reared successfully by 1963 at Milford Laboratory (Connecticut) by Loosanoff & Davis. Large batches of embryos or larvae of more or less even quality might also be of value in genetical studies and in studies on the effects of environmental changes on organisms.

Larvae of several tellinacean bivalves have been more or less successfully reared in the laboratory: *Cumingia tellinoides* Conrad (Morris 1917), *Tellina crassa* Pennant and *Abra alba* (S. Wood) (Lebour 1938), *Tellina* spec. (Sullivan 1948), *Scrobicularia plana* (da Costa) (Lebour 1938, Frenkiel & Mouëza 1979), *Donax variabilis* Say (Chanley 1969), *Donax vittatus* L. (Frenkiel & Mouëza 1979) and *Tagelus plebeius* Solander (Chanley & Castagna 1971). Lebour (1938) either did not try or did not succeed in breeding *Macoma balthica* (L.) in the laboratory. The planktonic life of this species lasts about 2–5 weeks (Caddy 1969). The veliger larva of *M. balthica*, from plankton, has been described by Werner (1939), Jørgensen (1946) and Sullivan (1948). Caddy (1969) described its postlarval development, which takes place in the sediment. As a supplement to these descriptions of the later developmental stages of this tellinacean species, I here give an outline of its egg cleavage and early larval development as brought about in the laboratory. A note is also given on inflammatory response of the ovary and intraovarian egg cleavage in some *M. balthica* individuals from the wild. These studies form part of a larger series of eco-physiological investigations on *M. balthica* in the Baltic Sea, southwestern Finland.

2. Material and methods

Spawning, fertilization and larval development in *Macoma balthica* occurred first by chance in another experiment (an infection experiment with trematode cercariae).

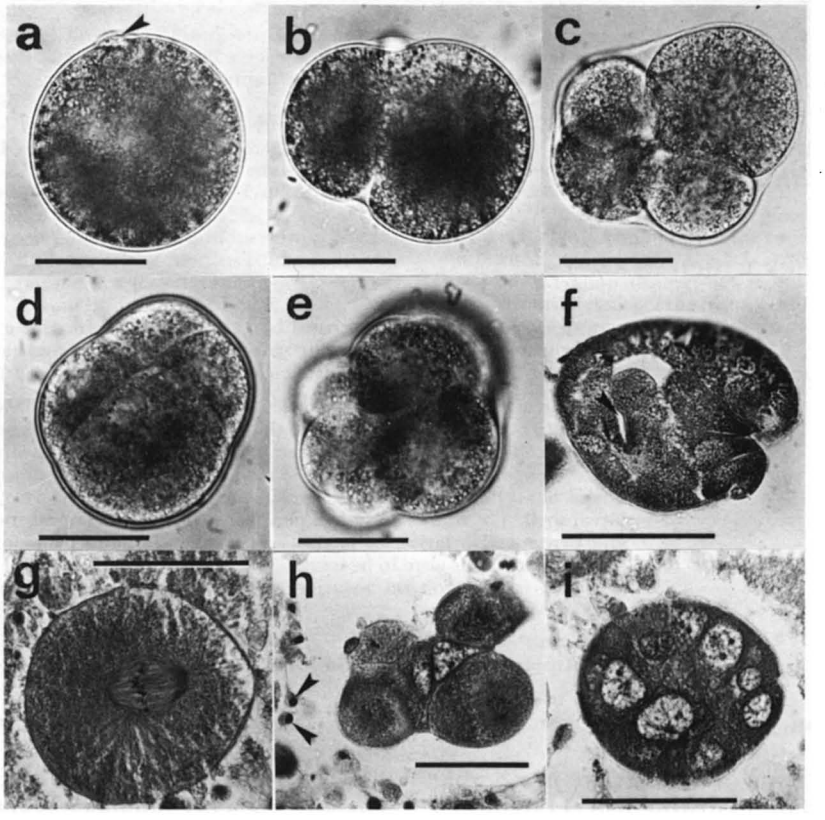

Fig. 1. Egg cell cleavage and gastrulation of *Macoma balthica*. – a. Egg with a polar body (arrowed). – b. Two-celled stage. – c–d. Four-celled stage. The egg membrane is separate at the furrows in c. – e. Six-celled stage. – f. Section of a gastrula, where the small hole (arrowed) is the archenteron and the large invaginated cells to the right will probably form the shell gland. – g–i. Intraovarian egg cleavage in a diseased female. The arrows in h point to amoebocytes. The sections (f–i) were stained with Masson–Gomori. Scale bars 50 μm in all pictures.

The clams had been collected from a depth of 35 metres in the neighbourhood of Tvärminne Zoological Station (SW Finland) in late summer 1984. They were kept in natural brackish water (S 6–7 ‰ at about 4°C until the commencement of the experiment in September. Later, spawning experiments were made in spring and early summer 1985. Clams from a nearby, shallower (depth 7–8 m), place were collected on 15 May and from the above-mentioned site (depth 35 m) on 15 June.

Clams (10–20) were put in brackish water with aeration in beakers (400 ml) with or without sand on the bottom. They were allowed to warm up to room temperature (20–22°C) a few times in the course of a few days. In some experiments an amount of sperm was added to the water. The eggs spawned were left in the beakers or pipetted into Petri dishes two hours after spawning. In the dishes the eggs/zygotes were washed with and left in clean brackish water. The beakers and dishes were kept at 4°C, although one batch of eggs was left at room temperature for one day. Microscopical examination of the cleavage and larvae was made at room temperature.

For histological preparations embryos and larvae were fixed in Bouin's fluid and stained with boraxcarmine, and using routine methods they were then embedded in paraffin. The 7–10 μm thick sections were stained with Masson– Gomori (Chromotrope–fast green, Gray 1954:339) or Pikroblauschwarz (Romeis 1968:374). Histological preparations had also been made from the gonads of clams taken from the two collecting sites in summer 1983. The sections from these were stained with Masson– Gomori.

3. Results and discussion

Raising the temperature is usually used as a means of conditioning and inducing spawning in bivalves (Thorson 1946, Loosanoff & Davis 1950, 1963). The presence of gametes of the same species also promotes spawning. Females, though ripe, will often retain their sexual products for a long time unless surrounded by a "sexual milieu" (Thorson 1946). Eggs spawned by *M. balthica* after the temperature was raised were not usually very numerous. At one time eggs appeared and some of

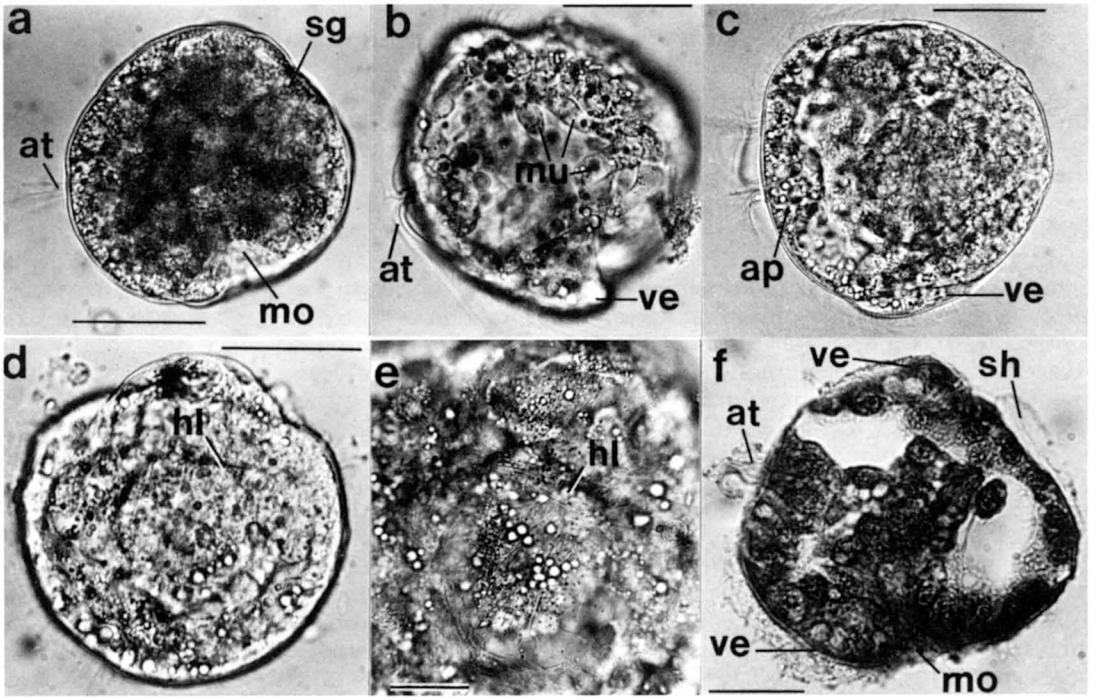

Fig. 2. Early larvae of *Macoma balthica*. — a. An early trocophore larva. at = apical tuft of cilia, mo = mouth, sg = shell gland. — b. A five-day-old larva with forming velum flaps (ve) and larval muscles (mu). — c. The same larva as in b, but with the inner part in focus. ap = apical plate. — d. Dorsolateral view of a one-week-old larva. hl = hinge line. — e. Detail of the laterodorsal part of the larva in d. The larval shell consists of two membranous sheets with minute dark spots. Folds due to pressing are visible in the sheets. — f. Section of a larva, where the apical plate with the apical tuft, mouth, velum and larval shell (sh) are visible. The section was stained with pikroblauschwarz. Scale bars 50 μm in a—d and 20 μm in e and f.

them began to cleave within four hours (the first four-celled stages appeared). Cleavage did not begin so soon in all batches of eggs. Often many eggs did not begin to cleave at all. These may have been immature. If an attempt was made to fertilize them again one or two days later, the success was still poor. Not all eggs discharged by a spawning female of *Venus mercenaria* possessed the same vitality (Loosanoff & Davis 1950). According to Loosanoff & Davis probably a strong temperature stimulation compelled some clams to abort immature eggs. Such eggs could develop into larvae, but these were feeble and soon died. *V. mercenaria* normally spawns at a temperature of over 20°C. Room temperature (20—22°C) may be physiologically too high for *M. balthica*, which normally spawns at about 10°C or much lower (Pekkarinen 1983) and hardly ever encounters such high temperatures here in its natural habitat. Also the last batches of eggs discharged by virtually spent females of *V. mercenaria* produce feeble larvae that grow slowly and have a high mortality (Loosanoff & Davis 1950). The majority of *M. balthica* from the shallower site had already spawned when the experiments were made in spring 1985. Clams at a depth of 35 m spawn later. Some of them were able to spawn in the laboratory even in September, 1984, and up to one-week-old larvae were produced. It is not known whether the penetrating cercariae in this experiment (see methods) contributed to spawning by irritating the clams.

Before the polar body formation the shape of the *Macoma* egg altered. The first cleavages take place within the vitelline (egg) membrane. The first cleavage is unequal (Fig. 1a—b), as it is in most bivalves (Raven 1958). Sometimes equal cleavage also occurred. Too early breakage of the egg membrane was also frequent. The blastomeres then separated to form a loose cell cluster instead of a compact embryo. These abnormalities often happened

when the eggs were examined under the microscope between a slide and cover glass in spite of a sufficiently thick layer of water. In this respect the first cleavage was most difficult to examine under the microscope. Concentrated sperm suspensions and extracts can give rise to lytic activities against the egg membrane. Sperm extract of *Mytilus edulis* causes the cleaving blastomeres to separate, especially during the first cleavage (Berg 1950). An overconcentration of the sperm of *Pecten maximus* causes polyspermy and abnormal development, as well as a decrease in the number of larvae (Gruffydd & Beaumont 1970). The thickness of the vitelline membrane varies between species. Even within Tellinacea the egg of *Scrobicularia plana* has a thick chorion, while the egg membrane of *Donax vittatus* is thin (Frenkiel & Mouëza 1979); it is thin in *M. balthica*, too. Raising the temperature to 32–37°C and the presence of hypertonic sea water can cause artificial parthenogenesis in *Cumingia tellinoides*. The polar body nuclei remaining within the egg contribute to cleavage (Morris 1917). Such eggs may develop into fairly normal swimming larvae. Cleavage was, however, slower than normal and slight differences, like equal divisions, occurred. Many other interventions, e.g. abnormal ionic composition of the medium, cause the first cleavage to become equal (Raven 1958).

The formation of the polar lobe, which is evident in several species (e.g. in *Modiolaria marmorata* Forb. described by Lovén 1879 and in *Mytilus edulis* described by Berg 1950), could not be noticed in the eggs of *M. balthica*. The four-celled stages (Fig. 1c,d) resemble those of *Cardium exiguum* Gm., described by Lovén (1879), and *Cumingia tellinoides*, described by Morris (1917).

Morulae developed at room temperature within nine hours. They developed into swimming ciliated larvae within 20 hours. Those at 4°C did not begin to swim within this time. Probably the shell gland invagination takes place at the time of gastrulation (Fig. 1f). Trocophore larvae developed at 4°C within 2–3 days (Fig. 2). There is an apical tuft of long cilia at the anterior end of the larva. The prototroch is a broad ciliated zone almost covering the anterior half of the body except for the apical plate. The telotroch is scarce. Both the outer surface of the larvae and the inner surface of the gut were covered by a microvillous-like refractile border. In five-day-old larvae the velum flaps were forming (Fig. 2b,c). Gut organization had proceeded, and mesenchyme cells and functional (contractile) larval muscles had developed. In one-week-old larvae (Fig. 2d) a faint membranous larval shell could be observed (Fig. 2e,f). Defective larvae occurred and many larvae died.

Over half of the clams from a depth of 35 m were unspawned by 15 June 1985. Some clams harboured granulomas and parts of kinorhynchs in their tissues (cf. Pekkarinen 1985). One female spawned eggs of uneven size, including exceptionally large ones. Many eggs soon began to cleave but the egg membranes broke. Histological sections of the gonads of clams from this collecting site from 1983 (August) showed cleaved intraovarian eggs in six individuals among 11 females studied. Nuclear spindles were prominent in many eggs (Fig. 1g) and also multi-celled stages were detected (Fig. 1h,i). There were numerous inter- and intrafollicular amoebocytes. Harshbarger's (personal information) diagnosis based on a histological microslide of such a female was generalized inflammatory response and granulomas in the ovarian area. This state did not seem to be normal resorption of residual eggs. Were these females not able to spawn and if so why not? Was the reason too low a temperature at that depth, or foreign particles or diseases? Were the clams too sparsely distributed such that the "sexual milieu" was not created? It is not known whether this kind of gonad regression occurs in many spawning seasons.

The experiments, which commenced by chance, concerning the rearing of *M. balthica* larvae in the laboratory were promising. Thus, *M. balthica* does not differ from several other tellinaceans in this respect. General outlines of the egg cleavage and early larval development of *M. balthica* could be examined. Better results (more and older larvae) might be obtained if a more sophisticated culturing device were to be used and the physical, chemical and biological factors were more optimal and better controlled during culturing.

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