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Short Communication

VARIATION OF RELATIVE ORGANIC MATTER IN *MYTILUS EDULIS* L. LARVAE AND POSTLARVAE

ALBERT LUCAS, LILA CHEBAB-CHALABI and PETER BENINGER[1]
Laboratoire de Zoologie, Faculté des Sciences, Université de Bretagne Occidentale, 29287 Brest Cedex, France

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Abstract: The variations in relative organic matter (ROM: organic matter/total dry weight) were studied in larval cultures of *Mytilus edulis* L. reared at 15 °C and at 20 °C, using single and mixed algal diets. A characteristic inverted peak is observed prior to metamorphosis, with higher minimum values in cultures reared at 20 °C and fed a mixed algal diet. The shape of the ROM curve appears to reflect major physiological events in *M. edulis* larvae and is thus appropriate as a convenient condition index from the first larval stages to the post-larval phase.

Key words: organic matter; larvae; postlarvae; *Mytilus edulis*

In recent years, biochemical techniques have proved useful in the study of larval bivalve physiology and ecophysiology (Holland & Gabbott, 1971; Holland & Hannant, 1973; Bayne *et al.*, 1975; Gabbott, 1975).

Although such methods lead to a better understanding of the significant metabolic and physiological responses of bivalve larvae to environmental conditions, they do not provide a rapid assessment of the overal physiological state of the animals. In a recent review of physiological condition indices used in marine bivalve aquaculture, Lucas & Beninger (1985) noted that the ratio: dry ash weight/total dry weight (Walne & Millican, 1978) was the best quantitative static index which may be used with bivalve larvae. The present work was undertaken in order to develop a simple, rapid method for the evaluation of this ratio to allow the physiological state of cultured bivalves to be assessed from hatching until after metamorphosis and settling.

Sexually mature broodstock were collected in the Bay of Brest from March to August 1983. After cleaning the shells, stimulation of the broodstock was performed using temperature variations from 18 to 25 °C every half hour. Spawning animals were individually isolated in small containers filled with 0.2-μm filtered sea water. Fertilization was accomplished using a single pair of parents. The water was changed every two days, and from the second day the larvae were fed daily with a suspension

[1] Present address: Département de Biologie, Université de Moncton, Moncton – Nouveau Brunswick, Canada E1A 3E9.

containing 2×10^7 monocellular algae per litre. The larval density was maintained at 1.0×10^4 per litre. Algal cultures were grown using the batch culture technique, and fed to the larvae during the plateau phase of growth. The algal species used were *Pavlova* (*Monochrysis*) *lutheri* (Parke), *Isochrysis galbana* Parke and *Dunaliella primolecta* Butcher. The larvae were examined daily by stirring the culture vessels, pipetting 1 ml of larval suspension, and counting live and dead larvae using an inverted microscope.

A sufficient number of larvae to obtain ≈ 1 mg dry wt were sampled from the culture water column and recovered on a weighed 2.5 cm diameter Whatman GF/C filter. The filter and larvae were rinsed with a solution of 0.9% ammonium formate-distilled water in order to eliminate mineral salts, placed in an oven at 100 °C for 24 h, cooled in a desiccator at room temperature over activated silica gel for 12 h, and then weighed to a constant weight, thus obtaining the total dry weight of the larvae (TDW). The filter and the dried larvae were then incinerated in a muffle furnace using a three-stage temperature cycle (4 h to 500 °C, 4 h at 500 °C, 4 h to room temperature). The filter was then reweighed following the procedure used for dry weights and the amount of inorganic matter (IM) was recorded. The amount of organic matter (OM) was obtained by substracting IM from TDW. The relative organic matter index was defined as OM/TDW. All weights were determined using a Mettler H34 precision balance to the nearest 10 μg.

All larvae used were the products of the same single pair of parents. The larvae were divided into six groups according to temperature and feeding regimes: (I) 15 °C, unfed larvae; (2) 15 °C, larvae fed with *Pavlova lutheri*; (3) 15 °C, larvae fed with all three algal species; (4) 20 °C, unfed larvae; (5) 20 °C, larvae fed with *P. lutheri*; (6) 20 °C, larvae fed with all three algal species.

The rearings at 15 °C were not maintained beyond 32 days. The unfed larval culture was stopped on the 12th day, as 50% or more of the larvae were found to be dead after microscopic examination and counting. The OM/TDW ratio showed a sharp and steady decline throughout this interval (Fig. 1). The fed larval cultures presented similar OM/TDW curves: an initial decline until the 12–20th day, followed by a steady increase until approximately the 30th day. The larvae fed with all three algal species showed minimum values occurring 1 wk later than the larvae fed with *P. lutheri* only.

At 20 °C, the cultures were maintained for 87 days. The unfed larval rearing showed the same OM/TDW as that of the corresponding 15 °C batch, the 50% mortality point being reached 2 days earlier. The fed larvae showed an initial decline followed by an increase which was more rapid than that observed in the corresponding 15 °C cultures. The highest minimum value was obtained in the larval cultures fed with all three algal species. After metamorphosis, the two curves of fed larvae showed a gradual and steady decline during the postlarval period, until the end of the study period.

A common and striking feature of the OM/TDW (or ROM) curves is the initial decrease, followed by a characteristic increase just prior to metamorphosis in fed larvae (Fig. 1). This pattern may also be observed in ROM calculated from component data of Sprung (1984), in which OM and IM were determined for *Mytilus edulis* larvae of

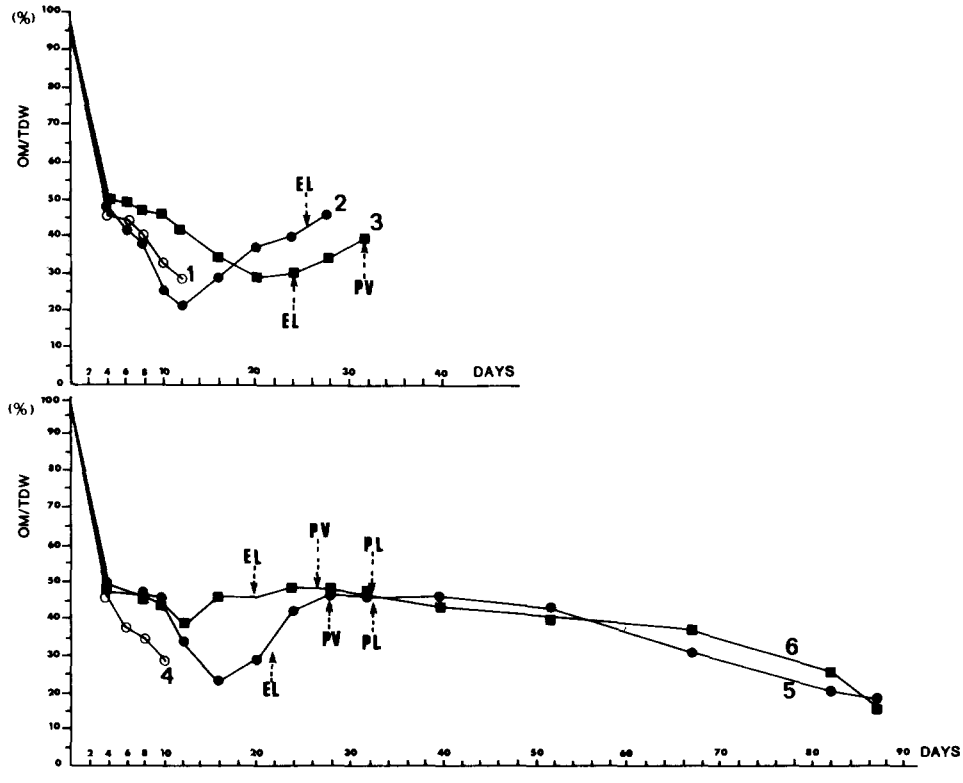

Fig. 1. The index OM/TDW (%) in *Mytilus edulis* larvae reared at 15 °C (curves 1,2,3) and at 20 °C (curves 4,5,6): curves 1 and 4, unfed larvae; curves 2 and 5, larvae fed with *Pavlova*; curves 3 and 6, larvae fed with *Pavlova*, *Isochrysis* and *Dunaliella*; EL, eyed larvae; PL, post-larvae; PV, pediveliger larvae.

different stages. Indeed, the pre-metamorphic minimum calculated from Sprung's data is 18.3%, compared to 20–23% in the present work. Such a phenomenon may also be implicit in the results of studies relating larval shell length and total dry weight (Nascimento, 1980; Jespersen & Olsen, 1982). The observations of Holland & Spencer (1973) on *Ostrea edulis* are more explicit: the proportion of inorganic matter to total dry weight decreased from 83 to 73.5% prior to metamorphosis. Conversely, Bayne (1983) cites a personal communication from M. Helm, indicating that there is an increase in dry organic weight of *Ostrea* larvae from 21–30% (shell length 180–190 μm) to 37–40% (300–310 μm). In a study on *Mytilus edulis* larvae, where absolute values of organic matter were calculated, Aldana-Aranda (1984) showed that there is a sudden increase in the weight of organic matter some days prior to metamorphosis, while the rate of shell growth and hence inorganic matter increment is constant. The characteristic ROM variations preceding metamorphosis may thus be explained on the basis of differential production of soft tissues and shell.

It is noteworthy that the unfed larval cultures did not show the characteristic ROM increase. Indeed, the cultures fed a mixture of three algal species showed higher ROM minimal values and less pronounced premetamorphic increases compared to larvae fed only with *Pavlova lutheri*, suggesting a better physiological condition in the former. This is in accord with several studies which have demonstrated superior larval growth on mixed algal diets (see Bayne, 1983 for references). It is thus likely that the pre-metamorphic increase in ROM is a good indicator of metamorphic competence in bivalve larvae.

In the larval cultures maintained past the postlarval stage (20 °C), metamorphosis occurred only after the ROM had risen to a value of 45–50, regardless of food type. Although Sprung's (1984) OM and IM data do not extend beyond the pediveliger stage, the ROM value at this pre-metamorphic phase was calculated to be 48.2. Further studies are needed in order to ascertain whether this is a critical value for metamorphic competence in *Mytilus edulis*.

Temperature may influence the shape of the ROM curve. Those larvae reared at 20 °C and fed a mixture of all three algal species showed a higher minimum ROM value, as well as an earlier pre-metamorphic ROM increase than the corresponding larvae reared at 15 °C (Fig. 1).

Following metamorphosis and settlement, the ROM undergoes a steady and gradual decrease (Fig. 1). This is probably due to the increased amount of shell production compared to tissue production in settled postlarvae. A similar observation has been made for *Ostrea edulis* larvae, where the percentage of shell increased from 73.5% (ROM = 36.5%) to 94% (ROM = 6%) of total dry weight after 25 days (Holland & Spencer, 1973). This compares to a ROM of 37–39% for *Mytilus edulis* 25 days after metamorphosis, and 16–18% 52 days after metamorphosis in the present study. The difference in ROM values after metamorphosis reflects the difference in calcification in these two species, and underscores the need for baseline evaluation of ROM in each species under investigation.

The results of the present study demonstrate that the ROM is a meaningful and appropriate index for the monitoring of physiological condition in bivalve larvae, especially in the critical pre-metamorphic phase. Its simplicity of measurement renders it particularly suitable as a routine condition index in the context of experimental cultures.

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