Larval Development of the Mussel Mytilus edulis galloprovincialis Cultured under Laboratory Conditions

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Laboratory cultures of the mussel (Mytilus edulis galloprovincialis) larvae have been attempted, in order to obtain large amounts of pediveliger larvae which are used for studies on mechanisms of larval settlement. Adult mussels were collected from either Mutsu Bay, Aomori, or Aburatsubo Bay, Kanagawa, Japan, from which D-shaped larvae were obtained by artificial fertilization and successfully cultured to the pediveliger stage. In a typical case, 7-10 individuals/ml of D-shaped larvae were cultured in 1 l and 10 l scales at 19 ± 1°C by feeding 1.0-1.5 x 10^5 cells/ml of Isochrysis galbana. They reached the pediveliger stage 14-21 days from the start of culture. Shell heights at the time of appearance of pediveligers were 171.16 ± 14.35 - 228.34 ± 24.55 μm. Survival rates when larvae reached the pediveliger stage were 48.5-86.7%. Competent pediveliger larvae were allowed to attach and metamorphose on glass slides on which microbial film had formed. Metamorphosis was completed in 2-3 days, as confirmed by secretion of the adult shell. The rate of post-larval shell growth was approximately 22 μm/day. Thus, we were successful in developing a culture method for the mussel larvae.

Key words: Mytilus edulis galloprovincialis, culture, pediveliger larvae, metamorphosis

Mussels are one of the most significant macro-fouling organisms as viewed by the many problems caused by their settlement on submerged man-made structures. Toxic antifouling organo-tin compounds have been used to control these fouling animals. However, recent increasing awareness of harmful effects on the marine environment of these toxic chemicals have prevented their use, and non-toxic antifouling substances are urgently needed.

In order to develop non-toxic antifouling agents it is necessary to understand the mechanisms of larval settlement and metamorphosis, which at present are scarcely understood. Larvae of barnacles and polychaetes can be successfully cultured for bioassay purposes; however, laboratory culture techniques of the mussel larvae are poorly developed in Japan. Bayne described in detail culture techniques for the Mytilus edulis larvae in the laboratory in consideration of various environmental factors on growth.

In the course of our studies on mechanisms of larval settlement and metamorphosis, we attempted to culture larvae of the mussel Mytilus edulis galloprovincialis in the laboratory. During our culture experiments, we observed the behavior of the larvae in detail, including their growth and survival rates, as well as the growth of the post-larvae (plantigrade) immediately after settlement and metamorphosis initiated by microbial film (the effect of microbial film to be published elsewhere). In this paper, we describe these results along with a discussion of problems encountered during culture of larvae to the pediveliger stage.

Materials and Methods

Culture of Larvae to the Pediveliger Stage

Adult mussels were collected from either Mutsu Bay, Aomori Prefecture or Aburatsubo Bay, Kanagawa Prefecture, Japan. They were kept at 4°C overnight and then transferred to a container filled with seawater at ca. 23°C, after which the temperature of the seawater decreased to ca. 20°C. Upon these treatments, mussels started spawning; adult mussels were then immediately removed from the container.

The seawater containing sperm and eggs was gently mixed and left undisturbed for 1 to 3 h. Excess sperm was washed off by filtering through a nylon plankton net (mesh opening: 20 μm), resuspended in filtered seawater, and left undisturbed for two days. Next, swimming D-shaped veliger larvae were decanted and gently washed prior to culture experiments.

A series of cultures were conducted on April 22 and July 9, 1992 in 1 l scales using glass beakers, and on March 25 and April 29 of 1993 in 10 l scales using 50 l polycarbonate tanks. Cultures of the larvae were conducted basically according to the methods described in Bayne. Culture was commenced at a larval density of 7-10 larva/ml by feeding with Isochrysis galbana at concentrations of 1.0-1.5 x 10^5 cells/ml. Every other day, larvae were washed by filtration through a nylon plankton net (mesh opening: 50 μm) and transferred to clean containers containing Isochrysis galbana suspension. Larvae were cultured to their pediveliger stage.

Culture was performed at 19 ± 1°C under a light intensity of less than 100 lx. Seawater used for cultures was filtered seawater (Whatman glass fiber filter, GF/C) with a salinity of ca. 28 ppt.

Growth and Survival Rates of Larvae

Larvae were randomly sampled from the culture before being transferred to newly prepared food suspensions. The size of each larva was measured according to definitions in Tanaka. Shell height (SH) was measured along the antero-posterior axis, while shell length (SL) was measured as the width with respect to the shell height. In most cases in this paper, the growth of the larvae was expressed by the increase in shell size of the individual. Survival rates of larvae during culture were also measured at each stage of development.

Growth Rates of the Post-larvae

When more than 90% of the larvae reached the eyed-veliger stage (30-day-old culture), they were sampled and allowed to attach and metamorphose by exposing them to microbial film coated glass slides in order to obtain post-larvae (plantigrade). Microbial film coated glass slides were prepared by immersing clean glass slides in an established aquarium for 2-4 weeks, thereby acquiring a film consisting mainly of bacteria, benthic diatoms and protozoa. Metamorphosis was confirmed by secretion of the adult shell as described by Bayne. Shell height (SH) and shell
length (SL) were measured daily after 48 h from exposure to microbial film. Only the growth rates of individuals which metamorphosed within the first 48 h after exposure to microbial film were recorded. Similarly, post-larval growth was expressed by the increase in shell size of the post-larvae.

Results

Figure 1 shows the typical stages of development of the mussel cultured in the laboratory, from larvae to newly metamorphosed planigrade (post-larvae). Figure 1-a shows a 3-day-old D-shaped larva with dimensions of 86.80 μm × 110.50 μm (SH × SL); Fig. 1-b, a 15-day-old veliconcha with dimensions of 154.26 μm × 117.71 μm; and Fig. 1-c, a 22-day-old pediveliger with dimensions of 209.90 μm × 224.10 μm and with a functional foot. Figure 1-d shows a 72-hour-old planigrade with a shell size of 344.19 μm × 269.79 μm (SH × SL). The newly secreted adult shell can be confirmed by the difference in color from the larval shell.

Growth and Survival Rates of the Larvae

Figure 2 shows the growth and survival rates of the larvae to the pediveliger stage in 1 l culture batches. The average increase in shell height during the culture period was measured for culture batches on April 22 and July 9, 1992, while the survival rate of the July 9 batch was recorded. The appearance of veliconcha was first observed on Days 14 and 15 from the start of culture for the batches of April 22 and July 9, respectively. Shell height during this period were 157.81 ± 22.33 μm for the April 22 batch and 148.60 ± 11.12 μm for the July 9 batch. The appearance of pediveligers was observed on Day 21 for both batches. Shell height during this period for the April 22 batch was 201.90 ± 15.17 μm and that for the July 9 batch was 228.34 ± 24.55 μm. In the July 9 culture batch, the survival rate of larvae decreased to 51.3% at the time when veliconcha larvae appeared, and decreased slightly to 48.5% at the appearance of pediveligers on Day 21. The final survival rate of the larvae during the 30-day culture period was 40.0%.

In 10 l scale cultures started on March 25 and April 29, 1993, growth and survival rates improved to some extent as shown in Fig. 3; the appearance of veliconcha was observed on Day 7, while pediveligers appeared on Days 14 and 15, respectively. Shell heights were 131.48 ± 9.57 μm for the March 25 batch and 120.01 ± 11.98 μm for the April 29 batch when veliconcha larvae appeared, and they increased to 182.44 ± 24.31 μm and 171.16 ± 14.90 μm, respectively at the appearance of pediveligers. In the March 25 culture batch, the survival rate of larvae at the appearance of veliconcha was 88.3%, and this was 86.7% at the

Fig. 1. Growth stages of the mussel.
D-Shaped larva (1-a), veliconcha (1-b), pediveliger (1-c), and plantigrade (1-d).

Fig. 2. Growth and survival of M. edulis galloprovincialis larvae when cultured in 1 l scales.
Circles represent April 22 batch; squares, July 9 batch; and shaded marks, survival rate. Vertical bars indicate standard deviation.

Fig. 3. Growth and survival of M. edulis galloprovincialis larvae when cultured in 10 l scales.
Circles represent March 25 batch; squares, April 29 batch; and shaded marks, survival rate. Vertical bars indicate standard deviation.
appearance of pediveligers. After 19 days from the start of culture approximately 80% of the pediveligers were observed to possess an eye-spot; the survival rate was 76.7%. From this stage, most of the larvae swam around the bottom of the culture containers. High mortality was sometimes observed when culture was further continued. This may be due to stress from over-stocking and the eventual propagation of bacteria and protozoa in the culture. Culture density beyond this stage was therefore decreased to 3–5 larvae/ml.

Growth Rates of the Post-larvae (Plantigrade)
A marked morphological change was observed between pre-metamorphosed pediveliger larvae and 72-hour-old post-larvae (plantigrade) as illustrated in Fig. 4. Pediveliger larvae had slightly larger shell length than shell height ($y = 0.72X + 66.15; r = 0.76$), whereas plantigrades had greater shell height than shell length ($y = 1.16X + 28.67; r = 0.72$). This change of shape occurred with the secretion of the disconch shell. The growth rate of the adult shell is represented in Fig. 5, which indicates that the shell height of the plantigrade increased proportionally with the lapse of time at a rate of approximately $22 \mu m/day$.

Discussion
In a series of cultures conducted in both 1992 and 1993, we were able to obtain pediveliger larvae within 14 to 21 days from the start of the culture, which is comparable to the results obtained by Bayne (1991) (16–20 days at 16°C). The shell heights at the appearance of pediveligers ranged from 171.16 ± 14.35 to 228.34 ± 24.55 μm. These values were smaller than those measured by Bayne (259.5 ± 4.7 μm when 50% of larvae in his culture reached the pediveliger stage). Naturally, larvae still continue to grow, whileremaining at the pediveliger stage of development. Bayne observed that individual larvae seldom reached the pediveliger stage before 250 μm. Our observations also indicate a large variation in size at the pediveliger stage, which is in contrast to the uniformity of size of the pediveliger larvae as reported by Bayne. The survival rates in our cultures varied from 48.5–86.7% at the pediveliger stage. However, Bayne did not mention the mortality rate of larvae during his cultures. It should be noted that newly developed pediveligers were still not competent to metamorphose and were unsuitable for use in settlement bioassays.

Generally, specific morphological features, such as a functional foot, the presence of an eye-spot and/or shell size, are important indicators for the competence of metamorphosis in mussel larvae. However, none of these features were applicable to our case. Instead, we were able to induce about 90% metamorphosis of 26-day-old larvae cultured at 19°C by exposing them to microbial films. While the potency of microbial film to induce metamorphosis is an important point to consider, it is beyond the scope of this paper and is therefore excluded from discussion. Eyster and Pechenik discussed that, for the time being, competence of metamorphosis can be verified only by successfully inducing larvae to metamorphose. Hence, we found it necessary to extend the culture period to about 4 weeks, in order to obtain "metamorphosis-competent" pediveliger larvae for bioassays using microbial film.

Bayne described the gross morphological changes which occurred during metamorphosis, the most obvious of these events being the disappearance of the velum and the consequent change in shell shape. The entire process of metamorphosis was completed within 2 days. We also obtained similar results. The rate of post-larval shell growth was high (22 μm/day), and therefore newly secreted adult shell was easily observed under the microscope within 2 to 3 days after exposure to microbial film. Hence, confirmation of adult shell secretion can be an accurate indicator of metamorphosis during bioassays.

In conclusion, we were able to constantly produce pediveliger larvae using the culture method described above. During the culture of larvae, we observed that problems, such as over-stocking at the initial stage of culture and...
propagation of bacteria, may cause detrimental effects to the larvae. These problems must be considered in order to reduce mortality when culturing the pediveliger larvae until the stage in which they are competent to metamorphose. Finally, other indicators to verify the condition of larvae cultured should be established to standardize the results of bioassays.

References