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Aspects of the technology of phyllosoma rearing and metamorphosis from phyllosoma to puerulus in the Japanese spiny lobster *Panulirus japonicus* reared in the laboratory

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Abstract Japan has a long history of more than 100 years in the development of rearing technology for phyllosomas of the Japanese spiny lobster, *Panulirus japonicus*. The first attempt to rear newly hatched phyllosomas was made in 1899, and it took about 90 years to succeed in rearing them to the puerulus and juvenile stages. The Minamiizu Station of the National Center for Stock Enhancement, Fisheries Research Agency, started to develop its technology for rearing the phyllosomas of this lobster in 1989, when it succeeded in rearing juveniles in the laboratory for the first time, although researchers at the station anticipated that the task would be difficult. We divided the technological development into two subject areas: development of rearing methods and development of diets. We worked on issues such as methods of treating the rearing water, optimum water temperature for rearing, new devices for mass-rearing, and measures to prevent bacterial disease, and we also set up tasks such as exploring foods and developing artificial diets to replace the gonad of the blue mussel, *Mytilus galloprovincialis*. We primarily used rearing tanks with a capacity of less than 50 L. As the technology developed, we were able to gradually increase the numbers of juveniles produced each year from several tens in 1994 to a few hundred in 2005.

Because of recent advances in the technology covering the period from hatching to metamorphosis into pueruli, we succeeded in observing 270 processes of metamorphosis in 2005, using final-stage phyllosomas reared for 228 to 429 days after hatching. The process of metamorphosis from phyllosoma to puerulus was divided into five stages: contraction of eyestalks, contraction of pereiopods, molting of pereiopods, molting of abdomen, and molting of antennae. We also clarified the apparent characteristics of metamorphosis and the time required for metamorphosis at each stage, and we revealed that the metamorphosis process took about 10 min from the very beginning to the end.

Key words: *Panulirus japonicus*, phyllosoma, rearing technology, metamorphosis, puerulus

Japan has a long history of artificial rearing of phyllosomas of the Japanese spiny lobster, *Panulirus japonicus*, and a rearing trial of newly hatched phyllosomas was first reported in 1899, more than 100 years ago (Hattori and Oishi, 1899). In 1958 phyllosomas were reared until they grew into third instars by being fed *Artemia salina* nauplii (Nonaka *et al*., 1958), and in 1981 they were reared to the final stage immediately before metamorphosis into pueruli by being fed *Artemia*, arrow worm (*Sagitta* spp.), and fish larvae and juveniles (Inoue, 1981). In 1989 Japan succeeded in rearing phyllosomas up to pueruli and lobster juveniles for the first time in the laboratory (Kittaka and Kimura, 1989; Yamakawa *et al*., 1989). Thus 90 years of research elapsed before newly hatched phyllosomas were reared successfully for the first time. It was the Mie Prefectural Fisheries Research Station (currently the

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Mie Prefectural Science and Technology Promotion Center - MPSTPC) and Kitazato University that first succeeded in rearing phyllosomas to the juvenile stage. MPSTPC used stagnant conditions, whereas Kitazato University took advantage of a closed recirculating system. Both institutions utilized *Artemia* and gonads of the blue mussel, *Mytilus galloprovincialis*, as food, and succeeded in rearing phyllosomas up to pueruli and juveniles.

The Minamiizu Station of the Japan Sea Farming Association (JASFA), which is currently called the Minamiizu Station of the National Center for Stock Enhancement, Fisheries Research Agency (NCSE, FRA) was established in 1989, the same year in which artificial rearing of phyllosomas up to juveniles was first reported. JASFA started to move on technological development to establish a mass-culture technology for Japanese spiny lobster phyllosomas. This station has specified rearing methods and natural and artificial diets as its two research subjects. In 2000 Minamiizu station joined a project team to develop a seed-production technology for decapod crustaceans; this project was designed to develop a mass-production technology for rearing 10 species of decapod crustaceans under study at seven out of the 16 stations of NCSE, FRA. In the initial stages of the research our station produced only a few pueruli and juveniles. Subsequently, as a result of technological development, the number of juveniles produced in our laboratory each year gradually increased to over 50 in 1994 and to over 200 in 2005 (Fig. 1). In addition, the survival rate from puerulus to juvenile stage was 30% to 50% before 2001, but it stabilized at a high level (60% to 80%) after 2002 (Fig. 1).

Here, we summarize both the technological aspects of phyllosomal rearing developed at our station and the process of metamorphosis, which we clarified during the development of a technology for mass rearing of final-stage phyllosoma larvae.

Technology of phyllosoma rearing at Minamiizu Station, NCSE, FRA

The technological development of lobster phyllosoma rearing has been extremely hard, for the following reasons:

1) The larval period lasts for nearly a year: the

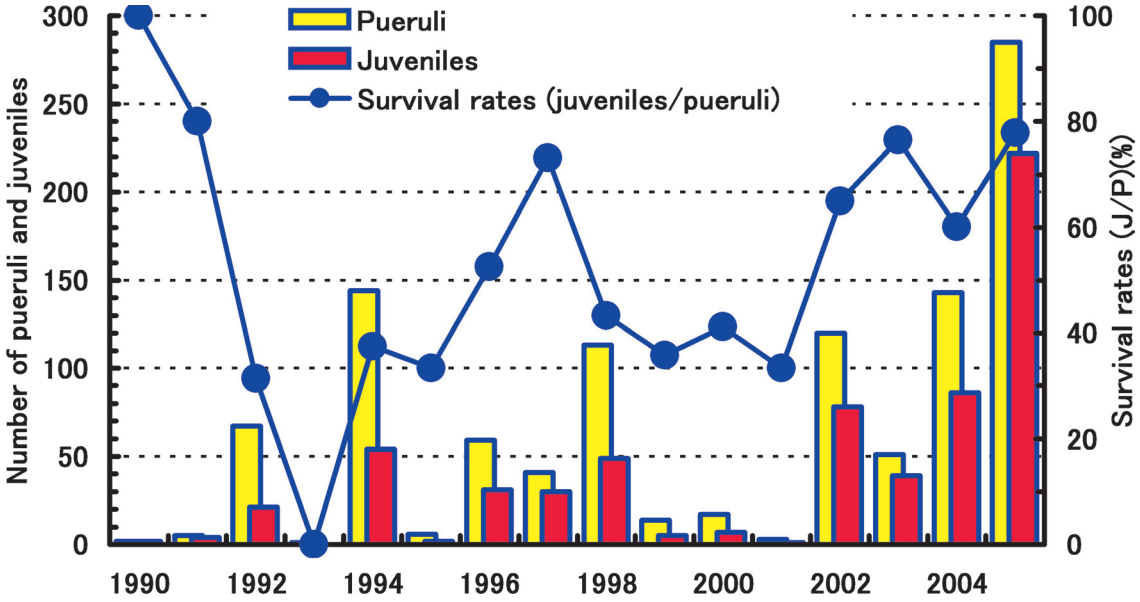

Fig. 1. Numbers of pueruli and juveniles and survival rates from pueruli to juveniles of *Panulirus japonicus* produced at Minamiizu Station, NCSE, FRA. J, juveniles; P, pueruli.

lobster grows from about 1.5 mm to 30 mm in body length (BL) from hatching to final-stage phyllosoma.
2) The larva has a transparent and flat body form and extremely long maxillipeds and pereiopods. Because of this extraordinary form, larvae are liable to injure their pereiopods and body surfaces through contact with other larvae.
3) The water temperature suitable for rearing is as high as 24 to 27℃ , at which temperature larvae are prone to infection with bacterial diseases.
4) The only food that can be used to rear phyllosomas up to the puerulus stage is the gonad of the fresh blue mussel.

Since it was established in 1989, the Minamiizu Station of NCSE, FRA has been carrying out research on the following two subjects to solve these difficult problems.

Development of technical skills to rear phyllosoma larvae

We have been researching the following technological factors to allow stable rearing of phyllosomas: methods of treating water for rearing; appropriate water temperatures; development of rearing tanks; and measures to prevent bacterial disease.

We tried three ways of pretreating the seawater used for rearing of phyllosoma: filtration by sand, filtration by 0.2-μm hollow fiber membrane that could physically eliminate bacteria; and sterilization by ultraviolet irradiation after filtration by simplified 0.45-μm cartridge filter. Comparative analysis of the three methods revealed that filtration by sand allowed larvae to survive for about 30 days after hatching, but the other methods enabled them to grow to the puerulus stage (Fig. 2). These results indicated the need to rear phyllosomas in clean seawater from which as many bacteria as possible had been eliminated. We also found that addition of antibiotics to the rearing water was essential to prevent bacterial disease, horizontal infection, and contamination of the body surface by bacteria while the phyllosomas were being reared.

After preliminary testing of several water temperatures to clarify which was best for rearing phyllosomas, we used rearing water at 24 and 27 ℃ to compare growth to the puerulus stage. At 27 ℃ all phyllosomas had died by about 220 days after hatching, whereas at 24℃ they grew to pueruli (Fig. 3). These survival curves crossed between 140 and

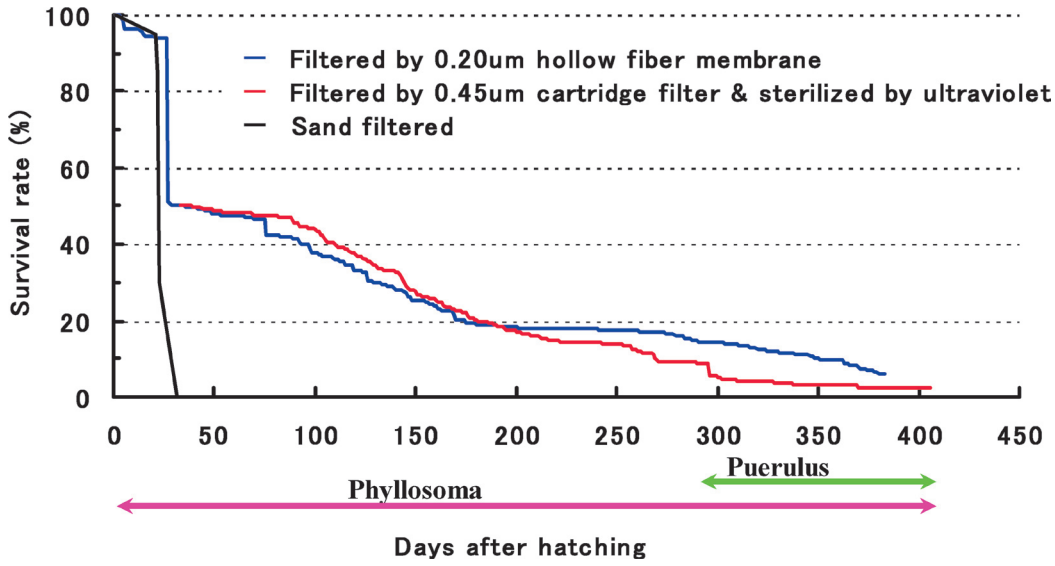

Fig. 2. Survival rates of *Panulirus japonicus* phyllosomas reared in 50-L acrylic semispherical tanks containing seawater treated as follows: filtration by hollow fiber membrane (pore size: 0.20 μm), sterilization by ultraviolet after filtration by cartridge filter (pore size: 0.45 μm), and filtration by sand.

150 days after hatching. We therefore concluded that 27℃ was appropriate for the period 140 to 150 days after hatching and 24℃ was appropriate after that period. This water temperature setting is still our standard for rearing phyllosomas.

The basic approach to developing a rearing tank is to construct a system that, by taking into account the species' body form and feeding behavior, allows efficient feeding and prevents the phyllosomas from damaging their pereiopods and other body parts. Efficient feeding increases the chances of encounters between the phyllosomas and the food and prevents the loss of pereiopods. In particular, it is necessary to decrease the degree of contact among phyllosoma larvae just before and after molting. A tank with a spherical bottom, which Nonaka and Inoue had already tried in the 1960s (Nonaka, personal communication), had been found to be effective in decreasing contact and dispersing phyllosoma larvae. Our station modified this tank design to create a bowl-type flow-through tank (600 mm in diameter, 500 mm high, actual capacity of 50 L; Fig. 4; Sekine, 1995), and we continued our rearing experiments using this type of tank. It thus became possible to rear many phyllosomas from hatching to puerulus, although the rearing results still lacked stability (Sekine *et al*., 2000). Nevertheless, this flow-through tank is still the standard for rearing phyllosoma. Fresh gonad of the blue mussel is an essential food for rearing phyllosomas up to the puerulus stage. Because small pieces of mussel gonads fall to the bottom of the tank, the conventional method creates a situation that allows phyllosomas to pick them up and eat them accidentally. In 2000, we started to develop a vertically revolving rearing system (VRR System; 300 mm wide, 600 mm in diameter, actual capacity 70 L; Fig. 5) that could create water flow of any strength inside the tank by revolving the whole rearing tank vertically; the aim was to increase the chances of encounters between the phyllosomas and the small pieces of mussel gonads (Murakami, 2004a). We used the VRR system to rear phyllosomas on an experimental basis and found that it encouraged their swimming activity; the survival rate to pueruli almost tripled in a few cases (Murakami, 2004a, b). However, this early-type VRR system tended to increase the number of contacts between middle- and late-stage phyllosomal larvae of more than 15 mm BL and to thus increase the number of phyllosomas with injured pereiopods. Improvement is still under way.

Bacterial diseases that occur during the rearing of

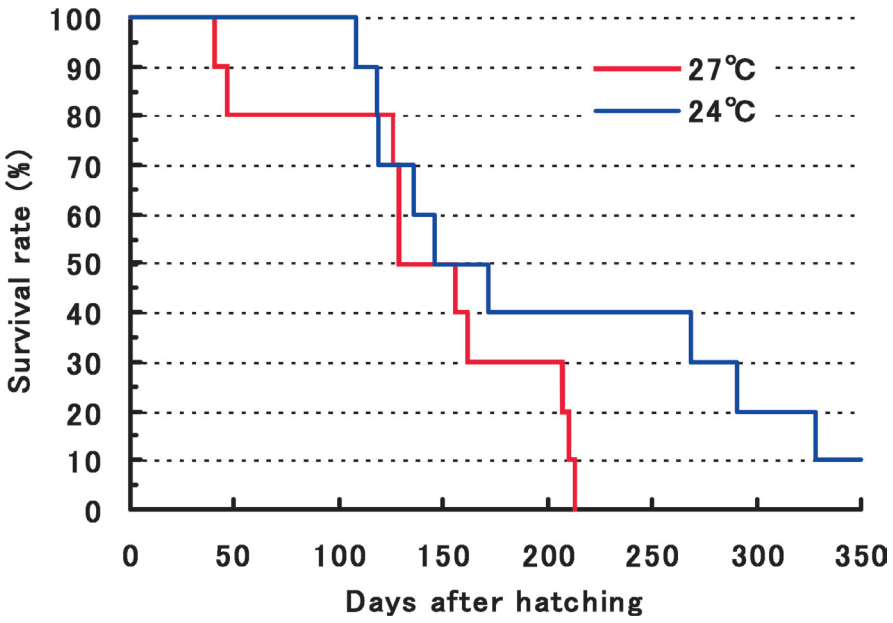

Fig. 3. Survival rates of *Panulirus japonicus* phyllosoma larvae reared in 1-L glass bowls at 24℃ or 27℃ .

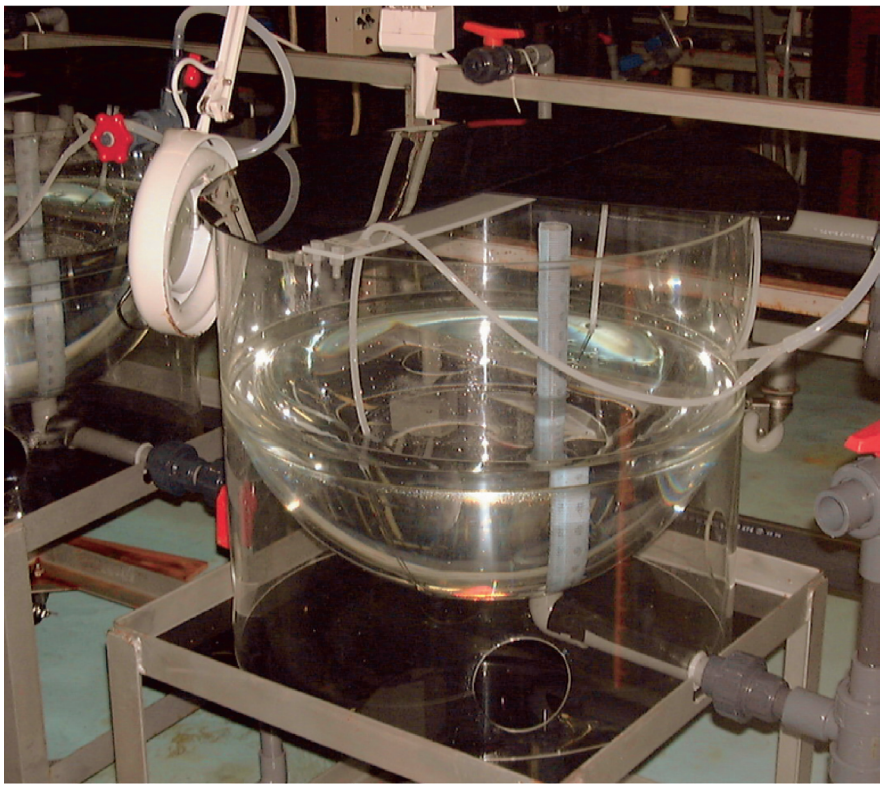

Fig. 4. An acrylic semispherical tank with a volume of 50 L. This is the type used mainly for *Panulirus japonicus* phyllosoma rearing.

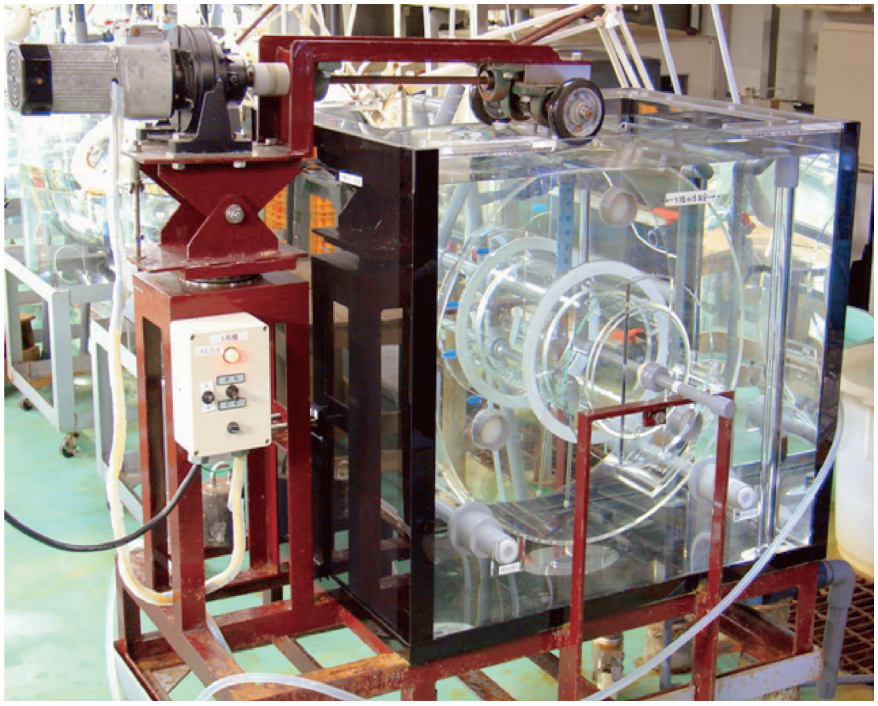

Fig. 5. The vertically revolving rearing system (VRR System), newly designed for *Panulirus japonicus* phyllosomas.

phyllosomas cause increased opacity due to necrosis of such parts as the antennal gland, hepatopancreas, hindgut, mandibles, maxillae, maxillipeds, and pereiopods, in addition to incomplete molting. The latter is caused by the filamentous bacterium *Leucothrix mucor*, which presumably originates from the egg and becomes implanted on the body surface of the phyllosoma. Use of an antibiotic-medicated bath is effective in preventing secondary infection with these diseases. It is effective to immerse the larvae in streptomycin sulfate or ampicillin 10 ppm for at least 15 h to prevent filamentous bacterial disease, which occurs predominantly in early-stage phyllosomas. Immersion of the phyllosomas in chloramphenicol 10 ppm for 24 h was effective in preventing increased opacity of the maxillipeds, pereiopods, and hepatopancreas. Furthermore, such bacterial diseases could be prevented effectively by making it a rule to change the rearing tank and immerse the phyllosomas in ampicillin 20 to 40 ppm for 15 to 18 h about once a week (Murakami and Hashimoto, 2003a). Under this rearing protocol, the number of bacteria in the rearing water can be expected to decreased to $<10^3$ CFU/mL (Murakami, 2004b). Adding antibiotics to the rearing water is essential for rearing phyllosomas from hatching to juveniles.

Exploitation of foods for phyllosoma larvae

Artemia enriched by the diatom *Phaeodactylum tricornutum* is used alone to feed phyllosomas up to 30 days after hatching; the gonad of the blue mussel, together with grown *Artemia* whose size is adjusted by culture, is used after this larval period. At this point, it is impossible to rear phyllosomas to pueruli only on *Artemia*, and only addition of the mussel gonad can enable them to grow into healthy pueruli.

To rear phyllosomas, we tried a total of more than 40 food species, including 15 species of zooplankton; five species of fish larvae and juveniles; the muscles, livers, and gonads of two crustaceans; six shellfish and three fish species; and the livers of cows and pigs (Sekine, 1999). We found that no food could be substituted for the gonad of the blue mussel in phyllosoma rearing. In feeding experiments tried recently, spontaneous ingestion of frozen sergestid shrimp (*Sergia lucens*) and frozen sand lance, *Ammodytes personatus*, by late-stage phyllosomas made it possible to rear them for half a month to pueruli, but these pueruli did not live long enough to become juveniles (Murakami and Hashimoto, 2003a). We used frozen sergestid shrimp to replace the mussel gonad for rearing early-stage phyllosoma larvae, and we succeeded in rearing them for more than 250 days. However, the phyllosomas did not grow as well on frozen sergestid shrimp as they did on mussel gonad (Murakami and Hashimoto, 2003b). At present, the fact remains that the gonad of the blue mussel is still the best food for phyllosoma rearing (Murakami and Hashimoto, 2004).

We examined a few food liaisons to prepare artificial diets, but we found that artificial diets that were palatable were made mainly of sea urchin, *Hemicentrotus pulcherrimus*, or mussel gonad with 2% alginate sodium additive. Each of them could be used for short periods of about 2 months, but phyllosomas reared on either of them had considerably lower growth rates that those reared on fresh mussel gonad. Even now, we are making efforts to develop artificial diets that ensure stable supply in terms of volume and preservation for long periods.

Metamorphosis from phyllosoma to puerulus

Highly active final-stage phyllosoma larvae just before metamorphosis are seldom captured in the wild, because they inhabit the pelagic ocean and their long pereiopods are easily injured by the sampling gear (Yoshimura, 2005). Similarly, it is extremely hard to rear large numbers of highly active final-stage phyllosoma larvae, as indicated by the results obtained during the long history of research on the rearing of phyllosomas. Therefore, until now, the process of metamorphosis from phyllosoma to puerulus had remained unknown. As a result of the recent improvements in the technology of phyllosoma rearing, in 2005 it became possible to rear relatively large numbers of final-stage phyllosoma larvae, and we succeeded in observing the metamorphosis process in detail under laboratory conditions.

We used 270 final-stage phyllosoma larvae reared for 228 to 429 days after hatching to observe their

Fig. 6. Photograph of first stage (contraction of eyestalks) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

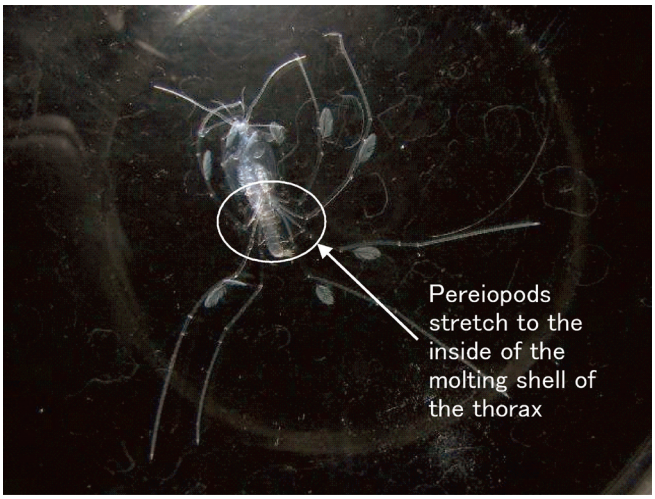

Fig. 7. Photograph of third stage (molting of pereiopods) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

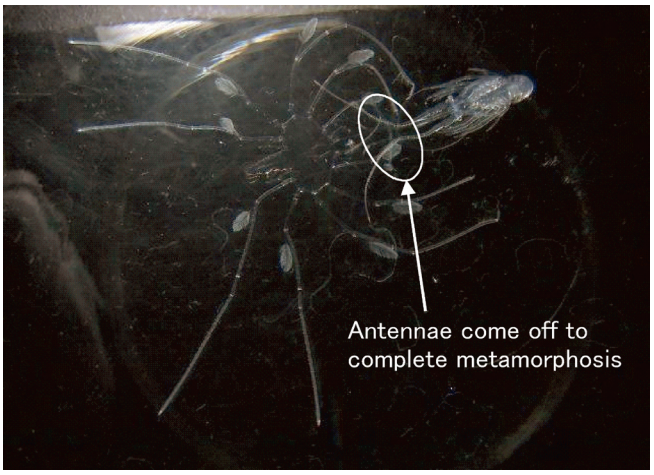

Fig. 8. Photograph of fifth stage (molting of antennae) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

metamorphosis to pueruli. These phyllosoma larvae became pueruli in 245 to 449 days after hatching; the average time taken was 319.5 days, with a standard deviation of ±37.8 days. From the very beginning to the very end, the metamorphosis process could be divided into five stages of visible change: contraction of eyestalks, contraction of pereiopods, molting of pereiopods, molting of abdomen, and molting of antennae.

We were able to confirm the beginning of metamorphosis from the time when the V-shaped eyestalks gradually broke off at their bases toward the cephalon side to become linear. In the first stage (contraction of eyestalks), the eyestalks further break down toward the cephalon side to become a reverse V shape, and the motion of the exopodites stops (Fig. 6). In the second stage (contraction of pereiopods) the pereiopods contract to the bases of the exopodites, and the tips of the dactylopodites can be confirmed inside the molting shell. In the third stage (molting of pereiopods) the pereiopods completely molt inside the thorax shell and the thorax creeps inside the cephalon to form the cephalothorax of the puerulus (Fig. 7). The eyestalks that began contracting in the first stage contract further to the bases of the antennae to become V-shaped again. In the fourth stage (molting of the abdomen) the abdomen rises inside the molting shell of the thorax to make inroads between the cephalon and thorax, and the cephalothorax and abdomen of the puerulus spring outside the shell almost simultaneously to molt. In the fifth stage (molting of antennae) the puerulus jumps backward by its abdomen, and the eyestalks, antennules, and antennae molt, in that order (Fig. 8). When the antennae have finished molting, the process of metamorphosis to puerulus is complete. The total time required for metamorphosis from phyllosoma to puerulus was 10 min and 39 s (N = 178; standard deviation ±2 min and 10 s).

Perspectives

Minamiizu Station of the NCSE, and the MPSTPC, are the only two institutions in Japan that are conducting research on the rearing of phyllosomas of the Japanese spiny lobster. The two institutions hold review meetings on a regular basis to deepen their exchange and thus improve the technology of rearing phyllosomas. For the 2005-2008 plan, the FRA initiated a national project commissioned by the Agriculture, Forestry and Fisheries Research Council. Tokyo University of Marine Science and Technology and MPSTPC are participating in the project. They aim to develop an optimum food and to confirm suitable rearing conditions for the healthy growth of phyllosomas; they also aim to greatly increase the survival rate of phyllosomas from hatching to juveniles. Hopefully the fruits of this project will resolve the remaining problems and improve larval rearing technology in seed production of the Japanese spiny lobster.

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