

## Development of technology for larval *Panulirus japonicus* culture in Japan: A review

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**Abstract** Of six *Panulirus* lobsters that occur in the shallow waters of Japan, the Japanese spiny lobster *Panulirus japonicus* is the most abundant and the most important to Japanese coastal fisheries. Hence, Japan has had a long and great interest in the propagation and aquaculture of this lobster. Research on larval (phyllosoma) lobster culture in Japan commenced in 1898, so there have now been over 100 years of research and development. However, specific biological characteristics of phyllosoma, such as their peculiar body form, protracted lifespan (ca. 1 year), and pelagic open-ocean life, have hindered significant progress in culture. The first complete culture from hatch to juvenile stage occurred in 1988 at Mie Prefectural Science and Technology Promotion Center, exactly 90 years after the first trial. Subsequently, the number of juveniles produced in the laboratory per year has increased gradually up to ca. 300 in 2003, a result that reflects the increasing availability of information on optimal culturing conditions, such as optimal environmental parameters, feeding, and tank design. Still, there are significant problems to overcome in the establishment of large-scale culture of phyllosoma. These further challenges include the control of bacterial diseases and excessive aggregation of larvae, the use of prepared diets such as artificial foods, and the reduction of high operating costs. At present, these problems are being examined in an effort to increase the number of larvae cultured in a single tank and to stabilize larval culture by controlling bacterial levels.

**Key words:** *Panulirus japonicus*, phyllosoma, culturing conditions, tank design

### Introduction

The Japanese spiny lobster *Panulirus japonicus* is distributed along the central and southern Pacific coasts of Japan and in the waters of southern South Korea and northern Taiwan (Sekiguchi, 1997). This lobster is highly valued as seafood and holds an important position in the coastal fisheries of Japan; the total annual catch in Japan ranged from 969 to 1691 tonnes during the period 1951-2002 and generated US\$52 million in 2002. However, the lobster fishery is fully exploited (Yamakawa, 1997) and the development of aquaculture is required for further expansion of production. The greatest barrier to the establishment of aquaculture is the difficulty in producing large numbers of juveniles

through larval culture. Despite numerous efforts to improve the technology of larval culture, large-scale larval culture has not been achieved (Saisho, 1962, 1966; Inoue, 1981; Matsuda and Yamakawa, 1997; Sekine *et al.*, 2000). Factors that have hindered phyllosoma culture are:

- 1) the peculiar form of the body (extremely long and thin maxillipeds and pereopods)
- 2) the tremendously long life (almost 1 year)
- 3) frequent occurrence of bacterial diseases, probably because of the larval pelagic open-ocean life
- 4) induction of excessive aggregation as a negative photoreaction
- 5) availability of few food regimes and little information on optimal feeding conditions

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succeeded in producing pueruli of a hybrid between *Jasus novaehollandiae* and *Jasus edwardsii* (Kittaka *et al.*, 1988) and of *Palinurus elephas* (Kittaka and Ikegami, 1988).

Yamakawa *et al.* (1989) cultured about 1000 newly hatched larvae of *P. japonicus* in 1-L glass vessels under stagnant conditions, and they successfully obtained a juvenile lobster in 1988. Shortly after this success, Kittaka and Kimura (1989) obtained two juveniles. Phase III in the development of phyllosoma culture started with these successes in 1988. Thereafter, complete larval cultures of this species were achieved by the Tazaki Pearl Co., Ltd. (cited in Kittaka, 2000) and the Japan Sea Farming Association (Sekine, 1995). Information began to accumulate on the optimal culturing conditions, such as optimal environmental parameters and feeding, and on the biological and physiological aspects of larvae; this resulted in increasing survivorship in small-scale cultures in 100- to 1000-mL vessels (*e.g.*, Matsuda and Yamakawa, 1997; Matsuda *et al.*, 2003). When phyllosoma were cultured communally in larger tanks, however, rates of survival from hatching to the juvenile stage were still low because there was little information on the optimal conditions for culturing the middle and late stages in large tanks. Recently, the use of new devices for mass culture has been proposed (Murakami, 2004; Matsuda and Takenouchi, 2005), and the number of juveniles produced in the laboratory has been increasing gradually, up to ca. 300 in 2003 (Matsuda, 2004).

At present, research on the culture of *P. japonicus* larvae is under way at two research institutes in

Japan: MPSTPC and the Minamiizu Station, National Center for Stock Enhancement, Fisheries Research Agency. A national project aimed at further developing phyllosoma culture commenced in 2005, and other institutes joined the project to develop feeding methods and clarify optimal environments. This project is expected to produce further advances toward large-scale phyllosoma culture.

### Phyllosoma culture technologies

There are many factors that affect phyllosoma survival and growth. These factors can be classified into four categories: environmental parameters, nutrition, culture management, and equipment and facilities (Table 1). This section will explain some of the factors that have marked effects on growth and survival.

#### Temperature

Temperature is one of the most influential and important factors in phyllosoma culture. Low temperatures can cause larval mortality, because the intermolt period is prolonged and consequently the larval body becomes tainted with foods given and debris floating on the surface of the culture tank. On the other hand, high temperatures induce deterioration of seawater and an imbalance between energy uptake and consumption. Hence, it is crucial to determine the optimal temperature for larval culture. Fig. 2 shows the relationship between larval growth efficiency (as calculated from the amount of carbon used for growth as a percentage of the total amount of carbon consumed by the larvae) and

**Table 1.** Factors that affect phyllosoma growth and survival

Category	Factor
Environmental parameters	Temperature; Salinity; Photoperiod; Light intensity; Water flow rate; Water current; Larval density
Nutrition	Kind and quality of food; amount of food; Food size
Culture management	Frequency of tank water change; Flow-through or re-circulation systems
Equipment and facilities	Tank design; Filtration and sterilization of seawater

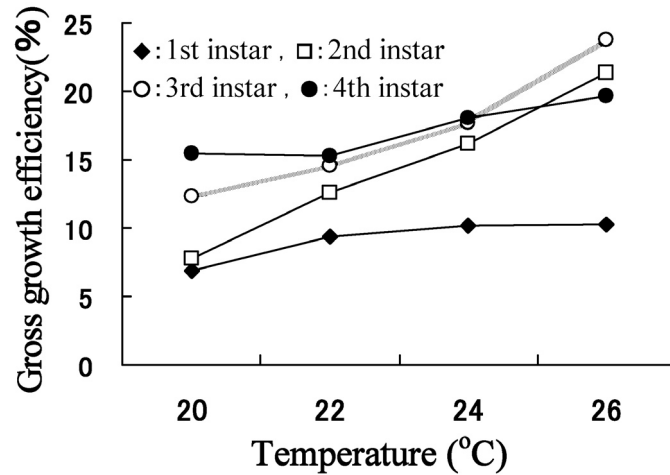


Fig. 2. Effect of temperature on gross growth efficiency (as calculated from the amount of carbon used for growth as a percentage of the total amount of carbon consumed by the larvae) of first to fourth instar phyllosoma of *Panulirus japonicus* (Matsuda, 2005).

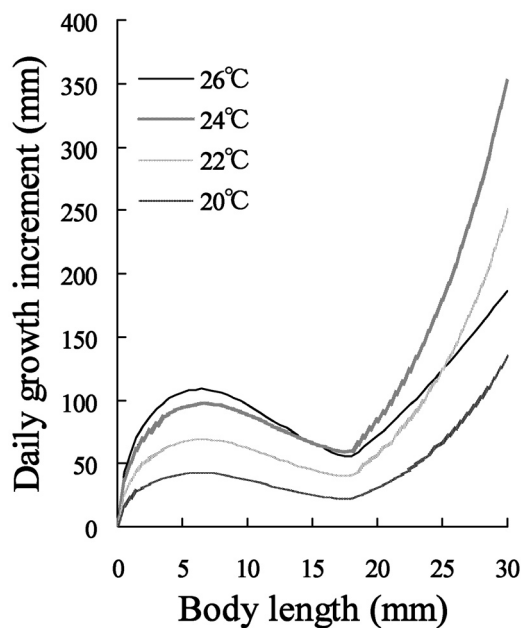


Fig. 3. Effect of temperature on daily growth increment, as calculated from molt increment in body length divided by intermolt period, throughout the entire phyllosoma phase of *Panulirus japonicus* (Matsuda and Yamakawa, 1997).

temperature in the early stage phyllosoma (Matsuda, 2005). Growth efficiency increased as temperature increased. On the assumption that the temperature that gives the highest efficiency is optimal, 26°C is the optimal temperature for these early stages.

Matsuda and Yamakawa (1997) examined the effect of temperature on daily growth increment (DGI), calculated from the molt increment in BL divided by the intermolt period, throughout the entire phyllosoma phase. Smaller larvae (<ca. 15 mm) showed the largest DGI at 26°C; for larvae that were around 15 mm long the 26 and 24°C lines were very close together; larger larvae showed the largest DGI at 24°C (Fig. 3). Larval mortality rates showed a trend similar to that of DGI: survival rate decreased as temperature increased for larger larvae (Matsuda and Yamakawa, 1997). These results indicate that the optimal temperature is 26°C for larvae smaller than around 15 mm BL and 24°C for larger larvae.

#### Light

Photoperiod and light intensity are also important in culturing phyllosoma because they affect behavior and physiological conditions and, in turn, growth and survival. When phyllosoma are cultured under a natural light-dark cycle they molt synchronously at around dawn, except during a period of about a month after hatching (Fig. 4); the timing of molting is regulated by an endogenous rhythm that is induced by the light-dark cycle (Matsuda *et al.*, 2003). This suggests that a stable light-dark cycle is important for culturing *P. japonicus* phyllosoma,

because a sudden change in photoperiod may disturb the molting rhythm of the larvae, as has been seen in a scyllarid lobster (Mikami, 2005). However, phyllosoma cultured under light phases of three lengths (10, 12, and 18 h) in a day showed no significant differences in growth and survival (Matsuda *et al.*, 2003).

Newly hatched larvae of *P. japonicus* display a strongly photopositive reaction. This photopositive reaction gradually disappears with development, and beyond 4 to 5 mm BL they generally show a photonegative reaction (Saisho, 1966). These positive and negative reactions to light lead to aggregation and tangling of the larvae when they are cultured communally in tanks. The tangling can cause loss of long maxillipeds and pereopods, resulting in slow growth and low survival rates. To avoid tangling, the phyllosoma of some palinurid lobsters have been cultured at low light intensities or in the dark (Moss *et al.*, 1999, Matsuda and Takenouchi, 2005). Low light intensities also help maintain an even distribution of food (*Artemia*) in the tanks so that no behavioral separation between larvae and food occurs (Moss *et al.*, 1999). Stage I phyllosoma of *J. edwardsii* reared at a photon flux density of  $0.001 \mu \text{mol s}^{-1} \text{m}^{-2}$  or in the dark grew larger than those reared at  $0.1$  or  $10 \mu \text{mol s}^{-1} \text{m}^{-2}$ .

#### Culture tank

In many studies of *P. japonicus* phyllosoma culture, cylindrical tanks with concave or flat bottoms were used, but rates of survival to the puerulus stage were low, at less than 10% (Inoue, 1981; Kittaka,

2000; Sekine *et al.*, 2000). Newly designed tanks were recently introduced to phyllosoma culture, and survival rates have been gradually increasing. Matsuda and Takenouchi (2005) designed a 40-L culture tank and obtained high survival rates of 37% to 54% to the puerulus stage from the middle phyllosoma stage (mean BL, 11.5 mm). This tank is elliptical and shallow, with a concave bottom and smoothly curved corners; it was designed to prevent excessive aggregation of larvae and to allow the larvae to be conveniently observed (Fig. 5). Takushi Horita of the Toba Aquarium introduced to phyllosoma culture a tank with a modified upwelling system (called the “planktonkreisel” and originally developed by Greve in 1975 for the maintenance of planktonic animals), and produced several pueruli

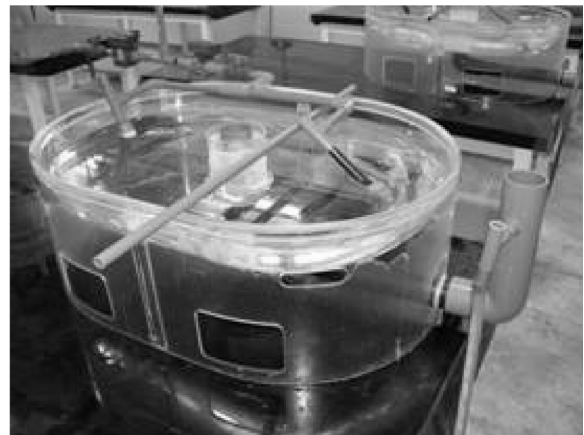


Fig. 5. Culture tank for *Panulirus japonicus* phyllosoma used at the Fisheries Research Division, Mie Prefectural Science and Technology Promotion Center (Matsuda and Takunouchi, 2005).

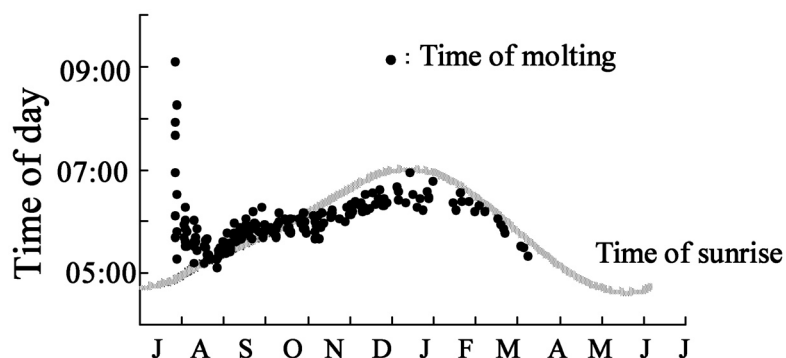


Fig. 4. Diel timing of molting of *Panulirus japonicus* phyllosoma cultured under natural light-dark cycle (Matsuda *et al.*, 2003).

(Horita, T., personal communication) (Fig. 6). A constant circular current is provided in the tank by water input at the top; the current keeps the larvae suspended in the water column and prevents them from aggregating and tangling with each other. Murakami (2004) further improved the upwelling system and designed a rotatory tank with a capacity of 70 L; the tank is placed in a large water bath and rotates by itself to provide a more moderate circular current (Fig. 7). His attempt was successful, and he obtained a survival rate of 28.3% to the juvenile stage from hatching.

Table 2 lists the types of phyllosoma culture tanks that have so far been used for palinurid lobsters; they can be classified into three types on

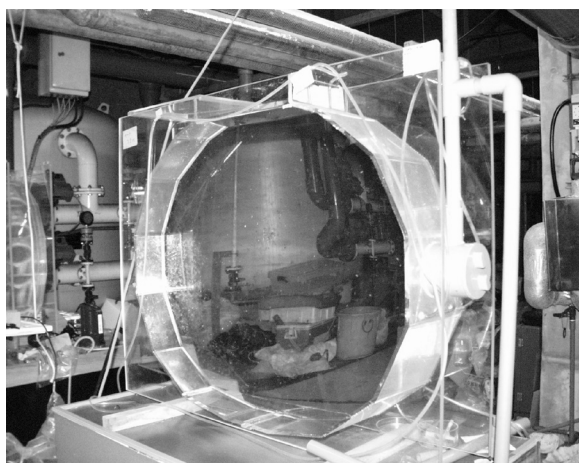


Fig. 6. Culture tank for *Panulirus japonicus* phyllosoma used at Toba Aquarium (Courtesy of T. Horita).

the basis of their configurations: horizontal, vertical, and intermediate. The horizontal type has the advantages of enabling easy operation of the culture systems and convenient observation of larvae. However, it also has disadvantages in that the larvae are always in contact with the bottom of the tank, and this appears to cause diseases and mortality. Moreover, it is difficult to scale up the larval culture because these types of tank need large amounts of space. The vertical type has advantages in that it is possible to maintain the larvae in suspension with a moderate vertical water current and it is easier to scale up the larval culture because the tanks do not need large amounts of space. However, the culture systems used with this type of tank are generally

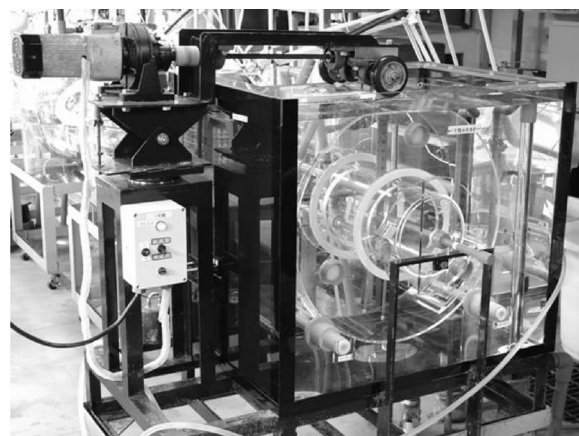


Fig. 7. Culture tank for *Panulirus japonicus* phyllosoma used at Minamiizu Station, National Center for Stock Enhancement, Fisheries Research Agency, Japan (Murakami, 2004).

Table 2. Culture tanks for palinurid phyllosoma

Tank type	Shape of tank	Volume (L)	Species	Reference
Horizontal	Elliptical	40	<i>Panulirus japonicus</i>	Matsuda and Takenouchi (2005)
	Circular	30	<i>Jasus edwardsii</i>	Ritar (2001)
Vertical	Square tube	72	<i>Jasus edwardsii</i>	Illingworth <i>et al.</i> (1997)
	Drum	90	<i>Panulirus japonicus</i>	Horita (pers. comm.)
	Drum	70	<i>Panulirus japonicus</i>	Murakami (2004)
Intermediate	Cylinder	16-100	<i>Panulirus japonicus</i>	Kittaka (2000)
	Cylinder	40	<i>Panulirus japonicus</i>	Sekine <i>et al.</i> (2000)

complicated, and their operation and management need more attention than those used with horizontal types. Intermediate-type cylindrical tanks have been used in many studies to examine the optimal conditions for flow-through culture of palinurid phyllosoma (*e.g.*, Inoue, 1981; Kittaka, 2000). Such studies have been used as a basis for the development of culture tanks, but this type of tank has disadvantages in that the larvae huddle together in a small area because the tank generally has a small bottom, and it is difficult to provide a moderate water current. At present, it is difficult to decide on which type is best for scaling up phyllosoma culture; further study of tank design will be required for the establishment of large-scale phyllosoma culture.

#### Future work

Culturing techniques for *P. japonicus* phyllosoma have been improving step by step; these improvements now enable us to produce more than 100 juveniles a year through phyllosoma culture. However, there are many problems to be solved in establishing large-scale phyllosoma culture, including the need to reduce the use of antibiotics in preventing bacterial diseases and the need to increase the efficiency of the culture procedure. The prevention of bacterial diseases is of paramount importance in larval culture. Hereafter, various new pathological and nutritional approaches and new culture techniques are needed.

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