Mass mortality and its control in the larval rearing of brachyuran crabs: Implications for mass culture techniques of phyllosoma larvae

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Mass mortality and its control in the larval rearing of brachyuran crabs: Implications for mass culture techniques of phyllosoma larvae

Katsuyuki HAMASAKI [*1], Takayuki KOGANE [*2], Keisuke MURAKAMI [*3], Tadao JINBO [*3] and Shigeki DAN [*4]

Abstract Larval mass mortality has been reported during the large scale seed production of brachyuran crabs in Japan, but rearing larvae of brachyuran crabs is relatively easy in small vessels. In this paper, we discuss the gaps between rearing methods of larval snow crabs using large tanks and small vessels, and show the results of a preliminary study to control the mass mortality of snow crab larvae in large tanks. Based on these results, we identify the direction for research on the mass culture of phyllosoma larvae of the Japanese spiny lobster *Panurilus japonicus*.

Key words: Brachyuran crabs, seed production, larval rearing, mass mortality, behaviour, phyllosoma

The Fisheries Agency of the Japanese Government initiated marine stock enhancement programmes in 1963 to augment and stabilize fishery resources. Brachyuran crabs were targeted in the stock enhancement programmes and we studied the seed production technologies of brachyuran crabs. These crabs included: horse hair crabs *Erimacrus isenbekii*, which are distributed in northern parts of Japan, especially in Hokkaido; snow crabs *Chionoecetes opilio*, which inhabit cold waters in the deep sea; swimming crabs *Portunus trituberculatus*, and mud crabs *Scylla serrata* and *S. paramamosain*, which inhabit warm waters (Hamasaki, 2002, 2003; Hamasaki *et al*., 2002a, b; Arai *et al*., 2004; Kogane *et al*., 2005; Jinbo *et al*., 2005). In the seed production of these species, newly hatched larvae are stocked in large tanks with flow-through water systems. Rotifers and *Artemia* sp. are daily supplied, at designated densities, to the tanks as food for the larvae. Phytoplankton are also added to the tanks as food for rotifers and *Artemia*.

Studies on the artificial rearing of larvae examined the ecology and physiology in the early life history of many brachyuran species all over the world (see Anger, 2001). In these studies the general method of larval rearing is as follows: newly hatched larvae are stocked in small vessels with seawater and prey, such as rotifers and *Artemia*. Larvae are daily transferred into new vessels with new seawater and prey using a large mouthed pipette.

We have experienced larval mass mortality during the seed production of brachyuran crabs, but rearing larvae of brachyuran crabs is relatively easy in small vessels. For example, in our rearing experiments, the survival rate of snow crab larvae in 500 L tanks rapidly decreased and few larvae moulted to the second zoea (Kogane and Hamasaki, unpublished data); however, in one litre beakers, mean survival rate to the first crab stage reached *ca*. 30 % (Kogane *et al*., 2005) (Fig. 1). Differences between survival rates in large tanks and small vessels were also observed for other brachyuran species (*e.g*., Hamasaki, 2003; Hamasaki *et al*., 2002a).

Why does mass mortality occur in large tanks? In this paper, we discuss the gaps between rearing methods of larval snow crabs using large tanks and

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[*1] Tokyo University of Marine Science and Technology, Minato, Tokyo 108-8477, Japan E-mail: hamak@kaiyodai.ac.jp (K. Hamasaki)
[*2] Obama Station, National Center for Stock Enhancement, Obama, Fukui 917-0117, Japan (Present address: Yashima Station, National Center for Stock Enhancement, Takamatsu 761-0111, Japan)
[*3] Minamiizu Station, National Center for Stock Enhancement, Minamiizu, Shizuoka 415-0156, Japan
[*4] Ishigaki Tropical Station, Seikai National Fisheries Research Institute, Ishigaki, Okinawa 907-0451, Japan

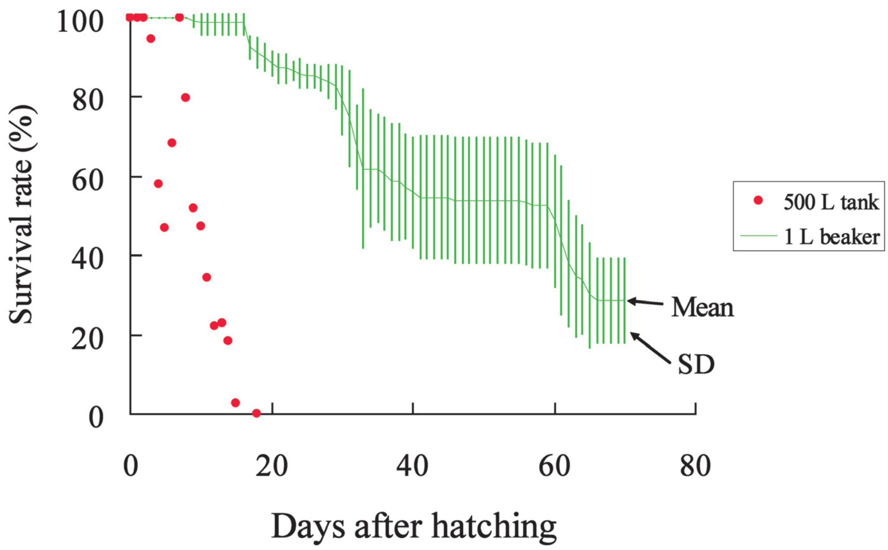

Fig. 1. Survival rates of snow crab larvae reared in a 500 L tank and 1 L beakers. Unpublished data from Kogane and Hamasaki are shown for a 500 L tank. Survival rates of larvae reared in five 1 L beakers at 14℃ are shown (Kogane *et al*., 2005).

small vessels and show the results of a preliminary study to control the larval mass mortality in large tanks. Based on these results, we identify the direction for research on mass culture of phyllosoma larvae of the Japanese spiny lobster *Panurilus japonicus*.

Gaps between large tanks and small vessels

During larval rearing in tanks, organic matter such as dead larvae, prey, and faecal pellets gradually accumulate on the tank bottom. Based on our observations, snow crab larvae are distributed in the upper water for a while after hatching. Then, they gradually lose their planktonic habit and sink to form patches on the tank bottom. Patches may be formed by water currents in the tanks. Larval snow crabs have long dorsal and ventral spines. We can assume that these spines are easily broken when the crabs are in patches on the tank bottom. Pathogenic bacteria increase due to decaying organic matter and infect injured larvae, causing mass mortality.

On the other hand, in small vessels, mass mortality does not occur because we can keep the rearing environment almost constant by transferring the larvae into new vessels with new seawater and prey and remove the dead larvae. In order to control the larval mass mortality in large tanks, we should prevent the larvae from sinking to the tank bottom and control bacterial disease.

Larval behavioural characteristics

To prevent the larvae from sinking to the tank bottom by controlling larval behaviour, we examined the phototaxis and geotaxis of larval snow crabs (Kogane and Hamasaki, unpublished data). Phototaxis was examined as follows. Five light-adapted larvae were put in the centre of a horizontal rectangular chamber (100 cm long, 10 cm wide and 10 cm deep). The length of the chamber was marked at 5 cm intervals. Each larval position was checked after five minutes under light and dark conditions. Light (500 lx) was turned on from the end of the chamber. This procedure was repeated three times. Geotaxis was examined by a similar method to that used in the phototaxis experiments. Five light-adapted larvae were placed into the top of a vertical rectangular chamber (45 cm long, 10 cm wide, and 10 cm deep) with marks at 5 cm intervals. Each larval position was checked after five minutes under light and dark conditions. Light (500 lx) was turned on from the top of the chamber.

In the phototaxis experiments, larvae did not move from the section where they were placed under dark conditions. Under light conditions, newly hatched larvae moved to the light source, i.e., showing strong positive phototaxis. Then, phototaxis gradually became weak with growth and the megalopa did not move under light conditions. In the geotaxis experiments, under both light and dark conditions, newly hatched larvae were positioned in the upper sections, i.e., showing negative geotaxis. Then, geotaxis became positive when late first zoea, second zoea and megalopa sank to the bottom. Thus, snow crab larvae sink according to their growth stages and it is difficult to prevent larvae from sinking to the tank bottom by controlling their behaviour.

Effects of water agitation and bath treatment with drugs on larval survival of the snow crabs in large tanks

To control the possible causes of mass mortality of larval snow crabs in large tanks, i.e., larval sinking and bacterial disease, we examined the effects of water agitation and treatment of tank water with drugs on survival of larvae (Kogane and Hamasaki, unpublished data).

Two rearing trials were conducted using 500 L tanks. In the first trial, newly hatched larvae were stocked in two 500 L tanks, one equipped with an agitator (Fig. 2) and the other without an agitator. The agitator rotated once per minute. The tanks were provided with a flow-through water system (turnover rate: once/day) and aeration. Water temperature was regulated with a heating system at 14℃. Larvae were fed with rotifers and *Artemia*. In the second trial, we used the same 500 L tanks with and without the agitator used in the first trial and added sodium nifurstyrenate to both tanks at a concentration of 2 mg/L weekly.

In the first trial, all of the larvae in the tank without the agitator died within 20 days after hatching. In the tank with the agitator, the survival rates were higher than the tank without the agitator (Table 1). In the second trial, the survival rates were higher than the first trial, and those in the

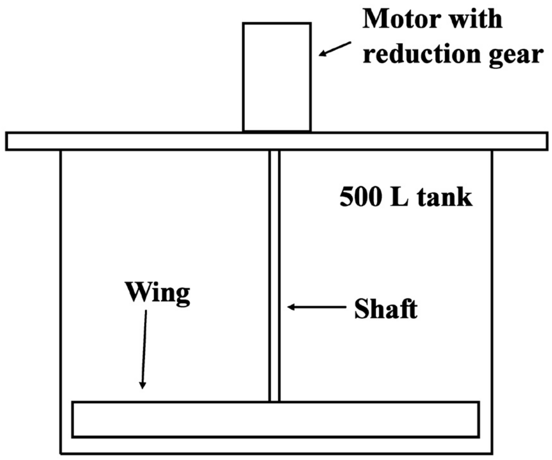

Fig. 2. Lateral schematic illustration of the agitator used for rearing experiments of the larval snow crabs in 500 L tanks.

Table 1. Survival rates of snow crab larvae reared in 500 L tanks

Expt.	Tank	Survival rate (%)			
		10 DPH	20 DPH	30 DPH	39 DPH
I	Agitator	53.3	11.3	2.5	-
	Control	47.3	0.0	-	-
II	Agitator	72.7	63.6	25.5	22.3
	Control	42.7	52.7	13.6	11.6

DPH: days post hatching

Bath treatment with sodium nifurstyrenate (2 mg/L) was conducted once every week in Expt. II tank with the agitator were higher than the tank without the agitator (Table 1). In both trials, rates of swimming larvae were higher in the tank with the agitator than the tank without the agitator (data are not shown).

Thus, the survival rates were improved using the agitator and sodium nifurstyrenate. Since 2002 we have applied this rearing system to larger tanks of 20 kL and shown the effectiveness of the agitator and tank water treatment with sodium nifurstyrenate. Larval survival rate was improved in the mass seed production tanks and several thousand larvae survived to the first crab stage (Fig. 3).

We improved the survival rate of snow crabs using the agitator and drugs but survival rates in larger tanks were still lower than in small vessels (Fig. 1 and Table 1). Some larvae sank to the tank bottom after the second zoeal stage even when using the agitator. In future studies, to prevent the larvae sinking to the bottom and to improve survival rates, we should study the water currents in the tanks, larval behaviour and bacterial disease in greater detail. Further, use of drugs might not be recommended for long because it can result in the selection of drug-resistant pathogenic strains. Hence, we should develop alternative measures to control bacterial diseases, *e.g.*, biological control, which is being studied by our research group for mud crabs (Hamasaki, 2003).

Research direction for mass culture of phyllosoma larvae

The problems encountered in seed production of brachyuran crabs have also existed in the rearing of phyllosoma larvae. Their larval development period is quite long, *e.g.* >300 days (Sekine *et al.*, 2000), and they have long pereiopods that are easily injured and damaged by interactions between individuals and by physical stress. Periodical tank antibiotic treatments and change of rearing vessels are essential to rear phyllosoma larvae to the juvenile stage (Murakami, personal communication), restricting the culture scale of phyllosoma to tanks about 100 L in volume.

To improve the survival rate and expand the scale of culture of phyllosoma larvae, firstly, behavioural characteristics such as phototaxis and geotaxis, and sinking speed; then, control measures to regulate larval behaviour such as using an agitator similar to that which improved the larval survival rate in the snow crabs, should be investigated. Further, it is important to study bacterial diseases and their control measures.

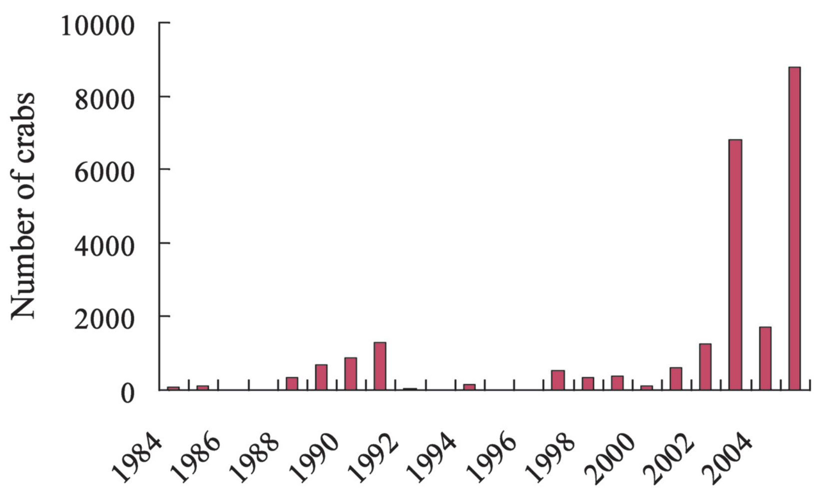

Fig. 3. Number of snow crab juveniles (first-stage crabs) produced at Obama Station of the National Centre for Stock Enhancement, Fisheries Research Agency.

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