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Mass Production of Artificial Seed of the Japanese Common Sea Cucumber (*Apostichopus japonicus*) in Hokkaido, Japan

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Abstract: Seed production and release of *Apostichopus japonicus* is a key approach to increase and maintain their natural stocks in Japan. Nevertheless, in many trials conducted mainly in Honshu and Kyushu by the end of the 1990's, the amount of seed production fluctuated annually, and the survival rate of released artificial juveniles was uncertain because of the difficulty of distinguishing them from wild ones. Due to these issues, some hatcheries discontinued *A. japonicus* enhancement projects. However, due to increase in Chinese demand, the price of *A. japonicus* dramatically increased after 2003 in Hokkaido. Facing the decline in the price of sea urchin and abalone, which are major high-value catches in the coastal areas, fishermen were very interested in *A. japonicus* stock enhancement by releasing artificial seeds. Accordingly, sea urchin and abalone hatcheries in Hokkaido began to produce *A. japonicus* seed after 2006. This paper will introduce the recent mass production techniques developed in Hokkaido.

Key word: *Apostichopus japonicus*, artificial seed production, larval rearing, post-larval rearing, control of predation

Japanese common sea cucumber *Apostichopus japonicus* is found in shallow coastal bottoms of Japan, Kuril Islands, Sakhalin, northeastern China and Korean peninsula. Among these countries, Japanese and Korean people prefer to taste this mainly in raw or picked as a seafood, but Chinese people prefer dried sea cucumber as a tonic rather than a seafood. Due to the economic development of China, demand for this species has increased. Unit price in Hokkaido peaked at 43.2 USD/kg in 2010. Facing the decline in the price of sea urchin and abalone, fishermen were very interested in stock enhancement by releasing artificial seeds.

After Imai *et al.* (1950) made the first trial of artificial seed production in Japan, many trials were conducted in Honshu and Kyushu. Small juveniles (<2mm) were mainly produced by 1990, and the productions peaked at 26.6 million year^{-1}. Thereafter, due to increase of production of larger juveniles, the total number of juveniles decreased to around 2–3 million year^{-1} (Fig. 1).

Mass mortality during post-larval rearing resulted in fluctuations in the number and size of seed produced. Furthermore, the survival rate of released artificial seeds had not been properly

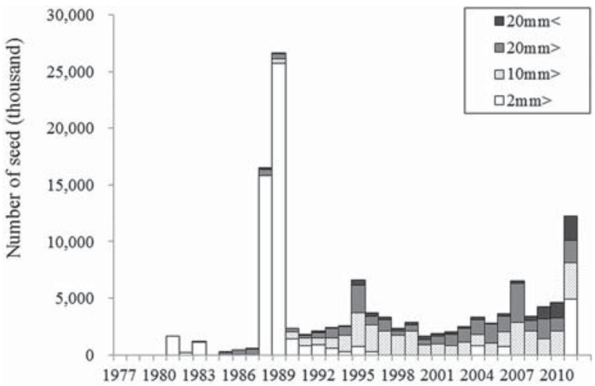

Fig. 1. Number of seed produced in Japan. Drawn from the statistical data published by Fisheries Agency, Fisheries Research Agency and National Association for Promotion of Productive Seas.

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evaluated, because of difficulty in distinguishing cultured from wild individuals. Therefore, some hatcheries in Honshu and Kyushu discontinued this stock enhancement project. However the demand for artifical seed production by fishermen, began to increase after 2006 in Hokkaido and the production of artificial seed increased again and reached to 12.3 million in 2011. From this experience and background, the improvement of artificial seed production is necessary for stock enhancement to be successful.

Seed production of *A. japonicus* is divided into 5 processes: broodstock collection, fertilization, larval rearing, settlement and post-larval rearing. I review the recent mass production techniques mainly developed in Hokkaido for each process.

Brood stock collection

Genetic impacts due to release of artificially produced organisms are considered in broodstock collection. Polymorphic microsatellite allele frequency of artificial seed tends to decrease to compare with wild ones due to small number of their parents (Sakai and Kanno, 2013). So to keep enough broodstock from wild stocks is necessary especially in the case of survival rate of released seed is high enough to contribute its reproduction on the released bottoms. Furthermore, to maintain genetic diversity, broodstock should not be used repeatedly.

Maturation of this species begins after they grow to ca. 50g in body weight, so larger broodstock are necessary. They are normally collected in the spawning season, late June to August in Hokkaido, to avoid excess labor and cost to rear them.

Fertilization

Gametes are obtained from fully matured broodstock by two methods: thermal stimulation or injection of gonadotrophic hormone (CUBIFRIN) through the body wall (Kato *et al.*, 2009; Fujiwara *et al.*, 2010). To induce to release gametes by thermal stimulation, broodstock are placed in 15 L volume containers individually and the seawater temperature is raised by 5-7℃ above ambient temperature. Mature broodstock begin to release their gametes into the container ca. 0.5-1.5 hours after the stimulation. Alternatively, broodstock injected with gonadotrophic hormones (CUBIFRIN) will also begin to release their gametes.

For insemination, 10-20 ml of sperm is added to a liter of seawater containing 0.1 - 0.2 million eggs. To prevent polyspermy, sperm concentration should not exceed 1,000,000 sperm ml^{-1} (Sakai and Konda, 2006). After insemination, eggs are transferred onto a 45μm mesh sieve immersed in a shallow tray and gently rinsed with filtered seawater to wash the excess sperm through the sieve.

Larval rearing

Hatched blastulae swim to the surface and are transferred to larval rearing tanks at a density of 1-2 individuals ml^{-1}. About 2 days after hatch, the larvae grow to the auricularia larvae and begin to eat *Chaetoceros gracilis*, unicellular phytoplankton. Daily feeding rate is near to 10 000 cells $individual^{-1}$. The fully developed auricularia larvae grow to ca. 1 mm body length in 5 to 10 days. After this, they begin to shrink to 0.4-0.5mm doliolaria larvae in 2 days. Then about 1 day after, doriolaria larvae grow to pentactula larvae which are ready to metamorphose to young juveniles and begin to settle onto the tank bottom or collectors.

The growth rate of larvae is regionally different in Hokkaido, fast in southwestern Sea of Japan areas and slow in Pacific areas. It takes only 9 days in southwestern Hokkaido and 14 days after hatch in the Pacific area.

Larval rearing tanks are static through the rearing period to minimize the loss of food and satisfy their food requirement. However, to avoid precipitation of larvae, food and feces on the bottom, aeration is very important; the optimum rate is 1.5-2.0 L min^{-1}. The water temperature should be kept at 18-20℃ .

Settlement

Doriolaria and pentacutula larvae are transferred to post-larval rearing tanks. They settle on corrugated polyvinyl chloride (PVC) plates or balled 1mm mesh polyethylene screens of which surface are covered with natural attached diatoms or algal powder which are used as the juvenile diet. To avoid the loss of these larvae, water exchange is initiated 10 days after the larvae are stocked, by which time most larvae complete settlement.

Post-larval rearing

Settled juveniles are reared up to 5 to 10 mm body length on corrugated PVC plates and/or polyethylene screens without temperature control. Daily water exchange rate varies from 1–7 times day^{-1} with aeration at the center of the tank to stir the rearing water well.

Various diets during this post-larval rearing have been examined (Yanagibashi *et al*., 1984; Yanagibashi and Kawasaki, 1985; Ueki and Ikeda, 1989; Konda and Sakai; 2005). The naturally occurring attached diatoms and LIVIC-BW, a commercially sold diet made from a mixture of dried algal powder of *Undaria pinnatifida* and *Ascophyllum nodosum* are used as inducers for larval settlement and as food for post-larval stages. Phytoplankton *C. gracilis* is also a good initial food for juveniles for the first month (Sakai and Konda, 2008). Proper feeding of LIVIC-BW has been examined according to juvenile size (Ikeda *et al*., 1992; Sakai *et al*., 2009). Juveniles fed LIVIC-BW grow to 6.0 mm in about 3 months (Sakai *et al*., 2009). Growth rate affects rearing density (Hatanaka, 1996; Sakai *et al*., 2009) and food densities (Ito and Kawahara, 1993; Sakai *et al*., 2009).

During the Great East Japan Earthquake, the factory of LIVIC-BW was destroyed. Argin-Gold (Andes-Trading Co., Ltd.) made from dried powder of *Ascophyllum nodosum* began to be used. This diet compensates the same growth of juveniles at least 3 month (Sakai, unpublished data). Further experiments are necessary to find the proper diet to increase the growth rate of artificial seeds.

In this stage, the predation of settled juveniles by *Tigriopus japonicus*, which is commonly found in the coastal area of western north-Pacific Ocean, was a major problem to produce the artificial seeds in Japan (Kobayashi and Ishida, 1984). This species grow to only ca. 1 mm in its body length as adults and produce more than 100 eggs at each spawn (Fig. 2). Even just hatched out nauplius larvae, 50 μ m in its body length, begin to eat *A. japonicus* juveniles.

The predatory impacts by *T. japonicus* are serious during post-larval rearing. The predation begins after the density of this animals increase to ca. 10–20 individuals 10ml^{-1} (Sakai and Konda, 2008;

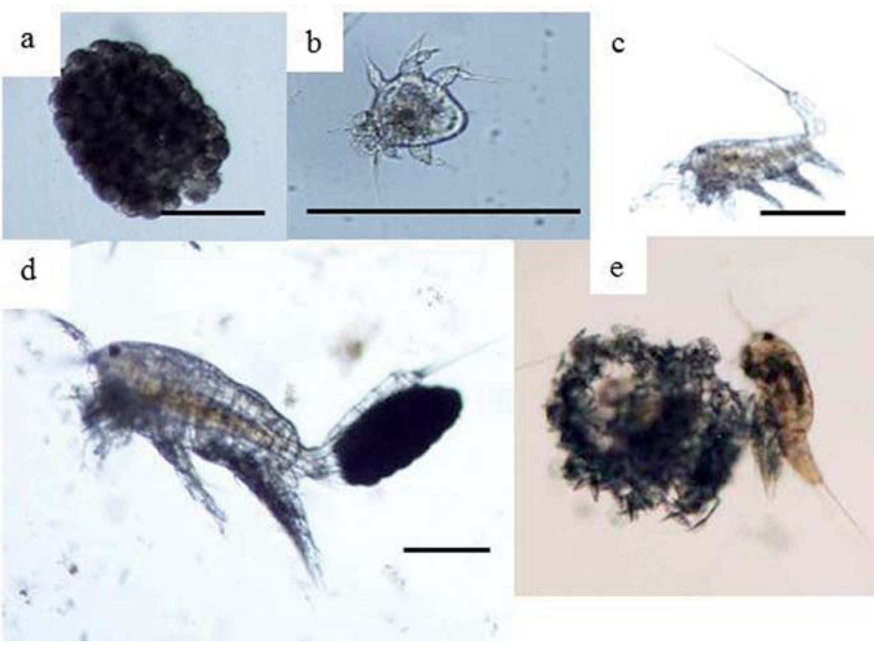

Fig. 2. Development of *Tigriopus japonicas*. a: eggs in the sac, b: higher magnification of fertilized nauplius larva, c: copepodid stage larva, d: adult female holding egg sac, e: pradation of *A. japonicus* juvenile. bar : 250μm.

Kobayashi and Yamaguchi, 2011; Noguchi and Noda, 2011). To prevent predation of artificial juveniles, it is necessary to decrease the density of *T. japonicus*. Trichlorphon, organo-phosphorous compound at a concentration of 1–2 ppm has been used to control this animal (Kobayashi and Ishida, 1984). However, after 2006, this medicine was prohibited by the Pharmaceutical Affairs Law in Japan.

After this, I developed 3 methods to prevent the predation: to immerse the plates into salt enriched seawater (ca.50‰), pumping the rearing water containing this predatory animal and filtered out with a 45μm mesh net to decrease copepod density in the water, and using a balled 1 mm mesh as the settlement material which allows the juvenile sea cucumbers to escape from the copepods (Fig. 3).

Immersing salt enriched water: Hypersaline seawater induces paralysis of the copepods and removes them from the plates, thereby decreasing predation (Sakai and Konda, 2008). Daily observation to confirm the increase of *T. japonicus* and to determine the timing of this treatment is necessary. Furthermore, a new tank free of copepods to set the collectors in is also necessary.

Pumping up to filter copepods: To reduce labor, I set an underwater pump on the bottom of the rearing tank to pump the copepod rich rearing water. The pumped water discharged into a 45 μm mesh net set at the surface of the rearing tank. *T. japonicus* were filtered out by this method and the density of copepods in the rearing tank decreased automatically.

Balled screen collector: The balled polyethylene screen is also available as the collector and rearing device to prevent predation. Sea cucumber juveniles can escape into the center of the balled screen if the

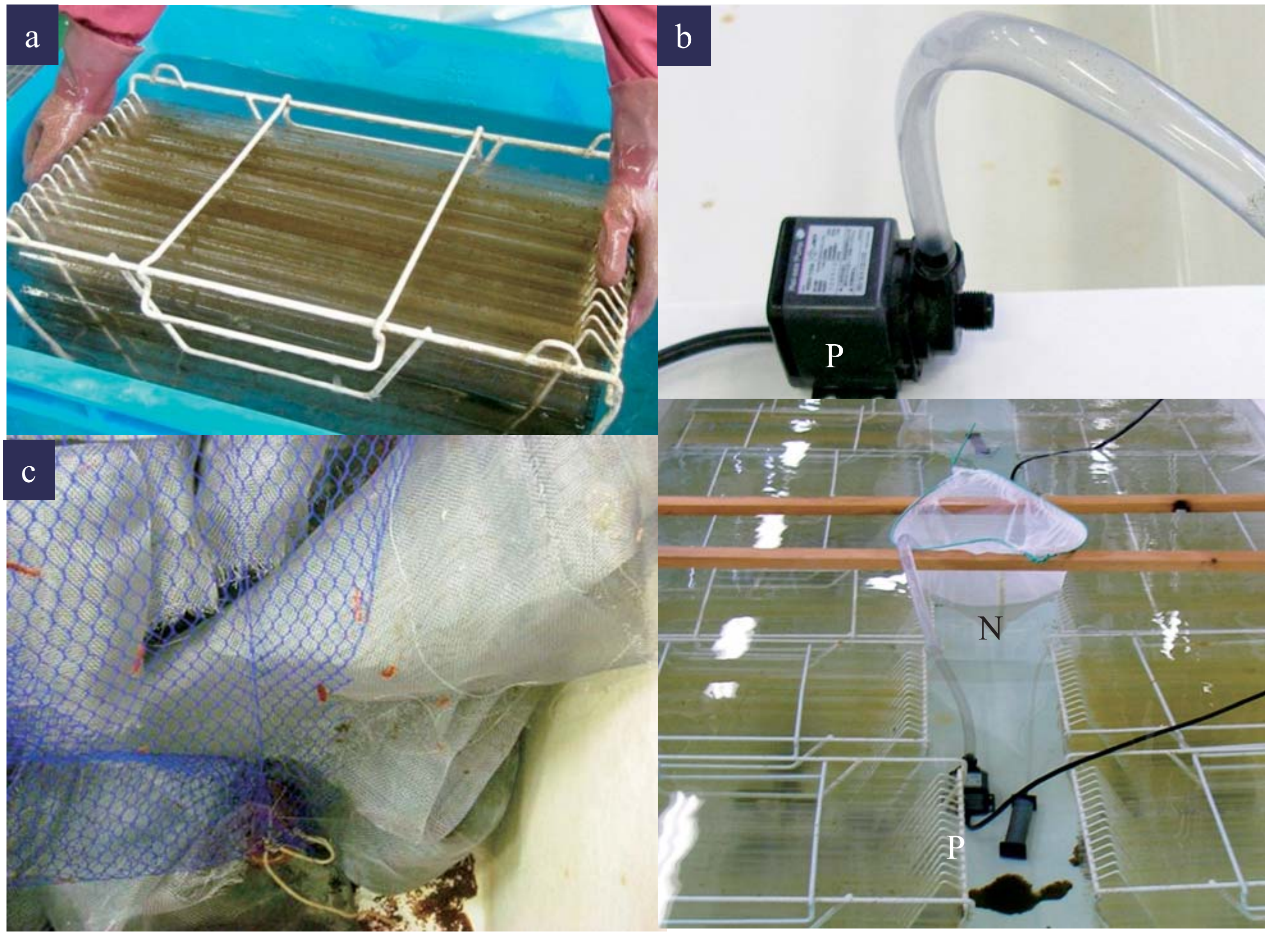

Fig. 3. Three main methods to control copepod density in the post-larval rearing tanks. a: Immersing the plates into salt enriched sea water, b: Pumping up and filter out the copepods, c: Balled screen collector. N: 45μm mesh net, P: under water pump.

density of copepods increases and allows them to escape from predation.

Release of artificial seeds

Juveniles are released onto the reefs by divers. Mitsunaga and Matsumura (2004) reported that large juveniles (26mm) survive better than smaller ones (14mm). However, the survival rate one year after releasing was only 3.8% and 0.1%, respectively.

Sakai and Kanno (2013) estimated the residual rate in an experimental area using msDNA marker. The residual rate of 8mm juveniles was only 3.9% four years after releasing, but the released juveniles had also observed in the catches from the fishing area neighbor to this experimental area.

Future perspective

To succeed in increasing the stock of sea cucumbers, further improvement in seed production, especially improving feeding to promote growth during post-larval rearing, is necessary. In addition, evaluating the effectiveness of artificial seed release strategy is indispensable.

The cheapest juvenile for release onto the fishery ground is the newly settled size, 0.4 mm in body length. Fishermen can produce these small juveniles themselves even in the corner of the port without many devices, technicians or long term labor. We can now distinguish even such a small seed from wild ones by msDNA markers. We will now determine if this small seed contributes to increase the natural stocks. The size dependence of the recapture rate should be evaluated as soon as possible for this project to succeed.

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