Experimental Captive Breeding and Exhibition of Post-Larvae "Shirasu" of the Japanese Anchovy *Engraulis japonicus*

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ABSTRACT: In Japan, variety of fish species are considered valuable food sources. Post-larval stage of Japanese Anchovy *Engraulis japonicus* is one of common traditional ingredients used in Japanese cuisine known as "Shirasu". In this study, we revisited current captive breeding techniques of the Japanese Anchovy and developed optimized techniques for culturing this species. Initially, live adult fishes caught in Sagami Bay were transported to the Enoshima aquarium. A fiberglass reinforced plastics tank, containing approximately 3500 L seawater was used to hold adult fishes. The lights on the tank were kept on for approximately 16 h/day. The anchovy in the tank received and consumed sufficient amount of food (over 5% of fish weight/day) for stimulating their growth. Under this condition, they matured and spawned eggs after approximately one month. The eggs (axis length: about 1.3 mm) were collected in plankton nets (0.5 mm mesh) and floated in tanks containing 600 – 1400 L sea water until hatching. The hatched larvae (total length: about 3.0 mm) received *Brachionus plicatilis*, *Artemia salina*, and pellets as food. They grew to over 30.0 mm TL after 30 – 40 days and gradually metamorphosed into juveniles. When they were kept under the same conditions as the adults, they grew to over 70.0 mm TL and matured after 5 – 7 months. We displayed the different development stages of the Japanese anchovy larvae, with explanatory information about the species and video footage of the moment of egg hatchings on small monitors at the Enoshima Aquarium from April 16, 2014, to the date. There are, however, some aspects related to cluture of this species that have not yet been completely resolved. For example, maintaining stable culture of the most important larval food, *Brachionus plicatilis*, is relatively difficult to achieve.

INTRODUCTION

Japanese anchovies (*Engraulis japonicus*) are distributed in the seas around Japan off the coast of Honshu, and around Taiwan, the Philippines, and Sulawesi Island (Miyachi *et al*., 2013). "Shirasu" is a common name designating to the larvae of several fish species found in Japanese water, such as anchovy, sardine, ayu, and eel. Larvae harvested in Sagami Bay near the Shonan region of Japan are specifically called "Shonan Shirasu" and are an important traditional Japanese food ingredient (Mitani, 1987). Several studies have been done on their culturing and breeding. However, it is difficult to exhibit live larvae in an aquarium over a long period of time.

In this study, we revisited the current breeding techniques commonly used with Japanese anchovies in captivity then developed more suitable methods for culturing this species. We here present this process and discuss topics emerging from it.

THE NEW EXHIBITION "SHIRASU SCIENCE"

We started to study breeding techniques of Japanese anchovy at the Enoshima aquarium in February 2013. The first experimental display "Exhibition of live whitebait" was opened in April 2014. A series of displays focusing more on their development ("Shirasu science") opened three years later in May 2017 (Fig. 1A-B, Fig. 4). A large tank displays juvenile and adult anchovies together with some other species of local fish (e.g., *Pleuronichthys japonicus* and *Sillago japonica*).

Fig. 1. The facilities of "Shirasu Science" A: Frontview of exhibition. B: Behind of exhibition tanks (culture tanks for the anchoby larvae). C: Culture tanks of live food, rotifers.

Two smaller tanks display exhibits two different stages of anchovy's larval development. Information labels and looped videos were placed nearby in order to explain the life cycle and ecological context of the anchovies. The exhibition remains active to this day, with the sixth captive bred generation larvae being now on display. Next, we discuss some studies we performed as well as issues relating to the maintenance of the exhibition.

ADULTS AND EGGS

Maturing adult fish

In June 2013, live wild adult anchovies that had been captured in Sagami Bay were transported to the Enoshima aquarium. Captured adult anchovies were kept in a fiberglass-reinforced plastic tank containing approximately 3500L of seawater. The lights in the tank were kept on for approximately 16 h/day. The fish were fed and consumed sufficient amount of food (in excess of 5% of fish weight/day) to promote their growth. They matured and began spawning after approximately one month

(Kawaguchi *et al.*, 1990; Yoneda & Kitano, 2012). Larvae were kept in different tank systems from adults till it reaches to juvenile stage. Juveniles matures five to seven month after they moved into the adult fish system. These processes were repeated over generations of captive breeding.

Egg development

Eggs (approximate axis length 1.3 mm) (Fig. 2A) were collected in plankton nets (0.5 mm mesh size). Because adult fish spawn at night, the nets were fastened to the drain pipe connecting from the main tank to the filtration tank in the evening and the eggs were recovered in the next morning. The eggs were moved to tanks containing 600-1400L of sea water until hatching. The eggs hatched about 48 hours in water temperature around 20 ˚C (Fig. 2B).

Egg toughness

Fertilized eggs are relatively resistant to minor physical shocks and can therefore be safely transported from the collecting net to the tank before hatching. (This transfer is not possible with live larvae.) Fertilized eggs may also be transported over long distances. We also studied relationship between the water temperature and the hatching rate of the eggs (Fig. 3). Eggs hatched at temperatures between 13 ˚C and 32 ˚C, however, more than 70% of all eggs hatched between 15 ˚C and 28 ˚C. The hatching rate around 20 ˚C was generally high.

FROM THE PRE-LARVA TO THE JUVENILE STAGE

Culturing at the pre-larva stage

Anchovies at the pre-larva stage are very small, with a total length of approximately 3 mm (Fig. 2B). They consume nutrient from large yolk sack for approximately 48 hours. During this stage, they do not have ability of swimming and rather suspend in the water column. Therefore we used kreisel tank (approx. water volume 20 L - specifically designed to keep jelly fish) to keep pre-larva. In order to grow pre-larva to juvenile stage, however, we need to use much larger tank (approx. water volume 1400 L) for both culture and exhibit purpose (Fig. 1B).

Culturing at the post-larva stage

At the initial post-larva stage, total length typically reaches 4 mm TL. Because of their small mouth, they can only eat small zooplankton in the first three weeks after hatching (Imai & Tanaka, 1996). It is therefore necessary to culture small live food sources, such as sea rotifers (*Brachionus plicatilis*), which have typical size of 0.1 mm. Kept density of rotifer at 15 rotifers/cm^3 in the tank was normally sufficient to feed the post-larvae. After consuming rotifers over about two weeks in 20□C environment, the post-larvae grow to 10 mm. At this stage, they can then eat larger organisms, e.g., nauplius of *Artemia* (small strain from Vietnam, approximate size 0.3 mm) fed along with rotifers (Fig. 2C).

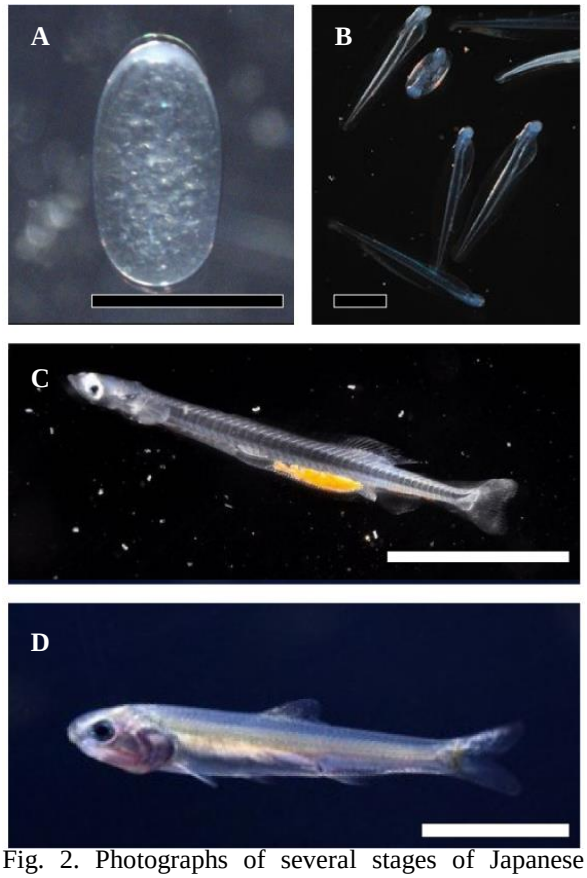

Fig. 2. Photographs of several stages of Japanese anchovy.
A: egg (immediately after spawning). B: egg and pre-larvae. Black scales indicate 1 mm. C: Post-larva. D: Juvenile. White scales indicate 10 mm.

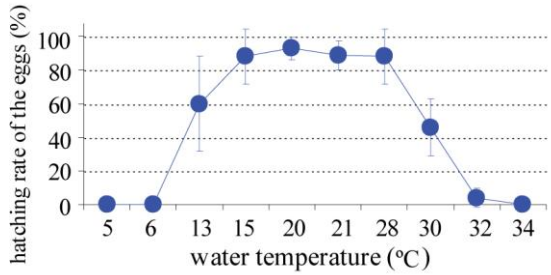

Fig.3. Relationship between water temperature and hatching rate.
Spots indicate average hatching rate.
Vertical bars indicate standard deviations.

Brackish water is arguably a good medium for larval cultures. Japanese anchovies occasionally enter river streams and larvae has been recorded that caught around the estuary in the wild (Mitani, 1987). We therefore studied their tolerance to salinity. When the water salinity was reduced gradually to a level just above 3 per mil, most fish survived for more than three days. The salinity of water in the tank was reduced further and then kept to approximately 25 per mil (Fig. 4). At this salinity, larvae looked healthy and their live rotifer food was also likely to survive well. The Approximately two months after hatching post-larvae become juveniles (typical larger than 30 mm TL) and could be transferred carefully to the larger tank (Fig. 2D). At this stage, they could eat small feed pellets such as the New Artech K-2 (Chlorella Industry Co.). They were grown in the exhibition tank or mature in the tank at behind the scene.

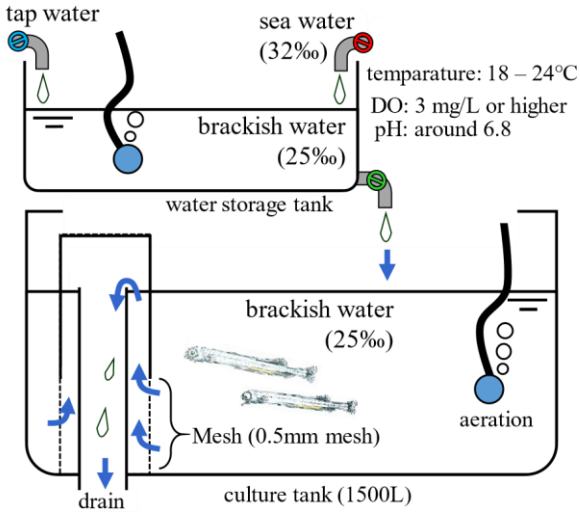

Fig. 4. The culture tank of "Shirasu Science"

ROTIFER CULTURING

Culturing large numbers of anchovies in the post-larva stage requires culturing suitable small live food, rotifers as well at our aquarium (Fig. 1C). Live rotifers are very popular in fish and invertebrates breeding facilities, and various studies have focused specifically on its culturing techniques (Ishibashi *et al*., 2003). We have adopted current culturing practices, such as extensive continuous culturing and batch culturing, on a daily basis. Rotifers are fed with live phytoplankton, *Chlorella*. When the conditions of the tank water are adequate and with right *Chlorella* concentration, rotifers breed rapidly. Maintaining stable culture of the most important larval food (*Brachionus plicatilis*) is relatively difficult to achieve. We culture the rotifers carefully using proven techniques and state-of-the-art knowledge (Yamauchi, 1993; Yoshimatsu, 2001; Yoshimura, 2002; Tomoda *et al*., 2007; Tanaka, 2013).

CONCLUSION

We succeeded in breeding and exhibiting Japanese anchovies from post-larva stage to adult. The exhibition of this kind is currently only presented at the Enoshima aquarium. Our research has also yielded useful insight into larval culturing. For example, the eggs were found to be relatively durable, whereas the larvae themselves are very fragile and require careful handling. Maintaining a stable culture of live food rotifers is relatively difficult but important nonetheless. We aim to continue improving our breeding and culturing techniques for the "Shirasu". Important new information on the life cycle and biology of Japanese anchovies may also be gathered by this aquarium setup.

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