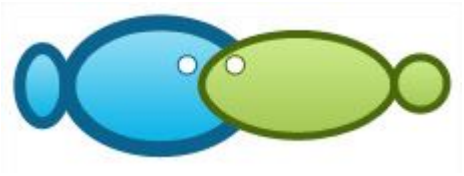

Artificial breeding and larval rearing techniques to conserve ayu, *Plecoglossus altivelis* (Plecoglossidae)

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Abstract. Ex-situ conservation, which is important to protect critically endangered species, requires special artificial breeding and rearing techniques during the early life stages of a species. This study describes the artificial breeding and larval rearing techniques to conserve ayu (*Plecoglossus altivelis*), an important fish that is endangered in some locations. Fish eggs and milt were acquired from mature fish by gently pressing the fish abdomen; subsequently, artificial insemination was performed by gentle mixing. The fertilized eggs were then incubated in running freshwater (for approximately 14 days). After hatching, the larvae were placed in brackish water (ca. 10 ‰ salinity; 7-8 mg L^{-1} dissolved oxygen) to maintain live food to the larvae. From the 1st to 35th day after hatching (DAH), the larvae were fed with live rotifers. Subsequently, from the 15th DAH onwards, the larvae were fed with brine shrimp nauplii along with rotifers and were familiarized with artificial food. After the fish gained a body length of 4-5 cm and started consuming 0.5-0.8 mm formulated food grain, they were transferred to a fingerling nursery with freshwater for further cultivation. This study also noticed that the susceptible stages of ayu larvae were at approximately 14-16th and 40-45th DAH. This work provides necessary information to design a conservation plan for the ayu population in the southern distribution range of this species where it is currently facing threats of extinction, such as in Vietnam. Additionally, the study describes the rotifer culturing technique that can assist in conducting larviculture.
Key Words: artificial breeding, ayu, larval rearing, larviculture, Ryukyu-ayu.

Introduction. Owing to climate change, many species have been declared extinct in the several past decades, while the populations of some other species are declining drastically. Thus, conserving species that sustain functions of ecosystem is urgently necessary. Ideally, species should be protected in their own habitat where they were encountered (in-situ conservation). However, relocation of a threatened species to a protected area suitable for conservation (ex-situ conservation) should also be considered if the natural habitat of the species is severely degraded or if the wild population has declined sharply (McGowan et al 2017).

Ayu (*Plecoglossus altivelis*) is distributed across the Japanese Archipelagoes, Korean Peninsula, Chinese coast, Taiwan, and north of Vietnam. It is an amphidromous fish and has a lifespan of one year, during which it moves across three habitat types: salt water, brackish water, and freshwater ecosystems. Adult fish spawn at the lower river courses from around mid-autumn to winter. Newly hatched larvae drift downstream to the river mouths or coastal areas, where they grow further by feeding on zooplankton (Senta & Kinoshita 1985). During spring, young fish ascend to the upper courses of rivers or streams, where they spend their remaining life, and feed on attached algae (Azuma et al 2003). Ayu is an essential resource in Japan for food and acts as a recreational fishing target (live decoy fishing and cormorant fishing). It is a keystone species in freshwater ecosystems because it consumes benthic algae, a primary producer in the riverine environment. However, some ayu populations have become locally extinct in low latitudinal areas [e.g., ayu in Taiwan and Ryukyu-ayu on Okinawa Island (Kawanabe 1972; Nishida et al 1992)], while some are critically threatened [Ryukyu-ayu on Amami-

oshima Island (Ministry of the Environment of Japan 2017); ayu in northern Vietnam (Tran et al 2014)]. Therefore, these ayu populations urgently need to be conserved.

Local populations living at the edge of species distribution range (marginal populations), such like ayu populations in the low latitudinal areas, are more vulnerable to threats, and thus, need to be given particularly more attention than those at the center of the range (Kolzenburg 2022). Generally, the responsibility of conservation of local species populations is assigned to local governments who have several financial limitations. Thus, to undertake conservation activities at a local scale under limited funds, many special techniques are required to address any unforeseen accidents that may occur. In the Amami-oshima Island of Japan, the Ryukyu-ayu population has been successfully conserved at a small scale for nearly a decade (less than 5000 adult fish are cultured per year) (Taniguchi & Ikeda 2009). Therefore, the techniques used in this region provide significant insights to conserve not only ayu but also other species at a local scale.

Accordingly, this study aimed to describe artificial breeding and larval rearing methods for ayu to promote its conservation. Additionally, as live food is vital for larviculture of tiny larvae fish, such as ayu, we described the process of maintaining and harvesting rotifers.

Material and Method. The method described below has been applied to conserve Ryukyu-ayu (*Plecoglossus altivelis ryukyuensis*), an endangered subspecies of ayu and endemic in the Ryukyu Archipelagoes of Japan. Conservation work is conducted at the Ryukyu-ayu Observation Center, which was established in 2002 in Amami-oshima City with aim to conserve Ryukyu-ayu. Several generations of hatchery-reared Ryukyu-ayu have been successfully cultivated at this center, where rearing work is presently ongoing.

Facilities required for artificial breeding and rearing of fish. The facilities required for conservation may vary in capacity, quality, and equipment. However, the minimal necessary conditions are listed below (the area and number of equipment described below are applicable for less than 5000 adult fish cultured annually).

A roofed room that can be used as a hatchery and a laboratory is needed (approximately 40 m^2, e.g., ca. 5 m × 8 m; Figure 1a). The room should be ventilated and supplied with electricity. An outdoor nursery with a corresponding area is required to cultivate the fish at fingerling and adult stages. The nursery should be covered with sunshade nets to avoid overheating and direct heavy rains (Figure 1b). The hatchery should be located near both clean seawater and freshwater sources. Two electric water pump systems (one each for seawater and freshwater) are required to continuously provide water to the indoor hatchery and outdoor nursery (Figure 2a). The water supply system for the indoor hatchery should also include a parallel pipe system to supply seawater and freshwater to the larval rearing tank (Figure 2b).

Figure 1. A roofed hatchery (a); an outdoor nursery (b).

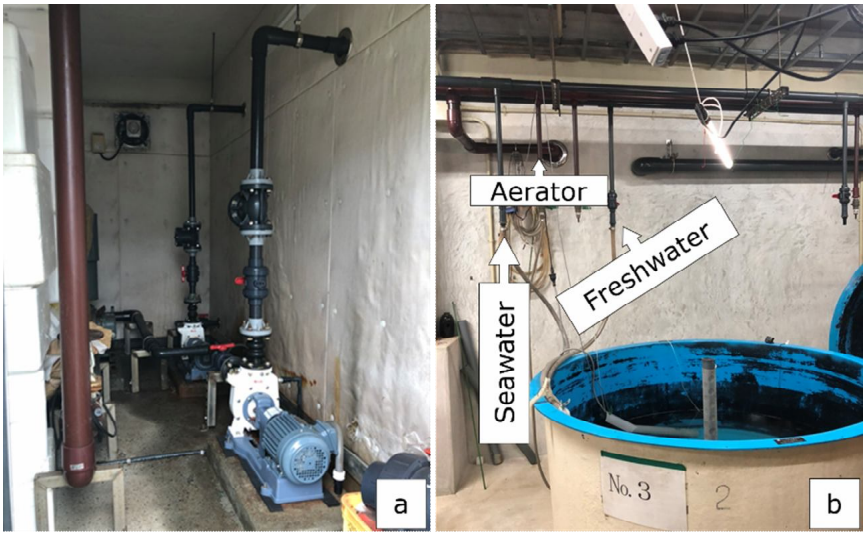

Figure 2. Water supply system: (a) Automatic water pump system; (b) Parallel pipe system.

The equipment required for an indoor hatchery are as follows: a rectangular egg incubation tank made of fiberglass that ensures continuous water supply in a specific direction and that can be easily cleaned (L × W × H = 300 cm × 50 cm × 50 cm; Figure 3). The tank should be placed near a natural light source (near the doors or windows). Four circular larvae rearing tanks made of fiberglass (volume = 1000 L, diameter = 100 cm, depth = 100 cm; Figure 4) should be supplied with seawater and freshwater parallelly, aerated by an aeration system, and illuminated by a neon light source attached with a timer to adjust the photoperiod.

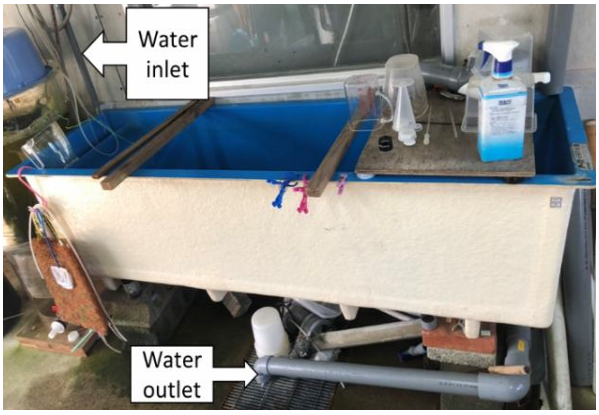

Figure 3. Egg incubation tank (300 L).

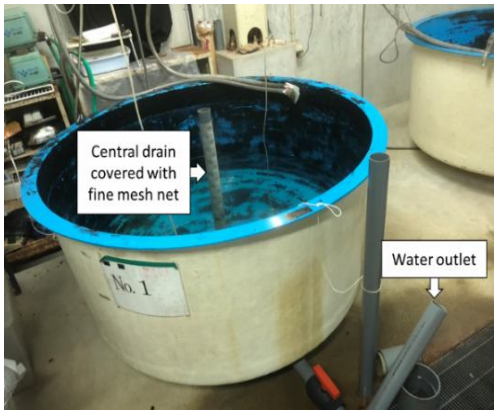

Figure 4. Larval rearing tank (1000 L; depth =100 cm).

Three juvenile and adult fish rearing tanks are required in the outdoor nursery (round polyvinyl chloride (PVC) coated tank, diameter = 3 m, volume = 3000 L; Figure 5). The tanks should be placed near natural light sources, supplied with clean freshwater, and sufficiently aerated.

To prepare live food, two rotifer rearing tanks (cylinder glass tanks or buckets that can be easily cleaned; volume = 100 L and 50 L; Figure 6) are required in the hatchery, along with brine shrimp (*Artemia*) incubating and rearing tanks (small aquarium size; Figure 7).

Other equipment required for the hatchery include portable aeration machines (equal to the number of tanks), portable water heaters (equal to the number of tanks), one water quality tester to measure water salinity, dissolved oxygen, and temperature, and a modified plankton net (a net of 120 µm mesh size, mounted on a hard square frame, as shown in Figure 8a. Additional requirements are digital scale, bowls (3 pcs), tablespoons (5 mL; 2 pcs), feather dusting brushes (2 pcs, Figure 8b), fish egg incubating mats (20 pcs, Figure 8c), watering-pot (1 pc), cleaning syphon (1 pc, Figure 8d), refrigerator, food cabinet, stereo microscope, fixed focus loupe (Peak Lupe 10X),

glass beakers (100, 1000, and 2000 mL), glass pipet, plastic mesh baskets (3 pcs), long-handle hand net (3 m long), and a plastic basin (1 pc, 30 L).

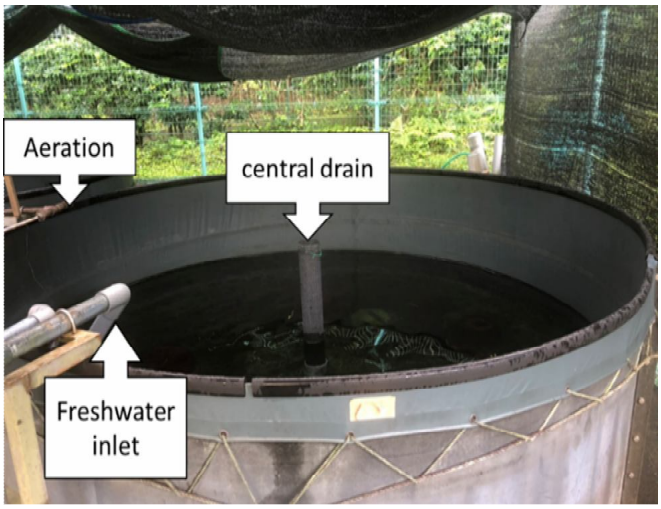

Figure 5. Outdoor rearing tank, V = 3000 L.

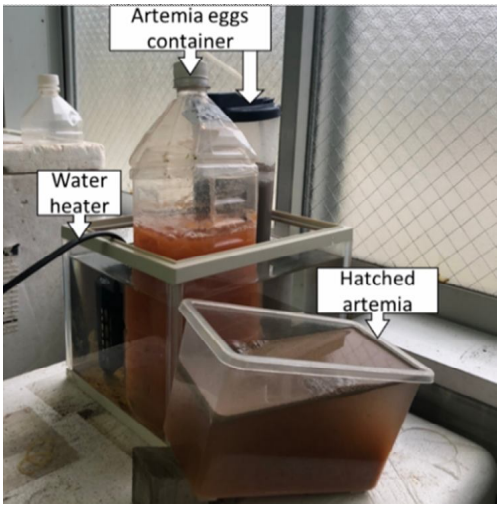

Figure 7. *Artemia* incubating tank.

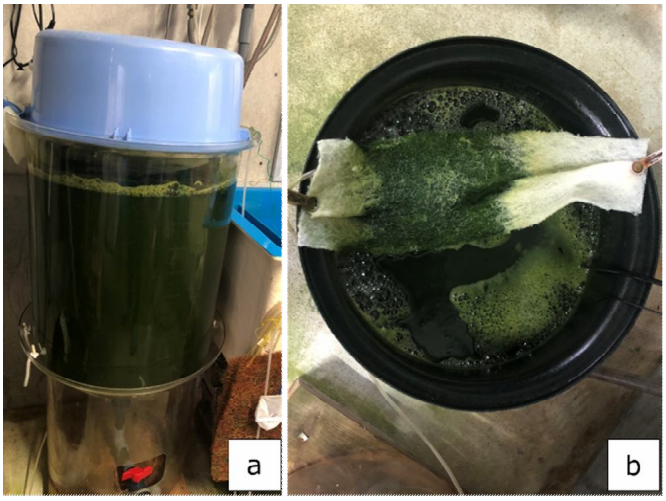

Figure 6. Two types of rotifer rearing tank: (a) Cylindrical glass tank (100 L); (b) Plastic bucket (50

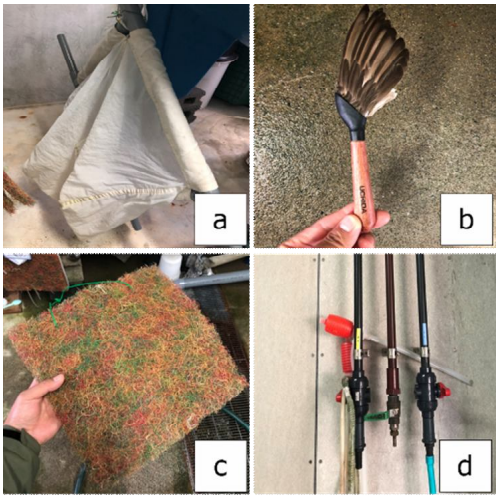

Figure 8. Other miscellaneous equipment required for the hatchery.

Artificial breeding techniques. The breeding season of Ryukyu-ayu in the natural rivers/streams in the Amami-oshima Island ranges from November to January (Kishino & Shinomiya 2004). However, during artificial rearing, the breeding season is slightly later (from mid-December to February; Matano (2020) - personal communication). The technician should observe and check the fish ovaries once every two days by gently pushing the abdomen near the anal area of female fish to check if eggs have reached an advanced stage that can assist in determining the breeding timing. Artificial breeding should be conducted under slightly sunny conditions. Eggs and milt can be acquired from the broodstock several times with an interval of two weeks.

Tools required for artificial breeding are as follows: hand-net (to catch the fish in the rearing tank); plastic mesh baskets; bowls; feather dusting brush; plastic basin; digital scale; fish egg incubating mat; egg disinfectant solution (Pyceze, Elanco Japan Co., Ltd.); soft towel.

Eggs and milt collection. It was done through the following steps:

(1) Starve the broodstock on the day of egg and milt collection (starving reduces mortality during gamete retrieval);

(2) Reduce the water level of the broodstock rearing tank to approximately 20 cm depth, which makes it easier to catch the fish;

(3) Catch the fish by using the long-handle hand net, and then place them in the plastic mesh baskets (at the origin of conservation work, the broodstock must be acquired from natural environments using a net, e.g., cast net);

(4) Place females (Figure 9a) and males (Figure 9b) separately into two different plastic mesh baskets. Separation is easy because ayu exhibits sexual dimorphism on reaching sexual maturity;

(5) Egg collection from female fish: dry the fish body with a soft towel. Bend the fish body at the anal area and gently push the abdomen downward the anal area using the thumb to remove eggs into a clean bowl (Figure 10a). Check whether the eggs are in good condition and are at an advanced stage (usually eggs are brightly yellow colored, and have a uniform size). Discard defective eggs. Weigh the good-quality eggs using a digital scale to estimate the amount of eggs collected;

(6) Milt collection from male fish: dry the fish body with a soft towel. Bend the fish body at the anal area and gently push the abdomen downward the anal area using the thumb, and spatter the milt into the egg bowl (Figure 10b).

Figure 9. Morphological differences between female (a) and male (b) fish.

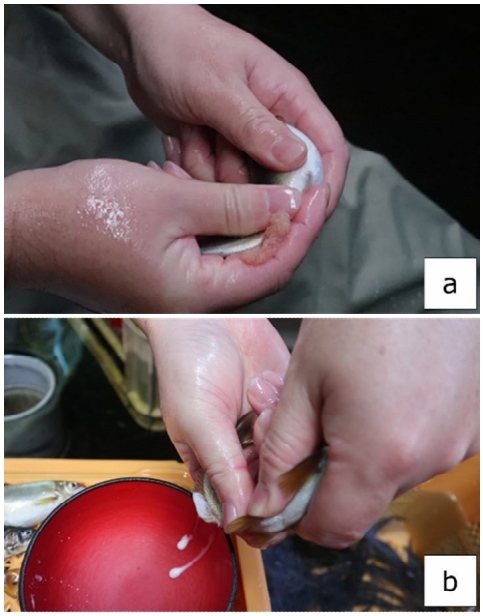

Figure 10. Collect eggs from female ayu (a) and milt from male ayu (b) by hand-stripping.

Fertilizing and fixing the eggs. It was done through the following steps:

(7) Gently mix the eggs and milt in a bowl using a feather brush (Figure 11a);

(8) Submerge the egg incubating mats into a clean freshwater basin, and spread 20 g of eggs on each mat using the same feather brush (Figure 11b). Make a small piece of the mat containing eggs as a sample for checking embryo development later.

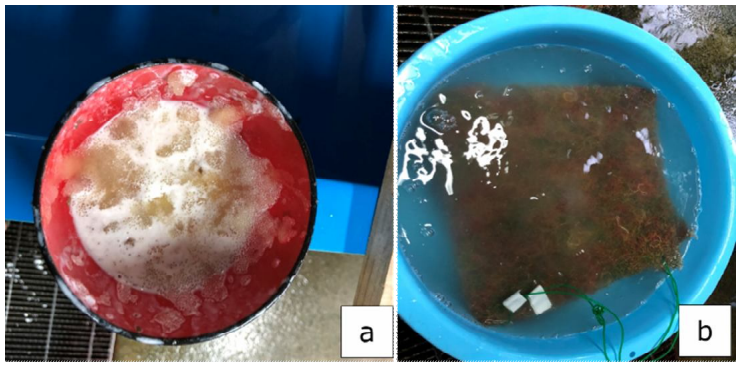

Figure 11. Artificially fertilize the eggs (a) and fix the eggs on the wet mat (b).

Egg incubation. It was done through the following steps:

(9) Hang the mats containing eggs on a stick and submerge them into the incubating tank so that the mats are approximately 5 cm from the bottom of the tank (Figure 12). Set the inlet velocity at approximately 20 L min^{-1} to ensure a uniform water current and increase the dissolved oxygen in the incubating tank;

(10) Dilute the egg disinfectant solution (e.g., Pyceze; 100 mL Pyceze/10 L freshwater) and pour the diluted disinfectant solution slowly into the egg-incubating tank. Submerge the mat containing eggs in the disinfectant for approximately 30 min to ensure complete disinfection (supply water at a rate of 20 L min^{-1}); subsequently, replace the water in the tank with clean freshwater;

(11) Set the outlet of the incubation tank so that the water level is kept at approximately 40 cm depth, which is sufficient to submerge the mats (Figure 13).

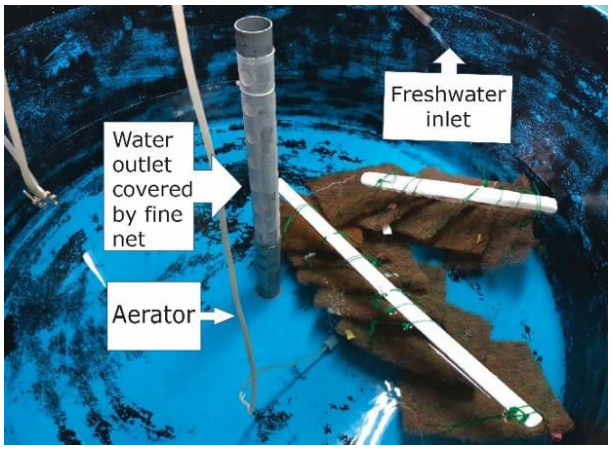

Figure 12. Larval rearing tank with 300 L freshwater, with the flow rate of the water inlet set at 1 L min^{-1}.

Figure 13. Submerge the mats containing eggs into the egg incubation tank.

Subsequently, submerge the mats containing the eggs in the diluted egg disinfectant solution once a day until the eyed stage (approximately 10 d after fertilization). Check the development of the eggs daily using a magnifying glass and identify the stages using a reference for embryo development (Tachihara & Kawaguchi 2003). The eggs hatch after nearly two weeks (at a water temperature of 10-15°C).

Post-hatching larval rearing techniques. Tools required for larval rearing were as follows: 1000-L round glass fiber tanks; freshwater supply; flicker-free fluorescent lamp (18 W); timer; aerator; water quality tester.

Prepare rearing conditions for early hatched larvae:

(1) Place the larval rearing tank in a position where freshwater and seawater can be easily supplied (Figure 2b);

(2) Supply the tank with freshwater, set the inlet velocity at approximately 1 L min^{-1} (to ensure a gentle water current in the tank), and set the outlet drain so that the water volume is approximately 300 L (30 cm depth; Figure 12); subsequently, submerge the aerator in the water and make sure that the dissolved oxygen concentration is maintained at 7-8 mg L^{-1};

(3) Place a fluorescent lamp above the tank, and set the timer to sync the photoperiod as a natural photocycle (14 L: 10 D);

(4) Submerge the mats containing eggs at the nearly hatching stage into the tank (Figure 13);

All eggs hatch after some days, and once all eggs are hatched, the mats are removed from the tank.

Regulating the rearing conditions. Under natural conditions, the newly hatched larvae of ayu drift directly to coastal areas with brackish or saline water, and feed on zooplankton. Therefore, the water salinity in larval tanks should also be regulated to conditions suitable for the growth of ayu larvae and rotifers, which are supplied in the tank as live food for larvae. After all larvae are hatched, and fish egg incubating mats are removed from the tank, the following process should be followed:

(1) Set both the seawater and freshwater inlets into the tank, and cover the two inlets with a piece of floss (adjust the water velocity of seawater and freshwater to 1 L min^{-1} so that the salinity is approximately 10 ppt; Figure 14).

(2) Submerge the water heater and set the temperature at 18°C.

(3) Place a thermometer to record and control the water temperature (Figure 14);

(4) On the 15th day after hatching (DAH), submerge a PVC tube into the tank and attach it with an aerator, as shown in Figure 15, to ensure a gentle water current in the tank.

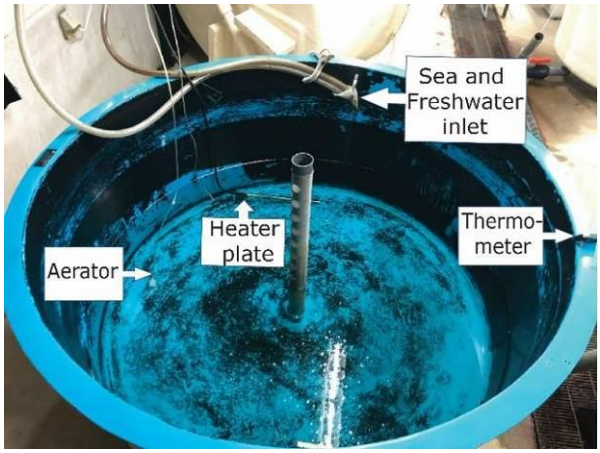

Figure 14. Prepare rearing conditions for newly hatched larvae.

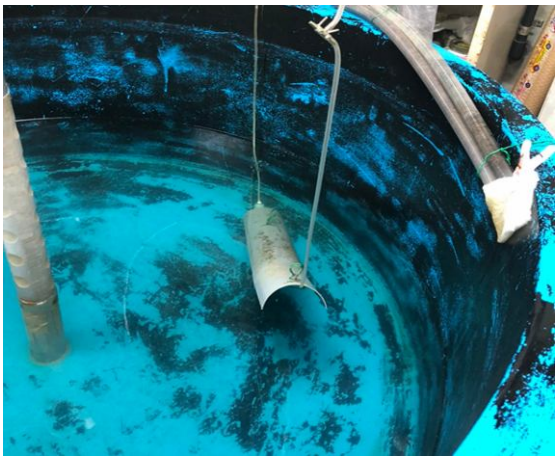

Figure 15. Attach a plastic tube to aerator to make a water current.

Cleaning of the larval tank. The tank should be cleaned every 3 d to remove dead fish, feces, and food leftovers. Use a plastic tube (Figure 8d) to syphon water from the bottom of the tank into a fine-mesh (1 mm) hand net (to collect the dead larvae). Count and record the number of dead larvae and assess their condition.

Feeding the larvae with live rotifers. The mouths of newly hatched larvae are closed. The larvae consume the yolk during an endogenous feeding period. The mouth opens and starts functioning after approximately 3 d of hatching. There may be some larvae hatched early, therefore, rotifers should be supplied to the larval tank immediately after all larvae hatch. The time and frequency of feeding with each type of food are shown in Table 1. The method for culturing live food, including rotifers and *Artemia* nauplii, is described in the subsequent section.

Table 1

Time table of feed management for ayu larvae

Feed management	*Larvae age (DAH)*										
	3	*5*	*10*	*14*	*15*	*30*	*35*	*40*	*45*	*50*	*100*
Rotifers											
Artemia nauplii											
Formulated food											

Darker color indicates higher feeding frequency.

Cultured rotifers (*Brachionus* sp.) are added into the rearing water tank as green water (water containing rotifers and chlorella has bright green color). Using a watering pot, spatter approximately 1 L of green water into the larval tank hourly during daytime.

Frequently check the density of the rotifers in the larval tank (extract 1 mL of the tank water into a glass pipette, and then count the rotifers using the fixed focus loupe). Maintain the rotifer density at 5 individuals mL^{-1} or more (shortage of rotifers may cause malformations in fish). Feed the larvae with rotifers until 35 DAH, when larvae can consume larger prey (*Artemia*) or formulated food.

Feed the larvae with Artemia nauplii. Larvae after the 15th DAH can consume larger food items, such as *Artemia* nauplii. Accordingly, feed the larvae once a day in the morning by spattering 1 L of water containing *Artemia* nauplii into the tank. Increase the frequency of feeding when the fish grows larger. The density of the remaining *Artemia* nauplii should be continuously observed to adjust the feeding frequency.

Familiarize the larvae with formulated food. Preparing the larvae to eat formulated food is necessary and can be achieved as follows:

(1) On the 14th DAH, spread one tablespoon (volume = 5 mL) of formulated food (e.g., ayu food Gold No. 1-powder type, Marubeni Nisshin Feed Co., Ltd.) on the water surface of the larval tank;

(2) Repeat step (1) once a day in the morning before feeding the fish with rotifers or *Artemia* nauplii. Observe the reaction of the larvae to the formulated food (whether or not the food is consumed);

(3) When the fish grows and can eat formulated food, use 0.5-0.8 mm of formulated grain food (Ayu soft No. 1 and No. 2; Nosan Corp.; see Table 2 for the food ingredients). Gradually increase the feeding frequency of formulated food while reducing the frequency of live foods.

Table 2

Ingredients of the formulated food for ayu

Food item	*Feed size (weight, g)*	*Grain size (mm)*	*Ingredient*				
			Animal protein[a]	*Cereals*[b]	*Vegetable oil residue*	*Rice bran*	*Other*[c]
Gold No. 1	1-3	0.08-0.16	71%	14%			15%
Ayu soft No. 1 and No. 2	3-8	0.5-0.8	68%	22%	3%	2%	5%
Ayu soft No. 3 and No. 4	8~	1-1.6	58%	25%	8%	5%	4%

[a]Fish meal and krill meal; [b]Wheat flour, flour, and pre-gelatinized starch; [c]Vegetable gum substance, licorice extract, calcium phosphate, calcium carbonate, and salt.

Transferring fish to an outdoor nursery and taking care of the fish until maturity. After approximately three months of rearing larvae and juveniles, the size of juvenile fish increases to 4-5 cm length. These fish would now depend completely on the formulated food. Subsequently, the fish are transferred to the outdoor nursery for the later rearing stages. This process can be conducted as follows:

(1) Gradually close the seawater inlet to reduce the salinity to zero;

(2) Fill clean freshwater into a 3000-L tank in the outdoor nursery and set the outlet so that the water level is approximately 40-50 cm;

(3) Place an aerator and a plastic tube in the tank to ensure a water current and maintain the water dissolved oxygen to aproximately 7-8 mg L^{-1};

(4) Transfer the juvenile fish into the 3000-L tank;

(5) Feed the fish four times a day by spreading formulated food (Ayu soft No. 3, No. 4; Nosan Corp.) on the water surface.

Preparation of live food

Culturing method of rotifers. It required the following tools: two cylindrical glass tanks (100 L), or buckets (for simple culturing system); rotifer stock culture (1 million, S-type, Reed Mariculture Inc.); seawater (10-35 ppt); aerator; water heater; supper fresh

Chlorella V12 (Chlorella Industry Co. Ltd., Japan), maintained at 4°C; rotifer floss (waste trap); beaker; glass pipet; fixed focus loupe; modfied plankton net.

Rotifer culturing should begin 3 d before the hatching date of larvae because rotifers take time to grow. The procedure for culturing rotifers is as follows:

(1) Prepare clean cylindrical glass tanks (100 L) or buckets and add 30 L seawater in each tank (water salinity ca. = 10-35 ppt, optimum salinity = 15-20 ppt; water gravity = 1.0075-1.026);

(2) Put a bag of rotifer stock culture (1 million rotifers) in the tank;

(3) Set the temperature of the water heater at 30°C to gradually increase the warmth of the water;

(4) After 5 min, open the rotifer stock bag and begin culturing;

(5) Add 20 mL of supper fresh *Chlorella* in the tank;

(6) Hang a floss across the water surface in the form of a curtain and weigh down the bottom so that it does not float (as shown in Figure 6b). This will accumulate solid wastes and thus, keep the culture clean. Clean the floss daily by rinsing in tap water, and place it again in the culture;

(7) Add 10 L of seawater on each of the following two days; thus, the water column rises to 50 L;

(8) Feed the rotifers once a day (add sufficient supper fresh *Chlorella* to maintain a light green color between feedings, e.g., add 20 mL of this solution for the first two days and 50 mL from the third day onwards);

(9) Check the density of rotifers in the culture by roughly estimating the number of rotifers in a glass pipet under a fixed focus loupe;

(10) Harvest rotifers by scooping them using a beaker and feed them to the larvae. The harvesting frequency should be equal to the feeding frequency of the fish larvae (described above). Harvest at least 20-30% of the culture tank daily;

(11) After the last harvest of the day, replenish the seawater volume to 50 L before adding supper fresh *Chlorella* (50 mL);

(12) Change the water in the rotifers culture tank every 3 or 4 d to prevent detritus over accumulation and ammonia poisoning. This can be done as follows:

- turn off the aerator and water heater, and clean them with a sponge under tap water;
- pour the culture tank water through a modified plankton net (rotifers are remained in the net), wash the tank gently under tap water, and then pour rotifers again into the culture tank;
- replenish the seawater up to 50 L;
- feed the rotifer with 50 mL of supper fresh *Chlorella*.

Preparation of live Artemia nauplii. This implied using the following tools: brine shrimp eggs (Ocean Star International. Inc., USA); brine shrimp incubating tanks (5 L); egg container (2-L plastic bottles); seawater (10-35 ppt; gravity 1.01-1.02); aeration; water heater; syphon (plastic tube); *Artemia* fortification diet (Bio-Chromis, Pacific Trading Co., Ltd.).

At approximately 15 DAH, larvae can eat larger food items (e.g., brine shrimp). Therefore, brine shrimp should be incubated one day prior to feeding according to the following procedure:

(1) Add approximately 3 L of water into the 5-L incubating tank. Submerge the water heater in the tank and set its temperature at 30°C;

(2) Add two teaspoons of brine shrimp eggs into a plastic bottle;

(3) Add 1.5 L seawater (salinity 10-35 ppt);

(4) Submerge the aerator into the plastic bottle containing brine shrimp eggs;

(5) Submerge the plastic bottle containing brine shrimp eggs into the incubating tank (Figure 16a). The brine shrimp will hatch after 20-36 h;

(6) After hatching, turn off the aerator and syphon the nauplii from the bottom of the tank into a new container using a plastic tube (Figure 16b);

(7) Feed the brine shrimp nauplii with one teaspoon of *Artemia* fortification diet (Bio-Chromis)

(8) Feed ayu larvae with the newly hatched brine shrimp as instructed above.

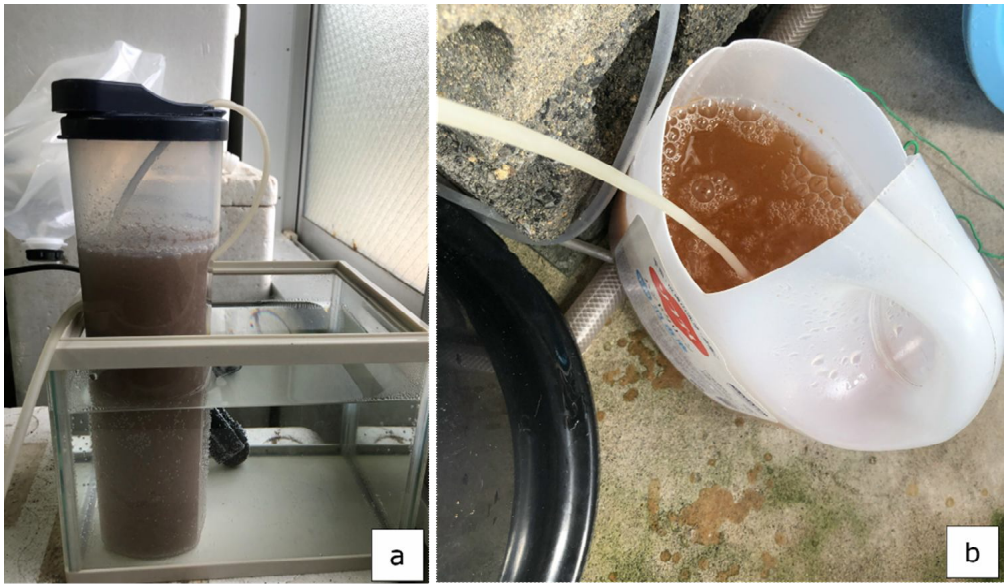

Figure 16. Brine shrimp incubation.

Special notes on vulnerable stages. On the 14-16th DAH [6.3-8.0 mm of standard length (SL)], we observed that many larvae sank at the bottom of the tank and died (larvae at this stage also reacted strongly when the water temperature reduced). At the 40-45th DAH (13.1-17.3 mm SL), larvae exhibited a strange behavior; they jumped out of the water and attached themselves to the objects on the water surface (such as the tank wall or outlet drain; Figure 17). This behavior caused mortality in many fish.

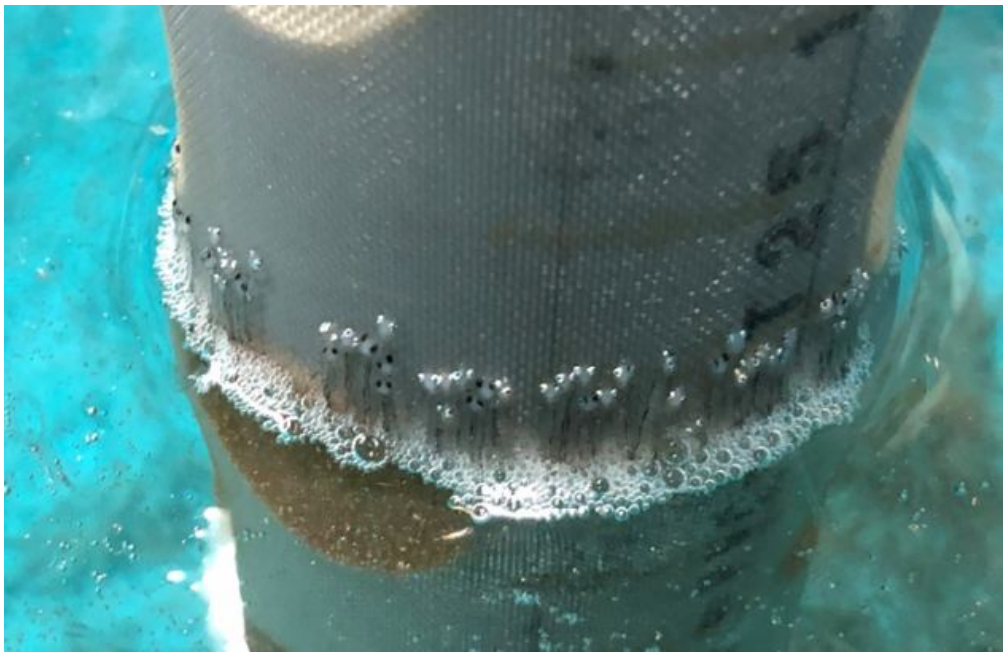

Figure 17. Fish exhibit a strange behavior as attach to outlet drain out of water.

We found that the two stages mentioned above were similar to the ages at which breaking points (when the growth rate of fish drops sharply) occurred in the growth rate model developed for Ryukyu-ayu individuals collected from the natural environment. The growth rate of these fish decreased sharply at the age of approximately 15th to 20th DAH and 40th to 50th DAH (Murase 2022, personal communication). The breaking points in the growth rate models usually occur during metamorphosis (Victor 1986; Kuroki et al 2010), which in turn is accompanied by high mortality in many fish species (Planes & Lecaillon

2001; Geffen et al 2007). This suggests that the high mortality and strange behavior of ayu, as observed in this study, may be related to metamorphosis during the early developmental stages of the fish.

To reduce the mortality rate during these two vulnerable stages, maintaining a stable water temperature may solve this problem because ayu larvae are extremely sensitive to temperature. Additionally, the 40-50th DAH are close to the age of the fish when they begin their upstream migration in the natural environments (Kishino & Shinomiya 2003). At this age, fish swim against the water current and jump out of the water surface, as described above. Therefore, generating a strong water current in the larval tank so that fish can swim against the current, similar to their natural behavior, may reduce the number of fish that attach to objects on the water surface, thereby reducing the mortality rate.

Conclusions. The artificial breeding and larval rearing techniques described in this paper can be applied to conserve ayu in southern areas which exhibit weather conditions similar to those in Amami-oshima (such as in the north of Vietnam, where the ayu population is at the edge of extinction). The techniques will also be useful in ayu rearing experiments. Additionally, the general rearing process can be applied to other species of the anadromy category as well. Future studies should focus on clarifying the mechanisms that trigger fish mortality at vulnerable stages.

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