THE CLOWNFISH (*Amphiprion* spp.) LARVICULTURE TECHNIQUE WITH RECIRCULATING AQUACULTURE SYSTEM (RAS) IN BULELENG, BALI

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Abstract

Clownfish is one of the most demanding-marine ornamental fish. Due to the increased demand of the clownfish, wild clownfish catch has also been increasing, until reaching the overexploitation condition. One way to preserve the existence of this fish species and to sustainably fulfil the market demand is by conducting a culture technique with advanced application using a recirculating-aquaculture system, specifically larviculture. Thereby, this paper reported the procedure of the clownfish larviculture and determined the growth performance of the larvae during the culture activity. This paper reviewed the direct observational operation for 40 days at Rizky Mitra Karya Hatchery, Buleleng, Bali. All observational data were analyzed descriptively. During the culture, the process was composed of rearing media preparation, clownfish egg-clutch collecting and stocking, and larval rearing. This process also included the feeding management in each rearing part (broodstock and larval rearing), water quality control by setting the RAS standard and and harvest for juvenile rearing. However, the hatching rate was lower than the standard given. Egg collection with scrapping method produced a lower hatching rate due to more human error discovered. To gain a better improvement, rotifer as a live feed for clownfish larvae and fluctuated weather condition due to the seasonal transition, which impacted on the availability of live feed for clownfish larvae should be handled intensively. Therefore, proper live feed culture with indoor system, thorough live feed intake observation on larvae, and proper larvae stocking by directly taking from the broodstock tank are necessary to stabilize this culture production with high sustainability level.

Keywords: *Breeding, Broodstock, Clownfish, Larvae, and Recirculation*

Introduction

Demands on marine ornamental fish in several decades have been increasing rapidly. These demands come from most ornamental fish hobbyists. Increasing demands occur as ornamental fish have colourful and interesting body shape. The global marine ornamental fish trade has also been increasing at more than $50-250 millions, whereas 1.5-2 billion of aquarium have filled with ornamental fish (Dhaneesh, et al., 2012). Akmal et al. (2020) also added that the marine ornamental fish trade in Indonesia has reached 33 billion USD with the volume up to 3 million. According to Pountney (2023), 1600 species of ornamental seawater fish has been traded internationally. One of the marine ornamental fish perceived by fish hobbyists is clownfish (*Amphiprion* spp.).

Clownfish can be easily maintained in an aquarium due to easily adaptable to different habitat condition from its original habitat and one of euryhaline fish species that has wide salinity tolerance among 10-30 ppt (Johan, et al., 2019). Moreover, this fish has high aesthetic value and often becomes a primadonna for fish hobbyists. The most expensive clownfish type is *Picasso* (*Amphiprion percula*), which has whiter colour covering almost of its body due to genetical disorders. This fish costs 59.99-199.99 USD, while *Ocellaris* clownfish (*A.*

ocellaris) is the cheapest clownfish type that costs at 20.99-59.99 USD with three white irregular bands surrounding the fish body (Aquarium creations online, 2020). Due to high market demand and one of the exporting fishery commodities, a direct catch of clownfish from nature becomes uncontrollable (overexploitation). This condition emerges as fishermen prefer catching this fish with explosives and destructive fishing gear that damages the coral reef as a habitat for many marine ornamental fish (Blondo & Burki, 2020).

A way to preserve the clownfish existence and fulfil the market demand is by conducting a culture activity. Clownfish culture has long been developing in Indonesia. The clownfish culture activities contain grow-out rearing, broodstock rearing, spawning, larval rearing, feeding management, water quality control, disease handling, harvesting, and marketing. One of the most critical parts in clownfish culture is larval rearing. Larval rearing should provide good fish seed quality and quantity, that can supply the market demand. Larval rearing activities contain stocking, media setting, feeding management, water quality control, and harvesting. To support larval rearing success, the water quality condition can be maintained through recirculating-aquaculture system (RAS). This system has long been applied in many culture activities, including the clownfish culture (Pietoyo, et al., 2020). However, a further observation should be performed to clarify whether the application of RAS in clownfish culture can be used well. Therefore, this article reviewed the application of RAS in clownfish larval rearing management at Buleleng, Bali and determined the growth performance of the larviculture with RAS.

Methods

This article described the clownfish larval rearing with RAS by following the culture activities in Rizky Mitra Karya *Hatchery*, Penyabangan Village, Gondol, Buleleng, Bali for 45 days. Therefore, the study method used in this article was a descriptive method. Data collected during the larval rearing activities were performed by direct observation and active participation. For confirmation, data were also ensured through interviews with the hatchery owner and staff, besides data comparation with the related literatures. Collected data were composed of egg stocking, larval tank construction and preparation, larval growth, water quality condition, feed types and feeding method, and harvesting.

Results and Discussions

Tank Preparation

The rearing tank used for clownfish larviculture (larval rearing) was a concrete tank with 9 m^3 volume (3 m × 3 m × 1 m). This tank was cleaned and washed with freshwater until all dirt and remaining waste were completely withdrawn. Tank was stood and dried for 24 hours. After drying for 24 hours, tank was filled with sterile seawater from the main seawater reservoir. During the filling period, seawater was also filtered with a 10 µm-net sized filter bag. Seawater filling was terminated, when the water height reached 75-80 cm. After water filling, rearing tank was aerated for good dissolved oxygen supply in the water and circulation, thus providing an optimum water condition for egg development, before hatching (Ghosh et al., 2012).

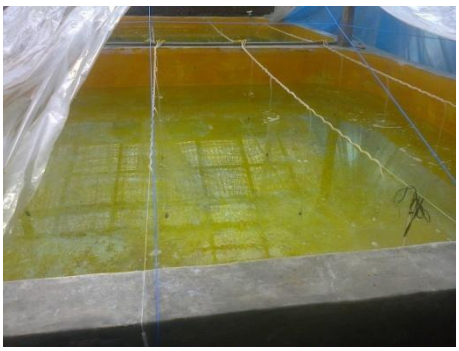

Figure 1. Larval rearing tank

Rearing tank was then stocked with phytoplankton *Nannochloropsis oculata* at 20-40 L. This volume contained the inoculant dose of phytoplankton at 3.507×10^5 cells/ml. The addition of phytoplankton onto the rearing media was applied as a nutrient enrichment action for clownfish live feed, rotifers. Also, this action provides a green-water condition to sustain the good water quality control in the rearing tank. For preventing the hatched larvae to starve, rotifers (*Branchionus plicatilis*) were also prepared in the rearing tank at 40-50 individual (ind)/ml. This activity was performed as the newly-hatched larvae had

less yolk volume at about 0.1027 mm^3 (Liew et al., 2006). Thus, larvae soon require exogenous feed that can be supplied from rotifers.

Egg Stocking

After broodstock spawning, eggs were attached to the substrate. Eggs were shaped likely as capsules with 1-2 mm length. According to Dhaneesh et al. (2012), the clownfish egg has 2.0-2.3 mm length and 0.9-1.2 mm width. Egg size in clownfish depends on the dissolved oxygen supply (Kunzmann & Diemel, 2020), temporal fanning behaviour as part of the parental care performed by male broodstock (Ghosh, et al., 2012), and the fulfilment of broodstock nutrient requirement (Subash, et al., 2020). Fertilized eggs mainly had white and yellowish colour, then turned to black and greyish colour, when ready to hatch, after 7-8 days of incubation period through parental care (broodstock pair collaborated together to maintain their eggs). Meanwhile, unfertilized eggs had solid white colour, which were then gulped by male broodstock clownfish.

Egg collection was performed by taking away the substrate from the broodstock pair. Then, eggs attached to the substrate were scrapped with a cutter and moved to a container filled with seawater. Total mature and collected eggs, that were distributed to larval rearing tank, were 400-1200. These eggs were then hatched as larvae with the hatching rate of 60-75%. According to Dhaneesh et al. (2009), scrapping method can also be performed by ink-filler. Another egg collection method is by directly moving the substrate to the larval rearing tank. Another way to collect the eggs were by waiting for the egg clutch to hatch in the broodstock rearing media, then moving the hatched larvae with artificial siphon and collecting them in a bucket filled with seawater. This method obtained the optimal hatching rate above 90% (DJPB-KKP, 2014). Thus, direct egg collection was less applicable due to more human error occurred during the collection. Besides egg collection method, other factors influence the hatching rate value in clownfish are broodstock feed types, egg-clutch position, and rearing environment (Kunzmann & Diemel, 2020). These factors will directly affect the protein concentration and physiological enzymes for optimal egg development.

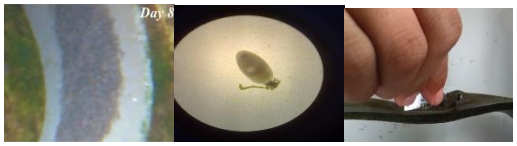

Figure 2. Clownfish eggs and collection: (A) Fertile egg clutch on the 8-th day of incubation, (B) Infertile egg, (C) Egg-scrapping method

Feeding Management

Larvae were reared to reach the juvenile stage for 28 – 30 days in the rearing tank. In brief, the feeding management of larval rearing can be shown in Table 1.

Table 1. Larval Feeding Management

Rearing Period (day)	Live Feed	Dosage	Volume (L)	Feeding Period
0	Phyto-plankton	3.507×10^5 cells/ml	40	Egg-stocking
	Rotifer	40-50 ind/ml	2	
1-20	Phyto-plankton	3.507×10^5 cells/ml	20	Two times a day
	Rotifer	40-50 ind/ml	1	
21-30	Phyto-plankton	3.507×10^5 cells/ml	20	2-3 times a day
	Rotifer	40-50 ind/ml	1	
	Nauplii *Artemia*	84 ind/ml	1.5	

During the rearing period, larvae were fed with rotifers. Rotifers has 100-300 µm size that matches with the clownfish larvae mouth-opening at 200-250 µm (Kostopoulou, et al., 2012). Rotifers also have high unsaturated fatty acids, such as DHA (22:6n-3) and EPA (20:5n-3) (Olivotto, et al., 2011). Rotifers were fed to the larvae at 40-50 ind/ml for 1-2 L with the feeding frequency at two-times a day at 08.00 WITA and 14.00 WITA. Rotifers were fed to the larvae until reaching

the juvenile stage or after 28-30 days of rearing. Before feeding the larvae, the rotifers were enriched with phytoplankton *N. oculata* in the rearing tank by supplying the phytoplankton at 3.507×10^5 cells/ml for 20-40 L. Nutrient enrichment is necessary for providing a good live fee quality and supporting the growth and development of the larvae.

When the rearing period reached on 21-st day, nauplii *Artemia salina* were supplied to the rearing tank as a nutrient supplement for larvae. This feed was stocked to the rearing tank at 84 ind/ml for 1.5 L. Nauplii *A. salina* contains high unsaturated fatty acids, mainly oleic acid (18:1) and EPA (20:5n-3), that are good for seawater fish (Herawati, et al., 2014). In addition, nauplii *A. salina* also has 400 µm size, which is suitable for clownfish mouth-opening at 300-500 µm (Sorgeloos, et al., 2001). After reaching 28-30 days of rearing, larvae have transformed into juvenile and should be moved to the juvenile rearing tank. Thus, live feed feeding was replaced by commercial feed feeding with more nutrient contents and stable supply.

Water Quality Control

Water quality control during the clownfish larvae rearing and other culture activities were supported by good water quality management with recirculating-aquaculture system (RAS) method. This system applies an irrigation system that supplies the clean seawater by recycling the wastewater from the rearing tank with low water quality condition, either physically, chemically, and biologically. Water recycling can be performed using filters. In addition, this system minimizes the operational energy cost in terms of water usage, more practical, and more environmentally-friendly (Ahmed & Turchini, 2021).

For RAS method, two water reservoir tanks were prepared as an inlet tank and an outlet tank. One of the tanks were organized with respective mechanical filters, namely sponge, sand, thatch, gravel, and stones. Another tank was organized with physical filters, namely heat blower and neon light as the UV-light source. RAS method applied in the larval rearing activity could maintain the water quality condition well and followed the water quality standard, as presented on Table 2.

Table 2. Water quality in rearing tank

Parameter	Value	Standard
Temperature (°C)	29-30	27-31*
Salinity (ppt)	32-34	25-35**
pH	8-8.5	7-8*
Dissolved oxygen (ppm)	4	3-5.45*
Ammonia (ppm)	0-0.15	< 3*

Note: * = (DJPB-KKP, 2014), ** = (Dhaneesh, et al., 2012)

Based on Table 2, all water quality parameters were conformed with the standard, except pH value. However, the pH value measured above was still tolerable for clownfish larvae, as there was no significant death found after harvesting.

Larval Growth and Development

For 28-30 days, larvae were observed their growth performance and development. The length growth of the larvae per day can be shown in Figure 3.

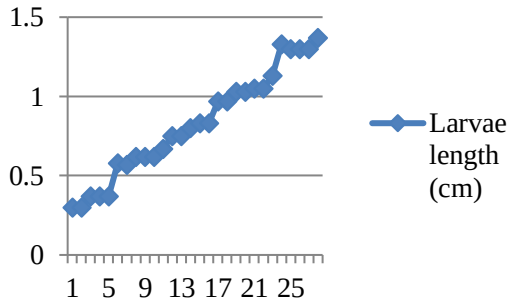

Figure 3. Larval length growth (27 days)

The initial length of the newly hatched larvae (1-day after hatching/DAH) was 0.3 cm. In this stage, larvae had a transparent body with small yolk and several organs formed, namely digestive organs and eyes. The 7 DAH larvae had less transparent and slightly orange color. In this phase, larvae have reached 0.5-0.6 cm length. The orange color in the larval body was solid and clear when the larvae have reached 14 DAH with 0.7-0.8 cm length. In the 21 DAH, larvae have reached 1-1.1 cm length with a white band surrounding their head. Also, larvae have started to spend more of their time at the tank base. At the final phase in 28-30 DAH, larvae had 1.2-1.4 cm length and transformed into juvenile, whereas their morphology is similar to the adult fish. Thus, they need to be

harvested and distributed to the juvenile rearing tank.

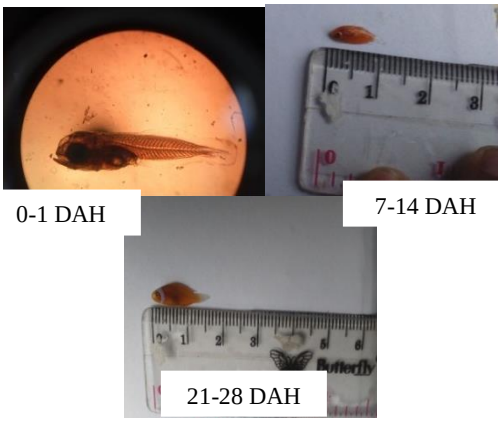

Figure 4. Larval Development (0-28 DAH)

Harvesting

Larvae has developed as juvenile, when the rearing has reached 28-30 days. This juvenile candidate was harvested from the rearing tank. Harvesting was performed by lowering the water height until 40-45 cm. Then, the juvenile candidate was caught with a 20-µm sized net and collected in a plastic container. When the harvesting has finished, the juvenile candidate was distributed to the prepared juvenile rearing tank with the stocking density of 400-600 fish/m^3.

After harvesting, growth performance of the larvae was calculated. The growth performance data contain absolute length growth (ALG), specific length growth rate (SGR), and survival rate (SR), as presented in Table 3.

Table 3. Growth Performance of Larvae

Parameter	Value	Standard
ALG (cm)	1.07	0.9-1*
SGR (%/day)	5.42	5.37^
SR (%)	60-70	60-70#

Note: * = Moore et al. (2023), ^ = Roux et al. (2019), # = Nass et al. (2016)

Based on Table 3, all values obtained the optimal value for growth performance in clownfish larvae, as all data were similar to the standard given. This condition means that the larviculture practice in this article can provide a good quality and quantity for juvenile rearing to fulfil the clownfish market demand. Yet, a further improvement can be regulated through live feed intake observation to supply a better amount of live feed for the larvae and increase the larval growth performance. Also, fluctuated weather condition that affected the live feed supply can be minimized by conducting an indoor live feed culture.

Conclusion

The larviculture process was composed of rearing media preparation, egg collection and stocking, larval rearing, feeding management, water quality control by setting the RAS standard, and harvesting. However, the hatching rate was lower than the standard given. Egg collection with scrapping method produced a lower hatching rate was discovered. For better improvement, proper live feed culture with indoor system, thorough live feed intake observation on larvae, and proper larvae stocking by directly taking from the broodstock tank are necessary to stabilize the culture production with high sustainability level.

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