THE FEEDING MANAGEMENT OF GROUPER (*Epinephelus* sp.) LARVAE FROM THE CROSS-BREEDING SPAWNING PRODUCT IN BULELENG BALI

Arina Saramoutia[1]*, Annur Ahadi Abdillah[2], Shobrina Silmi Qori Tartila[3]

[1]Department of Fisheries and Marine, Sumenep, East Java Province
[2]Marine Department, Faculty of Fisheries and Marine, Airlangga University
[3]Aquaculture Study Program, Department of Animal Science and Fisheries Technology, Faculty of Agriculture, Tidar University
*Email: arinamoutia3@gmail.com

Abstract

High mortality in larvae emerges due to less suitable size and nutritional contents of the feed for larval requirement. Therefore, live feed management is one of the successive factors in many grouper hatcheries to reduce high mortality risk in larval stage and provide good nutritional value in fish. This article reviewed the feeding management of grouper larvae and the obstacles occurred during the feeding management within the larval rearing period in Buleleng, Bali. The grouper larvae were produced from the cross-breeding process between female tiger grouper (E. fuscoguttatus) and male marbled grouper (E. polyphekadion). Rotifers (Branchionus plicatilis) were enriched with marine green microalgae (Nannochloropsis oculata) at 2×10^5 ind/ml. *Rotifers were then fed to the larvae at 1-3 ind/ml. This feeding application was performed when the larvae was at 2-7 DAH (days after hatching). Artemia salina was fed to the 17-DAH larvae at 0.2-0.5 ind/ml, then increased at 8 ind/ml as the larvae reached 27 DAH with four-time feeding frequency. Also, commercial feed was provided for the larvae at 15 DAH and small shrimp (Acetes sp.) was fed to the 40-DAH larvae for supplement. This feeding management could produce a good absolute length growth rate at 22.3 mm on 35 days of rearing. However, high mortality rate was still occurred due to cannibalism, high stocking density of rotifers, and fluctuated weather that decreased the live feed supply. Thus, additional nutrient enrichment and optimal larval consumption observation should be performed to reduce high mortality rate, cannibalism, and weather condition influence.*

Keywords: *Enrichment, Feeding, Grouper, and Larvae*

Introduction

Fishery is one of the developmental sectors in Indonesia that mostly supplies the country's income. This condition is also supported by the abundant water area in Indonesia that provides many kinds of fishery commodities. The fishery commodity that has been cultured in seawater condition with high economical value domestically or abroad is grouper fish (*Epinephelus* spp.). According to Sofiati et al. (2021), the total coral fish trade in Southeast Asia is about 30,000 ton per year with 15,000-21,000 ton is exported to Hong Kong. The grouper production from culture activities contributes only 8.6% of 52,000 ton of total grouper fish catch in Asia with more than 238 million USD value worth. From this condition, a good culture technique that contains breeding, nursery, and grow-out activities should be developed further.

A cross-bred grouper fish (*Epinephelus* sp.) has become the most genetical improvement of the cultured grouper fish to increase the growth performance and reduce the production activity (Jiet & Musa, 2018). Also, this fish commonly has a lower deformity level than the original grouper, such as camouflage grouper (*E. polyphekadion*) and tiger grouper (*E. fuscoguttatus*) (Ismi, et al., 2013). A culture activity will become unsuccessful, when there are no excellent fish seeds. However, high mortality level in grouper larvae becomes the main obstacle to fulfil good seed quality and quantity. Yulianti et al. (2012) reported that the survival rate of the grouper larvae on 1-day after hatching (DAH) to 30-40 DAH was up to 5-10%. This condition was occurred due to unsuitable live feed and nutrients required by the larvae. Thus, excellent seed quality

with high production level can be supplied by providing good feed nutrients fed to the larvae. Moreover, proper feeding time, feed quality and quantity are necessary to improve during the grouper fish larval feeding management.

Live feeds, namely phytoplankton *Nannochloropsis oculata*, zooplankton *Branchionus plicatilis* and *Artemia* sp., are common feed given to the larvae. The *B. plicatilis* enrichment with *N. oculata* has been confirmed to increase the nutrient contents of *B. plicatilis*. Adequate live feed feeding with good quality of phytoplankton and zooplankton will reduce the larval mortality level (Budianto, et al., 2014). The live feed fed to the cross-bred grouper larvae is carried out because live feed has complete nutrient contents to meet the nutrient needs of the larvae. Live feed feeding can be performed by providing good live feed needed by the grouper larvae first on an ongoing basis. Therefore, this paper reviewed the feeding management of grouper larvae from the cross-breeding spawning and discovered the obstacles found during the feeding management, thereby capable of providing proper feeding time with good quality and quantity of the live feed for grouper larvae.

Materials and Methods

This paper was based on the field observation of grouper larval rearing in Rizky Mitra Karya Hatchery, Buleleng, Bali for 60 days. All data obtained were analysed descriptively, by reporting the field activity related to the feeding management of cross-bred grouper larvae (*Epinephelus* sp.). The data were composed of live feed supply, feeding frequency and dosage, live feed stocking density in larval rearing tank, and water quality condition. By reporting the data with descriptive method, literature studies were also attached in this paper related to the larval feeding management.

Results and Discussion

Samples observed in this paper was *cantik* grouper larvae, produced from the cross-breeding of female tiger grouper (*E. fuscoguttatus*) and male camouflage grouper (*E. polyphekadion*) through an artificial spawning program. Larvae were hatched after 19-20 hours of incubation period in larval rearing tank. Meanwhile, the live feeds used in rearing the *cantik* grouper (*Epinephelus* sp.) larvae were rotifer (*B. plicatilis*) and *A. salina*. There were also *N. oculata* used as a nutrient enrichment material for rotifers and a green-water condition for maintaining the temperature balance in the rearing tank (Palmer, et al., 2007).

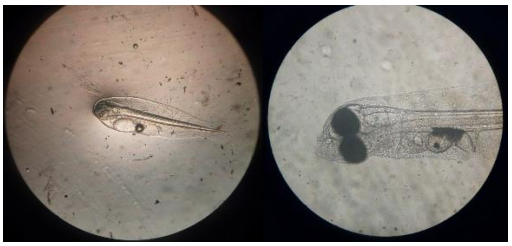

Figure 1. The newly-hatched grouper larvae (left); Larvae at 2-5 DAH (right)

Providing the live feed was initially prepared by cleaning the live feed culture tank from dirt and deposits found at the bottom of the tank. Cleaning was performed by brushing and rinsing the tank with freshwater or seawater, then dried for 24 hours. The culture media, that were applied for live feed culture must be sterile or uncontaminated by microorganisms. The sterilization process in the culture media was performed by filtering the seawater through mechanical filters, namely sand and gravel. Through the installation pipe, seawater was distributed into the live feed culture tank.

During the larval rearing, the 1 DAH larvae were remained unfed, as larvae still had yolk eggs and moved by following the water current in the tank. Feeding was initially performed on *cantik* grouper larvae in 2 DAH, as the yolk began to absorb by the larvae. The first live feed fed to the larvae was rotifer. Before applying this activity, the larval rearing tank was initially supplied with *N. oculata* at 2×10^5 cells/ml as an enrichment action for rotifers (*B. plicatilis*). This action was conducted once a day, so the nutrient quality of the rotifers remained well.

Nannochloropsis sp. contains52% saturated fatty acids, 25.5% poly-unsaturated fatty acids, and 22.2% monounsaturated fatty acids (Santhar, et al., 2021). Based on the proximate analysis, *Nannochloropsis* sp. has 33% protein, 21% lipid, and 16% carbohydrates (Sutomo, et al., 2007). The vitamin B12 in *Nannochloropsis* sp. is very important for increasing the rotifer population, while EPA is important for the

quality of nutrient value for rotifers as the main live feed for seawater fish larvae.

The rotifers used for feeding the *cantik* grouper larvae were small rotifers. According to Sugama et al. (2013), the small-sized rotifers are classified in type-S rotifers, which have a size of 120-180 µm. Rotifers have high protein content of 38.42% and 17.28% fat (Aslianti & Setyadi, 2014). Initially, rotifers were fed to the grouper larvae at 1-3 individual (ind)/ml. This feeding activity was carried out on 2-7 DAH larvae. Five days after the first feeding activity, it was suspected that the 2-7 DAH larvae did not feed too much and their movements were still limited because their organs were imperfect. In addition, it is suspected that the rotifers will grow larger both in terms of size and number. The rotifer density in the rearing tank must be observed to determine the next feeding activity. Observations can be made using the magnifying glass and a microscope. Based on the observation results, the rotifer density was elevated in the rearing tank by 4-6 ind/ml for 8-30 DAH larvae. If the rotifer density in the rearing tank was less than 4-6 ind/ml, then rotifers were added again, and *vice versa* to prevent the increasing ammonia level in the rearing tank.

Artemia is one of the widely-used feeds in the fish hatchery process, that can adapt to various environmental changes (Jubaedah, et al., 2006). In addition, *Artemia* also fulfils the nutrient requirements as a live feed, including being easy to prey on and fully digested, because *Artemia* swims slowly and has high nutrient value. This live feed contains high protein and essential fatty acids, namely 4.52% and 53.30%, respectively, while the lipid and carbohydrate contents in *Artemia* are 20.40% and 16.50%, respectively (Zaidin, et al., 2013). Compared to other zooplankton, the provision of nauplii *A. salina* is easier and more efficient, whereas *Artemia* cysts can be stored for several years whenever needed. These cysts can be taken for nauplii hatching, before being applied as a live feed for fish larvae.

Artemia was fed when the larvae were in 17 DAH with a density of 2-5 ind/ml. This feeding activity was performed twice a day. When the larvae were 27 DAH, the feeding density of *Artemia* was increased to 8 ind/ml with 4 times a day frequency to supply a highly nutrient provision for larvae properly. In 27 DAH, the rotifer consumption in larvae also began to decrease, evidenced by the high density of rotifers in the larval rearing tank among 10-14 ind/ml. Thus, rotifer feeding was slowly reduced, while *Artemia* and pellet feeding were increased. According to Ismi et al. (2013), the amount of *Artemia* feeding was adjusted for the larvae in the rearing tank, whereas this feed type must be eaten within 1-2 hours. The effect of *Artemia* administration on *cantik* grouper larvae provides a significant growth, as seen in Figure 2. The total length of grouper larvae at 20 DAH reached 11.3 mm, which was previously at 5 mm in 15 DAH. Therefore, *Artemia* has a complete nutrient content for the growth of *cantik* grouper larvae.

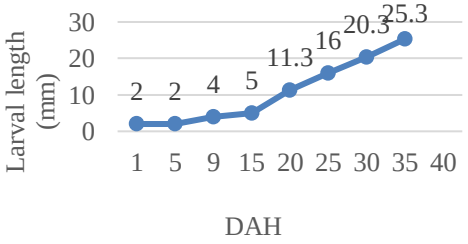

Figure 2. Grouper larval growth

In 15 DAH, larvae were also fed artificial feed in the form of pellets (*Otohime B1*) with a size of 250-360 µm. The provision of artificial feed was intended to avoid high mortality rate and an exercise for larvae to consume pellets as often as possible, thus larvae that transformed as juveniles could consume pellets very well (Sugama, et al., 2013). The nutritional contents of the pellets are 56.3% protein, 15.9% lipid, 2.5% calcium, and 2.3% phosphorus (PTAqua, 2022). In 40 DAH, another feed type was added, namely *Acetes* sp. This feed type was fed until apparent satiation for twice a day. The provision of *Acetes* sp. was performed due to the high nutrient content, whereas 100 g of sun-dried *Acetes* sp. contains 49.29 g protein, 3.62 g lipid, and 10 g chitin (Balange, et al., 2017). In brief, the feeding activity performed during the *cantik* grouper larval rearing is presented in Table 1.

Table 1. Larval feeding management

DAH	Live Feed	Dosage	Feeding Period
0-1	Phyto-plankton	2×10^5 cells/ml	-
	Rotifer	-	
2-7	Phyto-plankton	2×10^5 cells/ml	3-4 times a day
	Rotifer	1-3 ind/ml	
8-14	Phyto-plankton	2×10^5 cells/ml	
	Rotifer	4-6 ind/ml	
15-16	Phyto-plankton	2×10^5 cells/ml	
	Rotifer	4-6 ind/ml	
	Pellet	At satiation	
17-26	Phyto-plankton	2×10^5 cells/ml	2-3 times a day
	Rotifer	4-6 ind/ml	
	Pellet	At satiation	
	Nauplii *Artemia*	2-5 ind/ml	
27-39	Phyto-plankton	2×10^5 cells/ml	once a day
	Pellet	At satiation	
	Nauplii *Artemia*	8 ind/ml	4 times a day
40-45	Phyto-plankton	2×10^5 cells/ml	1-2 times a day
	Pellet	At satiation	
	Acetes sp.	At satiation	twice a day

To maintain the survival of the grouper larvae, the water quality was managed. The water quality management was carried out by siphoning and changing the water media in the rearing tanks to avoid high ammonia level in the rearing tank. Siphoning was carried out when the larvae reached 17 DAH in the afternoon. Siphoning was performed to clean dirt and remaining feed that settled at the bottom of the rearing tank. This activity was carried out gradually in each part of the rearing tank to avoid stress on the larvae. In 17-25 DAH, siphoning was carried out only a quarter part of the rearing tank. In 27-37 DAH, siphoning was carried out half part of the rearing tank. In 38-45 DAH, siphoning was carried out in all parts of the rearing tank. In addition to siphoning, water changes were also carried out, when the larvae reached 16 DAH. Along with the siphoning, water changes were carried out gradually within a few days, then increased as the larvae became older. The water quality measurement is shown in Table 2.

Table 2. Water quality condition

Parameter	**Value**	**Standard**
Temperature (^oC)	31-33	28-31*
Salinity (ppt)	34	33-34*
DO (ppm)	4	4.7-6.4*
pH	7.5-8.5	7-8.5^
Ammonia (ppm)	0.03-0.27	$<0.1^+$

Note: * = Ismi et al. (2013), ^ = Budianto et al. (2014), $^+$ = Sugama et al. (2013)

High ammonia values were occurred when the larvae reached 15-19 DAH at 0.15 ppm. This condition was caused by massive feeding activity, that caused the remaining feed, both live and artificial feeds, became higher. In addition, siphoning in this period was not performed, as siphoning was started at 18 DAH with only a quarter part of the rearing tank. After siphoning, the ammonia level was lower, namely 0.03-0.05 ppm. Excess rotifer feeding can also cause the ammonia level in the tank becomes higher. This was proven by the value of ammonia in 25 DAH larvae at 0.11-0.27 ppm and the rotifer density in each rearing tank was observed at 11- 12 ind/ml, whereas the rotifer density in each tank should be maintained at 4-6 ind/ml.

After harvesting the larvae at 45 DAH, growth performance of the larvae was calculated. The growth performance data during the larval rearing at 0-25 DAH are presented in Table 3. Growth performance data in the table are composed of absolute length growth (ALG), specific length growth (SLG), and survival rate (SR).

Table 3. Growth performance of the larvae

Parameter	Value
ALG (mm)	22.3
SLG (%/day)	321.1
SR (%)	5-6

All growth performance data showed a significant value, except the SR value. This condition was occurred due to high mortality rate during the larval rearing. The occurrence of high mortality is one of the obstacles that often occurs in grouper hatcheries. The high mortality is caused by several problems, including the presence of critical periods in the larvae and cannibalism. The critical period for *cantik* grouper larvae was observed at the beginning of larval rearing, namely at 1-8 DAH, due to dorsal spine development, and at 30-35 DAH, when the larvae entered a period of metamorphosis into juveniles. Moreover, high intensity of rain that occurred during the larval rearing that interfered the supply of live feed for larval growth and development support. The availability of live food for grouper larvae is very important to meet the nutritional needs of the grouper larvae, whereas these nutrients are used for the growth and development of the larvae. If the nutrients needed by the larvae are not met, the growth of the larvae will run slowly and can increase the mortality rate of the larvae.

Conclusions

The grouper larval feeding management began with the provision of rotifer (*B. plicatilis*) and nauplii *Artemia*. Live feed feeding on *cantik* grouper larvae was performed when the larvae reached 2 DAH. Feeding stage began by supplying phytoplankton *N. oculata* as a rotifer feed. Rotifers were fed during 2-27 DAH. *Artemia* was started to feed on 17-40 DAH. Obstacles found in the feeding management of *cantik* grouper larvae are high rainfall which can inhibit the availability of live feed and high mortality rate of the larvae due to critical metamorphosis stage and cannibalism.

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