

Outdoor larval rearing of golden rabbitfish (*Siganus guttatus*): observation on embryogenesis and early development stage

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Abstract. The outdoor culture system is an extensive larval rearing system used to produce fingerlings using a large volume of facilities in outdoor conditions. This study was aimed at investigating the embryogenesis and early-stage development of larval golden rabbitfish reared in outdoor concrete tanks. Selected broodstocks were spawned in a tank equipped with a substrate for egg attachment. The larvae were reared in a 64 m³ concrete tank with sand substrate at the bottom, at a density of 5 individuals per liter for 25 days. The larvae were fed with rotifers and commercial powder feed during the experimental rearing. The results showed that female broodstock weighing 712 – 755 g had a fecundity of 413,400 – 585,000 eggs. Embryo development from the 2-cell stage to hatching took 20 hours and 10 minutes. The hatching rate was 44 – 66% at an egg diameter of 450 – 661 µm. D-1 larvae had a total length of 2.4 mm, and mouth opening occurred at late D-2. At the end of the experimental period, larval growth achieved a total length of 21.0 mm.

1 Introduction

The demand for rabbitfish was still supplied by capture from the wild. Increased fishing activity for a long time may endanger the rabbitfish population in nature. In order to meet market demand, aquaculture activities must be carried out [1]. The availability of seeds, on the other hand, is a barrier to aquaculture activities in general [2], including culture development for golden rabbitfish, where seed production of the species still faces several constraints.

In particular, there are low survival rates [3, 4]. The critical larval phase occurs during a short period of starvation following egg yolk absorption or during the long period from hatching to metamorphosis. During this time, the larvae require enough food that is appropriate for their needs [5].

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As for other tropical marine fish species, rabbitfish larvae transition from endogenous nutrition to feeding on live prey between 2 and 3 days after hatch (DAH) [6]. The lifecycle has been closed for multiple rabbitfish species; however, the hatchery stage remains a bottleneck for the commercial production of rabbitfish fingerlings [7]. Several studies have been carried out to increase rabbitfish seed production, such as the use of several natural feeds, improvement in water quality, and others [8, 9].

Marine fish hatcheries can be conducted indoors or outdoors. Indoor larval rearing for rabbitfish *Siganus argenteus* has been reported to produce survival rates until 28 DAH, ranging from 1-5% [10]. A new approach to improve the seed production of the golden rabbitfish is to rear the newly hatched larvae in the fertilized pond [11] or outdoor tank. The system is commonly applied in ponds to produce seeds of freshwater fish and is less used for marine species. Although larval survival in extensive rearing systems is generally lower, and more variable than in intensive rearing systems, a study in Australia showed that it was more cost-effective for production fingerlings [12]. This system was applied for the larval rearing of golden rabbitfish using a big-volume outdoor tank. This study aimed to investigate the embryogenesis and early-stage development of golden rabbitfish larvae in an outdoor tank as the preliminary assessment of seed production of the species in the outdoor system.

2 Methods

The research was conducted in the Rabbitfish Hatchery Station of the Research Institute for Coastal Aquaculture and Fisheries Extension, Barru located in Barru Regency, South Sulawesi, Indonesia.

2.1 Broodstock spawning

Cultured broodstocks of golden rabbitfish used in this research were selected from a population with an average body weight of 506 g for males and 731 g for females. The broodstocks were maintained in a concrete tank with dimensions of 1.5 x 1.5 x 1.5 meters. Every tank has two separate water intake (inlet) and output (outlet) pipes. Since the eggs of golden rabbitfish are adhesive, shelters were set up on each side of the tank the day before spawning as a substrate for the eggs to adhere to. Before the new moon, the broodstocks spawned between midnight and dawn between 0:00 and 4:00. It takes 3–4 days for rabbitfish to spawn. After spawning, the eggs are carefully transferred from the shelter into the incubation tank. The incubation tank has a running water system of about 200% exchange seawater to ensure the eggs develop properly.

2.2 Larval rearing of golden rabbitfish

Larval rearing of golden rabbit fish was conducted in an outdoor system, using a 90 T concrete tank with sand at the bottom. The tank was filled with water to reach a volume of 64 m³, and the larval rearing lasted for twenty-five days.

2.2.1 Preparation of the larval rearing

Preparations for larval rearing included drying the tank, filling water, and applying fertilizer. The tank was dried for four days, and the seawater was filled for two days. On the first day, water was filled to 50% of the tank volume, and then fertilizer was applied. The types of inorganic fertilizer used were ZA 80g, TSP 60g, and Urea 100g. On the second day, water was filled up to 80% of the tank volume.

2.2.2 Stocking of larvae

The selection of the larvae stocked for this observation was uniform in size, actively moved, had no deformity, and was free of parasites. Larvae with the age of one day after hatching were stocked, where egg yolk as a food reserve for the larvae was still available. Larvae were harvested using a 100 - 150 µm scoop net. The larvae were harvested carefully and placed in a 10 L bucket equipped with aeration. Larvae stocking was carried out in the morning and acclimatized before they were stocked in larval rearing tanks. The stocking density of rabbitfish larvae is 5 individuals/L.

2.2.3 Feed and water quality management

During the rearing period, larvae were fed with live feed rotifer, *Brachiouneus rotundiformis*, and artificial feed (commercial powdered feed). *Nannochloropsis* as a green water system was given one day before the larvae were stocked until 25 days old (Table 1). A total of 10–15 individuals/mL of rotifers were given twice a day at 06.00 and 14.00 local time. The size of the artificial feed was adjusted to the age of the larvae, with feeding rate four times a day, at 08.00, 10.00, 12.00, and 16.00. The water addition starts at 7 days of age slowly with modest flows, and water changes of 15 - 25% begin at 10 days of age.

Table 1. Management of feeding and water during larval rearing of golden rabbitfish in an outdoor system.

| Rearing Management | DAY | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------------|-----|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | | | | | |
| Preparation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Drying tank | ■ | ■ | ■ | ■ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Filling seawater | | | | ■ | ■ | ■ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fertilization (Urea, TSP, ZA) | | | | ■ | ■ | ■ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Broodstock spawning | | | | | | | ■ | ■ | ■ | ■ | ■ | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Larvae Rearing | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Age of Larvae (DAH) | | | | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | | | | |
| Larvae stocking | | | | | | | | ■ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nannochloropsis | | | | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| Rotifer | | | | | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| Artificial Feed | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Micropellet (powder) | | | | | | | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | |
| Micropellet (< 0.4 µm) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Micropellet (0.4 - 0.7 µm) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Water management | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Slow circulation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Water exchange (15-25%) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harvest | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Partial | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Total | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ■ |

2.3 Data collection and calculation

Data were collected directly by monitoring the parameters observed during rabbitfish larval rearing. Data were analyzed descriptively, quantitatively, and qualitatively before being presented in the form of tables, graphs, and pictures.

2.3.1 Gonad maturity index (GMI) and broodstock fecundity

Six female rabbitfish were weighed and dissected, and the gonads were weighed. Determining the Gonad Maturity Index (GMI) uses the following formula:

$$\text{Gonad Maturity Index (\%)} = 100\% \times (\text{gonad weight/body weight}) \quad (1)$$

The weight of the sub-sample was weighed and the number of eggs in the sub-sample was counted from the three gonads of the female rabbitfish. The following formula was used to calculate broodstock fecundity.

$$\text{Fecundity} = \text{number of eggs in sub-sample} \times (\text{gonad weight} / \text{sub-sample weight}) \quad (2)$$

2.3.2 Embryogenesis, egg diameter, and hatching rate.

Ten rabbitfish eggs were observed for embryo development using an Olympus microscope coupled to a camera and computer at a magnification of four times. The embryogenesis was observed from the time after the broodstock spawned until they hatched. Egg diameter was observed in 100 rabbitfish egg samples. The hatching rate of rabbitfish eggs was observed in a 20 L aquarium filled with 400 rabbitfish eggs and an oxygen source provided by aeration. After 24 hours, the hatched larvae are counted to determine the hatching rate of rabbitfish larvae according to the following formula:

$$\text{Hatching rate (\%)} = 100\% \times (\text{number of larvae/number of eggs}) \quad (3)$$

2.3.3 Development of rabbitfish larvae

The development of rabbitfish larvae was observed using an Olympus microscope connected to a camera and computer. Ten samples of larvae were measured every day, and parameters observed included total length, eye diameter, and larval mouth opening.

2.3.4 Water quality

The water quality parameters observed were temperature, dissolved oxygen, salinity, and pH. Water quality observations were measured every day at 07.00 and 16.00.

3 Results and discussion

3.1 Gonad maturity index (GMI) and broodstock fecundity

The mean weight of rabbitfish broodstocks used in this study was 731 g for females and 506 g for males. According to the result, male broodstock had the highest GMI value of 4.0% and the lowest 0.4%, while female broodstock had the lowest value of 7.3% and the highest value of 9.9%. Meanwhile, broodstock rabbitfish had fecundities of 413,400, 483,600, and 585,000. Table 2 displays data on the Gonad Maturity Index and fertility of the golden rabbitfish broodstock.

Table 2. Gonad maturity index and fecundity of golden rabbitfish broodstock.

| No | Sex | Body weight (g) | Gonad Weight (g) | GMI (%) | Fecundity (eggs) |
|----|--------|-----------------|------------------|---------|------------------|
| 1 | Female | 717 | 53 | 7.4 | 413,400 |
| 2 | Female | 722 | 62 | 8.6 | 483,600 |
| 3 | Female | 755 | 75 | 9.9 | 585,000 |
| 4 | Male | 471 | 19 | 4.0 | |
| 5 | Male | 660 | 3 | 0.6 | |
| 6 | Male | 680 | 3 | 0.4 | |

The GMI of males was frequently lower than females. The difference in this index was related to differences size of the gonads of male and female fish, with female fish having larger gonads than male fish. Female fish gonads are larger due to the presence of egg cells. The egg cells of female fish in gonads become larger, followed by an increase in the GMI until spawning time [13]. The GMI rises to its highest value during spawning and then falls rapidly until spawning is complete [14]. The number of eggs released during spawning is referred to as fecundity. In this study, the fecundity values were 413,400, 483,600, and 585,000, respectively (Table 2). The findings of this present study supported the previous studies that one rabbitfish broodstock can generate between 245,000 and 500,000 eggs [15].

The diameter of rabbitfish eggs in this study ranged from 450.7 to 661.9 μm . The egg diameter obtained in this study was relatively similar to that found in the previous study which was 580-640 μm [16]. The diameter of rabbitfish eggs ranged from 546-550 μm depending on the size of the fish weight [15]. Egg productivity was influenced by the availability of natural food, the living environment and body size [17]. Egg diameter can be determined by the level of gonad maturity [18]. Based on egg diameter and histological spawning rabbitfish are included in the total spawning type or fish spawning in one period and releasing the eggs at once in a short time. The existence of non-uniform diameter size was suspected to be influenced by the condition factor of the broodstock [19]. Condition factors are influenced by environmental conditions, diet, age differences, food availability, and gonadal maturity [20]. Egg diameter varies between species and individuals within the same species. As previously mentioned, egg diameter is also influenced by the level of gonadal maturity. The greater gonad maturity, the greater the diameter of the eggs obtained or the greater the spread of egg diameter [21]. The same evidence was reported by previous studies that the more developed the gonad, the greater the center line of the egg as a result of the deposition of oil grains that goes along with the development of the level of gonadal maturity [14].

3.2 Embryogenesis, egg diameter, and hatching rate

The embryogenesis of golden rabbitfish occurs in multiple developmental sequences. The first stage is cell cleavage into two cells, which happens about 30 minutes after spawning. Every 10 minutes, further development is detected, notably cell division from 2 to 4 cells, then division from 4 to 8 cells, and so on until the development reaches many cells.

The next division, the high-stage phase was the division of very many cells. The time required from the division of 1 million cells to the high stage was 15 minutes. The following phase was the germ ring stage, which is the phase of division forming a ring around the

dividing cell, taking 45 minutes. Entering the shield stage was the phase where the division formed an anprization around the egg wall. The time required was more than 60 minutes.

The next stage was the formation of epiboly or the stage where the layers of epithelial cells move to spread according to their axis. This phase was divided into 2, namely 30% epiboly cleavage and 50% epiboly cleavage. From the shield stage phase to the 30% epiboly stage took about 1 hour, while from 30% epiboly to 50% epiboly took about 30 minutes. The Blastoderm Extension phase was a critical phase where embryonic cells entered the organ formation phase. The time required from the 50% epiboly phase to the blastoderm extension phase was around 1 hour 30 minutes. The eye lobes phase or eye formation took 40 minutes.

Next was the somites phase or the formation of spine divided into two, namely 10 somites and 18 somites. The time span required from eye lobes to 10 somites was 2 hours. From 10 somites to 18 somites takes > 3 hours. The heartbeat phase or the phase in the heart starts to look and the beat also starts to take about 30 minutes. This phase is the last phase before the time the eggs hatch, where in this phase, the eyes and body shape can already be seen. Entering the hatching phase took about 7 hours from the heartbeat phase or 20 hours 10 minutes after spawning. The complete embryogenesis of rabbitfish is illustrated in Figure 1.

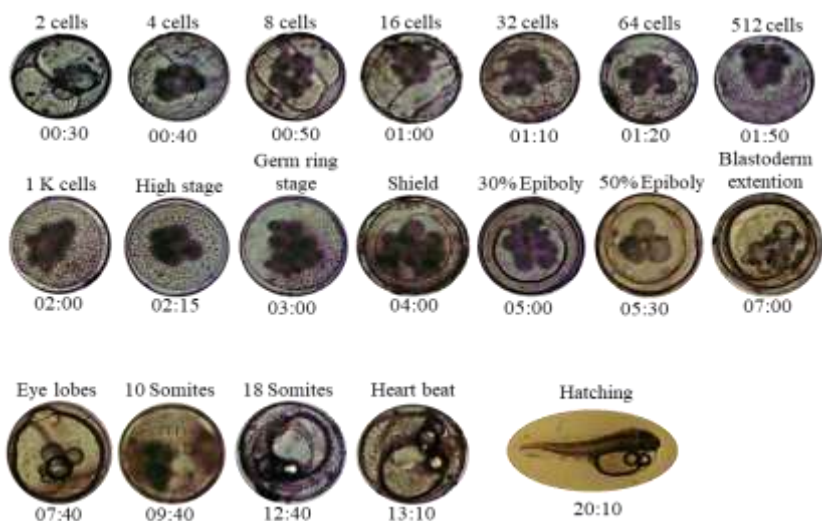


Fig. 1. The embryogenesis of golden rabbitfish.

The egg diameter of golden rabbitfish ranged from 450.7 - 661.9 μm , with the largest distribution of eggs at a diameter of 541.3 - 571.4 μm . The hatching rate of rabbitfish in this study ranged from 45.3 - 66.0% (Figure 2).

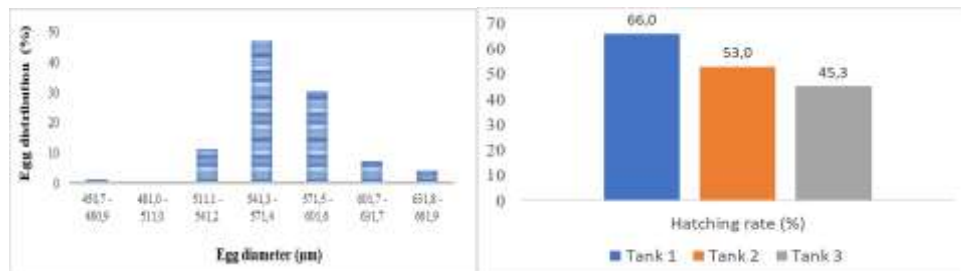


Fig. 2. Distribution of egg diameter and hatching rate of golden rabbitfish eggs.

The initial development phase of the embryo in rabbitfish species *Siganus guttatus* broadly includes the cleavage phase, morula, blastula (blastoderm formation), gastrula (yolk closure), and hatching. The critical period of rabbitfish embryo development occurs in the development phase of many cells and the phase of gastrula formation until hatching [22]. In this phase, embryonic development often stops if the eggs are subjected to suboptimal handling or shocking environmental conditions. The hatchability of rabbitfish eggs obtained in this study was lower than the previous research which was 78.6% - 84.7% [22]. Low hatchability is thought to be due to high salinity and temperature. The salinity in this study was 30 ppt and the temperature ranged from 30-31 °C. The optimum salinity for egg-hatching tanks was 25-28 ppt, and temperatures ranged from 27- 29 °C [23]. The hatching of fish eggs was influenced by several factors, namely internal (egg quality and hormones) and external factors (water quality) [24].

3.3 Larval development

Observations of larval development showed that starting from 0 DAH (day after hatching or shortly after hatching), larvae had a body length of 1.71 mm, with a clear (transparent) color, and had a full yolk sac. On 1 DAH, the larvae had a body length of 2.14 mm; the yolk began to shrink, and the color was transparent. The larvae 2 DAH were 2.42 mm in length; the yolk sac was starting to run out; the eyes had formed; the mouth was present but not yet open; the pectoral and caudal fins were getting clearer, and the digestive tract had started to form. On 3 DAH, larval length was 2.50 mm; larval mouth opening was clearer with a size of 152.7 µm; eye diameter was 201.1 µm; yolk was depleted. On the 5 DAH, the larvae reached 2.66 mm in length, with a mouth opening of 161.2 µm and an eye diameter of 219.8 µm. On the 7 DAH, body length reached 3.05 mm, with a mouth opening of 259.7 µm and eye diameter of 231.9 µm.

The body length of larva on 11 DAH was 4.99 mm with a mouth opening of 574.2 µm. At this stage dorsal fins are already visible on the larvae. The length of larva on 16 DAH was 8.75 mm, mouth opening of 1.064 mm, and eye diameter 606.2 µm, dorsal fins, and dorsal fins were developing, colors begin to appear, and the typical of *S. guttatus* dots begin to appear. On 18 DAH, fish larval length reached 12.38 mm, mouth opening 1.377 mm and eye diameter 700.2 µm, 25th length. On 21 DAH, larval length reached 17.50 mm, eye diameter 1.611 mm, mouth opening 1.349 mm. Larva length on 25 DAH was 21.00 mm with eye diameter of 1.753 mm, and mouth opening 1.725 mm. The fish color and chromatophore was getting clearer. At this early juvenile stage, the internal and external organs are fully developed as adult fish. The development of total length, eye diameter, and mouth opening are presented in Figure 3. Meanwhile, the early development stage of rabbitfish larvae reared in an outdoor system is presented in Figure 4.

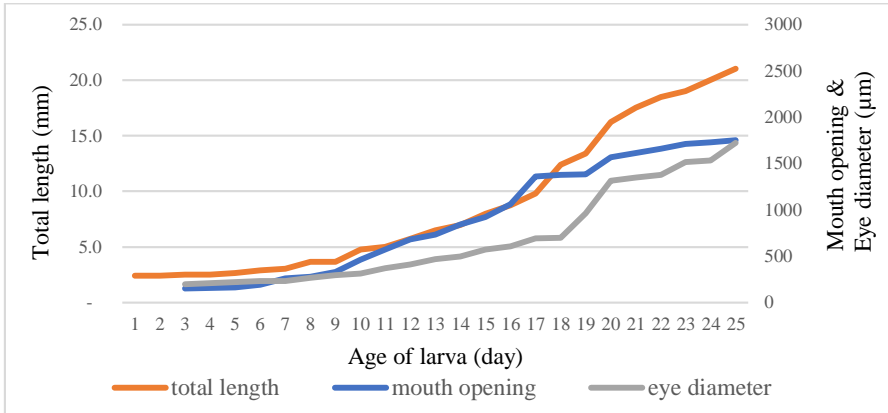


Fig. 3. Total length, mouth opening, and eye diameter of golden rabbitfish larvae reared in an outdoor system.

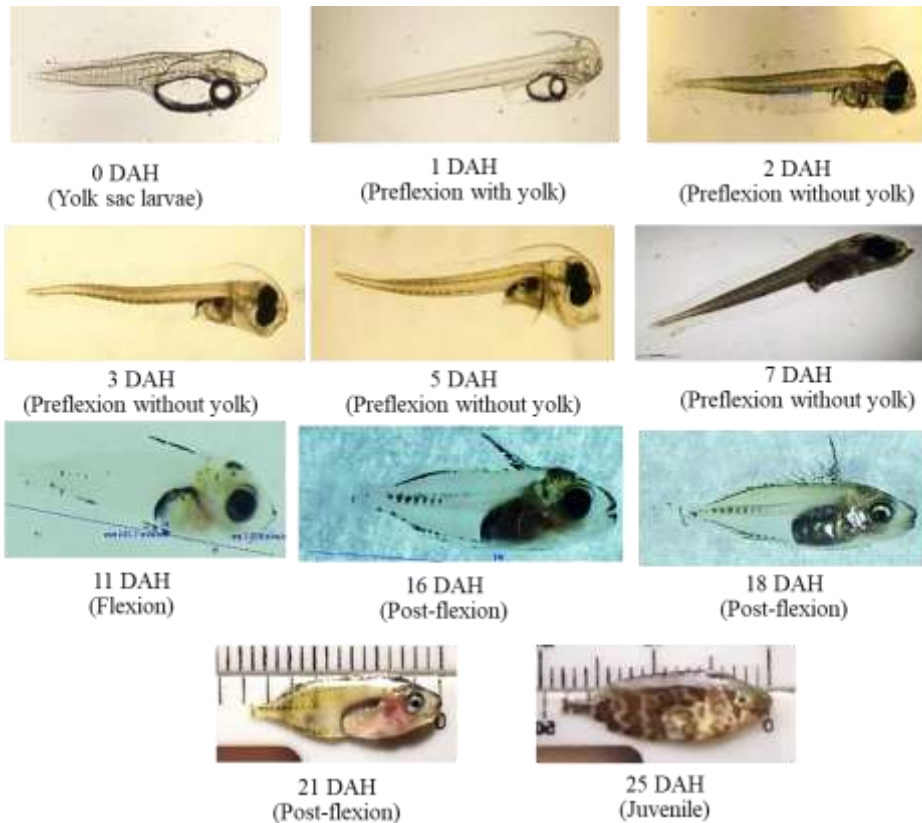


Fig. 4. The early development stage of golden rabbitfish larvae reared in an outdoor system.

Newly hatched rabbitfish larvae have eyes and mouths that are not yet functional. Based on observations of rabbitfish larvae, eye pigmentation was fully developed, and the mouth opened when the larvae were 3 days after hatching. Complete absorption of egg yolk happened when the larvae were 3 days after hatching. The egg yolk was reserved energy in development before the larvae eat external food [20]. The color of the larvae from hatching

until 15 days old was clear. At the age of 8 to 45 days after the eggs hatch, the larvae undergo a metamorphosis process, which changes the morphology or physiological function of the external and internal organs towards a more perfect stage [16]. Internal and external changes are influenced by various factors such as species, feed quality, and environmental conditions. The metamorphosis process is completed when the larvae reach a further developmental phase, the juvenile phase.

3.4 Water quality

During the study, the temperature ranged from 28.1 °C to 31.7 °C, the salinity ranged from 27.2 to 30.6 ppt, the dissolved oxygen levels ranged from 4.5 to 7.8 mg/L, and the pH ranged from 7.19 to 8.08 (Figure 5).

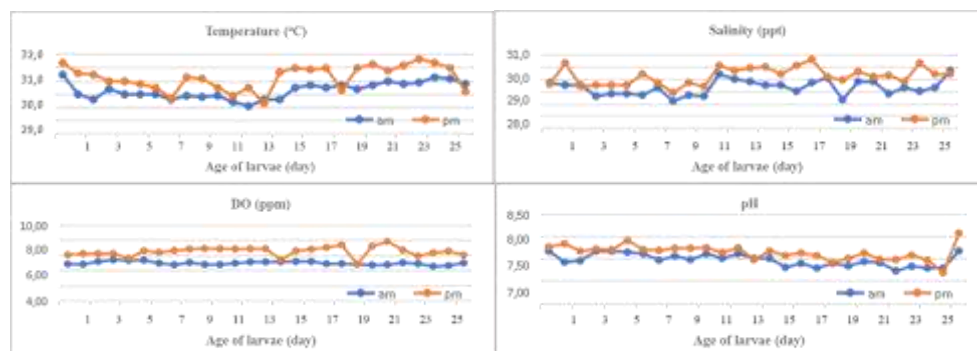


Fig. 5. Water quality parameter of outdoor rearing larvae of rabbitfish.

Water quality parameters measured during this study were temperature, salinity, pH, and dissolved oxygen within the optimum limits that support the growth of rabbitfish larvae. This indicates that rabbitfish larvae can be reared using outdoor facilities. The best temperature for rearing rabbitfish larvae was 26-31 °C, and the salinity for rabbitfish larvae was in the range of 25-30 ppt. The ideal dissolved oxygen value for rabbitfish larvae was > 4 ppm, while pH of water for rearing rabbitfish larvae was 7.0-8.6 [23, 25]. The temperature of the rearing medium affects the development of larvae after eggs; this is because temperature can affect the rate of absorption of egg yolk, which is a source of energy for the larval metabolic process. Temperature can affect metabolic rate and increase oxygen consumption and fish movement activity [26]. Salinity directly affects the fish body's metabolism, especially the osmoregulation process [27]. Dissolved oxygen is needed by all bodies for breathing, metabolic processes, or substance exchange which then produces energy for growth and reproduction [23].

4 Conclusion

Golden rabbitfish larvae can be reared in an outdoor tank facility. The embryogenesis performs well and begins to hatch 20 hours and 10 minutes after spawning. The golden rabbitfish larvae succeed and develop properly, with the length of the larvae increasing every day and the development of morphology and organs of the larvae body improving every day until metamorphosis into juveniles that look like adult fish. The water quality of the rearing media in the outdoor facility remains within the optimal range for rabbitfish larval development and growth.

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