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Development of Siganid (*Siganus guttatus*) larvae during the transition period

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ABSTRACT

Siganid is better known as rabbit fish. In hatcheries, constraint that is still faced is the low survival, which was assumed to occur because of the timing for initial feeding is not solidly known. This research aimed to examine the best initial feeding time for siganus, based on the evaluation of eyes and yolk reserves during the transition. The research was conducted from 24-29 March 2021 in IPUW Barru, South Sulawesi. Larvae were obtained from the second progeny (G2) of domesticated *Siganus guttatus*. Larvae were reared for 5-6 days without feeding. Evaluated parameters include eyes diameter and yolk reserves. Samples were observed with a microscope and will be explained descriptively. Water quality parameters were measured, namely DO, salinity, pH and temperature. Eyes diameter at 6 Hour After Hatching (HAH) ranges between 81.5-128.9 μm, 13 HAH= 125.5-167.7 μm, 24 HAH= 138.2-213.9 μm, two days after hatching 2 Day After Hatching (DAH) = 113.6-193.1 μm, 3 DAH= 163.1-219.2 μm, 4 DAH= 190.4-212.6 μm. Yolk reserves diameter ranged between 137-194μm (6 HAH), 13 HAH= 152-191μm, 24 HAH= 94.0-185μm, 2 DAH= 75.3-99.63μm, 3 DAH= 42.33-87.58μm, 4 DAH= 38.17-55.59μm. After age 5 DAH, there are no larvae found alive (dead). Eyes developed at age 6 HAH and experienced pigmentation at age 24 HAH. Conversely, yolk reserves diameters were getting smaller since age 24 HAH and completely disappeared at age 4 DAH. It indicates that eyes effectively see feeds at age 2 DAH. Therefore, initial feeding should be started. The water quality parameters measured were still in normal conditions according to the life of *S. guttatus* larvae. From this research, it can be concluded that eyes were well functioned at age 2 DAH and yolk reserves was finished at age 4 DAH. Therefore, the initial feeding should be done at the age of 2 DAH.

Introduction

Siganid is locally known as "baronang". The most significant aquaculture production is obtained from the Philippines (Laining *et al*., 2017; Gonzales *et al*., 2018). Siganid cultivation has begun in Indonesia, for example, in the Seribu Islands, Banten Bay, Riau Islands (Salampessy and Irawati, 2021), and the local species *Siganus guttatus* is now domesticated in the Barru Tiger Shrimp Farm (IPUW), South Sulawesi. This species is marked by the brown spots on its skin. A morphological feature which is the key identification of this species is a big yellow spot adjacent to the last few rays of its dorsal fin near the caudal peduncle, which distinguishes the fish from other siganids.

The production of healthy *S. guttatus* fingerlings is hindered because of the low larval survival rates. Only three percent survived the larvae stage in each hatchling cycle (direct field observations during 2020). The low survival rates were assumed to occur due to imprecise time to start exogenous feeding of the larvae. Accordingly, hatchery cannot sustainably supply fingerlings to the pond and sea cage farmers (Kamaruddin *et al*., 2019; Usman *et al.*, 2021). Therefore, research on an appropriate time to initiate feeding the larvae is crucial.

Appropriate initial feeding the of larvae largely

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depends on the availability of the yolk reserves brought from the embryonal stage of the larvae. Fish depends largely on the eyes to support its feeding activities (Yufera *et al.,* 2014), including *S. guttatus*. Feeding activity is is an essential factor in fish farming (Az Zahra *et al.*, 2019). Feeding success is positively correlated with larval growth and survival (Stuart, 2013). Feed will be preyed if they are visible (Riyanto *et al.,* 2011). Eyes development was essential for living organisms survival. Therefore, it is crucial to have research on larval eyes development to be a reference in determining an appropriate timing of feeding.

S. guttatus eyes are started to form on the first day after hatching, yet it has not been fully functioned. They also have limited coverage. These capabilities improve in terms of distance and range as the larvae grow. Feed viability in rearing tanks must be given on time before the yolk reserves, and oil globule are completely resorbed (Yufera *et al.*, 2014). Hence, it is important to have information on eyes development and the time when the yolk reserve is fully resorbed so that it can be a reference to initiate feeding and finally achieve better growth, development and survival.

Therefore, this research has aimed to evaluate the eyes development of *S. guttatus* and measure the yolk reserves availability as food reserve attached since the embryonal stage of the fish. The result of this research can be utilized to determine the timing for initial feeding, so that feed can be exploited by larvae and not be wasted that can become pollutants in the rearing tanks. Also, accurate timing to start feeding ensures that the feed can be digested optimally by the fish.

Materials and Methods

Location and time of research

The research was done in the Tiger Shrimp Hatchery Installation, Barru, South Sulawesi from 24-29 March 2021. The eyes diameter development and yolk reserves resorption was done in IPUW Biotechnology Laboratory.

Sampling techniques

To obtain data on eyes development, and yolk reserves resorption as well as oil globules, a series of preparation were done including:

a. Rearing tank preparation

The rearing tanks that were used in this study consisted of two cylindrical fiber tanks of size 1000 L. The tanks were filled with 700 L of seawater with 25 ppt salinity. Each tank was equipped with aeration to supply oxygen. Seawater was collected from Lawallu Village, Barru Regency, South Sulawesi, that has been treated (filtered and UV sterilized for 6 hours).

b. Larval transfer

Larvae used in this research were obtained from *S. guttatus* brood stocks which were the second progeny (G2) kept in the IPUW facility. Larvae transferred to rearing tanks were aged 1 day after hatching (D1) from the incubation tank. Transfers were done in the morning (06.00 am) with a density of 20 individuals/liter (according to technical guidelines from Juario *et al.,* 1985). The number of larvae stocked was 20,000 in the rearing tank, and each observation was carried out by harvesting 30 larvae.

c. Larval rearing

Larvae were reared for six days without feeding to evaluate duration of yolk reserve utilization until all larvae died. During the period, samplings were done every day and were done randomly. Samples were collected from each stage of age which are 6 Hours After Hatching (HAH), 13 HAH, 24 HAH and 2 Days After Hatching (2 DAH), 3 DAH, 4 DAH, 5 DAH and 6 DAH.

Tested parameters

Samples obtained from the tank were brought to the laboratory to observe. Those parameters include:

a. Eyes

To measure the diameter of the eye of *S. guttatus,* a microscope with a magnification of 4X was used. The microscope was connected to the computer to ease observation and documentation. The eye's diameter was measured by taking a straight perpendicular line from a point at the eye circle edge to a point on the other edge's side. Measurements were taken every morning (07.00) at the age of 6 HAH, 13 HAH, 24 HAH, 2 DAH, 3 DAH, and 4 DAH.

b. Yolk reserves diameters and oil globules

Measuring yolk reserves and oil globules was done using a similar method to measuring eyes diameter.

c. Water quality parameters

Water quality parameters that were measured during the rearing include DO, salinity and temperature. Dissolved oxygen was measured using DO meter, salinity was measured using a hand refractometer, pH was determined using a pH meter while temperature was measured using a thermometer.

Data analysis

Data on the eyes diameter and yolk reserves which was derived in the form of picture (documentation) will be described descriptively. Water quality parameters will also be described descriptively by comparing water quality parameters against the standard reference values of those of *S. guttatus* larvae.

Results

Eyes development

S. guttatus larvae's eyes development from 6 HAH, 13 HAH, 24 HAH, two days after hatching (2 DAH), 3 DAH, 4 DAH are described in Figure 1. In Figure 1, eyes were differentiated at 6 HAH, but they were not pigmented and were still transparent. Pigmentation took place at 24 HAH and the color was getting darker as they grew up (at 2-4 DAH). Eyes diameter measured at 6 HAH was 81.5-128.9 µm. Diameter at 13 DAH ranged from 125.5-167.7 µm. At 24 HAH, it was between 138.6-213.9 µm. The diameter was getting larger on 2 DAH (113.6-193.1 µm), 3 DAH was between 163.1-219.2 µm, and on 4 DAH it was measured to have a range between 190.4-212.6 µm. On 5 DAH, living larvae were nil.

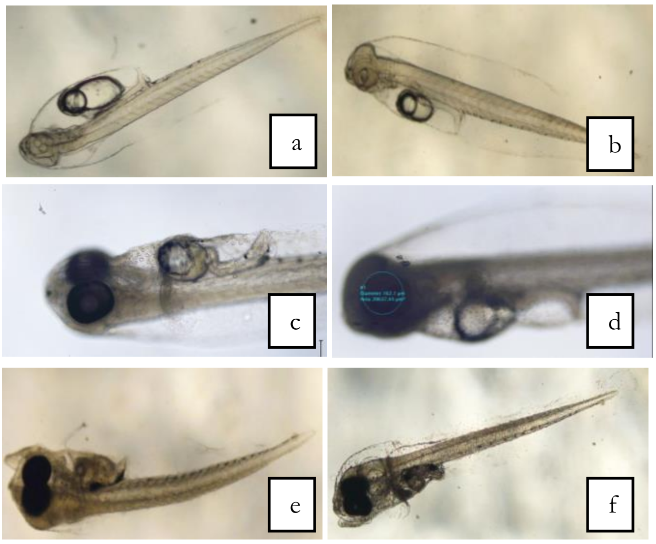

Figure 1. *Siganus guttatus* larvae's eyes development (blue arrow); (a) 6 HAH, (b) 13 HAH, (c) 24 HAH, (d) 2 DAH, (e) 3 DAH, (f) 4 DAH.

Yolk reserves and oil globules utilization (Food Reserves)

Food reserves utilization of *S. guttatus* from 6 HAH, 13 HAH, 24 HAH, 2 DAH, 3 DAH and 4 DAH are described by Figure 2. Figure 2 shows larvae development in terms of yolk reserves utilization. In Figure 2a, the reserve food is still complete. The size of its yolk reserves is larger than the fish head. In figure 2b, the size of the food reserve is almost similar to larva age 13 HAH. The yolk sac size was measured between 137-194 µm, while at the age of 2 DAH the size was measured between 75.3-99.63 µm. The size of the yolk sacs on 3 DAH are 42.33-87.58 µm, and age 4 DAH 38.17-55.59 µm. After 5 DAH of this experiment, there was no surviving larvae.

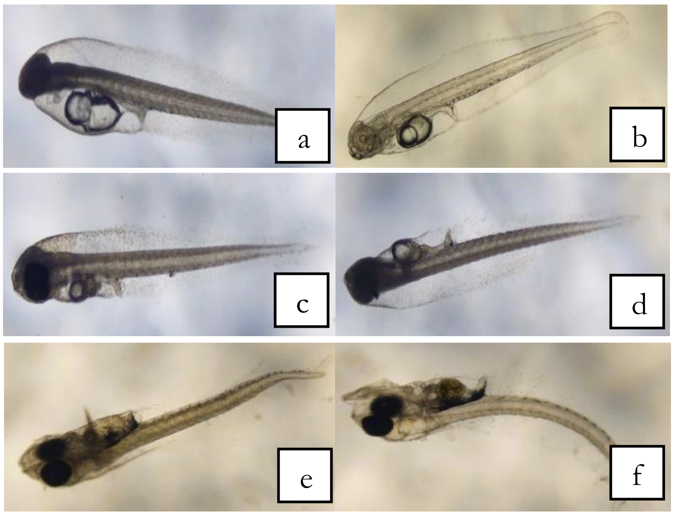

Figure 2. *Siganus guttatus* larvae morphology at particular age (blue arrow); (a) 6 HAH, (b) 13 HAH, (c) 24 HAH, (d) 2 DAH, (e) 3 DAH, (f) 4 DAH.

Water quality parameters

Water quality parameters measured during *S. guttatus* larval rearing are described in Table 1. Water quality parameters measured during *S. guttatus* larval rearing (Table 1) were DO, ranging between 2.9-8.0 mg/L, salinity (22-25 ppt), pH between 7-8.24, and temperature ranged between 27.5-30.1°C. The range of those water quality parameters is still under the normal living conditions of *S. guttatus* larvae.

Table 1. Water quality parameters measured during *S. guttatus* larval rearing.

Water quality parameters	Ranges measured in this study
DO (mg/L)	2.9-6.0
Salinity (ppt)	25-29
pH	7.00-8.24
Temperature (°C)	27.5-30.1

Discussion

According to (Figure 1), eye diameter increased proportionally with larval growth. This is by the opinion that the larger the size of the fish, the larger the eye diameter, and the greater the ability to see objects (Fitri and Asriyanto, 2009; Riyanto *et al.*, 2011). It was further added by Yufera (2014), that fish age increment will influence their ability to detect prey/feed/predator/threats. Guntur and Muntaha (2015) also revealed that almost all fish,

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use the sense of sight to respond to the environment, including detecting feed or preys and predators.

Eye circles in *S. guttatus* are differentiated since 6 HAH, and pigmentation was seen at 24 HAH (1 DAH). This observation is consistent with Hara *et al.* (1986) that pigmentation in *S. guttatus* occurs at 24 HAH but their visibilities are not maximum. Sighting process does not only depends on eyes development but is also supported by lighting. Eyes need particular lighting to see objects in front of them (Stuart, 2013). Lighting condition is one of the factors that fish can see well (Fuad *et al.*, 2019), and each species has interests in different colors of light (Fuad *et al.*, 2020).

Riyanto *et al.* (2011) and Yufera *et al.* (2014) added that eyes differentiation can be detected by the number of photoreceptors (cone cell and rod cell). At the initial larvae stadium, cone cells will develop faster than rod cells. At older age, cone cells will be decreased and replaced by rod cells in adequate quantity (Yufera *et al.*, 2014).

Eye development in fish will affect their behavior (Riyanto *et al.*, 2011), including hunting prey or feed. Feed will be easily preyed on when supported by appropriate eyes development (Yufera, 2014). Preying success positively correlated with larvae survival (Stuart, 2013). Eyes development can be improved by enriching rotifers using enrichment contents such as beta-carotene (pro-vitamin A) (Yunarsa and Adiatmika, 2018; Widomska and Subczynski, 2018; Medina *et al.*, 2019). Further research will test the use of beta-carotene as a feed enrichment material (Rotifera) to support the development of the eyes of *S. guttatus* larvae, so that the larvae will grow fonter and ready to receive the initial feed.

Based on the data on yolk sac diameter (Figure 2) from this research, age increment negatively correlated with the diameter of the yolk sac (yolk reserves). This condition occurs because larvae use the food reserve as their energy source to meet their energy needs to grow and develop. Food reserve utilization had been observed from 2 DAH, and the size was getting smaller until the fourth day it was almost completely resorbed. Furthermore, on the 5 DAH, all larvae have died. The same thing happened in different species, namely the larvae of *Epinephelus fuscoguttatus*, reported by Wulandari *et al.* (2020), that egg yolk energy is used for development.

Exogenous feeding was notin this experiment to ensure that larvae relied entirely on the yolk reserves and oil globules as an energy source. Yolk reserves and oil globule were completely resorbed on 5 DAH which resulted in larvae mortality due to starvation. Quinitio and Sa'an (1988) stated that during the transition from endogenous to exogenous feeding, larvae are vulnerable to mortality, particularly on 2-3 DAH. The mortalities were assumed to be due to excessive level of energy utilization in the condition of lack of feeding (hunt for prey). Therefore it is suggested that the feeding is started before the yolk reserves is completely resorbed to avoid the risk of stress during prey hunting. The time of utilization of egg yolks depends on the size of the yolk diameter of the larvae and also affects the size of the body tissue formed (Hariyandi *et al.*, 2020).

Furthermore, Pardo *et al.* (2016) added that acceptable feed for larvae at the initial feeding is natural feed which was suitable for their needs and their mouth size. One of the natural feeds suggested for the fishes is rotifers (Haas *et al.*, 2015) and nauplius copepods (Darsiani *et al.*, 2017). The use of natural food cannot be eliminated in the handling of early larvae (Li *et al.*, 2017) or in other words replacing them with artificial feeds. Artificial feeding can be done when the digestive system is developed (Parma *et al.*, 2013). The period of artificial feed introduction is known as weaning (Parma and Bonaldo, 2013).

Based on the information on eyes development and duration of yolk reserves utilization, the time for feeding initiation can be determined (Gomes *et al.*, 2014). Understanding feeding management can save production costs (in the hatchery) up to 80% (Parma and Banaldo, 2013).

Based on Table 1, dissolved oxygen measured during the study was in the range of 2.9-6.0 mg/L. This range is still in accordance with the range of dissolved oxygen that the *S. Guttatus* can tolerate. Similar opinions were expressed by Lante and Muslimin (2012); Suci *et al.* (2020); Latuconsina *et al.* (2020); Paruntu (2015); Lante *et al.* (2011); Kamaruddin *et al.* (2019), that *S. guttatus* can live in waters with oxygen concentrations between 4.0–7.7 mg/L. Salinity measured during the study was 25-29 ppt. According to Lante and Muslim (2012); Suci *et al.* (2020); Latuconsina *et al.* (2020); Paruntu (2015); Sarisma *et al.* (2017); Lante *et al.* (2011); Kamaruddin *et al.* (2019), the range of salinity that can be tolerated by the *S. guttatus* is between 10-36 ppt.

While the measured pH ranged from 7.00-8.24 and the temperature ranged from 27.5-30.1 °C. The measured pH and temperature ranges are also still considered suitable for the *S. guttatus* life niche (Lante and Muslimin, 2012; Suci *et al.*, 2020;

Latuconsina *et al*., 2020; Paruntu, 2015; Sarisma *et al*., 2017; Lante *et al*., 2011; Kamaruddin *et al*., 2019).

Conclusion

Based on the results, it can be concluded that *S. guttatus* eyes start differentiation at 6 HAH, and pigmentation takes place at 24 HAH (1 DAH) which develop as the age increase. While yolk sac and oil globule (larval food reserve) decreased as the age increased and completely resorbed on 4-5 DAH. Feeding should be done before the food reserves run out and the eyes have differentiated at the age of 2 DAH.

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