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# First hatchery bred streaked spinefoot rabbitfish (*Siganus javus*) in Sabah, Malaysia

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## Abstract

The present study aims to expand the market potential of the streaked spinefoot rabbitfish (*Siganus javus*) in Malaysia and provide consumers more choices of affordable protein source from aquaculture. Spawning hehavior of *S. javus* was recorded in a week after the new moon in October 2019 and January 2020. Spawning occurred between midnight and early morning, and the eggs hatched about 16 to 18 hours later. Throughout the incubation and larvae rearing period, water temperature was maintained at 30 to 31 °C. Water salinity, pH and DO were 31 to 33 ppt, 7.8 to 8, and 5 to 7 mg/l, respectively. Eggs diameter ranged from 0.550 to 0.603 mm. At 1 day after hatched (d AH), eyes of the larvae were formed but unpigmented and mouth structure was not developed. At 2 d AH, the pigmentation of eyes darkened, digestive tract formed, and anus and mouth opened. At 3 d AH, the lower jaw began to show movement, indicating that the ability of larvae to indulge in feeding. The buds of dorsal and ventral fins started to develop and elongate from 9 d AH and were covered with melanophores. At 25 d AH, the body of the larvae turned brownish and abdominal cavity was deeply pigmented, indicating the onset of metamorphosis. By the age of 30 d AH, the larvae completed development into juvenile stage. At 270 d AH, the juvenile reached maximum size of 21 cm in total length. This study was the first successful attempt at naturally spawning and hatchery rearing of *S. javus* into juvenile stage in the country. The development of a complete larval rearing protocol for *S. javus* could provide information of practical importance in hatchery breeding of other rabbitfish species.

Keywords: *Siganus javus*, Hatchery, Captive breeding, Sabah

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## Introduction

Rabbitfish (*Siganus* spp.), or spinefoot are nutritionally important fish with economic value in several Indo-Pacific countries and Eastern Mediterranean nations (FAO, 2020). Their main identical features are the venomous spines along the fins and changing colour to a mottled pattern during rest and when under stressed condition. The venomous spines can cause sharp pain, bleeding and numbness but there are probably no reported cases of death of the victims of attack. They are often seen in a school when foraging the seagrass beds and algal flats, whereas some pair-bonding species are often seen on coral reefs (Seale and Ellies, 2019).

Rabbitfish has received attention in aquaculture due to its environmental resilience and ability to tolerate conditions of captivity. It has a remarkable tolerance to crowded conditions, readily accepts artificial diets, and due to its low tropic level, the fish requires less protein or inexpensive feeds (Tabugo et al., 2012). Rabbitfish can be cultured in either brackish water ponds or in marine floating net cages (Rachmansyah et al., 1997). Attempts to breed rabbitfish in captivity were first reported by Manacop (1937) and then by Fujita and Ueno (1954). Many studies on breeding of rabbitfish reported difficulties in larval rearing especially during early stage of development (Popper et al., 1973; Soh and Lam, 1973; Westernhagen and Rosenthal, 1975; Bryan and Madraisau, 1977), due to unavailability of suitable feed for the initial larval stage (Juario et al., 1985; Rachmansyah et al., 1997; Huang et al., 2018).

Traditional farming of rabbitfish has existed in Guam and the Philippines (Duray, 1998). In Malaysia, rabbitfish are often caught using trawls and traps, and are mostly landed in Sabah (MYAgro, 2020). However, no hatchery or farming of rabbitfish has been reported in Malaysia. The private sector aquaculture farms are focusing on the culture of highly commercial value species that are carnivorous fish, such as grouper and seabass. Rabbitfish is considered a favourite fish and is consumed by the local population, particularly in the state of Sabah. It is considered palatable and affordable. It has tender flesh and less bones (Ben-Tuvia et al 1973; Lam 1974; Putro et al. 1985; Darsono, 1993). The fish contains rich amounts of highlyunsaturated fatty acids or HUFA) (Huang et al., 2018). According to Wahyuningtyas et al. (2017), rabbitfish flesh contained 77.79% moisture, 15.93% protein, 0.93% fat, and 16 amino acids, including nine essential and seven non-essential amino acids.

Rabbitfishes are not considered a high market value fish in most countries. However, in some remote South Pacific islands such as New Caledonia, it is sold for USD 14-18/kg (FAO, 2020). In Singapore, rabbitfish can fetch up to 20 SGD (equal to 60 MYR) per kilo and the price may increase to 80 SGD per kilo during Lunar New Year period (PickMe, 2021). This shows that the market demand for rabbitfish can be developed not only for local consumption but also for export. The present study by Bayu Aquaculture Sdn. Bhd. aims to expand the market potential of rabbitfish and provide consumers more choices of affordable protein from farmed fish.

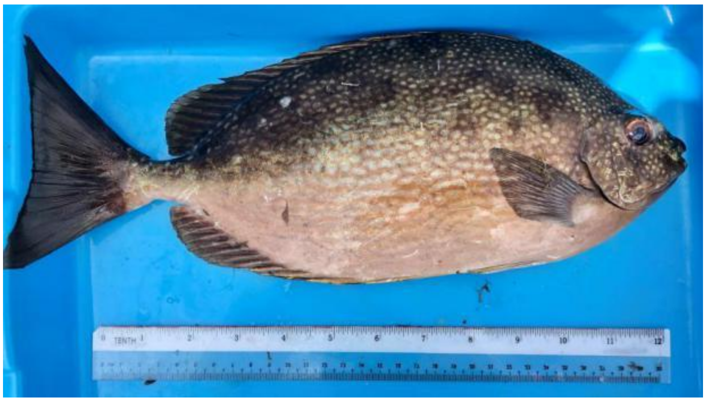


**Figure 1.** Wild caught Streaked spinefoot rabbitfish (*Siganus javus*).

*Siganus javus* (Figure 1) is one of the more abundant and commercially important rabbitfish species in Malaysia. It is locally known as "ikan dengkis jawa" or "belais". This fish has many small white spots on the upper side of the body and fine longitudinal lines on the lower part. The head and fins are often yellow, and the tail fin is dark. Adult size ranges between 25 and 55 cm in total length. They live in marine and brackish water, and some may enter river, lakes and harbour (De Beaufort and Chapman, 1951; Herre, 1953; Kurup and Samual, 1985; dela Paz and Aragones, 1990). Chemical analysis of *S. javus* shows relatively high protein content (Peiris and Grero, 1972), supporting the potential of this species being cultured commercially.

## Materials and Methods

### *Broodstock and spawning*

Adult male and female specimens of the fish were collected about a week before new moon from Gaya Island, Sabah by using fish traps. Only the bigger size of the brood fish (each weight 100 to 250 g and above, 30 cm in total length) was selected for the breeding purpose. The broods were kept in the hatchery of Marine Ecology Research Centre in Gaya Island and conditioned in a three-meter diameter and one meter depth round tank, with 24-hour aeration and continuously running seawater (water exchange rate above 100% per day) to maintain good water quality and circulation. Water temperature, pH and salinity were maintained at 28 to 30 °C, 7.8 to 8, and 31 to 33 ppt, respectively. About half of the water in tank was released daily in the morning and refilled back in the evening to trigger the broods to spawn.

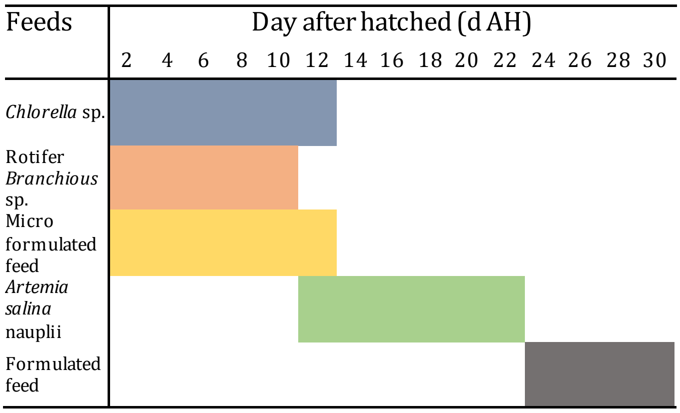
The broods were fed twice a day with enriched diet such as squid, trash fish and formulated feed. Prior to spawning, females with swollen abdomen and males with milt were selected and separated evenly into different tanks. In this study, nine pairs of broods (sex ratio 1:1) were selected and separated into three similar round tanks (three pairs per tank). Since the eggs are demersal and adhesive, few pieces of old PVC pipes and nylon net (half inch mesh size) were placed inside each tank for eggs attachment.

After conditioning for about 2 weeks, spawning activities were recorded in a week after new moon in October 2019 and January 2020. The eggs were laid between midnight and early morning. The broods were removed from the tank after spawning to avoid filial cannibalism. The fertilized eggs were left in the spawning tank for incubation. Slow running water (approximately one litre per hour) was allowed to remove the excess milt and turned off in the evening to prevent the hatching larvae from overflowing out from the tanks.

### *Eggs incubation and larval rearing*

Fertilized eggs were incubated in the same tank used for spawning. The tanks were filled with approximately 1500 litre of water with 24 hours aeration. Throughout the incubation period, a heater was placed in each incubation tank to maintain the water temperature at 30 to 31 °C. Water salinity, pH and DO were 31 to 33 ppt, 7.8 to 8, and 5 to 7 mg/l, respectively. Upon hatching, the larvae were packed and transferred to the fish farm of Bayu Aquaculture Sdn. Bhd. in Tuaran, Sabah.

**Table 1.** Feeding schedule of *S. javus* during larvae rearing.




The larvae were adjusted to the desired stocking density and reared in the 15 m x 6 m x 1.3 m concrete pond with 24-hour aeration, and later graded by size and transferred to round tank filled with two to four tonne of seawater after grading. The feeding schedule of the *S. javus* in larval stages is shown in Table 1. The larvae were fed with different diets three times a day, including C*hlorella sp.*, rotifer *Brachious sp.*, commercial brine shrimp *Artemia salina* nauplii and formulated feed, Otohime (Marubeni Nisshin Food). Newly hatched larvae relied on egg yolk sac on day 1 to 2 day after hatched (d AH), and start feeding from day 2 after the mouth and digestion system is fully developed. Total length (TL) of larvae were measured from 0 d AH. Eggs and larval development were observed under stereo microscope (Motic SMZ-171) and photographs were taken with a digital microscope camera (Moticam 5+).

## Results

### *Eggs development*

The female laid small, spherical, transparent eggs that are externally fertilized by sperm. The eggs contain multiple oil globule that coalesce as development proceeds and only one or two oil globules remain before hatching. Eggs diameter ranged from 0.550 to 0.603 mm in this study. Spawning occurred between midnight and early morning, and the eggs hatched about 16 to 18 hours later. The adhesive demersal fertilized eggs were attached to the wall of the tank, on the PVC pipe and nylon nets placed in the tank, and to the aeration tube. This caused difficulty in estimating the number of eggs that had been laid. Hatching rate was roughly estimated at 90 %.

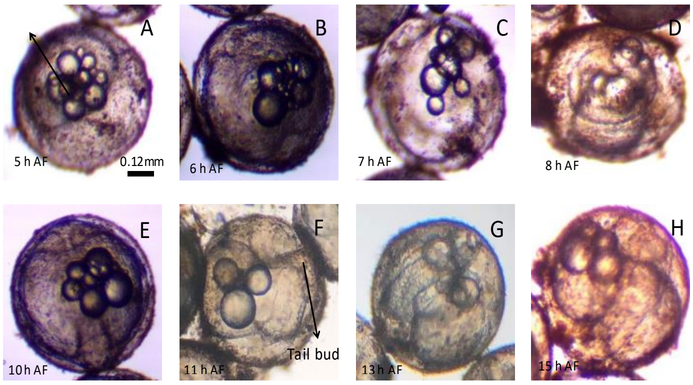



**Figure 2.** Embryonic development of *S. javus* after fertilization. A. Mid blastula; B. Late blastula; C. Early grastula; D. Late grastula; E. Neurula; F. Tail bud formation; G. Organ formation; H. Muscle contraction; all scale are the same as shown in A at bottom right corner.

The embryonic development of *S. javus* was shown in Figure 2. After fertilization, the eggs undergo rapid cell divisions. Upon reaching the 16-cell stage in about 1 hour after fertilization (h AF), blastomeres reduced in size and the eggs entered the morula stage. In this period, successive cleavage occurs, blastomeres became crowded cells and blastodisc looked more like a ball. After the cleavage has produce over 100 cells, the eggs entered the blastula stage (4 h AF). In this period, the epiboly was formed and continued until grastrula stage (7 h AF). During gastrulation, germ ring and embryonic shield were formed. The blastomeres extended to cover the epiboly, with number of oil globules greatly reduced and embryo body was formed. The development continues with neurulation and the formation of organ until hatching at 16 to 18 h AF.

### *Larval development*

The larvae morphological changes and juvenile development of *S. javus* are illustrated in Figure 3 and 4, respectively. Total length (TL) changes of larvae are shown in Figure 5. At 1 day after hatched (d AH), the larvae eyes were formed but unpigmented and mouth still unformed, thus relying on yolk sac for nutrient (Figure 3A). Larvae have melanophores on the snout, yolk and oil globules, and along the ventral side of body. At 2 d AH, the larvae eyes pigmentation darken, digestive tract formed, anus and mouth opened (Figure 3B). At 3 d AH, yolk sac was absorbed and the oil globule diminished to negligible size, the lower jaw began to move, indicating the larvae ability to start feeding. At 3 d AH, the larvae TL averaged 2.47 mm and mouth was 0.10 mm in width. *Chlorella sp.*, rotifer *Branchious sp.* and micro formulated feed were being introduced as their starting diet. At 4 d AH, the larvae's stomach was full and golden-brown in colour, indicating the larvae were well fed and the feed given were accepted.

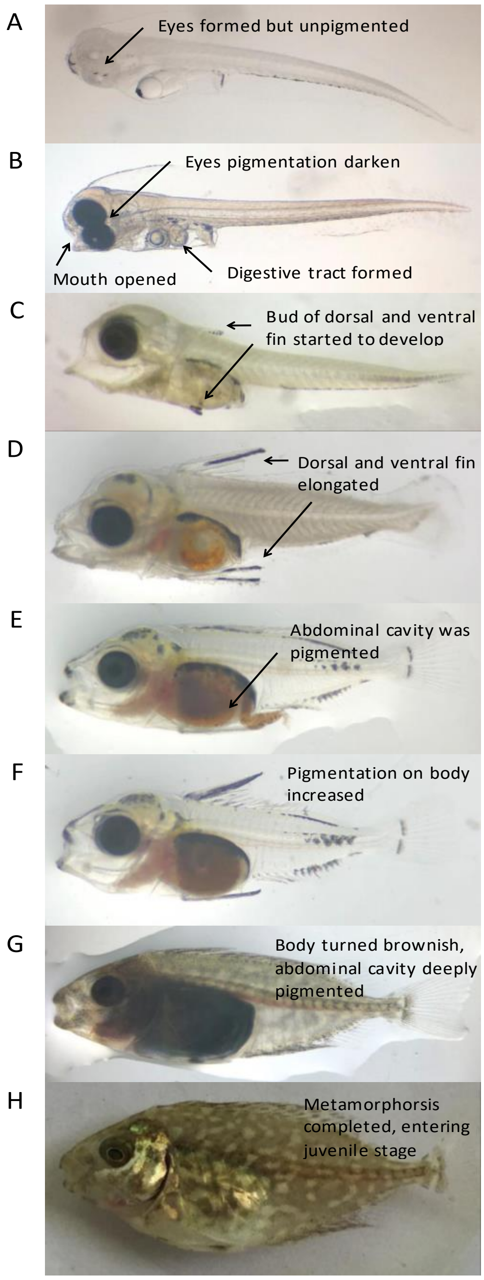



**Figure 3.** Morphological changes of *S. .javus* in larvae stage. A. 1 d AH, 2.37 mm TL; B. 2 d AH, 2.47 mm TL; C. 9 d AH, 4.22 mm TL; D. 11 d AH, 5.0 mm TL; E. 15 d AH, 7.92 mm TL; F. 18 d AH, 13.5 mm TL; G. 25 d AH, 23.0 mm TL; H. 30 d AH, 26.0 mm TL

The bud of dorsal and ventral fin started to develop and elongate from 9 d AH and were covered with melanophores (Figure 3C). At 13 d AH, the larvae reached 6.27 mm in TL and actively fed on *Artemia salina* nauplii. The abdominal cavity was pigmented and coloured. Pigmentation or melanophores were seen on lateral line and posterior part of dorsal and anal fins. Melanophores increased from 15 d AH (Figure 3E) onwards and spread to other parts of body, including the head part, lateral line, and caudal fin in 18 d AH (Figure 3F). Larvae started to exhibit schooling behaviour and were observed swimming in group. At 25 d AH, the larvae body turned brownish and abdominal cavity was deeply pigmented, indicating the onset of metamorphosis (Figure 3G).

By the age of 30 d AH, the larvae completed metamorphosis and started showing the juvenile morphological features. The fish body was no longer transparent, the body shape and coloration resembled those of the adult, and all the fins were complete well developed (Figure 3H). The completion of metamorphosis indicated the fish is entering juvenile stage. Survival rate of larvae reared to metamorphosis was approximately 15 %. At 120 d AH, the juvenile reached 11 cm in TL with small white spots on the upper side of the body and fine longitudinal lines on the lower part of the body clearly seen (Figure 4A). At 150 d AH, the juvenile reached 13 cm in TL, active and responding well to formulated feed given (Figure 4C). At 270 d AH, the juvenile reached 21 cm in TL (Figure 4D).

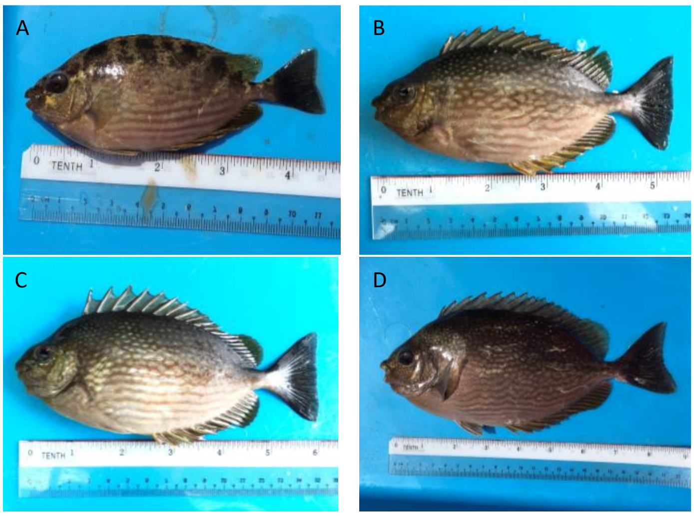



**Figure 4.** Juvenile development of *S. javus*. Body colour and pattern resemble of the adult. A. 120 d AH, 11cm TL; B. 150 d AH, 13cm TL; C. 200 d AH, 15cm TL; D. 270 d AH, 21cm TL.

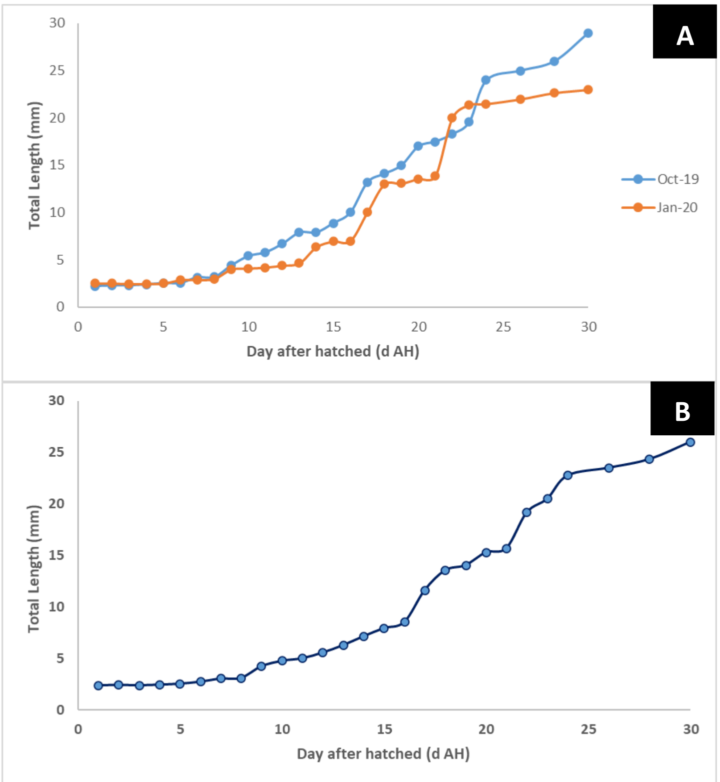



**Figure 5**. A) Average growth of *S. javus* from 2 different batches (October 2019 and January 2020). B) General average growth (TL) of *S. javus* larvae from 0 to 30 d AH.

## Discussion

### *Spawning*

In this study, *S.javus* broods were collected before new moon and natural spawning occurred a week after new moon. Rabbitfish are lunar cycle spawner and exhibit lunar spawning rhythm (Lam, 1974). According to Rahman et al. (2004), the gonadal development and the hormonal fluctuation of the rabbitfish are repeated at an interval of one month according to the lunar cycle. However, the lunar phase for timing of synchronous spawning is different among species (Takemura et al., 2004). Thus, hormonal treatment may not be necessary if spawning is attempted on the right days of lunar month (Darsono, 1993). In nature, each rabbitfish species exhibits a definitive reproductive season, which differs by tropical region.

Water in tank holding *S. javus* broods was released in the morning and refilled back in the evening to trigger spawning. According to Duray (1990), other than specific lunar phases, the spawning of rabbitfish is also synchronously linked to tides. McVey (1972) mentioned that tidal level is the most important factor in the spawning of rabbitifsh. Laviña and Alcala (1974) reported spawning of rabbitfish at night in the open water near the surface of Southen Negros, Philippines. The advantage of these strategies may minimize immediate egg and larval predation, by facilitate their transportation to offshore by the strongest outgoing tide (Thresher, 1984).

The broods of *S. javus* in this study were fed with squid, trash fish and commercial pellet during preparation for spawning. Rabbitfish are mainly herbivores in nature. They feed on benthic algae, seaweed and seagrass. However, in captivity, rabbitfish can be easily weaned on to formulated feeds (Li et al., 2018). Lam (1974) stated that rabbitfish, which are herbivorous in nature, will become omnivorous in captivity. Early maturity and year-round spawning has been recorded in captivity where rabbitfishes were fed with nutritional artificial diets. (Lam, 1974; Hara et al, 1986; Takemura et al., 2004).

***Embryonic and larval development***

Similar to other rabbitfish species, fertilized eggs of *S. javus* observed in this study were small, spherical, transparent, demersal and adhesive, and contained multiple oil globules. Unlike other marine fin fishes which produce mostly pelagic and non-adhesive eggs, the characteristics of the rabbitfish eggs are similar to most freshwater fish that produce adhesive eggs which stick on rock or gravel, weed or sand. The fertilized eggs of *S. javus* in this study were ranged from 0.550 to 0.603 mm in diameter and took about 16 to 18 hours to hatch at 30 to 31°C. In general, rabbitfish eggs measured 0.42 to 0.70 mm in diameter and took 18 to 35 hours to hatch at 22 to 30 °C (Lam, 1974; FAO, 2020). Hatching time of rabbitfish eggs is variable, depending on temperature and perhaps on the species and locality (Lam, 1974). For example, *S. canaliculatus* eggs measured 0.58 mm in diameter took 39 to 42 hours to hatch at 23.2 to 24.5 °C (Huang et al., 2018), *S. vermiculatus* eggs measured 0.56 mm in diameter took 25 hours 30 minutes to hatch at 27 to 29 °C (Anuraj et al., 2019), and *S. randalli* eggs measured 0.51 to 0.58 mm in diameter took 18 to 21 hours to hatch at 26 to 28 °C (Nelson and Wilkins, 1994). The temperature for eggs incubation in this study was slightly higher compared to the above studies, which probably contributed to the shorter time *S. javus* eggs took to hatch.

Newly hatched larvae were pelagic, and they have small yolk sac containing one oil globule. The larvae began to develop into juveniles stage at 25 to 30 d AH, with 23 to 26 mm in total length. The results are similar with the study conducted by Darsono (1993), stating duration of the planktonic stage of rabbitfish is about 25 days, with juvenile fish between 20 and 26 mm long. However, the time taken for the larvae to metamorphose into juveniles depends on the species; 24 to 35 days in *S. guttatus* (Juario et al., 1985), 23 to 30 days in *S. canaliculatus* (May et al., 1974), 23 to 25 days in *S. vermiculatus* (Gundermann et al., 1983) and 29 to 35 days in *S. lineatus* (Bryan and Madraisau, 1977). The process of metamorphosis include changes in colouration, behavior, and feeding habit (Gundermann et al., 1983). The end of the larval stage is marked by a metamorphosis process, more or less abrupt depending on the species, during which the larva becomes a juvenile that is morphologically similar to the adult and has the meristic characters of the species.

In this study, *Chlorella sp*., rotifer *Branchious sp.* and micro formulated feed were introduced as the diet starter for *S. javus* larvae. The larvae mouth was formed in 2 d AH with the opening size of only 0.10 mm (100 μm). Thus, the larvae only able to consume the smallest rotifer *Branchious sp*. with size less than 100 μm. This poses a problem as only small amount of the rotifer fed to the larvae constitutes this size group. Due to this, *Chlorella sp.* (3 to 8 μm) and micro formulated feed (75 to 150 μm) were also added as starter feed.

According to Rachmansyah et al. (1997), the main constraint faced in rabbitfish larval rearing is the availability of suitable size and quality of feed for day one to five larvae. Similarly, Stephanou and Georgiou (2000) also encountered the same problem during feeding of early stage of larvae (day 2 to day 6) due to their small mouth size. They concluded that the size of the prey is the limiting factor and that rotifer and phytoplankton alone cannot support larval survival. The mouth size of newly hatched rabbitfish larvae were smaller compared to other commonly cultured marine fin fish larvae such as grouper and seabass. Thus, diet particles must be chosen with consideration to the small mouth size of fish larvae. Appropriate feed is important to increase the survival rate and quality of the juveniles (Rachmansyah et al., 1997). Success of larval rearing depends mainly on the availability of suitable diets that can be readily consumed, efficiently digested and that provide the required nutrients to support good growth and health (Giri et al., 2002).

Although rabbitfish has long been identified as appropriate candidates for aquaculture, hatchery breeding of rabbitfish in captivity is still consider relatively new. Rabbitfish is abundant during its recruitment periods, making it easy to catch in the shallows and thus keeping down the costs of acquiring fingerlings (Teitelbaum et al., 2008). Due to this, fish farmers preferably practice capture-based aquaculture to collect the rabbitfish fingerlings rather than obtain the fingerlings through full breeding cycle. Capture-based aquaculture does not rely on controlling the reproduction and breeding of farmed species, but it could be considered as an unsustainable aquaculture practice, due to the increasing pressure on fish stocks that could cause successive stock depletion, low recruitment, stock collapse, and reductions in genetic biodiversity (Ottolenghi et al., 2004). Full cycle breeding, on the other hand, provides genetic improvement, food security and an additional income source to rural communities (Lovatelli and Holthus, 2008). Besides, the fact that rabbitfish are herbivorous fish given advantages in terms of energy transformation (high nutrient quality) and resource utilization (low production cost) compared to carnivorous fish species (Li et al., 2018), making rabbitfish a promising aquaculture candidate.

## Conclusion

This study concluded the early successful naturally spawning and hatchery rearing of *S. javus* to juveniles stage in the country. This is a major breakthrough achievement in the seed production of rabbitfish. The successful development of a complete larval rearing protocol for *S. javus* could provide information for hatchery breed of other rabbitfish species in the country. A continuous and sufficient supply of hatchery-breed rabbitfish would support market demands and reduce dependence on wild-caught juveniles to achieve more sustainable aquaculture practice.

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