

Reproductive behavior and larval development of the striped blenny *Meiacanthus grammistes*

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Abstract

This study aims to describe aspects of reproductive behavior and larval development for the striped blenny, *Meiacanthus grammistes*. Altogether 8 broodstock fish (8.5 cm to 10 cm) were maintained in two 400 l tanks. The first spawning occurred 45 days in one tank and 65 days in another tank after fish acquisition. Egg clutches were only found attached to inside walls of the 50 mm capped PVC pipes with a single 25 mm reduce entry hole while the male took full responsibility for egg care. The fish spawned routinely every 8–10 days in both tanks throughout experimental period. The fecundity ranged from 500–4200 eggs per spawning with an average of 1837 ± 1197 eggs/clutch. Newly extruded eggs were spherical and incubation period lasted 203 - 207 h at $27 \pm 1^\circ\text{C}$. Newly hatched larvae measured 3.11 ± 0.14 mm in standard length (SL) and 0.87 ± 0.08 mm in body depth (BD) with average mouth-gape height and width at 272.42 ± 61.03 μm and 187.50 ± 36.46 μm , respectively. Under such a feeding regime, most of larvae had settled out water column onto the bottom around 23 DPH but not yet displayed the full colouration pattern of adults. In period of 24 to 29 DPH, the colouration pattern developed with alternating black and yellow stripes running through the entire length of body and the newly settled juvenile measured 12.91 ± 0.35 mm in SL and 3.36 ± 0.12 mm in BD around 30 DPH.

Keywords: Reproductive behavior, larval development, fecundity, mouth-gape.

INTRODUCTION

The value of the global marine ornamental fish and invertebrate trading was estimated between 20–40 million US dollars in the early 1980s but this number had risen rapidly to 200–300 million US dollars in recent years [1, 2]. Unfortunately, the vast majority of marine ornamentals are captured from the wild, mainly from coral reefs [1, 3, 4]. Capture of tropical marine ornamental fish negatively impacts coral reefs due to damaging collection methods (e.g. using sodium cyanide) that are commonly utilized in developing countries; moreover, stress due to poor handling and post-harvesting husbandry lead to high mortality of the wild caught fish (25–80%) before they reach consumers [1, 5–7]. Therefore, captive breeding of marine ornamentals provides a sustainable alternative to supply the rapid growing of marine ornamental industry as well as reduces impacts of marine ornamental collection on the wild populations and natural environments [1, 2, 8]. However, there are many bottlenecks such as broodstock management and larval rearing which affected the success of captive breeding of most marine fish including both marine food fish and ornamental fish [2]. To date, captive larval rearing successes have been largely limited to small, experimental, or hobbyist scales. Additionally, very few scientific publications contain documenting aspects pertinent to captive culture of ornamental reef fish species [9, 10].

The family Blenniidae is among 10 most popular families in marine ornamental fish trading (Moorhead and Zeng, 2011). The ornamental species belonging to the family generally reside on coral reefs and are small in size. They normally produce eggs that are demersal or semi-demersal and attached to various substrata, such as bivalve shells; it has also been reported that several females can spawn in a same nest, evident by substratum-attached eggs with different development stages [11]. The striped blenny *Meiacanthus grammistes* is one of such ornamental blenny species distributing in the Indo-Pacific shallow reefs [12]. The species is among commonly traded marine ornamentals and depending on

their size and other factors, the retail price tag for the species ranges from AUD\$ 20 to 70 per fish in Australian and global markets. However, the majority of specimens of the species traded in global aquarium markets come from the wild and are now becoming sporadic [12]. Therefore, studying captive breeding of the striped blenny *Meiacanthus grammistes* is necessary to supply a high-quality fish resource for marine ornamental fish trade as well as help to reduce pressures for coral reef environment. The aim of this paper is to highlight major bottlenecks/barriers to the development of marine ornamental aquaculture, with focus on the key issues of reproductive behavior and larval development.

MATERIALS AND METHODS

Broodstock culture

Altogether 8 adults *Meiacanthus grammistes* (total length: 8.5 cm to 10 cm) were obtained from Cairns Marine, a marine ornamental wholesaler, on 6 July 2015. Upon the fish being delivered to the Marine and Aquaculture Facility Unit (MARFU), James Cook University, Australia, then they were randomly allocated into two 400 l round tanks with 4 fish per tank without sex determination. Three types of shelters, including a giant clam shell, a 50 mm capped PVC pipes with a single 25 mm reduce entry hole and a half terracotta pot, were placed in each tank as nests for the fish to spawn. The broodstock tanks were located outdoor with natural photoperiod regime while water temperature was kept at $27 \pm 1^\circ\text{C}$ by connecting to a heated recirculating system. Water parameters were maintained relatively healthily with salinity 33–37 ppt, pH 8.0–8.2, $\text{NH}_4^+/\text{NH}_3^-$ and $\text{NO}_2^- < 0.02$ ppm. The recirculating system consisted of an 80,000 l sump, a 50 μm mechanical filter, a foam fractionation and a trickling biological filter. Broodstock were fed twice per day (at approximately 9 am and 4 pm) with a homemade wet feed, consisting of a mix of blended prawn, squid, fish and mussel meat supplemented by multi-vitamins and minerals, and with gelatin as the binder.

Defining of reproductive behavior and fecundity

The shelters were checked daily before morning feeding and afternoon feeding to determine spawning activity until the first egg clutch was detected. Reproductive behavior was observed daily 1-2 times prior to the morning feeding (07:00-08:00) and after afternoon feeding (16:00-17:00) for two months. The males' courtship and parental care behavior was focused on to observe reproductive behavior of this species. For the estimation of number of eggs produced by the females, a transparent plastic sheet was placed inside 50 mm capped PVC pipes for females to spawn on it. On daily check in the morning, if a new batch of eggs was found attached to the plastic sheet, it was taken out and photographed with a digital camera (Canon EOS 600D) before being put back into the broodstock tank for continuous incubation. The number of eggs of each spawn was subsequently counted based on the photo taken to obtain fecundity data.

Description of larval development

To describe larval development of *M. grammistes*, the 50 mm capped PVC pipe with attached eggs was removed from the broodstock tanks and placed in a 10 L hatching bucket around 6 pm. Gentle aeration was directed toward the egg clutch to facilitate egg hatching and the hatching bucket was wrapped up with a black plastic sheet. Newly hatched larvae in the next morning in the hatching bucket were transferred to a conical 150 L tank for rearing. The larvae were cultured adopting the optimized feeding regime established based on larval feeding experiments (unpublic data): i.e. larvae were fed 20 rotifers per mL initially (0-6 DPH), *Artemia* nauplii were then introduced on 7 DPH to be co-fed with rotifers (10 rotifers + 5 *Artemia* per mL) for 2 days (7-8 DPH). From 9 DPH onward, larvae were fed *Artemia* nauplii only provided at 5/mL while from 15 DPH onward, *Artemia* nauplii were replaced by enriched metanauplii being fed to the larvae at 5/mL. Throughout larval rearing, water temperature was maintained at $28 \pm 0.5^\circ\text{C}$ by a submergible heater. Among other physical parameters, salinity was between 33-35 ppt; pH 8.0-8.2; $\text{NH}_4^+/\text{NH}_3^-$ and $\text{NO}_2^- < 0.05$

ppm. Photoperiod was maintained at 24h light : 0h dark as recent studies showed the good survival and growth at this light regime for several ornamental fish, including the cleaner goby, *Gobiosoma evelynae*, and the lemonpeel angelfish, *Chrysiptera flavissimus* [13, 14].

On the day of larval hatching, a total of 20 larvae were sampled for the measurement of mouth-gape height and width at the first feeding. They were kept in 4% buffered formalin and photographed under a stereo microscope. Mouth gape width was measured as the distance between the left and right postero-ventral tips of the articular bones of the jaw while the mouth gape height (GH) was calculated using the formula:

$$GH = \sqrt{UJL^2 + LJL^2}$$

Where: *UJL* and *LJL* are upper and lower jaw lengths, respectively [15].

To describe larval development, ten larvae were randomly collected daily from the conical 150 l tank and anaesthetized with 0.05 ml L^{-1} Aqui-S, they were then observed and photographed with a stereo microscope (Olympus S2-CTV, Japan) fitted with a digital camera (Olympus DP21, Japan). Larval development was observed and described until 30 DPH when they completed metamorphosis and became juveniles.

RESULTS AND DISCUSSIONS

Reproductive behavior and fecundity

The first spawning was found occurring 45 days after broodstock acquisition in one tank and 65 days in the other tank. After the first spawning, spawning became routine every 8–10 days in both tanks throughout experimental period. The times of the day at which spawning occurred were observed to be during early morning between 6–9 am. Among various shelters provided, egg clutches were only found attached to inside walls of the 50 mm capped PVC pipes with a single 25 mm reduce entry hole. PVC pipe is selected as the nest by the broodstock in this study is also found similarly in another study that was conducted on this species [12] and forktail blenny *Meiacanthus atrodorsalis* [15], indicating a preference of this

shelter type for spawning. The study of Buchheim and Hixon, 1992 showed that Spinyhead blennies occupy abandoned worm holes in coral heads on Caribbean reefs, these small holes were hiding places and prevent larger fish from accessing the holes [16]. The PVC pipes have shape as a hole so they are suitable shelters for the blenny fish. It was observed that the courtship was always started by a female, a female would swim close to the entry hole of the male's preferred shelter and displayed her well rotund abdomen to attract male. After a short period of displaying, the female entered into the shelter, followed by male. The spawning and fertilizing processes generally last for approximately 45 to 60 minutes. After which, the female left the nest while the male was responsible for caring the eggs until hatching. During the incubation period, the male was often found guiding at the entry hole and agitated the water surround the eggs, only left the nest for a very short time for feeding.

During the two-month period from 30 August to 30 October, a total of 45 egg clutches were found from both broodstock tanks (30 egg clutches in tank 1 and 15 egg clutches in tank 2). The average number of eggs produced per spawning was 1837 ± 1197 eggs and ranged from around 500 eggs to 4200 eggs. Different females were observed to produce eggs with different colors, including yellow, pink and red. A male was commonly seen taking care of several egg clutches, suggesting that a male could pair with several females. On many occasions, newly laid batches of eggs were found with same or different colours in a same PVC pipe 2 to 5 days after the first spawning event. The incubation time generally lasted between 203 to 207 hours (averaged about 8.5 days) at water temperature of $27 \pm 1^\circ\text{C}$ and hatching normally occurred during night time. On the same day or within 1 to 2 days after a batch of eggs was hatched, a new egg clutch was often found laid in the nest.

Number of eggs spawned by females in present study fluctuated widely from 500 to 4,200 eggs per spawn and averaged $1,837 \pm 1,197$ eggs, which is comparatively higher than other demersal spawning ornamental species previously reported, including the Gobidd fish

Priolepis nocturna (268 to 3,121 eggs) [17], the folktailed blenny *Meiacanthus atrodorsalis* (315 to 2,575 eggs) [15], and the purple firefish *Nemateleotris decora* (400 to 500 eggs) [18]. The egg incubation period was found to be between 204–207 hours or around 8.5 days at $27 \pm 1^\circ\text{C}$, which is slightly longer than 7 days reported by Olivoto et al (2010) at 28°C . The difference is most likely due to lower water temperature during the incubation in this study since temperature is well known to exert decisively influence on embryonic development rate of fish [19]. A single male was often observed taking care of several eggs clutches, this means that no pair bond was established in this species [12]. Thus, it is different from the clownfish, within a single group, there are only a dominant breeding pair and nonbreeders [20, 21]. Moorhead and Zeng (2011) reported that the dominant male of *Meiacanthus atrodorsalis* has exclusive breeding with the females but the subordinate males were often observed taking care of the eggs.

Larval development

Newly hatched larvae (0 DPH) measured 3.11 ± 0.14 mm in standard length (SL) and 0.87 ± 0.08 mm in body depth (BD) (table 1); they still possessed a small yolk reserve, had transparent finfold and with dark stellate melanophores scattered at the anterior part of the body (fig. 1A). Immediately after hatching, larvae swam actively and were positive phototoxic near the water surface; their jaws were well developed with average mouth-gape height and width at 272.42 ± 61.03 μm and 187.50 ± 36.46 μm , respectively, and ready to feed.

On 5 DPH, SL of the larvae increased to 4.16 ± 0.17 and BD to 1.30 ± 0.12 . Eyes, mouth and finfold were well developed and pelvic fin could be seen. Melanophores appearing at anteroposterior elongation of the head and the anterior part of the body became darker due to increased pigmentation (fig. 1B).

On 9 DPH, larval stomach showed yellow colour due to ingestion of *Artemia* nauplii; more melanophores appeared along the body and upper and lower jaws (fig. 1C).

On 15 DPH, three black stripes appeared along the body and yellow colour developed on

the head; fin rays appeared within the dorsal, pelvic and caudal fins (fig. 1D).

On 20 DPH, the three black stripes became darker, wider and ran horizontally along the whole body while yellow colour covered the head and extended to half way of the body;

melanophores also started to appear on the dorsal, pelvic and caudal fins (fig. 1E). The majority of larvae started to change behaviour at this time, moving from water surface close to the tank wall and bottom.

Table 1. Standard length (mm) and body depth during larval development of *M. grammistes*

Days post hatching (DPH)	Standard length (mm) \pm SD	Body depth (mm) \pm SD
0	3.11 \pm 0.14	0.87 \pm 0.08
5	4.16 \pm 0.17	1.30 \pm 0.12
9	5.44 \pm 0.26	1.78 \pm 0.13
15	7.93 \pm 0.56	2.50 \pm 0.20
20	10.31 \pm 0.75	2.85 \pm 0.31
23	12.14 \pm 0.55	3.22 \pm 0.05
30	12.91 \pm 0.35	3.36 \pm 0.12

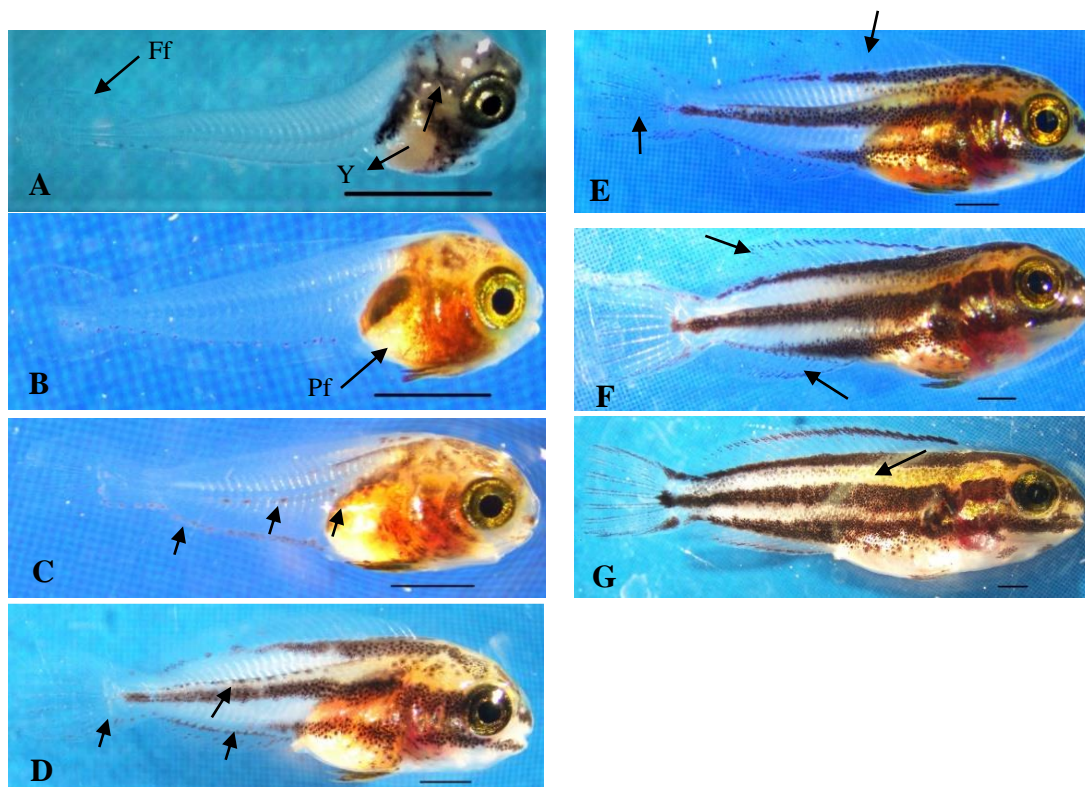


Fig. 1. Larval development of *M. grammistes* cultured using an established feeding protocol. Scale bars denote 1 mm. A) 0 DPH: Newly hatched larvae with a small yolk reserve (Y) and transparent finfold (Ff); B) 5 DPH: Pelvic fin (Pf) could be seen and anterior part of the body became darker due to increased pigmentation; C) 9 DPH: Showing increased stellate melanophores along the body (arrows); D) 15 DPH: Showing three distinct lines of melanophores running along length of the body (arrow); E) 20 DPH: Fin rays appearing within the dorsal, caudal and pelvic fins (arrows); F) 23 DPH: Melanophores appearing clearly on dorsal, pelvic and caudal fins; G) 30 DPH: Newly settled juvenile; showing adult coloration pattern with alternative black and yellow stripes running along the entire length of the body (arrow)

On 23 DPH, three black stripes ran along the entire body and melanophores well developed on the dorsal, pelvic and caudal fins (fig. 1F). Most of larvae had settled out water column onto the bottom but not yet displayed the full colouration pattern of adults.

On 30 DPH, the colouration pattern resembles adults with alternating black and yellow stripes running through the entire length of body; melanophores fully developed on the dorsal, pelvic and caudal fins (Fig. 1G). The newly settled juvenile measured 12.91 ± 0.35 mm in SL and 3.36 ± 0.12 mm in BD (table 1). In this study, the larvae entered metamorphosis and approached settlement on 15 DPH. This metamorphosis process is much earlier compared with the larvae of this species on 26 DPH which was reported by Olivotto et al (2010) and forktail blenny on 25 DPH [12, 15]. The possible explanations for such different results compared with Olivotto et al (2010) on this species could include: 1) Rotifer and *Artemia* strains used and their combination were different; in the study by Olivotto et al (2010), larger rotifer *B. plicatilis* and AF430 *Artemia* were used, and both of them were enriched by Algamac 3000s prior to being used for feeding larvae, meanwhile, ss rotifer strain and *Artemia* cyst from Great Salt Lake strain, INVE Aquaculture were used in this study; and 2) Other physical culture conditions and procedures are also different.

CONCLUSION

The first spawning was found in period of 45 - 65 days after broodstock acquisition. After the first spawning, spawning became routine every 8-10 days.

Time of spawning occurred during early morning between 6 - 9 am. The spawning and fertilizing processes generally last for approximately 45 to 60 minutes.

The incubation time generally lasted between 203 to 207 hours (averaged about 8.5 days) at water temperature of $27 \pm 1^\circ\text{C}$. Number of eggs fluctuated widely from 500 to 4200 eggs per spawn and averaged 1837 ± 1197 eggs.

The newly settled juvenile was observed on 30 DPH.

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