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Research Article

Growth Performance and Nutritional Condition of Marble Goby (*Oxyeleotris marmoratus*) Larvae Fed under Different Onsets of First Feeding

Siti Fairus Mohamed Yusoff,[1] **Fui Fui Ching**,[1] **Gunzo Kawamura**,[1] **and Shigeharu Senoo**[1,2]

[1]*Borneo Marine Research Institute, University Malaysia Sabah, Kota Kinabalu 88999, Sabah, Malaysia*
[2]*Aquaculture Technology and Production Center, Kindai University, Shirahama 649-2211, Wakayama, Japan*

Correspondence should be addressed to Fui Fui Ching; cfuifui@ums.edu.my

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The marble goby (*Oxyeleotris marmoratus*) is a valuable food fish, but its aquaculture production is often hampered by poor growth performance associated with starvation at an early stage. The objective of this study was to investigate the effects of delayed initial feeding on the growth performance and nutritional condition of the marble goby. Six different first feeding times were examined: 0, 12, 24, 36, 48, and 60 hours after the first feeding (HAFF), and their impacts on growth performances were evaluated based on larval final total length (mm) and survival (%), while nutritional condition was evaluated based on body morphometric changes, gut epithelium height (μm), and gut condition. The experiment was conducted for 15 days. All parameters were measured after larvae were collected at different sampling times, except survival and growth, which were measured at the end of the experiment. The results showed that the onset of the first feeding was 36 h after hatching (hAh) and that a short delay in the first feeding by 12, 24, 36, 48, and 60 HAFF significantly reduced the growth performance of the larvae and severely affected the larval nutrition condition with noticeable shrinkage in body morphometry and gut epithelium height. This study concluded that the onset of first feeding in marble goby occurs at 36 hAH and that first feeding beyond 36 hAH significantly worsens the nutritional condition and growth performance of the larvae.

1. Introduction

The marble goby (*Oxyeleotris marmorata*) (Bleeker, 1852) is a member of the family Eleotridae and is native to freshwater and brackish water in Southeast Asia. The marble goby is a sought-after food fish and has a high market value both domestically and internationally due to its excellent flesh quality. The marble goby is produced in small and irregular quantities in many parts of the Asian region [1–3]. Unfortunately, most of the supply still comes from wild-caught fish, and exploitation continues unabated, so a sharp decline in the natural population is to be expected [4].

Like other aquaculture species, the marble goby relies exclusively on its yolk sac as a source of food and energy to undergo various biological developments in the early stages [5]. Before endogenous reserves are depleted, larvae must successfully shift from endogenous to exogenous feeding to ensure their survival and growth in the subsequent stage, as has been reported in the larvae of tiger grouper (*Epinephelus fuscoguttatus*) [6], silver catfish (*Rhamdia voulezi*) [7], and African catfish (*Clarias gariepinus*) [8]. In addition, there are some other factors that are critical for the successful initiation of the first feeding, including the availability, density, and type of food [9, 10], the development of the digestive tract, and the swimming ability of the larvae [11]. If these factors are not properly established, many scientific reports have shown that larvae will fail to initiate first feeding and starve to death [12–14]. Unlike well-developed adult and juvenile fish, larvae are unable to tolerate food deprivation even for a short period of time [7]. Fish larvae are fragile and very susceptible to death once they reach an irreversible state of starvation. Even when food is provided in the later stages, larvae are unable to resume feeding to recover. Blaxter and Hempel (1963) referred to this critical stage as the point-of-no-return (PNR) [15].

Understanding how starvation affects larval development, growth and survival is crucial to determine the time frame in which fish can tolerate the absence of food before reaching the point-of-no-return [16], and this can be a very powerful tool to understand the causes of early larval mortality in fish farms [17]. Starvation also affects the nutritional condition of fish larvae, which often represent the health status of fish in captivity. To assess the nutritional condition of fish, morphometric changes in body proportions and histological analyses on gut condition particularly the changes on gut epithelium height are the most reliable scientific tools to evaluate the extent of starvation in fish. Several studies have shown the negative effects on the histopathological changes of the digestive system or specifically the intestinal or gut epithelium after starvation [18–20]. Measuring the cellular degeneration of the digestive system is considered a good indicator because the digestive tract thrives during the abundance of food and may atrophy during starvation [21]. In addition, during the starvation period, muscle fibers are responsible for providing energy for the hydrolyzed protein [22–24], hence resulted in the reduction of muscle mass.

In the larval rearing of marble goby, the main cause of mortality in the early life stages due to starvation is poorly understood, and the estimation of the frequency of starvation is not clear. The present study aims to investigate the effects of different onsets of first feeding on growth and survival, the nutritional transition period, and nutritional status, especially on the responsiveness of the intestinal epithelium, to better understand the gut condition of larvae. This study aimed to find the most ideal timing for the first feeding without adverse effects on the growth performance and nutritional condition of marble goby larvae.

2. Materials and Methods

2.1. Ethical Approval. All experiments were conducted in accordance with the researcher guidelines of the Code of Practice for the Care and Use of Animals for Scientific Purpose, University Malaysia Sabah.

2.2. Spawning, Eggs, and Larval Collections. This experiment was conducted in the fish hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. The larvae used in this experiment were obtained by the induced spawning method. A healthy and mature female (body weight (BW): 560.0 g; total length (TL): 31.20 centimeters (cm) and male (BW, 620.0 g; TL, 40.20 cm) broodstock were selected and injected with Human Chorionic Gonadotropin (Profasi, Laboratories Serono, Switzerland) at a dose of 1,000 and 500 International Units per kilogram (IU/kg), respectively in the peritoneal cavity.

The injected male and female broodstock were immediately transferred to a spawning tank consisting of a 700-liter (ℓ) glass aquarium with size of $0.8 \times 1.1 \times 0.8$ m with a water recirculation system connected to a 100-ℓ filter tank installed adjacent to the spawning tank. An artificial concrete substrate (45×8 cm) was placed in the spawning tank to allow the female to deposit eggs. During this time, the male was removed from the spawning tank to prevent both broodstock from becoming aggressive due to their naturally strong parental care behavior. A heater was installed, and the temperature was set to 29°C. The aeration rate was set at 250 mℓ/min. The spawning tank was covered with a black plastic sun protection net to minimize disturbances that could lead to spawning failure.

About 72 hours after injection, the female could be observed depositing eggs on the artificial substrate. Forty-eight hours after egg deposition, the newly hatched larvae could be seen in the tank. The water supply was stopped at this time to prevent the larvae from swimming into the filter tank. Approximately 30,000 hatched larvae were collected and counted as 0 hours after hatching (hAH). Size of larvae were 3.26 ± 0.02 mm. The larvae were carefully transferred to the experimental tanks.

2.3. Experimental Design and Larval Rearing. Twenty-four 7-liter plastic aquariums were prepared as a quadruple of 6 treatments representing 6 different times of first feeding (0, 12, 24, 36, 48, and 60 hours after first feeding (HAFF)). The control group is 48 HAFF, as is common in marble goby farming. Each treatment consisted of 4 aquariums, one of which was prepared and served as a sampling tank, while triplicate tanks were used to measure final larval survival and growth in total length at the end of the experiment.

To maintain the water temperature at 29 ± 0.5°C throughout the experimental period, a water bath system with a 150-ℓ plastic tank ($2\,m \times 10\,m \times 10\,m$) was used. Next, 50-ℓ water were filled into the tank, and a heater (Heto, HW –300W, China) and a cooling sensor (Iwaki Co. Ltd, AZ-251X, Japan) were immersed into the water bath system. A total of 6 water bath systems were prepared for 6 different treatments.

Throughout the experimental period, dissolved oxygen levels were maintained at 6-7 milligrams per liter (mg/L), temperature (29 ± 0.5°C), and pH (6-7) using the multiparameter water quality probe (550A; YSI Inc; Yellow Springs, OH, USA). Water quality was measured twice daily at 8 am and 4 pm. The tanks were cleaned mainly at the bottom to syphon out dead larvae and phytoplankton, if present, and the water was renewed by 10 percent (%) daily.

2.4. Onset of First Feeding and Feeding Protocol. The onset of first feeding was determined by larval morphological characteristics, including pigmented eyes, an open mouth with an upward and downward moving mandible, peristaltic movements in the digestive tract, and an open anus. The presence of food in the gut has also been used as further evidence of the onset of first feeding in the marble goby.

To determine the onset of the first feeding, 5 larvae were randomly collected at hourly intervals using a fine-mesh hand net and a cup from the sampling basin AH. The collected larvae were then anesthetized with a dose of 25 parts per million (ppm) (Transmore, NIKA, Malaysia) before being observed under a light microscope (Nikon, Eclipse E600). Larval eyes, mandibles, gut, and anus movements were observed hourly until all organs were functional.

At the first feeding, larvae were fed L-type brackish water rotifers *Brachionus plicatilis* at a density of about 20 individuals/ml (ind/mℓ) together with phytoplankton *Nannochloropsis* sp. at a density of 0.25 million cells/ml. The densities of rotifers and *Nannochloropsis* sp. in the experimental tank were calculated twice daily, and an adjustment was made to add or remove if either fell below or above the required densities. The same feeding protocol was used throughout the 15-day experimental period.

2.5. Larval Sampling Protocol

2.5.1. Nutritional Transition Period. The nutritional transition period of larvae was calculated from the beginning of the first feeding to the end of yolk sac absorption. Five larvae from each treatment were collected from the sampling tank and later anesthetized before beingplaced under a profile projector (Mitutoyo, PJ-3 000, Japan). The yolk sac of the larvae was observed at 6 h intervals, and the hours that elapsed until the yolk sac was completely absorbed were recorded.

2.5.2. Body Morphometrical Changes. Five larvae were taken from the sampling tank and anesthetized to measure their body height (BH), muscle height (MH), and gut height (GH) under a profile projector. BH was measured from the anterior to the dorsal fin down to the abdomen, while MH was measured from the base to the tip of the anus, and GH was measured from the base to the tip of the stomach cavity, as was done by Pena and Dumas [25]. Body morphometric changes were determined at 5-day intervals, starting at 0 dAH and ending at 15 dAH.

2.5.3. Larval Growth and Survival. Final larval growth in total length (mm) and survival (%) were counted at the end of the experiment in triplicate rearing tanks. For the final total length, ten larvae were taken from each treatment, while the survival rate was expressed as the percentage of surviving larvae compared to the initial number of larvae used. Larval total length was measured from the tip of the mouth to the end of the tail.

2.5.4. Gut Condition. Larval gut condition was measured based on the gut epithelium height. At the end of experiment, 3 larvae from each treatment were sampled and processed histologically. The specimens were preserved in Bouin's solution, followed by dehydration in series of ethanol, embedded in paraffin, cut into a 6 micron meter (μm) thick sagittal section, and proceeded with staining in hematoxylin and eosin. The slides were observed using a microscope (Nikon Corporation, Nikon Eclipse 80i, Japan) equipped with a camera (Nikon Corporation, Nikon digital sight DD-U2, Japan). The intestinal epithelium height of each larva was measured randomly at different points ($n = 10$) over the intestine using image analysis (ImageJ1.44P, Wayne Rasband, USA).

2.5.5. Statistical Analysis. Data were analyzed using software SPSS (Statistical Program for Social Science, version 22.0). One-way ANOVA was used to analyze the difference. The data were tested for normality prior to statistical analysis by using Shapiro Wilk's W test, and the homogeneity of variances was checked by Levine's test. The post hoc under Duncan's multiple range test was performed to ascertain any significant differences between the different treatments at significant level of $P < 0.05$.

3. Results

3.1. Onset of First Feeding and the Nutritional Transition Period. The onset of first feeding in the marble goby was recorded at 36 hAH. A total of 100% of larvae showed deeply pigmented eyes, an open mouth with functional mandible lower jaw moving up and down, peristaltic movements in the digestive tract and an open anus. Rotifer and *Nannochloropsis* sp. were also found in the digestive tract at the same time. Yolk sac was still visible ($519.4 \pm 23.0 \times 10^{-5}$ mm^3) at the onset of first feeding, and the longest nutritional transition period ($P < 0.05$) was observed in marble goby first fed at 0 HAFF compared to those first fed in 12, 24, 36, 48, and 60 HAFF (36, 28, 28, 26, and 24 h), respectively (Table 1). Meanwhile, larvae first fed at 60 HAFF had a significantly shorter ($P < 0.05$) nutritional transition period compared to other groups of larvae. No significant differences were found in the nutritional transition times of larvae fed at 24, 36, and 48 HAFF.

3.2. Growth Performance. The results showed that the different onset of the first feeding affected the growth performance of marble goby larvae. Larvae that initiated first feeding at 36 hAH (0 HAFF) attained significantly higher survival ($P < 0.05$) (59.7 ± 0.81%) compared to those first fed in 12 HAFF (41.3 ± 1.86%), 24 HAFF (37.1 ± 3.27%), 36 HAFF (28.2 ± 0.58%), 48 HAFF (28.8 ± 0.41%), and 60 HAFF (21.6 ± 0.63%), respectively (Figure 1).

In terms of growth, marble goby fed 0 HAFF also achieved significantly higher growth ($P < 0.05$) in final total length of 4.77 ± 0.05 mm, followed by 12, 24, 36, 48, and 60 HAFF (4.20 ± 0.05, 4.20 ± 0.09, 4.13 ± 0.05, 3.63 ± 0.05, and 3.5 ± 0.09) (Figure 1). The initial total length was 3.26 ± 0.02 mm.

3.3. Morphometric Changes

3.3.1. Muscle Height. The marble goby showed an increase in muscle height with age, although the onset of first feeding

Table 1: Nutritional transition period of marble goby fed at different hours after first feeding.

Age at onset of first feeding (hAH)	Hour after first feeding (HAFF)	Age at first feeding (hAH)	Age at end of yolk sac absorption (hAH)	Nutritional transition period (h)
36	0	36	84	48[a]
	12	48	72	36[b]
	24	60	64	28[c]
	36	72	64	28[c]
	48	84	62	26[c]
	60	96	60	24[d]

Lowercase letters represent statistical differences $P < 0.05$ in nutritional transition period.

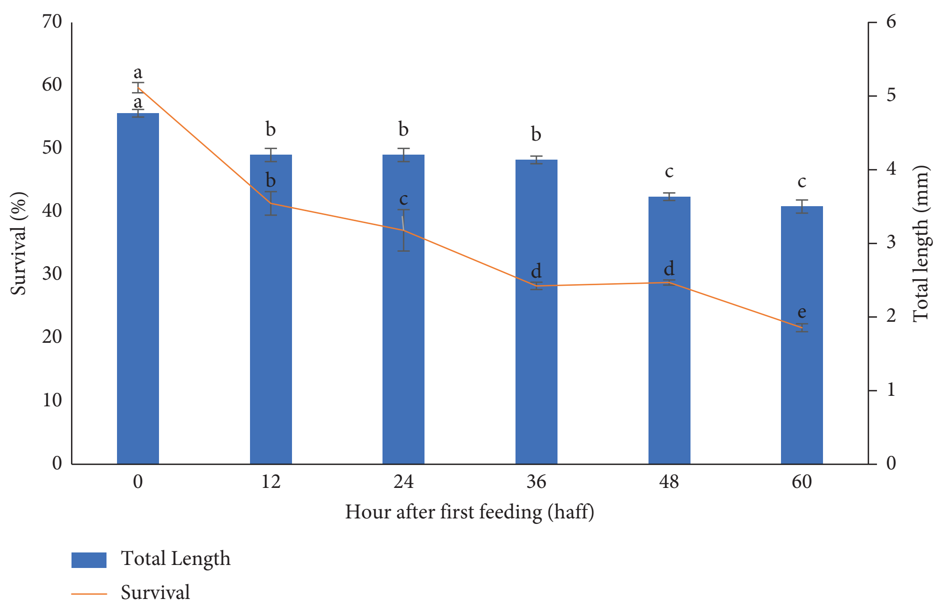

Figure 1: Growth and survival performances of marble goby fed at different hours after first feeding. The primary axis represents survival (%) ($df = 5$, $F = 0.946$), and the secondary axis represents growth in final total length (mm) ($df = 5$, $F = 4.827$). The mean values in the same column and line with different superscripts are significantly different ($P < 0.05$).

was variable (Figure 2(a)). At 5 dAH, larvae fed at 0 HAFF had significantly higher ($P < 0.05$) muscle height (0.183 ± 0.005 mm) compared to those first fed at 12 (0.17 ± 0.00 mm), 24, (0.17 ± 0.00 mm), 36 (0.077 ± 0.005 mm), 48 (0.02 ± 0.008 mm), and 60 (0.02 ± 0.008 mm) HAFF, respectively ($df = 5$, $F = 1069.74$). At 10 and 15 dAH, larvae fed 0 HAFF showed significantly higher muscle heights ($P < 0.05$) of 0.250 ± 0.008 mm and 0.283 ± 0.005 mm, respectively compared to the other groups of larvae (Figure 2(a)). Meanwhile, no significant differences were detected in the muscle height of larvae first fed at 12, 24, 36, 48, and 60 HAFF (Figure 2(a)).

3.3.2. Gut Height. The marble goby also showed an increase in gut height with age, although the onset of first feeding was variable (Figure 2(b)). At 5 dAH, larvae fed at 0 and 12 HAFF attained significantly higher ($P < 0.05$) gut heights at (0.160 ± 0.003 mm and 0.154 ± 0.003 mm) while a significant decrease in gut height ($P < 0.05$) was observed in marble goby first fed at 36 (0.120 ± 0.000 mm), 48 (0.116 ± 0.005 mm), and 60 (0.090 ± 0.002 mm) HAFF ($df = 5$, $F = 2.674$). At 10 and 15 dAH, the marbled goby fed 0 HAFF showed significantly higher ($P < 0.05$) gut heights of 0.183 ± 0.005 mm and 0.243 ± 0.005 mm, respectively, compared to the other larval groups (Figure 2(b)). No significant differences were observed in the muscle height of larvae first fed at 12, 24, 36, 48, and 60 HAFF.

3.3.3. Body Height. The marble goby displayed an increment in body height despite the different onset of first feeding (Figure 2(c)). At 5 dAH, a significant decrease in body height ($P < 0.05$) was witnessed in marble goby fed at 36 (0.39 ± 0.01 mm), 48 (0.32 ± 0.01 mm) and 60 (0.32 ± 0.01 mm) HAFF, respectively ($df = 5$, $F = 47.46$). Surprisingly, marble goby fed at 0 HAFF attained a significantly rapid growth in body height at 15 dAH (0.71 ± 0.01 mm) compared to larvae first fed at 12, 24, 36, 48, and 60 HAFF (0.63 ± 0.03, 0.60 ± 0.02, 0.52 ± 0.02, 0.46 ± 0.02 and 0.41 ± 0.01 mm), respectively.

3.4. Gut Condition. The gut condition of the marble goby was determined by the height of the gut epithelium, as

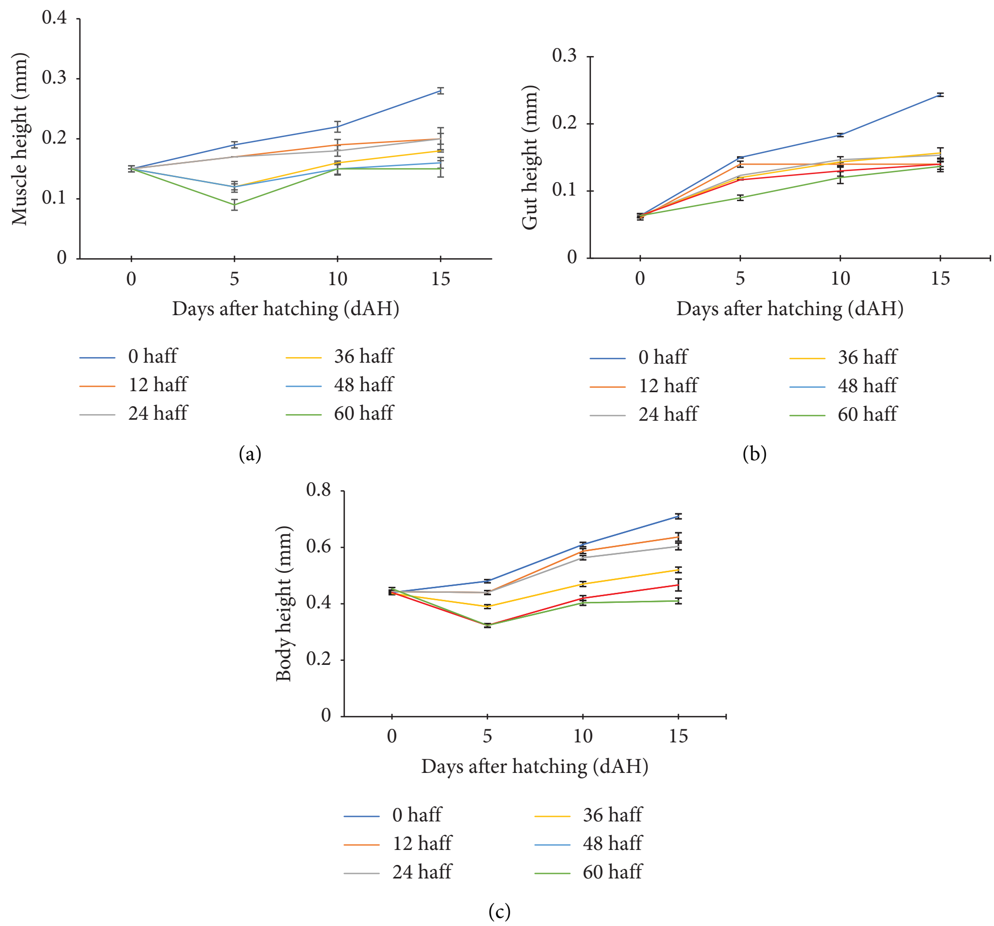

Figure 2: Body morphometric changes of marble goby fed at different hours after their first feeding. (a) Muscle height (mm), (b) gut height (mm), and (c) body height (mm).

shown in Figure 3. At the end of the experimental period (15 dAH), the marble goby fed 0 HAFF showed significantly higher ($P < 0.05$) intestinal epithelial height of 25.9 ± 0.9 µm compared to 12, 24, 36, 48, and 60 HAFF (18.1 ± 0.1, 12.9 ± 0.7, 12.9 ± 0.32, 11.4 ± 1.6, and 5.95 ± 00.38 μm, respectively). The differences in the histology of the gut of larvae fed at different times can be seen in Figure 4. Larvae fed at 48 and 60 hours after feeding showed severe damage to the gut, with tissues separated in the mucosa and submucosal layers (Figures 4(e) and4(f)). Larvae fed at HAFF's of 0, 12, 24, and 36 showed a well-developed gut with muscle folds and supranuclear vacuoles (Figures 4(a)–4(d)). However, it was noticeable that only larvae fed at 0 HAFF had the highest height of gut epithelium with a well-developed condition of gut.

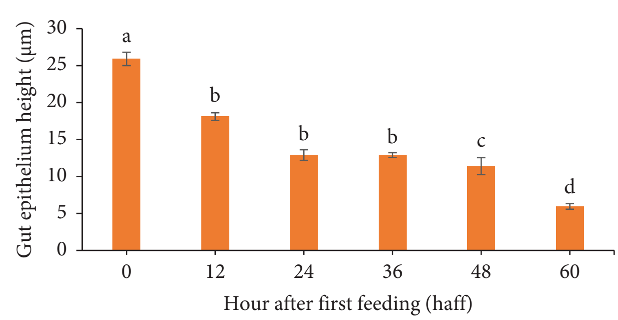

Figure 3: The gut epithelium height of marble goby fed at different hours after the first feeding. The mean values in the same column with different superscripts are significantly different ($P < 0.05$).

4. Discussion

4.1. Onset of First Feeding and the Nutritional Transition Period. First feeding time plays a crucial role in the overall growth performance of marble goby larvae, as is also the case for several freshwater fish species such as the silver therapon (*Leiopotherapon plumbeus*) [16], the African catfish [8], the silver catfish [7], and the river catfish (*Pangasianodon hypophthalmus*) [26]. Unlike marine fish larvae, freshwater fish are usually larger at hatching and have a larger yolk sac, which provides sufficient food and energy for various biological developments. However, they are still prone to mass mortality caused mainly by starvation in the early stages. This is especially true if the larvae have already been exposed to a point-of-no-return condition, where the starved larvae are too weak to initiate feeding, even if food is offered, as also described by Blaxter & Hampel, 1963 [15].

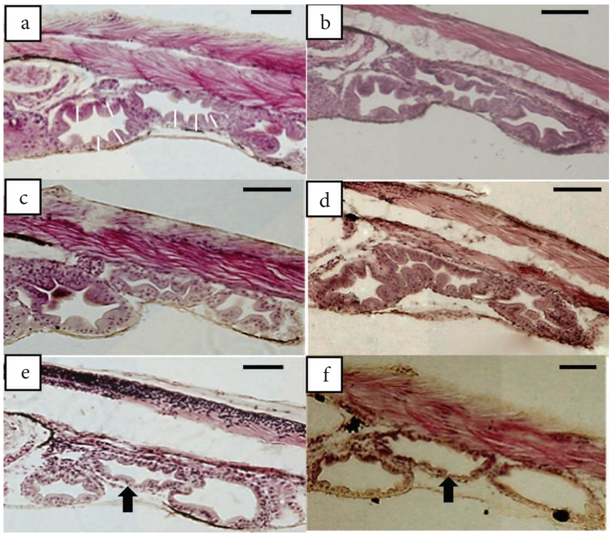

Figure 4: Histology features of the gut of a marble goby fed at different hours after first feeding. (a) 0 HAFF, (b) 12 HAFF, (c) 24 HAFF, (d) 36 HAFF, (e) 48 HAFF, and (f) 60 HAFF. Magnification at 40×. The scale bar represents 10 μm. Arrows in Figures 4(e) and 4(f) show disconnection of tissues in mucosal and submucosal layers in larvae first fed at 48 and 60 HAFF.

In this study, the onset of the first feeding was recorded at 36 hAH. In aquaculture, the first feeding does not begin until four organs have become functional for feeding. Larvae require (1) well-pigmented eyes to detect food by sight, (2) a functioning mandible that enables food to be taken into the mouth by creating suction to draw the prey into the oral cavity, (3) a peristaltic gut to assimilate, digest, and absorb food, and (4) an open anus to excrete digested food. The readiness of these four organs can be easily observed under a light microscope or profile projector and is therefore the most practical method of determining the first feeding time of a fish species. Failure to initiate the first feeding immediately after fish are morphologically prepared for feeding often leads to various complications such as deformities [27] and abnormal behavior [28], which may eventually suppress normal growth and even lead to mortality. However, the tolerance threshold for food deprivation is species-specific and should be determined to maximize the welfare of fish larvae in captivity.

The current study also showed that the larval nutritional transition period was affected when the first feeding was initiated at different times. The nutritional transition period is described by FHYN (1989) [29] as the period between the onset of exogenous feeding and the end of yolk sac absorption. A longer nutritional transition period is advantageous for larvae, as they have two sources of energy derived from the yolk sac and external food to support development in the early stages [27]. In contrast, a short food transition period can lead to mortality, especially if the larvae do not develop their feeding ability before the yolk sac is completely depleted.

The marble goby fed at 36 hAH without delaying the onset of the first feeding had the longest nutritional transition time (48 h) and was probably responsible for better survival and growth as seen in this study. It was assumed that the larvae have sufficient time to gain feeding experience and improve their feeding ability before they become completely dependent on external food in the later stages. Similar assumptions were made by Shan et al. (2008) for rock bream (*Oplegnathus fasciatus*) [14] and tiger grouper [6].

In contrast to the fed larvae, the starved larvae (12, 24, 36, 48, and 40 HAFF) experienced a much shorter nutritional transition period (26, 28, 28, 26, and 24 h). The yolk sac, which served as the only energy reserve for the starved larvae, was rapidly absorbed while they underwent numerous morphological and physiological changes in the early stages. In addition, the absence of an external food source around the completion of yolk sac absorption resulted in a rapid yolk sac absorption rate. Therefore, larval susceptibility to starvation increased, which in turn led to poor feeding performance during the early larval stage [30, 31]. Similar findings were reported in the Rohu (*Labeo rohita*) and Singhi *Heteropneustes fossilis* [32].

4.2. Growth Performance. This study also found that delayed first feeding negatively affected the growth and survival of marble goby larvae, which is consistent with previous studies on other fish such as Rohu, Singhi, and Malabar grouper (*E. malabaricus*) [31, 32]. In marble goby, a significant reduction in survival and growth was observed even with short delayed first feeding at 12, 24, 36, 48, and 60 HAFF. The declining of growth probably occurred after muscle mass was lost due to tissue synthesis as the metabolic rate was high during starvation [33, 34]. During starvation, muscle is considered one of the tissues that is affected to due to the reduction of muscle glycogen, which is a source of metabolic fuel [35]. Under these circumstances, the loss of lipid and muscle protein helps in providing mass energy to the larvae to sustain survival [36]. Like other animals, fish larvae, either in nature or captivity, are thought to have a natural survival mechanism when exposed to starvation. When fish starve, several adaptive physiological processes affect organs such as the brain, skeletal muscle, and digestive tract, causing changes in the intermediate metabolism of carbohydrates, lipids, and proteins to maintain homeostasis [37]. Although fish larvae have immature organs in the early stages, they catabolize proteins from muscles, which is later reflected in reduced growth, as seen in the marble goby when exposed to delayed first feeding.

Apart from affecting larval growth, delayed initial feeding significantly worsens larval survival, as similarly observed in obscure pufferfish (*Takifugu obscurus*) [17], silver catfish [17], and even an ornamental fish, the clownfish (*Amphiprion percula*) [38]. Larvae experience high mortality, particularly when they start to shift from endogenous to exogenous feeding [39]. During this time, many important biological developments take place such as the development of morphology and sensory organs, so that the larvae can function well in subsequent stages. Therefore, the transition between exogenous and endogenous sources is critical, and the successful initiation of the first feeding is imperative. Marble goby first fed at 12, 24, 48, and 60 HAFF attained significantly lower survival, although larvae also received both endogenous and exogenous food and energy sources similar to those first fed at 0 HAFF. However, considering that the nutritional transition period was much shorter and larvae were thought to be unable to complete some biological development due to energy and nutrient deficiency. This critical stage is often observed towards the end of yolk sac utilization, which was also confirmed by Sulaeman and Fotedar [40].

4.3. Body Morphometric Changes and Gut Condition. Changes in body morphometrics are commonly used to determine the nutritional status of larvae exposed to different periods of food deprivation [7]. In this study, the nutritional status of the marble goby was severely impaired, except for those that were first fed at 36 hAH. Muscle height, gut height, and body size are among the most sensitive body proportions in fish larvae and can be easily affected, especially when exposed to starvation, as reported in tiger grouper, Asian seabass (*Lates calcarifer*) [41], crucian carp (*Carassius carassius*) [42], loach (*Misgurnus anguillicaudatus*) [43], and rock bream [14]. During starvation, larvae begin to utilize proteins in the body to maintain homeostasis, which later leads to various complications in growth and survival.

In addition to muscle height, body height, and gut height, Peña and Dumas (2005) suggested the use of body angle [25], while Park et al. (2013) recommended changes in body angle and the ratios of eye height to head height, gut height to standard length, and gut height to myotome as indicators for assessing the magnitude of the starvation effect [44]. The nutritional status of the larvae was further deteriorated when it affected the development of the larval intestine, including the decrease in intestinal fold [45], intestinal length [27], and degeneration of the mucosal thickness of the gastric and gut epithelium [46]. The decrease in the height of the intestinal epithelium of starved marble goby larvae was noticeable from 12 HAFF onwards. Proteolysis, also known as degeneration of intestinal epithelium cells, is a common response of fish larvae to starvation [47, 48], as similarly observed in this study. Similar to the study by Gisbert et al. (2004), the histological organization of the different body regions gradually deteriorated with progressive flattening of the intestinal folds in the anterior intestine with shrinkage of epithelial cells and detachment of the brush border [49]. This is perhaps the reason for the reduced growth and survival of the marble goby fed at 12 HAFF onwards, as the larval digestive system ceases to function properly and nutrients can no longer be absorbed to be converted as energy to support various developments in a later stage [50].

5. Conclusions

This study concluded that the first feeding of marble goby started at 36 hAH and delayed first feeding beyond 36 hAH onwards has been shown to have adverse effects on larval growth performance and nutrition condition. Therefore, it is recommended to introduce the first feeding immediately to marble goby soon after they are morphologically prepared, even though the yolk sac is still visible and capable of providing food and energy sources to larvae, to maximize their overall growth performance and wellbeing in the early stage.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

Ethical review and approval were waived for this study because no fish were subjected to hazardous chemicals except those for histological analysis. However, research proposals and monitoring the conduct of projects were done for 6 times by the Universiti Malaysia Sabah.

Disclosure

Preliminary data for this project were presented in the 3rd International Symposium on Cage Aquaculture in Asia 2011 (Caa3) "Securing the Future", Kuala Lumpur, Malaysia.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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