

Original Article

Survival, growth, and feeding ability of marble goby, *Oxyeleotris marmorata* (Bleeker, 1852) Larvae under delayed initial feeding

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Abstract

This study evaluated the effects of delayed initial feeding (DIF) time [Control (fed at initial feeding), 1, 2, 3, 4, or 5 days delay] on growth, survival, feeding ability, point-of-no-return (PNR), and deformity of *Oxyeleotris marmorata* larvae. Findings revealed that the growth and survival of the larvae were significantly lower in all other treatments than the control. The mean percentage of the deformity was the highest in 5 d DIF. The PNR was between 3 and 4 d DIF, which indicated irreversible starvation at this point. The negative effect of DIF on the feeding intensity was significant in 3, 4, and 5 d DIF. Decreasing feeding intensity with the DIF time signified the deterioration of feeding ability, which subsequently affects the larval growth and survival. It is recommended to introduce initial feeding within one day after commencing the exogenous feeding (36 HAH) ($29 \pm 0.5^\circ\text{C}$) for optimum growth and survival.

Keywords: *Oxyeleotris marmorata*, starvation, growth, survival, PNR

1. Introduction

Larval growth and survival are strongly influenced by the early life history, especially during the transition from endogenous to exogenous feeding (Fotedar, 2018). Successfully established initial feeding or the transition to exogenous feeding once the yolk is exhausted is crucial for fish larvae to avoid progressive starvation (Dou, Masuda, & Tsukamoto, 2005). At this transition, the optimum time to introduce initial feeding, capacity to consume food, and accessibility to food are the primary factors for fish larvae to successfully establish initial feeding, as it is pertinent for their survival, growth, and next developmental stages (Sanderson & Kupferberg, 1999). Numerous studies have been conducted to

examine the starvation tolerance of fish larvae, since the ability to endure starvation varies by species (Dou, Tanaka, & Tsukamoto, 2002; Lima, Andrade, Pini, Makrakis, & Makrakis, 2017). Most findings revealed considerable harm from food deprivation to larvae at initial feeding, which in turn affects their survival and growth (Ching, Nakagawa, Kato, Murata, & Miyashita, 2011; Wang *et al.*, 2010) and feeding ability (Klimogianni, Pagoulitou, Trageli, & Hotos, 2013; Sheng, Lin, Chen, Shen, & Lu, 2007), causing morphological deformities (Park *et al.*, 2013), abnormal feeding behaviors (Gwak & Tanaka, 2001), and nutritional problems (Ching, Nakagawa, Miyashita, & Senoo, 2016).

Mortality during the larval stage has also been associated with low prey density or reduced capability of starved larvae to capture prey (Chambers, Witting, & Lewis, 2001). As previous findings revealed, delayed initial feeding after the yolk sac exhaustion caused a reduction in food utilization efficiency and feeding activity of fish larvae, which

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later affects their starvation tolerance (Zhang *et al.*, 2009). To investigate the starvation tolerance of fish larvae, point-of-no-return (PNR) was introduced as that time point when 50% of fish larvae were still alive but unable to feed even in the presence of food (Blaxter, 1963).

The marble goby, *Oxyeleotris marmorata* is a highly valued species occurring in many parts of Southeast Asian countries (Luong, Yi, & Lin, 2005). As the popularity and demand for this species increase by the day, this has led to the establishment of artificial breeding techniques for this species in captivity, for mass production (Senoo, 1994; Abol-Munafi, Liem, & Ng, 2002). Even though breeding techniques have been established, stable mass seed production is still inconsistent due to high mortality that occurs in the early larvae stages (Tavarutmaneegul & Lin, 1988). A previous study reported that *O. marmorata* larvae fully utilized their yolk sac at 82 HAH (27–29 °C), mortality started to occur from this point onwards, and they had fully died at 130 HAH (Amornsakun, Sriwatana, & Chamnanwech, 2002). Even though they have a long period before yolk exhaustion, how long they could tolerate starvation is uncertain. Understanding starvation tolerance and its effects on larval development is vital, especially during post yolk sac stage, to ensure that hatchery reared larvae do not starve during this critical development period. Thus, the current study was carried out to investigate the implications of delayed feeding on growth, survival, feeding ability, and deformity of *O. marmorata*.

2. Materials and Methods

2.1 Eggs and larval collection

This experiment was carried out at the hatchery of Borneo Marine Research Institute at Universiti Malaysia Sabah. The brood fish were bought in Penampang area of the state of Sabah and retained in the hatchery for approximately six months prior to the induced breeding on a diet of trash fish. A matured male (30.10 cm total length TL, 541 g body weight BW) and female (25.40 cm TL, 370 g BW) were induced with Human Chorionic Gonadotrophin (hCG, Profasi, Laboratories Serono, Switzerland) with the dosage of 1,000 IU per kg for female and 500 IU per kg for male (Senoo, 1994). The fertilization and hatching rates of the eggs exceed 90%. The eggs hatched 70–90 h after fertilization.

During the peak hatching, larvae were harvested and transferred to experimental tanks. In this experiment, hatching time was defined by 50% of eggs having hatched out: this was 0 hours after hatching (HAH) of the larvae. For the observation of larval morphological development for initial feeding, larvae ($n = 10$) were randomly taken from the experimental tank, anesthetized with 25 ppm transmore (NIKA, α -methylquinoline) and observed under a light microscope (Nikon, Eclipse E600, Japan). The onset of initial feeding was marked when over 50% of the sample larvae were morphologically prepared for their initial feeding, which was indicated by the eyes of the larvae being completely pigmented, mouth open, and lower jaw movable, intestine peristaltic, and anus open. Based on the morphological development, the initial feeding occurred at 36 HAH (1.5 DAH) (29.0 ± 0.5 °C).

2.2 Experimental design and larval rearing

Newly hatched larvae were collected from the spawning tank and carefully transferred using a 0.5 L glass beaker into each experimental tank. Six different onsets of initial feeding were set at 1, 2, 3, 4, and 5 days of delay in initial feeding (d DIF), and control treatment defined the time when the larvae commenced their initial feeding. All the experimental tanks had gentle aeration at approximately 250 ml min⁻¹, and the water salinity level was maintained at 10 ppt for each experimental tank. Rotifer, *Brachionus* sp. (L-type) (10–20 ind ml⁻¹) were supplied at the initial feeding until 12 DAH, and the feed was then changed to *Artemia nauplii* (10–15 ind. ml⁻¹) onwards up to 40 DAH. Green algae, *Chlorella* sp. (0.5 million cells mL⁻¹) was introduced into the larval rearing as a water conditioner and was maintained uniformly in all treatments throughout the experimental period. All the treatments were conducted in triplicates of similar conditions. Water quality was recorded daily at 08:00 am and 17:00 pm. DO level (6–7 mg L⁻¹), temperature (29 ± 0.5 °C), and pH (6–7) of stayed within the optimal range, measured using an oxygen probe (550A; YSI Inc; Yellow Springs, OH, USA). This experiment was conducted in a water bath system where the temperature was regulated by a thermostat controlling a heater (Heto, HW-300W, China) and a cooler (Iwaki Co. LTD, AZ- 251X, Japan).

2.3 Experiment I: Effects of delayed initial feeding on larval growth, survival, and deformity

The experiment was conducted to determine the growth, survival, and deformity for various delays in the initial feeding. Larvae were stocked at 40 L⁻¹ in 7-L plastic aquarium (18 × 26 × 17 cm). Measurements of the total length were done every 10 days starting from 0 until 40 DAH, and survival was observed on the days of measuring the total length. The deformity was observed at the end of the experiment. No bottom cleaning was done during the first ten days of the experiment to avoid mortality due to handling.

2.4 Experiment II: Effects of delayed initial feeding on feeding incidence, PNR and feeding intensity

The experiment carried out separately was similar to experiment I, and was designed to determine the feeding ability based on feeding incidence and intensity. The larvae were stocked at 60 L⁻¹ in 20-L plastic aquaria (30 cm × 17 cm × 20 cm) and larvae were sacrificed to count the rotifer number. Feeding incidence was determined as the percentage of sampled larvae ($n = 10$) in triplicate contained rotifers in the gut at their initial feeding time. The PNR was assessed based on the graph of larval feeding incidence, and the PNR was established as the time in days when the feeding incidence dropped to less than half (50%) from the highest feeding incidence recorded among delayed initial feedings (Wang *et al.*, 2010). Feeding intensity was defined as the total number of rotifers in the gut of sampled larvae ($n = 10$). Sampling was conducted in the morning (08:00 am), and larvae were fed with rotifer 1 h prior to that. The number of rotifers was counted under a microscope (Nikon Corporation, Nikon Eclipse 80i, Japan).

2.5 Statistical analysis

All data are presented as mean \pm SD and were subjected to one-way ANOVA to analyze the different variables by delay in initial feeding time for significant difference ($P < 0.05$) and *post hoc* Duncan test was performed to confirm significant differences between treatments. All statistical analyses were performed using SPSS version 22.

3. Results

3.1 Growth

The growth of *O. marmorata* was highly correlated with the time of initial feeding (Figure 1). At 10 DAH, there were no significance differences ($P > 0.05$) in TL between control (6.69 \pm 0.58 mm), 2 (6.41 \pm 0.53 mm), and 3 (6.36 \pm 0.08 mm) d DIF treatments but it significantly differed ($P > 0.05$) in 1 (6.27 \pm 0.38 mm), 4 (5.48 \pm 0.15 mm), and 5 (4.96 \pm 0.42 mm) d DIF treatments. Meanwhile at 20 DAH, larvae in control and 1 d DIF treatments grew significantly faster ($P < 0.05$) than those with 3 and 5 d DIF. Larvae in treatment with 4 d DIF seemingly were able to recover in growth at 30 DAH, showing almost similar size to the 1 d DIF treatment without significant difference ($P > 0.05$) from the other treatments, except for the 5 d DIF treatment. Control (13.13 \pm 1.21 mm) led the growth at 40 DAH with significantly high ($P < 0.05$) TL compared to the other treatments.

3.2 Survival and larval deformity

Larval survival was significantly affected by the delay in initial feeding time (Figure 2). In general, mortality sharply dropped in all treatments by the first 10 DAH, indicating that the larvae were fragile during this period. At 10 DAH, the survival of larvae noticeably declined with increasing DIF, being 45% in 4 d DIF and 60% in 5 d DIF treatments compared to control treatment. At 20 DAH and onwards, survival in all treatments (including control) decreased continuously. The percentage of deformity increased proportionally with the DIF, being highest in 2 (9.00 \pm 1.15%), 3 (9.00 \pm 2.31%) and 5 (10.50 \pm 2.89%) d DIF treatments. The percentage of deformed larvae was significantly lower ($P < 0.05$) at 0 d DIF (1.50 \pm 0.58%) treatment than in 5 d DIF (Figure 3); the deformation was a bend of the caudal peduncle (Figure 4).

3.3 Feeding incidence, PNR, and feeding intensity

Feeding incidence for the various DIF times of *O. marmorata* larvae is shown in Figure 5. Feeding incidence showed an increase from control up to 2 d DIF and decreased subsequently until 5 d DIF. The PNR was estimated as being between 3 and 4 d DIF (indicated by the arrow in Figure 5) as it is the time at which feeding incidence had dropped to approximately half of the highest feeding incidence; the larvae of *O. marmorata* were unable to survive beyond 4 d DIF.

The feeding intensity of *O. marmorata* larvae for the various DIF times is shown in Figure 6. In general, the feeding intensity gradually increased with age of the fish larvae. The highest feeding intensity recorded at initial feeding was for 3 d DIF, followed by the 1 and 2 d DIF

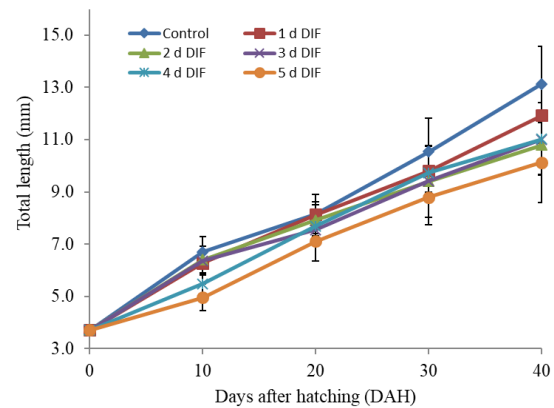


Figure 1. The mean total length of *O. marmorata* fed at various initial feeding times. Vertical lines indicate SD.

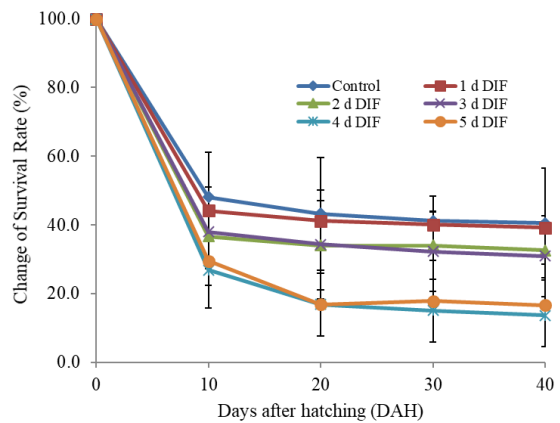


Figure 2. The survival rate of *O. marmorata* fed at various initial feeding times. Vertical lines indicate SD.

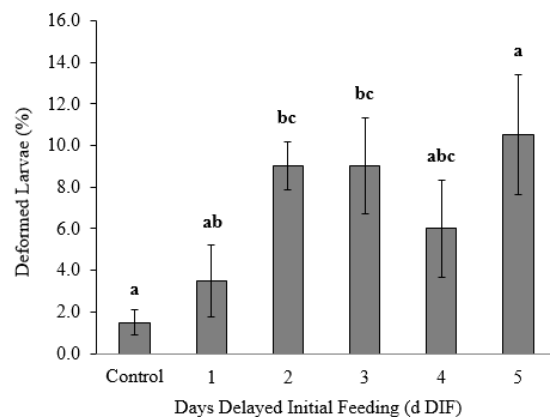


Figure 3. Percentage of deformed juvenile *O. marmorata* at 40 DAH. Vertical lines indicate SD.

treatments. The trend seemed similar to feeding incidence, which increased from control until 3 d DIF and subsequently decreased in 4 and 5 d DIF treatments. The significant decrease in feeding intensity for 3 d DIF and onwards was consistent with the decrease in feeding incidence, which



Figure 4. Bend (circle) at the caudal peduncle observed in a deformed *O. marmorata* juvenile at 40 DAH.

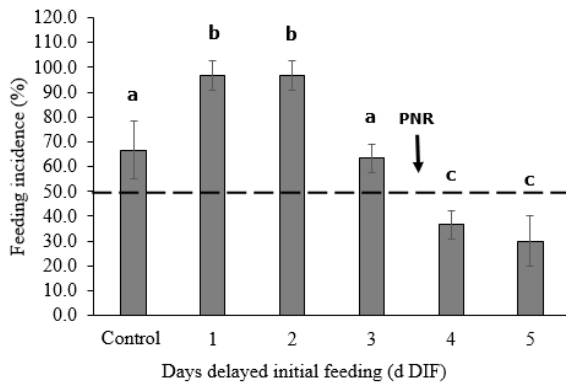


Figure 5. The mean feeding incidence of *O. marmorata* fed at various initial feeding times. Dotted line is for estimating the PNR (defined as 50% of maximal feeding incidence). Vertical lines indicate SD.

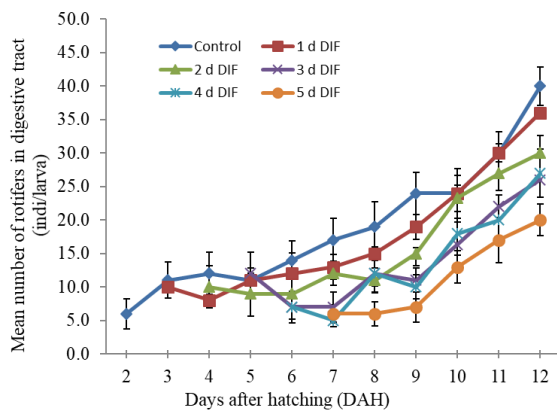


Figure 6. The time profiles of feeding intensity for *O. marmorata* fed at various initial feeding times. Vertical lines indicate the SD.

coincided with the starting point of the PNR. At the end of the experiment, the highest feeding intensity was observed in control (40.0 ± 2.88 ind.larva⁻¹) which was significantly higher ($P < 0.05$) than with 3 (26.0 ± 1.79 ind.larva⁻¹), 4 (27.0 ± 3.61 ind.larva⁻¹) or 5 d DIF (20.0 ± 2.37 ind.larva⁻¹) treatments.

4. Discussion

O. marmorata larvae were found to have less growth when the initial feeding time was delayed as compared

to the not delayed control. Larvae in the control treatment showed maximum growth and this could be related to their foraging ability, since the larvae had enough time to gain their feeding ability before the yolk exhaustion (Xiong *et al.*, 2013). The growth was apparently not recovered for the larvae fed at 1, 2, 3, 4, and 5 d DIF even up to the juvenile stage (40 DAH), as evidenced by significantly higher growth in the control treatment. Depressed growth with prolonging the starvation period presumably occurred as the energy allocation strategy of starved larvae for their survival was affected by energy limitations, especially when the energy gains solely came from the yolk sac. Larvae might respond to food concentration by altering the allocation of the resources between growth and development, in order to successfully establish their initial feeding (Shan, Quan, & Dou, 2008).

Delaying the initial feeding significantly affected larval survival in the present study, where larval survival was significantly higher ($P < 0.05$) in control group than with 4 or 5 d DIF. Various studies have reported similar findings, in freshwater species of loach larvae for which the initial feeding was needed early (3 DAH) or there was reduced survival (Wang *et al.*, 2010); and in tiger grouper *Epinephelus fuscoguttatus* that was affected if it failed to feed at 0 h after mouth opening (h AMO) and these larvae could not survive when the initial feeding was delayed beyond 6 h AMO (Ching *et al.*, 2011); and in coral trout grouper, *Plectropomus leopardus*, reportedly affected if they fail to initially feed within 1 day after first feeding time (Yoseda *et al.*, 2006). A number of factors could be implicated by poor larval survival and for affecting growth of fish larvae at an early stage, and this includes nutrients stored in the yolk sac, yolk sac size, temperature, food density, light intensity and time of initial feeding (Dou *et al.*, 2002).

In the present study, remarkably decreased survival was recorded during the first 10 DAH of *O. marmorata* larvae. It appeared that at this early stage the larvae were fragile to interference, such as handling and water changes, and proper handling is necessary to minimize the mortality. A similar trend has been reported for some other species such as survival of 41% in *Gradus morhua* (Puvanendran, Leader, & Brown, 2002), 46% in *Sciaenops ocellatus* (Brinkmeyer & Holt, 1998), and 38% in *Melaogrammus aeglefinus* (Hamlin & Kling, 2001) during the first few weeks after mouth opening under the optimum rearing conditions.

The PNR which occurred around 3 and 4 d DIF (4.5–5.5 DAH) in this study could be considered short, since the larvae had only 3 to 4 days before approaching the irreversible starvation, counted from commencing their initial feeding. According to previous studies, some temperate species can initiate feeding over a period of several days or longer after the yolk exhaustion (Gisbert & Williot, 1997; Gisbert, Conklin, & Piedrahita, 2004). Some subtropical species, however, have only one day or less after the yolk exhaustion to initiate their initial feeding or they starve (Garcia, Sayco, & Aya, 2020), indicating poor starvation tolerance of these fish larvae. There are many factors that influenced the PNR such as body size, the size of the yolk sac and oil globule, time of initial feeding, and temperature. As regards temperature, the PNR becomes shorter when the temperature is increased. For example, Japanese flounder took 2.0 - 3.7 days from the yolk exhaustion to PNR at 15–21°C (Dou *et al.*, 2005), while Atlantic herring took 3 to 5 days

from the yolk exhaustion to reach PNR at 7.5–13.1°C (Yin & Blaxter, 1987).

Delaying initial feeding to around the PNR or later would further deteriorate the feeding ability and cause deformities induced by early starvation (Dou *et al.*, 2002; Gisbert *et al.*, 2004; Yúfera, Pascual, Polo, & Sarasquete, 1993), and this is inconsistent with the current study in which some larvae were able to survive up to the juvenile stage but were deformed. The cause of this might be that the starved larvae did not have enough energy to undergo morphological development and skeletal formation, due to limitations in availability of nutrients from external sources (Cahu, Infante, & Barbosa, 2003). Yandi and Altinok (2016) also reported that larval stage of horse mackerel, *Trachurus mediterraneus*, with caudal deformity has difficulty in capturing live feed, and died because these larvae have to rely on their caudal fins to capture live feed. In this study, even if the deformed juvenile could survive and grow to adult stage, it had a disadvantage for use in the production as it will have reduced market value.

The feeding incidence was significantly higher at 1 and 2 d DIF compared to control, 3, 4, and 5 d DIF, and reached the PNR between 3 and 4 d DIF, and this pattern is in accordance with previous findings on several species that have reported active feeding after food restriction (Hart, 1997; Parra & Yúfera, 2000; Peña & Dumas, 2005; Klimogianni *et al.*, 2013). This presumably occurred because prolonged starvation may increase the feeding intake for a certain period to compensate for the nutrition limitations affecting growth. In the current study, decreasing feeding incidence from 2 d DIF onwards indicated decreased feeding ability of the larvae to capture their prey. Johnston (2006) states that swimming activity of larvae involves rhythmic contractions of arranged myotomes and is aided by the fins, and restricted feeding may affect these structures thereby reducing the locomotion and foraging capacity of the fish larvae. Besides, a short period of starvation could induce deformities, and permanent feeding and digestive problems that subsequently affect survival (Gwak & Tanaka, 2001). Even with the presence of food, Yúfera *et al.* (2007) added that there is no guaranteed ingestion of food that occurs beyond this point, and would not affect the survival up to the juvenile stage.

5. Conclusions

Delayed initial feeding time decreased the feeding ability of *O. marmorata* when the PNR was approached, and subsequently decreased the larval survival and growth. For optimum survival and growth, it is crucial to introduce initial feeding once the larvae are morphologically prepared for their initial feeding (1.5 DAH) ($29 \pm 0.5^\circ\text{C}$). The larvae could tolerate up to 3 d DIF (3.5 DAH) prior to reaching the PNR. Delays beyond that are not recommended as the significantly reduce growth, survival, feeding ability, and increase deformities in the *O. marmorata* juveniles.

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