EFFECTS OF DELAYED FIRST FEEDING ON THE SURVIVAL AND GROWTH OF MARBLE GOBY, *Oxyeleotris marmoratus* LARVAE

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THIS DISSERTATION IS SUBMITTED AS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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I hereby declare that this thesis is my own independent work, apart from some quotations and references which have been appropriately acknowledged.

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ABSTRACT

Larval observation was done in marble goby, *Oxyeleotris marmoratus* larvae to determine the morphologically prepared time for first feeding, time when presence of rotifer *Brachionus* sp. inside the gut and time when the yolk sac fully absorbed. After the observation step, experiment on the delayed first feeding was carried out. Thirty aquarium (18 x 26 x 17 cm) were prepared and divided into triplicate groups. The larvae were reared up to 10 day after hatch (d AH). The first feeding was given at 31 hour after hatch (h AH) in control tank fish while in the other tanks, the first feeding was delayed every 24 hours up to 247 h AH. Based on the observation, larvae are morphologically prepared for first feeding at 31 h AH and yolk sac was fully absorbed at 86 h AH at temperature 26-27 °C. Therefore, nutrition transition period (NTP) for *O. marmoratus* is from 31 to 86 h AH. Results showed that the mean survival rates were highest 64.00±3.72 % (31 h AH), and lowest 2.00±2.91 % (223 h AH). The mean total length were highest 4.97±0.26 mm (55 h AH), and lowest 3.50±0.16 mm (247 h AH). The results showed mean survival rate and total length was decreased when we delayed the first feeding timing. This proved that delayed first feeding will lead to low survival and growth. Interestingly, deformities were also observed in the treatment of 151, 175, 199, 223 and 247 h AH. The deformed larvae have bended body, and it showed absence of dorsal and anal fin. From the results, the morphology prepared for first feeding time of marble goby was happened at 31 h AH. The larval survival and growth can be improved by determining the optimum first feeding timing. The optimum time for first feeding of marble goby was achieved at 55 h AH because the best survival and growth was achieved at this time.

ABSTRAK

Permerhatian ke atas ikan ketutu, *Oxyeleotris marmoratus* larva telah dijalankan untuk menentukan waktu bagi pengambilan makanan pertama, kewujudan rotifer, *Brachionus* sp. dalam usus dan waktu ketika pundi telur diserap sepenunnya. Selepas process pemerhatian, kajian terhadap kesan penangguhan pengambilan makanan pertama dijalankan. Larva ikan ketutu dikultur dalam akuarium (18x26x17 cm) dengan tiga replikasi selama 10 hari dalam rawatan yang berlainan mengikut tempoh masa pengambilan makanan pertama bermula dari 31 sehingga 247 (jam selepas penetasan) h AH dengan selang masa 24 jam. Berdasarkan pemerhatian, larva secara morfologi telah bersedia untuk pengambilan makanan pertama pada 31 h AH dan pundi telur diserap sepenuhnya pada 86 h AH. Oleh yang demikian, tempoh transisi gizi (NTP) adalah dari 31 hingga 86 h AH. Kajian juga menunjukkan kadar kemandirian yang tinggi bagi rawatan 31 h AH (64.00±3.72 %) dan yang terendah bagi rawatan 223 h AH (2.00±2.91 %). Kadar pertumbuhan tertinggi mengikut kepanjangan badan larva ditunjukkan oleh rawatan 55 h AH (4.97±0.26 mm) dan terendah adalah rawatan 247 h AH (3.50±0.16 mm). Kadar kemandirian dan pertumbuhan menurun apabila kita menangguhkan masa pengambilan makanan pertama. Ini menunjukkan penangguhan makanan pertama akan menyebabkan kadar kematian yang tinggi dan melambatkan kadar pertumbuhan larva. Dalam kajian ini, kecacatan pada larva juga telah ditemui pada rawatan 151 hingga 247 h AH. Larva yang cacat mempunyai struktur badan yang bengkok, kehilangan sirip dorsal dan sirip anal. Dari hasil kajian ini, morfologi larva bersedia untuk pengambilan makanan pertama ketutu adalah pada 31 h AH. Tekink pengkulturan larva dapat dikukuhkan dengan menentukan masa optimal bagi pengambilan makanan pertama. Masa optimal bagi pengambilan makanan pertama bagi larva dicapai pada 55 h AH kerana ia menunjukkan kadar kemandirian dan pertumbuhan yang terbaik pada waktu tersebut.

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LIST OF SYMBOLS

°C	degree centigrade
%	percentage
BMRI	Borneo Marine Research Institute
BW	body weight
cm	centimeter
d AF	days after fertilization
d AH	days after hatching
DO	dissolve oxygen
ED	eye diameter
g	gram
h	hour
HCG	Human Chorionic Gonadotrophin
I.U.	International Unit
kg	kilogram
SPSS	Statistical Package for Social Science
L	liter
m	meter
mg	milligram
ml	milliliter
NTP	Nutrition Transition Period

ppt	parts per thousand
RM	Ringgit Malaysia
TL	total length
UMS	Universiti Malaysia Sabah
YSv	yolk sac volume

CHAPTER 1

INTRODUCTION

1.1 Marble Goby, *Oxyeleotris marmoratus*

Marble goby, *Oxyeleotris marmoratus* (Photo 1.1) is the largest freshwater Eleotridae which can grows to more than 50 cm in total length (TL) and 2 kg in body weight (BW). This species is mainly distributed in the Southeast Asia Region such as Thailand, Cambodia, Vietnam, Singapore, Indonesia, the Philippines, and Fiji (Cheah *et al.*, 1994; Senoo *et al.*, 1994). *O. marmoratus* is known as "Ikan Ketutu" or "Ikan Hantu" in Malay and " Soon Hock" in Chinese Fujian, and it is also called as "Bamboo Fish" (Senoo *et al.*, 1994). It is a very popular and highly demanded fish in the market because of its taste, non-bony flesh and high protein value (Amornsakum *et al.*, 2003). *O. marmoratus* is the most expensive freshwater table fish in Malaysia (Senoo *et al.*, 1997). The retail price of this species is RM 60 – 80/kg compare to other freshwater species such as Tilapia, African catfish and Common carp which prices are RM 2-12/kg (Department of fisheries, 2005).

Many fish farmers wish to produce *O. marmoratus* but the seed supply is inadequate in this country. Some middlemen in the Kuala Lumpur are importing this species from Indonesia, Thailand and Cambodia (Senoo *et al.*, 1994) because natural seeds have been over fished and artificial seed production techniques are not yet established. (Senoo *et al.*, 1994). Due to its high price and market demand, interest in the cultivation of *O. marmoratus* is gradually increasing. Peoples are using artificial seed production to get the seeds and cultivate in artificial closed system. However, there are several problems in culture of *O. marmoratus* such as high mortality and slow growth during larval and juvenile stage (Cheah *et al.*, 1994; Lin *et al.*, 2000). Besides, the feed and feeding scheme in the larval and juvenile *O. marmoratus* also have not been established (Amornsakum *et al.*, 2003). The low survival and growth of *O. marmoratus* during the larval and juvenile stage is due to the unknown optimum first feeding timing. There have many factors that will cause to the low survival and growth of *O. marmoratus*, but this studies will focus on the optimum first feeding time.

Photo 1.1 Marble goby, *Oxyeleotris marmoratus*. Largest freshwater Eleotridae which can grows to more than 50 cm in total length (TL) and 2 kg in body weight (BW).

1.2 First Feeding

. First feeding of the larvae can be defined as the initial of larval exogenous feeding (Pena & Dumas, 2005). First feeding is very important for survival and growth of marine and fresh water larvae after the yolk sac had fully absorbed. It is because after the yolk sac finished, the larvae have to depending on the external food to support their survival and growth. First feeding happens when fish larvae eye is pigmented and movable, mouth is opened and lower jaw is movable and intestinal tract is peristalsis (Senoo *et al.*, 1994). The onset of first feeding refers to the moment larvae start to feed at the exogenous phase. It is the source of nutrient and energy necessary to continue the larval development changes from the yolk reserves to the ingested food. Therefore, mouth size development is very important in the first feeding of larvae to match appropriate prey size. (Amornsakum *et al.*, 2002).

First feeding of fish larvae from endogenous reserves to exogenous feeding is very crucial for their subsequent growth and survival. Most of the fish larvae attain deformed growth, inability to swim and prey the feed if they fail to initiate successful first feeding soon after mouth opening (Houde, 1974; Dou *et al.*, 2002). High mortality of the fish larvae usually will occur at the early stage of the larvae if the yolk sac is exhausted and suitable food is not provided (Senoo *et al.*, 1994).

1.3 Nutrition Transition Period (NTP)

Nutrition transition period is defined as the time when larvae switch their feeding source from endogenous to exogenous (Rao, 2003; Turingan *et al.*, 2005). Fish larvae can be divided into yolk sac larvae and larvae. Yolk sac larvae are depending on endogenous feeding. Endogenous feeding means that the fish larvae are using the yolk sac to obtain nutrient for growth. For fish larvae, they are depending on the exogenous feeding. Exogenous feeding means that the fish larvae are rely on the external food which is available in rearing water. Nutrition transition period also refers to period of complete yolk sac absorption and the beginning of the first feeding (Bariganao, 1986).

At the beginning stage, larvae are sustained by their yolk supply while they gradually develops skills to capture feed in the water and physiological abilities to digest feed (Rao, 2003). Larval survival greatly depend on appropriate feed being available in the immediate environment particularly at the end of their nutrition transition period (Eldin *et al*., 1997; Rippigale & Payne, 2005).

The first exogenous feeding period is the most critical stage for fish larvae (Buskey, 2006). This is because larvae that fail to feed sufficiently at this time will die due to the starvation, grow slowly and increasing their risk to predation or disease (Rippingale & Payne, 2005). It is recognized that the critical period of larval rearing begins at the time yolk absorption is completed. If some larvae do not begin to eat during that period, then they become weak and eventually die. Different species of fish larvae have different nutrition transition periods. Marine fish larvae with a short nutrition transition are often considered as poor larval quality (Konho 1998; Williams *et al.*, 2004; Turingan *et al.*, 2005).

1.4 Significance of the Study

Complete aquaculture system has already been established on several species such as carps, African catfish, patin, tilapia, salmons, trouts, sea bream, and tiger grouper; thus, a lot of information is available in the cultural history of these species (Senoo *et al.*, 1994a). However, *O. marmoratus* still requires much study regarding the culturing system. Many studies have been conducted on the artificial seed production technique, deformation, egg collection, egg development and early larval stage of *O.marmoratus* (Senoo *et al.*, 1992; Senoo *et al.*, 1993; Senoo *et al.*, 1994; Senoo *et al.*, 1997; Senoo *et al.*, 2000). Despite this, there is still lack of knowledge on the early larvae stage of *O. marmoratus* , especially concerning first feeding timing of the larvae. Low survival and slow growth in the larvae stage are still remains as major constraints in aquaculture due to the unknown optimum first feeding timing..

Therefore, more information is needed for the purpose of cultivation. There is a possibility that *O. marmoratus's* survival and growth performance will be affected if larvae first feeding is poor. First feeding of fish larvae from endogenous reserves to exogenous feeding is very crucial for the larvae survival and growth. For an example, if larvae fail to begin successful first feeding, larvae will suffer from starvation, inability to swim and eat the feed if they fail to initiate successful first feeding soon after mouth opening. (Houde, 1974; Dou *et al.*, 2002). Therefore, it is very important to know the optimum first feeding timing of the *O. marmoratus*.

1.5 Objectives of the Study

This study was intended to elucidate the challenges of low survival and growth in the larval stage *O. marmoratus* that might influence by first feeding timing. The main objectives of this study are as follow:

1. To determine the optimum *O. marmoratus* larval first feeding timing.
2. To determined the Nutritional Transition Period (NTP) of *O. marmoratus* larval.
3. To know the effects of delayed first feeding on the larval survival and growth *O. marmoratus*.
4. To improve the larval survival and growth *of O. marmoratus*.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and Morphology

Marble goby belongs to the kingdom of Animalia, with phylum as Chordata and superclass as Pisces; the order is Perciformes and suborder is Gobioide*a*; with the family name as Eleotridae, genus name as *Oxyeleotris* and species name as *Oxyeleotris marmorata* (Komarudin, 2000). Bleeker (1874) reported that the Eleotridae or sleepers comprise some genera and 150 species of mostly tropical, marine, brackish, and freshwater fishes. Two genera and four species occur in the fresh waters of western Borneo. *Oxyeleotris* is unusual among eleotrid genera in being almost entirely restricted to fresh water species of the genus in Southeast Asia and others in the Austrian region. Three species known from Western Borneo are *O. urophthalmus*, *O. urophyhalmoides* and *O. marmoratus*. Kottelat *et al*. (1993) had stated that Eleotrididae is a small family similar to Gobiidae; however, it can be differentiated from other Goiidae by their separate pelvic fin and the six branchiotegal rays. Most of the members of this family can be found in sea, brackish waters and ; while *O. marmoratus* is found in freshwater. *O. marmoratus* has colour in alcohol dark brown above, pale brown below; body with a series of large, dark blotches; fins with black bands or dusky (Bleeker, 1877). The morphological change on the eggs and larvae of *O. marmoratus* were observed by Senoo *et al.*, (1994) who stated that the juvenile stage of *O. marmoratus* was after 40 days after fertilization (d AF) where the yellow-ocher pigmentation appeared on body.

2.2 Natural Habitat and Culture Condition

Koumans (1953) had reported that the *O. marmoratus* also occurred in river as well as estuaries. Kottlelat *et a.*, (1993) also reported that *O. marmoratus* was found in rivers, swamps reservoirs and canals among the Mekong and Chao Phraya Basins, Malay Penisula, Indochina, Philippines and Indonesia.

O. marmoratus was cultured in ponds and ex-mining ponds either in monoculture or in polyculture with tilapias. Cage culture of this species in lakes and rivers had also been successful done (Cheah *et al.*, 1994). Cage culture of *O. marmoratus* in floating bamboo or wooden cages had been carried out in Thailand since the early 1970s (Suwansart, 1979). There was a study done on the cove polyculture of *O. marmoratus* with silver carp, common carp and grass carp in Tri An Reservoir of Vietnam. This study revealed the result of improving productivity and economic performance both in *O. marmoratus* and carps. It had proven that this cove polyculture system has a prominent prospect ecologically, technologically and economic performance (Vu *et al.*, 2003).

Senoo *et al.*, (1994) elaborated the larval rearing method for *O. marmoratus* that require tank cleaning and 30-50% water exchange daily from 7 d AF. The water must be well aerated and treated with 20 W florescent lights above the tank daily from 0800-1800 h with 100-800 lux on the water surface until 30 d AF. This is because the larvae were negatively phototactic after 30 d AF. The water temperature, dissolved oxygen (DO) and pH during the larval rearing ranged from 27.0-29.5°C, 2.1-7.8mg/l, and 7.5-7.9, respectively.

Mok (2004) reported that the hatching rate for the eggs of *O. marmoratus* that were incubated in 10 ppt was the best among the 4 treatments (0, 10, 20 and 30 ppt). It also showed that least mortalities occurred both in eggs and larvae in 10ppt. Besides, the result also indicated that 10ppt can also shorten the hatching period of the eggs where the hatching peak occurred on the fourth day after spawning. With this, there was higher possibility to produce well hatched larvae due to its hatching period.

Raymond (2006) had reported that survival rate of juvenile *O. marmoratus* that reared in 0 ppt and 10 ppt was the best among the 4 treatments (0,10,20,30 ppt) where no mortality was found throughout the experiment while the TL gain was the highest for the fish reared in 0 ppt.

Tavarutmaneegul *et al.*, (1988) fed chicken egg slurry and rotifers (*Brachionus sp.*) to the larvae from 7-20 days after hatching (d AH). Thereafter, the larvae were transferred to outdoor tank and feed daily with live organisms, such as *Moina sp.*, *Brachionus sp.*, *Chironomid* (Blood worm) larvae and trash fish from 30-60 d AH .

Amornsakum *et al.*, (2003) had also reported on the feeding regime of larvae and juveniles of *O. marmoratus*. The author suggested that larvae at 3-18 d AF consumed only rotifer. 21- 27 d AH larvae with average total length of 0.44-0.65 cm consumed rotifer and *Artemia nauplii*. Whereas, 30-45 d AH larvae with average total length 0.69-21.5cm consumed only *Moina*. From 60 d AH, only the *Chironomus* larvae were given and most of the juveniles became used to feeding on it within a few days. At the later stage, the juveniles are fed on minced fish, shellfish and shrimp or artificial pellet.

2.3 Larval Development

Amornsakum *et al.*, (2002) had reported that newly hatched *O. marmoratus* larvae were 2.39±0.12mm in total length, and had yolk sacs of 55.32±14.85µm volume. The yolk sacs were completely absorbed within 82 hour (3.4 days) after hatching at water temperatures of 27.0-30.5 °C.

Senoo *et al.*, (1994a) reported the correlations between the morphological and behavioral changes in *O. marmoratus* larvae. Observation on the behavioral changes of the egg and larvae of *O. marmoratus* from 2-40 d AF had been reported by (Senoo *et al.*, 1994). Gobies usually change their habitat from pelagic to benthic as they change from the larval to the juvenile stages. Senoo *et al.*, (1994) stated that newly hatched larvae 2-3 d AF lay on the tank bottom and gradually exhibited the "swim up, sink down" behavior. On 4-5 d AF, they are positively phototactic and show active S-posture and horizontal swimming. They are negatively photo tactic after 25 d AF and make schooling at the bottom. At 30-35 d AF, the larvae show active swimming on the bottom. After that, they are change to juvenile after 40 d AF. At juvenile stage, they are not active and lay at the bottom.

2.4 First Feeding and Their Effects Towards Survival and Growth

The *O. marmoratus* larvae commenced feeding on phytoplankton at 3 d AF when the eyes were pigmented and the yolk sac had been absorbed to almost the same size as the air bladder (Senoo *et al.*, 1994).

Amornsakum *et al.*, (2002) had reported that yolk sacs of *O. marmoratus* larvae were completely absorbed within 82 hour after hatching. All larval mouth was open 36 hour after hatching but was not yet functioned. At 80 hour after hatching, the fish started feeding on the rotifer at which time the yolk sac remained at 6.16% of its initial volume. Without feeding, the larval *O. marmoratus* started to die at 84 hour and totally died with 130 hr (5.4 days) after hatching. Many researches had reported that poor larval survival during initial feeding periods (Kohno *et al.*, 1997; Kohno, 1998; Gisbert *et al.*, 2004; Pena & Dumas, 2005) and this is due to various factors such as temperature, light intensity, food supply, egg and larval size, yolk and oil quantity and resorption rate, time of onset of feeding and feeding behaviour (Blaxter, 1974; Dou *et al.*, 2000; Hecht & Appelbaum, 1988).

Kailasam *et al.*, (2007) had reported that the sea bass *Lates calcarifer* larvae started initial feeding at 48 h after hatching. Sea bass larvae after hatching can survive even up to 96 h after hatching without feed, since it can utilize the food from yolk reserves and complete yolk absorption was noticed at 96 h after hatching. European