REPRODUCTIVE BEHAVIOUR OF MARBLE GOBY, *Oxyeleotris marmoratus* AFTER HORMONE INJECTION UNDER ARTIFICIAL REARING CONDITION

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DECLARATION

I declare that this dissertation is the result of my own independent works, except where otherwise stated.

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ABSTRACT

The experiment was conducted to improve the artificial egg collection technique of Marble goby, *Oxyeleotris marmoratus* by understanding its reproductive behaviour. The experiment was done through observation of reproductive behaviour of the selected brood fish in a 700L aquarium following Human Chorionic Gonadotropin (HCG) injection. A pair of *O. marmoratus* brood fish was selected for each trial and from a total of six trials, three pairs of selected brood fish were successfully spawned. All successful spawning pairs showed similar reproductive behaviour at 26 - 32°C, and the breeding cycle completed within 142 - 165 hours. After hormone injection, male and female started to swim at the bottom and they always separated at least 20cm apart. After male occupied the nest, it started to swim out to court female and lead into the nest. Female that not ready to spawn rejected male without showing response to the male courtship. In the nest, the pairs showed pre-spawning behaviour prior to spawning. For the three successful spawning trials, spawning phase took place within 37 - 74hAI, and parental care also started at the same time. After female left the nest, the male guarded and fanned the eggs until the end of hatching. The results suggest that observation of the reproductive behaviour of *O. marmoratus* brood fish enables the culturists to predict whether the hormone treated fish will be able to spawn and also to estimate when the spawning will take place. Hence, the artificial eggs collection technique can be improved.

ABSTRAK

Tujuan eksperimen ini dijalankan adalah untuk memperbaiki teknik pengumpulan telur ikan Ketutu, *Oxyeleotris marmoratus* yang dihasilkan secara aruhan dengan memahami kelakuan reproduktif mereka. Kajian ini dijalankan dengan memerhati kelakuan reproduktif bagi pasangan yang dipilih dalam akuarium 700L selepas suntikan 'Human Chorionic Gonadotropin' (HCG). Daripada enam pasang *O. mamoratus* yang telah dipilih, tiga pasang telah bertelur. Ketiga-tiga pasangan yang bertelur menunjukkan kelakuan reproduktif yang sama pada suhu air 26 - 32°C dengan kitar pembiakan yang mengambil masa selama 142 - 165 jam. Selepas suntikan hormon, pasangan ikan mula berenang pada dasar akuarium tetapi sentiasa berada pada jarak sekurang-kurangnya 20cm antara satu sama lain. Selepas jantan masuk ke dalam sarang, jantan tersebut akan mula memikat ikan betina dan cuba untuk memimpin betina masuk ke dalam sarangnya. Sekiranya, ikan betina tidak bersedia untuk bertelur, ia tidak akan memberi sebarang reaksi terhadap pikatan jantan. Dalam sarang, ikan jantan dan betina akan menunjukkan kelakuan pra-bertelur. Dalam eksperimen ini, fasa bertelur bermula dalam 37 - 74 jam selepas suntikan hormon and pada masa yang sama ikan jantan and betina akan mula menjaga telur-telur dalam sarang. Selepas ikan betina meninggalkan sarang selepas bertelur, ikan jantan akan menjaga telur sehingga semua telur menetas. Keputusan eksperimen ini mencadangkan bahawa pemerhatian terhadap kelakuan reproduktif bagi *O. marmoratus* membolehkan pengkultur ikan meramal sama ada ikan yang telah dipilih itu akan bertelur atau tidak dan menganggar masa bertelur pasangan tersebut. Oleh yang demikian, teknik pengumpulan telur *O. mamoratus* secara aruhan akan dapat diperbaiki.

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LIST OF ABBREVIATION

APF	artificial powder feed
BH	body height
BL	body length
BR	body round
BW	body weight
BWd	body width
cm	centimeter
dAH	days after hatching
dAS	days after spawning
DO	dissolved oxygen
Exp	experiment
g	gram
HL	head length
HCG	human chorionic gonadotropin
IU	International Unit
kg	kilogram
km^2	square kilometer
L	liter
M	male
mm	millimeter
min	minute
mt	metric ton
µm	micrometer
P1	first pair
P2	second pair
P3	third pair
P4	fourth pair
P5	fifth pair
P6	sixth pair
TL	total length

YL	yolk length
°C	degrees centigrade
%	percent

CHAPTER 1

INTRODUCTION

Malaysia covers of an area of about 336,733km^2. It consists of the Malay Peninsular and the states of Sabah and Sarawak. Its temperature is high with heavy rainfall in all year round due to its location which is near to the equator. Malaysia has many mountains, heavy rainfall all year round, which gives rise to many rivers from the mountain. Rich in freshwater supply and warm weather make Malaysia a potential country for freshwater aquaculture.

Freshwater fish culture in Malaysia had started more than 50 years ago. The progress was very slow in the early years, however significant expansion took place after 1957. Malaysian government keens to develop aquaculture sector in the country because fish and other aquaculture products are the main source of animal protein for its population. The National Agriculture Policy formulated by Malaysian government in 1984 with the aim to modernize the agriculture sector benefited the development of Malaysia freshwater aquaculture in recent years (Ang, 1990).

There are some important freshwater aquaculture species, which include Marble goby *Oxyeleotris marmoratus*, African catfish *Clarias gariepinus*, River catfish *Pangasius* spp., Tilapia *Oreochromis* spp., Carp *Cyprinus* spp., and Giant freshwater prawn *Macrobrachium resenbergii*. Most of the fry of these fishes have come from hatcheries

while some are imported from foreign countries. However, fry for some species like *O. marmoratus* are still collected from the wild.

O. marmoratus (Photo 1.1) is the largest freshwater Eleotrididae in the world (Tavarutmaneegul *et al.*, 1988; Senoo *et al.*, 1994a; Kottelat *et al.*, 1993). The adult fish can grow to more than 2kg and up to 50cm in total length (TL) (Senoo *et al.*, 1994a). *O. marmoratus* is also known as "Sand goby". In Indonesia, it named as "Ikan malas" and "Plabu" in Thailand. In Malaysia, it is known as "Ikan ketutu" or "Ikan hantu, while Chinese like to call it "Soon hock" or "Bamboo fish" (Senoo *et al.*, 1994a).

O. marmoratus is a very profitable freshwater cultured species. It has high market demand and its price can fetch up to RM100kg^{-1}. Compare to other freshwater fishes such as Tilapia and Patin, which is about RM8kg^{-1} and RM12kg^{-1}, *O. marmoratus* is considered very expensive. Its tender and delicious meat makes it as a highly esteemed food fish especially among Chinese community. Besides local consumption, this fish is also exported to others Asian countries such as Singapore, Taiwan and China.

Overfishing and failure in artificial seed production cause higher price of the fish in market. Aquaculture production of *O. marmoratus* was 39mt in 1997 compare to 115mt in 1992 (FAO, 1999). Besides, production of *O. marmoratus* is the lowest since 1994 compare to other freshwater species such as Common carp and Catfish, (FAO, 1999) (Figure 1.1). Although scientists had attempted to produce artificial seed of *O. marmoratus* in captivity since 30 years ago, the successes are limited. The artificial seed production of *O. marmoratus* is still in infancy stage.

The low seed production from aquaculture is due to two reasons, difficulties in artificial egg collection and lower larvae survival rate. In captivity, induce spawning is necessary for *O. marmoratus*. During ovulation time, eggs can be collected through stripping method. However, due to the stickiness of eggs, small size and longer ovulation period, stripping will usually cause lower fertilization rate, development rate and hatching rate. Thus, artificial eggs collection by stripping method is not feasible.

Natural spawning is another way to collect egg in captivity. It can be done by introducing a nest into spawning tank after hormone treatment. According to Senoo *et al.*, (1993b), natural spawning is a more feasible for artificial egg collection of *O. marmoratus* in captivity. The authors found that natural spawning results higher fertilization, developmental and hatching rate. Hence, to be more confident to collect eggs through natural spawning method, understanding the reproductive behaviour of the fish is very important. Constant artificial egg collection of *O. marmoratus* through natural spawning in captivity can also be achieved.

O. marmoratus eggs have longer hatching period. Fertilized eggs commonly hatch from 2 - 5 days after spawning (dAS). Too early hatching (1 - 2dAS) larvae are not well developed (they are physiologically not functioning and morphologically not well develop); however late hatching larvae (after 4dAS) are over developed. Due to insufficient space in the egg shell to accommodate bigger size larvae, larvae deform in the shell. Besides, late hatching also causes death to the larvae due to starvation (Senoo *et al.*, 1993a). Through observation and understanding spawning behaviour of the *O. marmoratus* in captivity, the artificial egg collection technique for *O. marmoratus* through natural spawning can be improved.

In order to solve these problems in artificial seed production and to have a sustainable seed supply of *O. marmoratus*, understanding the behaviour of the fish is necessary. Through observation of the reproductive behaviour of the fish, it might be possible to give a new idea to create an alternative reproductive and larvae rearing techniques of *O. marmoratus* in captivity.

The objectives of this study are as follow:

1. To improve artificial egg collection technique of *O. marmoratus* through the understanding of reproductive behaviour.
2. Understanding the sequence of the reproductive behaviour of the brood fish of *O. marmoratus*.
3. To investigate the significance of parental behaviour to promote proper hatching of *O. marmoratus* eggs.

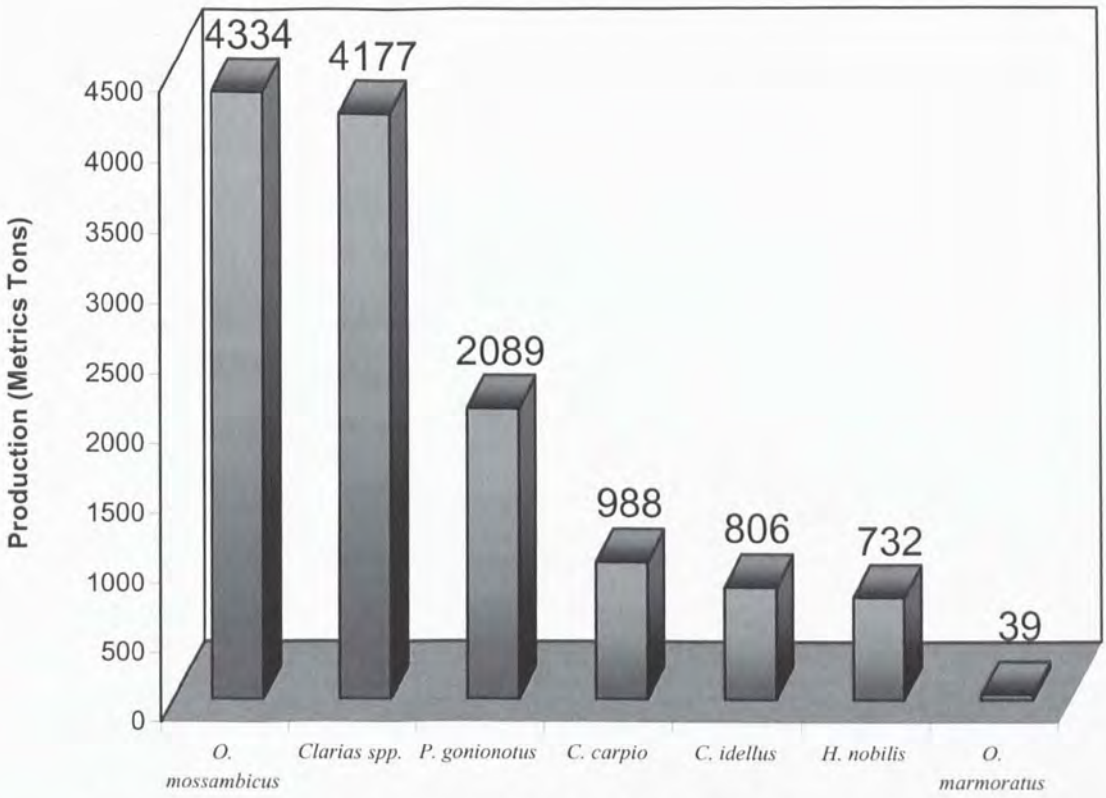

Figure 1.1 Malaysia freshwater fish production from aquaculture in 1997.

Source: FAO Fisheries Department, (1999)

Photo 1.1 Marble goby, *Oxyeleotris marmoratus*

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy, Morphology and Fish culture

O. marmoratus belongs to the Phylum Chordata, Family Eleotrididae, Order Perciformes and Class Actinopterygii. Eleotrididae is a small family similar to Gobiidae; it can be differentiated from other Gobiidae 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 estuaries, while *O. marmoratus* is found in freshwater (Kottelat *et al.*, 1993).

Aquaculturists had started to breed *O. marmoratus* in captivity about 30 years ago. However, the seed supply is still primarily dependent on wild seed. In 1973, Tan and Lam bred the fish in Singapore, however the larvae die within six days. In Malaysia, the first reported success on induced spawning of *O. marmoratus* was at the Universiti Pertanian Malaysia (UPM) in 1989. Studies on seed production of *O. marmoratus* were carried out by Cheah *et al.* (1994) and Senoo *et al.* (1992, 1993a, 1993b, 1994a, 1994b and 1994c) at the Faculty of Fisheries and Marine Science in UPM. Subsequently, several techniques of culturing *O. marmoratus* in captivity have successfully improved at UPM.

2.2 Reproductive Behaviour in Nature

O. marmoratus and other gobies are multiple spawners. They spawn throughout the year under suitable condition (Tavarutmaneegul and Lin, 1988). Male may receive eggs from several females in every spawning (Svensson *et al.*, 1998). During spawning, the male turns upside down and release sperm; sperm embedded on the ceiling of the nest followed by turning upside down to attach eggs to the ceiling of the nest in a single layer. The female will not stay in the nest after spawning, and the male guards and fans the eggs until hatching (Tavarutmaneegul and Lin, 1988; Senoo *et al.*, 1993b).

The male reproductive behaviour for many gobies is a size dependent. Male attracts female by courtship behaviour, where the male kept all fins erect, swimming rapidly close to the female and lead the female into the nest. Female is known to prefer larger and colourful males, perhaps for protection (Forsgren *et al.*, 1997).

Male *O. marmoratus* and other gobies are nest builders; they build nest using mussels shell and cover it with sand. Larger males are usually able to occupy large mussel shells, and also able to obtain more eggs in their nest while smaller males are less likely to build a nest and attract a female successfully (Svensson *et al.*, 1998). The nest with the most sand on top with the smallest entrance is preferred by the female to spawn. This type of nest is hardly exposed to egg predators (Jones *et al.*, 1999).

2.3 Induced Spawning

O. marmoratus can spawn naturally throughout the year under proper feeding regime and suitable water temperature and pH. In captivity, spawning can be induced either with provision of nest with hormone injection or without hormone injection. However, induced spawning with hormone treatment is preferred for the purpose of mass seed production (Tavarutmaneegul and Lin, 1988).

Hormone Injection

The most common and suitable method used to induce final maturation and ovulation in *O. marmoratus* is to inject the fish with Human Chorionic Gonadotropin (HCG) (Senoo *et al.*, 1992; Senoo *et al.*, 1993b; Tan and Lam, 1973). Tan and Lam (1973) were the first to use HCG for induced spawning of *O. marmoratus*. Cheah *et al.* (1994) also reported that induced ovulation in *O. marmoratus* using HCG is the most effective compare to acetone-dried common carp pituitary.

The two common administration methods of hormone are the intra-muscular injection at the dorsal muscle and intra-peritoneal injection under the pectoral fin. Senoo *et al.* (1994a) noted that intra-muscular injection is effective to induce spawning of *O. marmoratus;* it is safe and less likely to damage vital organs.

The frequency and the number of injection are depending on the stage of maturity of the recipient brood fish (Tan and Lam, 1973). Maturity stages of the fish can be determined by measuring average diameter and assessing egg morphology under a light microscope.

Oocytes can be sampled using a polyethylene cannula, where it is inserted into the genital papilla and the oocytes are mouth-drawn. However, this can only be applied for larger female that has larger genital pore for the cannulation tube to be inserted in (Head *et al.*, 1994). Tan and Lam (1973) mentioned that female at stage one maturity required two to three injections in a week interval and ovulation will occur within three days after the last injection. However, single injection is sufficient to cause ovulation for female at stage four maturity. According to Cheah *et al.* (1994), the second experiment they did in year 1989 succeeded to induce ovulation in six out of seven females by a single injection of $5IUg^{-1}$ HCG. While Senoo *et al.* (1994a) noted that spawning can be achieved by a single or double injection using only $3IUg^{-1}$ HCG at 28 - 30°C. Unlike female, hormone injection on male is mostly for the acquisition of better quality sperms and increased semen volume (Kohler *et al.*, 1994). Therefore, male is usually injected half of the dosage which female received (Tan and Lam, 1973; Senoo *et al.*, 1992; Senoo *et al.*, 1993b; Senoo *et al.*, 1994a).

Artificial Egg Collection in Captivity

Artificial egg collection of *O. marmoratus* in captivity is very difficult. This is because of stickiness of the eggs and difficulty in obtaining milt from male. The eggs will become very sticky and clump together when there is water on it. Besides, the volume of milt that can be obtained from a male is also very little (Senoo *et al.*, 1992).

Milt and eggs of *O. marmoratus* can be collected using collectors. The collector consists of a cylindrical plastic container and two plastic tubes with different length connected to the cover of the container. The shorter tube is inserted into the papilla of the fish while another one (longer tube) is used to put in to operator mouth to suck out the eggs

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