

**EFFECTS OF SALINITY ON THE SURVIVAL,
GROWTH AND DEVELOPMENT OF SENSORY
ORGANS, AND GILL CHLORIDE CELLS OF
MARBLE GOBY, *Oxyeleotris marmoratus*
LARVAE**



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ABSTRAK

PERBEZAAN PARAS SALINITI TERHADAP KEMANDIRAN, TUMBESARAN, PERKEMBANGAN ORGAN DERIA DAN SEL KLORIDA INSANG PADA LARVA IKAN KETUTU, *Oxyleotris marmoratus*

Ikan ketutu, *Oxyleotris marmoratus* merupakan ikan air tawar yang paling penting dalam industri akuakultur kerana mempunyai permintaan dan harga pasaran yang tinggi. Masalah utama dalam pengkulturan *O. marmoratus* adalah kekurangan sumber benih yang disebabkan oleh penangkapan berlebihan. Antara masalah lain ialah kadar kematian yang tinggi pada peringkat awal larva dalam keadaan pengkulturan tiruan. Eksperimen ini dijalankan untuk mendapat paras saliniti optima untuk pengkulturan larva serta mengkaji perkembangan organ deria dan sel klorida insang bagi larva yang dikulturkan dalam 0 dan 10 ppt. Eksperimen 1 dijalankan untuk menentukan paras saliniti optima bagi larva bermula 1-40 hari selepas penetasan (dAH) dengan paras saliniti berlainan (0, 5, 10, 15, 20, and 30 ppt). Keputusan menunjukkan 10 ppt merupakan paras saliniti optima untuk kemandiran dan tumbesaran terhadap larva bermula 1-40 larva. Tambahan pula, penggunaan *Brachionus* sp. untuk larva dapat menangani pemakanan pertama bagi larva. Dengan penemuan tersebut, kadar kematian yang tinggi pada peringkat awal larva dapat ditangani dan teknik pengkulturan dapat ditingkatkan. Eksperimen 2 dijalankan untuk menentukan saliniti optima bagi larva pada empat jangka masa pendek (1-10, 11-20, 21-30, 31-40 dAH) dengan paras saliniti yang serupa dalam Eksperimen 1. Larva untuk jangka masa 1-10dAH merupakan jangka masa yang paling kritikal dan larva mesti dikulturkan dalam 10 ppt. Keputusan juga menunjukkan larva 11-40 dAH boleh dikulturkan dalam tahap saliniti 0-30 ppt bergantung pada sumber air yang dapat diperolehi. Bagi Eksperimen 3, perkembangan organ deria diperhatikan secara histologi untuk larva yang dikulturkan dalam 0 dan 10 ppt. Perkembangan mata, telinga dalam, organ bau, tunas rasa dan neuromas bebas adalah serupa bagi larva yang diperlihara dalam 0 dan 10 ppt. Keputusan ini menunjukkan perkembangan organ deria tidak mempengaruhi kemandiran yang tinggi di 10 ppt. Bagi Eksperimen 4, perkembangan sel klorida insang ditentukan secara imunologikimia untuk larva yang diperlihara dalam 0 dan 10 ppt. Keputusan menunjukkan larva dalam 0 dan 10 ppt mempunyai pertaburan dan saiz sel klorida insang yang berlainan. Ini menunjukkan larva *O. marmoratus* berkemampuan untuk hidup dalam 10 ppt dengan bantuan sel klorida insang yang berfungsi untuk menyelaras tekanan osmotik kerana ia adalah ikan air tawar. Secara kesimpulan, 10 ppt boleh menangani kadar kematian yang tinggi pada peringkat awal larva. Keputusan kajian dapat menyumbang kepada peningkatan teknik pengkulturan larva untuk penghasilan benih *O. marmoratus*.

ABSTRACT

EFFECTS OF SALINITY ON THE SURVIVAL, GROWTH, DEVELOPMENT OF SENSORY ORGANS AND GILL CHLORIDE CELLS OF MARBLE GOBY, *Oxyeleotris marmoratus* LARVAE

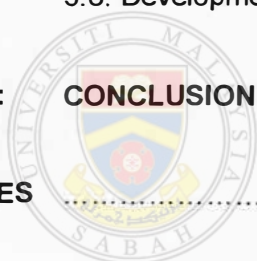
Marble goby, *Oxyeleotris marmoratus* is one of the most important freshwater fish in the aquaculture industry because it has high market demand and value. The main constraint in *O. marmoratus* farming is insufficient seed supply due to overfishing. Another constraint is high mortality during the early larval stage under artificial rearing conditions. The experiments were conducted to obtain the optimum salinity for larval rearing and to study of the development of sensory organs and gill chloride cells of *O. marmoratus* larvae reared in 0 and 10 ppt. Experiment 1 was conducted to determine the optimum salinity level for larval stage from 1-40 days after hatching (dAH) with different salinity (0, 5, 10, 15, 20, and 30 ppt). The results showed that 10 ppt is the optimum salinity for survival rate and growth for *O. marmoratus* larval stage from 1-40 dAH. The results also revealed that the larvae can feed on the seawater rotifer, *Brachionus* sp. and this has solved the problem of first feeding. With these findings, the high mortality during early larval stage can be overcome and larval rearing technique can also be developed. Experiment 2 was conducted to determine the optimum salinity for the larvae reared in four shorter periods (1-10, 11-20, 21-30, and 31-40 dAH) by testing the same salinity used in Experiment 1. The rearing period 1-10 dAH was shown as the most critical period for larval survival. The results revealed that the larvae in the rearing period from 1-10 dAH must be reared in 10 ppt. The results also revealed that 11-40 dAH larvae can be reared in the salinity range of 0-30 ppt depending on available water sources. In Experiment 3, the developments of sensory organs were observed histologically for the larvae reared in 0 and 10 ppt. The development of eyes, inner ears, olfactory organs, taste buds and free neuromasts of *O. marmoratus* larvae were similar in larvae reared in 0 and 10 ppt. The results indicated that the development of sensory organs does not affect the high survival rate in 10 ppt. In Experiment 4, the developments of the gill chloride cells were identified immunocytochemically for the larvae in 0 and 10 ppt. The results showed that the larvae reared in 0 and 10 ppt have different distribution and sizes of the gill chloride cells. This suggested that the larvae have the ability to adapt and survive in 10 ppt with the help of their gill chloride cells that can deal with the osmotic pressure despite it being a freshwater fish. As a conclusion, 10 ppt is the optimum salinity that can overcome the high mortality of *O. marmoratus* during early larval stage. Results from these experiments can contribute to improve the larval rearing techniques for the mass seed production of *O. marmoratus*.

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
ABC	Avidin Biotin Complex
BGAR	biotinylated goat anti-rabbit immunoglobulins
BSA	bovine serum albumin
C	cornea
cm	centimetre
COJ	cod oil juice
DAB	diaminobenzidine-4-HCl
dAH	days after hatching
dAO	days after ovulation
DO	dissolved oxygen
ed.	edited, edition, editor
ESD	equivalent spherical diameter
<i>et al.</i>	and others, and the rest
FAO	Food and Agriculture Organisation
g	gram
GCL	ganglion cell layer
h	hour
hAF	hours after fertilization
hAH	hours after hatching
HCG	human chorionic gonadotrophin
H & E	haematoxylin and eosin
HDPE	high density polyethylene
INL	Inner nuclear layer
IU	international unit
Kg	kilogram
KHz	kilo hertz
L	litre
M	metre
M ³	cubic metre
mg	milligram
min	minute

ml	millilitre
mm	millimetre
Mt	metric ton
NAP 3	Third National Agriculture Policy
NGS	normal goat serum
N.D	not detected
ON	optic nerve
ONL	outer nuclear layer
OPL	outer plexiform layer
PBS	phosphate buffer solution
PE	pigment epithelium
pH	hydrogen ion concentration
ppm	parts per million
ppt	parts per thousand
R	retina
RM	ringgit Malaysia
RNA	ribonucleic acid
s	second
sp.	species
μm	micrometre
UMS	Universiti Malaysia Sabah
UV	ultraviolet
VCL	visual cell layer



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KEY WORDS

Oxyeleotris marmoratus, optimum salinity, survival, growth, sensory organs, gill chloride cells.



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CHAPTER 1

INTRODUCTION

1.1. Aquaculture in Malaysia

The government of Malaysia is encouraging aquaculture under the Third National Agriculture Policy (NAP3 1998–2010) (Ministry of Agriculture Malaysia, 2000). Through aquaculture, the country can gain many advantages. Aquaculture can provide an alternative means to increase fish production to fulfill the country's protein needs instead of relying on capture fisheries (Galid, 2003; Sugiyama *et al.*, 2004). It also provides opportunities for the development of supporting industries such as the production of feed and fish fry (Ang, 1990). Furthermore, it helps to increase foreign revenue through the export market (Chua & Tech, 1990; Ministry of Agriculture Malaysia, 2000; Sugiyama *et al.*, 2004). From an environmental point of view, aquaculture not only reduces pressure on the natural resources due to overfishing, but also protects the unexplored underwater species of Malaysia (Utama & Nuruddin, 2005).

Malaysia has many good prospects to expand aquaculture industry (Ministry of Agriculture Malaysia, 2000; Galid, 2003). The country has vast potential inland areas and coastal areas, therefore both freshwater and marine aquaculture can be developed (Ang, 1990; Senoo, 2001; Department of Fisheries Malaysia, 2003). Malaysia is a tropical country, so aquaculture activities can be conducted throughout the year with little limitation of seasons (Senoo, 2001; Galid, 2003). The stable politics and economic growth have also encouraged and attracted long-term investments in aquaculture (Senoo, 2001; Sugiyama *et al.*, 2004). As a multiracial

country, the aquaculture products can meet the demand without any boundaries of race, religion or ethnic group (Ang, 1990; Senoo, 2001; Galid, 2003).

In 2003, the total production of aquaculture in Malaysia was 196,874 tons valued at RM 1,172 million. From this total production, it has contributed 13.3% of the total nation's fish demand (Department of Fisheries Malaysia, 2003). It is estimated that the total production from aquaculture will increase four times from its present level by 2010 (Department of Fisheries Malaysia, 2003).

In 2003, the marine sector contributed 74% of the aquaculture production in Malaysia and the freshwater sector 26% (Department of Fisheries Malaysia, 2003). The major cultured marine species are seabass (*Lates calcarifer*), groupers (*Epinephelus* sp.), snappers (*Lutjanus* sp.), tiger prawn (*Penaeus monodon*), mud crab (*Scylla serrata*) and shellfish. Meanwhile the major cultured freshwater species are tilapia (*Tilapia* sp.), big head carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*), river catfish (*Pangasius* sp., *Mystus* sp.), Javanese carp (*Puntius gonionotus*) and marble goby (*Oxyeleotris marmoratus*) (Senoo *et al.*, 1994a, 1994b; Senoo *et al.*, 1997; Department of Fisheries Malaysia, 2003).

As the Malaysian economy improves and people's standard of living escalates, it is noticeable that the demand for high economic value species is increasing (Senoo *et al.*, 1997; Senoo, 2001; Utama & Nuruddin, 2005). Among all the major cultured freshwater fishes, *O. marmoratus* is the most favoured and potential freshwater species due to its high market value and good taste (Cheah *et al.*, 1994; Senoo *et al.*, 1994a; Senoo *et al.*, 1997; Luong *et al.*, 2005).

2. Background of *O. marmoratus* Culture

Marble Goby, *O. marmoratus* (Figure 1.1) is the largest freshwater Eleotridae. It grows to 50 cm in total length (TL) and 2 kg in body weight (BW) (Mohsin & Ambak, 1983; Roberts, 1989; Inger & Chin, 2002). In Asian countries, this fish is also known as “Sand Goby” in English, “Ikan Ketutu” or “Ikan Hantu” in Malay and Soon Hock” or “Lam Kor” in Chinese (Senoo *et al.*, 1993a; Cheah *et al.*, 1994; Senoo *et al.*, 1997).

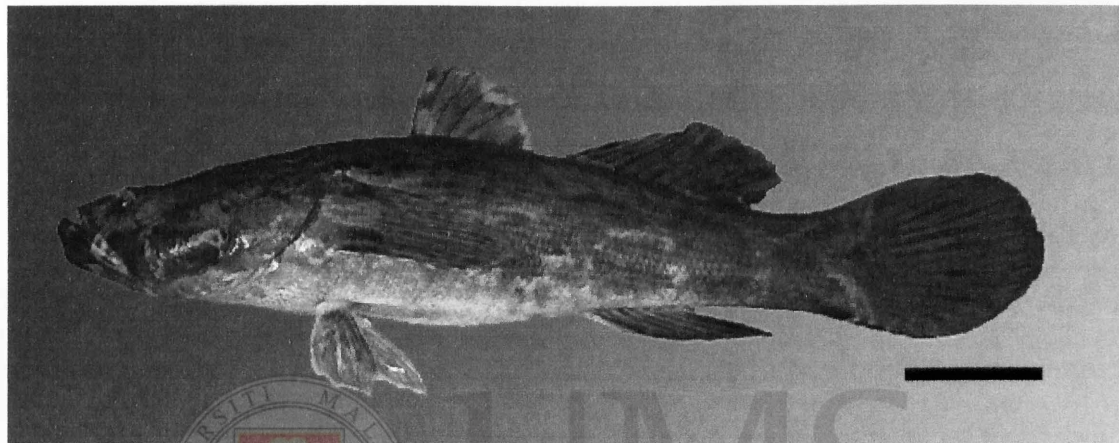


Figure 1.1: An adult female *Oxyleotris marmoratus*, 660 g in body weight and 34.5 cm in total length. Scale bar 5 cm.

O. marmoratus is distributed throughout the Southeast Asian region such as Malaysia, Singapore, Thailand, Indonesia, Vietnam, Cambodia, Philippines and Laos and also Fiji (Mohsin & Ambak, 1983; Inger & Chin, 2002). In Malaysia, *O. marmoratus* can be found in freshwater such as rivers, lakes, small streams, paddy fields, ponds, abandoned mining pools and reservoirs (Smith, 1945; Tan & Lam, 1973; Cheah *et al.*, 1994). Occasionally they can also be found in brackish water such as river mouths, swamps and mangrove areas (Kottelat *et al.*, 1993; Senoo *et al.*, 2004).

O. marmoratus is one of the most expensive freshwater fish in the Southeast Asian region due to its delicious taste (Mohsin & Ambak, 1983; Senoo *et al.*, 1993b;

Cheah *et al.*, 1994; Senoo *et al.*, 1997; Lin & Kaewpaitoon, 2000; Amornsakun *et al.*, 2003; Luong *et al.* 2005). In Malaysia, the retail price of *O. marmoratus* in major markets is RM 41.50 per kg, the highest among the other major cultured freshwater fish (Figure 1.2) (Department of Fisheries Malaysia, 2003). A survey in Kota Kinabalu, Sabah, Malaysia, showed that the price of *O. marmoratus* in seafood restaurants ranges from RM 100-120 per kg.

With the high market value and demand, *O. marmoratus* is considered a commercially important species for inland fisheries (Cheah *et al.*, 1994; Senoo *et al.*, 1994a; Amornsakun *et al.*, 2003; Luong *et al.*, 2005). In Malaysia, over 99% of the *O. marmoratus* culture is in earthen pond while only 0.56% is cultured in cages in reservoirs, lakes and rivers (Department of Fisheries Malaysia, 2003). However, in 2003, the production of *O. marmoratus* through aquaculture was only 683 tons valued at RM 8.9 million, which was the lowest (2.3%) among the major cultured freshwater fish (Figure 1.2) (Department of Fisheries Malaysia, 2003). Besides production through aquaculture, public water bodies such as rivers, ex-mining pools, dams and lakes also produced 48 tons valued at RM 1.8 million in 2003 (Department of Fisheries Malaysia, 2003).

O. marmoratus culture generally relies heavily on seed collected from the wild (Brohmanonda & Thanakumcheep, 1983). However, natural resource of *O. marmoratus* seed has decreased drastically due to overfishing while mass seed production technique has yet to be established due to high mortality at the early larval stage (Tavarutmaneeagul & Lin, 1988; Ang, 1990; Senoo *et al.*, 1994a, 1994b). Therefore, the insufficient and unreliable supplies of *O. marmoratus* seeds has become the main constraint to the farming of *O. marmoratus* culture, so usually the *O. marmoratus* culture is on a very limited scale (Tan & Lam, 1973; Ang, 1990; Senoo *et al.*, 1993a, 1993b; Senoo *et al.*, 1994a, 1994b; Senoo *et al.*, 1997).

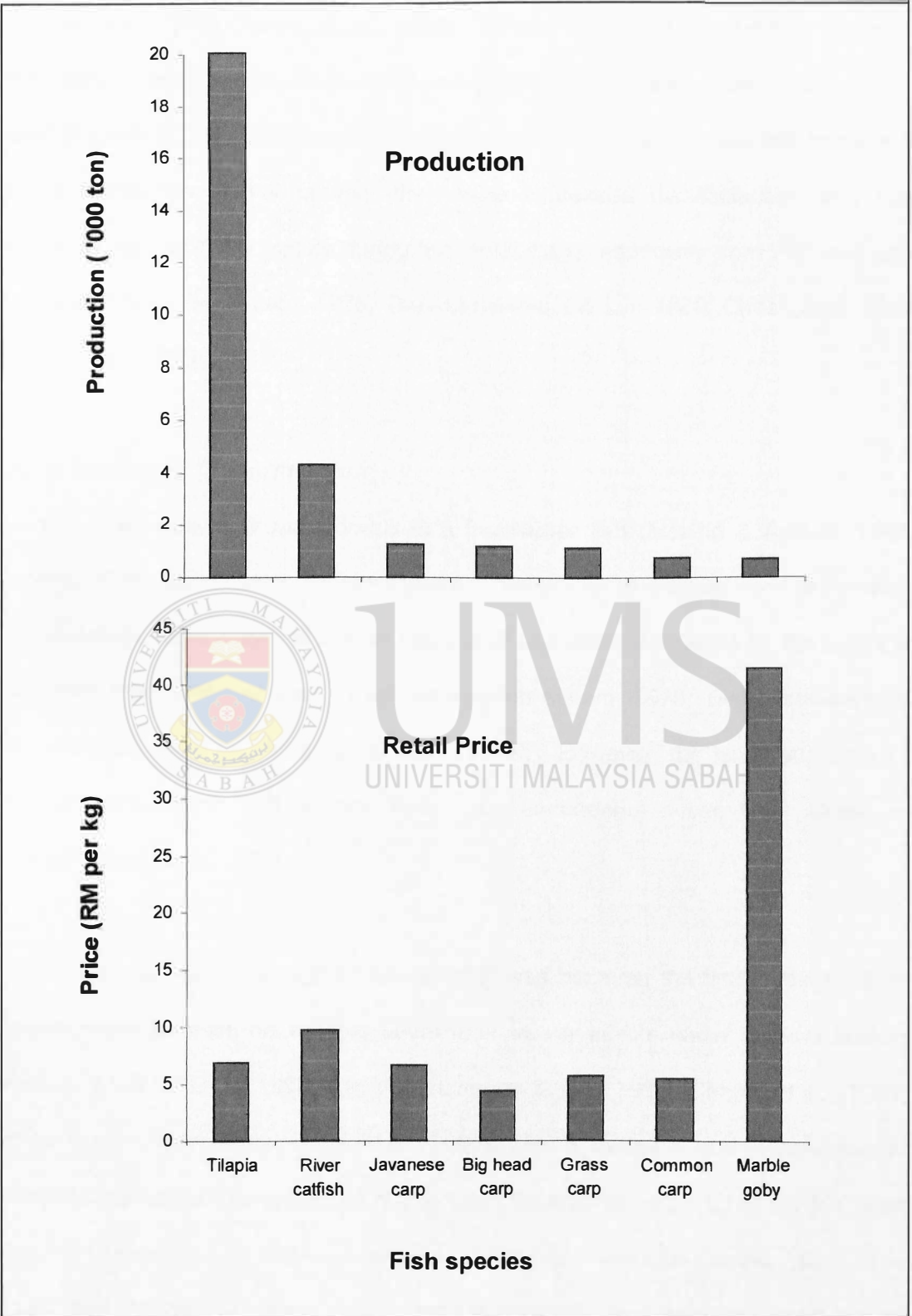


Figure 1.2: Comparison of production and retail price of some major cultured freshwater fish in Malaysia in 2003. (Source: Department of Fisheries, 2003).