FEEDING PROBLEMS IN EARLY LARVAL STAGE OF THE WHITE BLOTCHED SNAPPER, *LUTJANUS RIVULATUS*

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ABSTRACT

FEEDING PROBLEMS IN EARLY LARVAL STAGE OF THE WHITE BLOTCHED SNAPPER, *LUTJANUS RIVULATUS*

Efforts were made to produce the seeds of *Lutjanus rivulatus* in the Marine Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS Hatchery). The larvae showed a high mortality rate in the 10 days after hatching (dAH). This study was conducted to investigate the causes of high mortality and to observe the early larval development of *L. rivulatus*, in order to obtain information to improve the larval rearing techniques of *L. rivulatus*. Fertilized eggs were collected from the broodfish tank and were placed in the incubation tank. The newly hatched larvae were cultured with density 90,000 tails in 1-tonne tank and fed with algae, *Nannochloropsis* sp. (0.5 million cells/mL) and rotifer, *Brachionus* sp. (20 rotifer/mL). From 0 to 72 hours after hatching (hAH), the larvae were sampled every 6 hours and fixed in Bouin solution for histology and Karnosky's solution for scanning electron microscope observations. The larvae from 3 to 10 dAH were sampled everyday by the same solution. The total length (TL), body height, yolk sac length, and oil globule diameter of larvae were measured. *L. rivulatus* larvae (2.19 ± 0.09 mm in mean TL) hatched with unpigmented eyes, no taste bud and unopened olfactory pits. The mouth was not opened and the anus was not formed. A pair of free neuromasts with cupulae was found behind the eyes and the larvae were suspended head down in the water column. At 42 hAH, the larvae (3.09 ± 0.06 mm in TL) were sufficiently morphological develop to be fed and the volume of the yolk sac left was 0.0013 mm^3 (99% of yolk sac volume had been absorbed). The larvae had deeply pigmented eyes, opened mouth (80 μm) with moveable lower jaw, opened anus with movable intestine and demonstrated horizontal movement and S-posture for feeding. It was found that *L. rivulatus* larvae have only an 18-hours nutritional transition period, which is considered shorter than many other species such as *Lates calcarifer*, and because of their minute mouth, they are unable to ingest S-type rotifer (150-230 μm). From these results, it is suggested to start feeding the larvae at 42 hAH with starter feed of less than 80 μm to avoid death of larvae due to starvation.

ABSTRAK

Usaha telah dijalankan untuk menghasilkan benih ikan Lutjanus rivulatus dalam Hatceri Marin, Institut Penyelidikan Marin Borneo, Universiti Malaysia Sabah (Hatceri UMS). Larva-larva L. rivulatus telah menunjukkan mortaliti yang tinggi dalam 10 hari selepas menetas (hST). Kajian telah djalankan untuk memerhati perkembangan awal larva L. rivulatus agar dapat mengkaji sebab-sebab mortaliti yang berlaku dalam pengkulturan larva. Telur L. rivulatus diambil dari tangki ikan induk dan diletakkan di dalam tangki penetasan. Larva L. rivulatus dikultur dengan densiti 90,000 ekor dalam tangki satu ton dan diberi plankton, Nannochloropsis sp. (0.5 million cells/mL) serta rotifer, Brachionus sp. (20 rotifer/mL) sebagai makanan. Larva-larva tersebut disampel pada setiap enam jam dari 0-72 jST dan diawet dalam larutan Bouin untuk histologi dan larutan Karnosky untuk pemerhatian di bawah scanning electron microscope. Larva-larva tersebut juga disampel pada setiap hari dengan larutan yang sama dari 3-10 hST. Pengukuran panjang badan, ketinggian badan, panjang pundi telur dan pundi minyak larva direkod. Larva L. rivulatus menetas (2.19 ± 0.09 mm) dengan mata yang tidak berpigmen, tiada sel rasa dan lubang deria bau larva tidak terbuka. Mulut larva tidak membuka dan anus larva tidak terbentuk.. Sepasang free neuromasts (sejenis unit deria mekanikal) didapati di belakang mata larva dan larva-larva terapung sahaja di dalam ruang air dengan kepalanya menghadap ke bawah. Pada 42 jST, larva-larva tersebut (3.09 ± 0.06 mm) didapati bertumbuh agar dapat memakan, di mana isipadu pundi telur hanya tertinggal 0.0013 mm^3 (99% daripada isipadu pundi telur telah diserap). Pada masa yang sama, larva-larva tersebut mempunyai pigmen mata yang menebal, mulut terbuka (80 μm) dengan rahang atas yang bergerak, anus yang terbuka dengan usus yang bergerak, serta berenang secara melintang dan menunjukkan pergerakkan gaya-S yang sebagai tanda bersedia untuk mengambil makanan. Didapati bahawa larva-larva L. rivulatus mempunyai 18 jam untuk tempoh pengalihan nutrisi, di mana tempoh tersebut adalah lebih pendek adaripada spesies lain seperti Lates calcarifer, dan saiz mulut yang kecil telah menghalang larva-larva daripada memakan rotifer (150-230 μm). Berdasarkan keputusan yang didapati, adalah dicadangkan bahawa larva-larva tersebut mula diberikan makanan pada 42 jST, dengan saiznya kurang daripada 80 μm untuk mengelakkan larva daripada mati kelaparan.

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LIST OF ABBREVIATIONS

%	percentage
&	and
^{o}C	degree Celsius
п	p
%	percentage
A	anus
BH	body height
BW	body weight
cm	centimetre
dAF	day after fertilize
dAH	day after hatching
DO	dissolved oxygen
Ey	eye
ED	eye diameter
Ff	fin fold
Fn	free neuromast
FRP	fibreglass reinforced plastic
GnRHa	Gonadotropin and Releasing Hormone Analog
G	ganglion
g	gram
H	head
h	hour
hAH	hours after hatching
hAF	hours after fertilized
hAS	hours after spawning
Hc	horizontal cells
HCG	human chorionic gonadotropin
HDPE	high density polyethylene
Ie	inner ear
In	inner nuclear layer
Ip	inner plexiform layer
It	intestine
kg	kilogram
L	lens
l	litre
UJL	upper jaw length
m	metre
m^3	cubic metre
M	mouth
mg	milligram
ml	millilitre
mm	millimetre
mm^3	cubic millimetre
Np	nasal pit
NaCN	Sodium Cyanide
NAP3	Third National Agricultural Policy

Og	oil globule
OgD	oil globule diameter
On	outer nuclear layer
pH	potantra of hydogeni (power of hydrogen)
Pe	pigment epithelium
Pf	pectoral fin
ppt	part per thousand
SD	standard deviation
SEM	scanning electron microscope
Tl	tail
TL	total length
Tk	trunk
t	tonne
UMS	Universiti Malaysia Sabah
μm	micrometer
Xy	xylene
YsH	yolk sac height
YsL	yolk sac length
Ys	yolk sac
YV	yolk sac volume

CHAPTER 1

INTRODUCTION

1.1 AQUACULTURE IN MALAYSIA

Aquaculture in Malaya began in the early 1900s (Ang, 1990; Malaysia Fisheries Department, 2007) with Chinese carps such as grass carp, *Ctenopharyngodon idella* and bighead carp, *Aristichthys nobilis* cultured in mining pools (Kamaruddin, 1988; Ang, 1990; Malaysia Fisheries Department, 2007). Shrimp and cockle culture began in the 1950s, followed by marine fishes in net cages (Chua & Tech, 1990) as well as mussel culture in the early 1970s (Ang, 1990; Malaysia Fisheries Department, 2007). Currently, there are over 20 species of fish cultured in Malaysia, such as common carp, *Cyprinus carpio* and red tilapia, *Oreochromis niloticus* in freshwater, while Asian sea bass, *Lates calcarifer*, mangrove red snapper, *Lutjanus argentimaculatus* and groupers, *Epinephelus* spp. are some of the marine cultured species. Crustaceans such as the giant freshwater prawn, *Macrobranchium rosenbergii*, tiger prawn, *Penaeus monodon* and mud crab, *Scylla serrata* as well as shellfish such as cockles, *Anadara granosa*, mussels, *Perna viridis* and oysters, *Crassostrea* sp. are also cultured locally (Appendix A) (Malaysia Fisheries Department, 2004).

In Malaysia, aquaculture has been developed as a branch of the fisheries industry. Fisheries products provide a high level of protein to the diet of the population. However, naturl fisheries resources are unable to meet the protein demand due to over-fishing and population growth (Chuan, 2007). Aquaculture has been encouraged to fulfill the demand. Aquaculture contributes a continuous supply of protein (Bernama, 2003), as well as reduces over-exploitation of natural resources (Pillay, 2001). In term of employment opportunities, aquaculture has given a wide range of opportunities, particularly for people who depend on fisheries activities for their livelihood (Pillay, 2001; Malaysia Fisheries Department, 2004).

Aquaculture has become one of the major contributors to economic growth in Malaysia (Bernama, 2003). Aquaculture contributed 13% to the total fisheries production in 2004 (Malaysia Fisheries Department, 2004). In 1994, the aquaculture production amounted to 114,114 metric tonnes valued at RM0.37 billion (Malaysia Fisheries Department, 2003). This amount shows a steady increase over the next 10 years, the total aquaculture production amounting to 202,225 metric tonnes valued at RM1.26 billion in 2004 (Malaysia Fisheries Department, 2004).

The Malaysian government has intensively encouraged the development of the aquaculture industry. The third National Agricultural Policy (NAP3) has been formulated to encourage investment in food production including aquaculture (Ministry of Agriculture, 1999; Jabatan Penerangan Malaysia, 2007). The NAP3 attempts to improve aquaculture production by introducing new fish species, upgrading aquaculture management, strengthening research and providing training. Suitable locations such as lakes, ponds, ex-mining pools as well as rivers will be fully utilized for aquaculture production in the future (Ministry of Agriculture, 1999).

Malaysia is geographically suitable for aquaculture development (Senoo, 2001a). Since Malaysia is located near the equator; the tropical climate and high rainfall throughout the year greatly affects the richness and productivity of the fishes (Mohsin & Ambak, 1996; Senoo, 2001a). Malaysia surrounded by the South China Sea, Sulu Sea, Java Sea and Andaman Sea has a 4,800 km coastline (Mohsin & Ambak, 1996; Senoo, 2001a). Its marine resources are protected from typhoons and offer wide opportunities for the aquaculture industry (Senoo, 2001a; 2001b).

The political stability and economic growth in Malaysia also encourage aquaculture development. Conflicts happening in the Southeast Asian regions did not occur in Malaysia. The harmonious conditions make it attractive for long-term investments in the aquaculture industry (Senoo, 1997a; 1997b; 2001a).

As a country with diverse racial, cultural and religious background, certain religions may have some prohibited food items, for instance Muslims do not eat pork and

Hindus do not eat beef (Senoo, 1997b; 2001b). Fish products become the most demanded protein across different races, religions and ethnic groups (Senoo, 1997a; 2001a). These factors have led the aquaculture industry to become an important activity in Malaysia.

1.2 *LUTJANUS* SP. CULTURE IN MALAYSIA

Lutjanus spp. such as mangrove red snapper, *L. argentimaculatus,* golden snapper, *L. johnii,* common bluestrip snapper, *L. kasmila* and white spotted snapper, *L. stellatus* are widely accepted as excellent food fish in Asian countries (Sena, 2001) such as Taiwan, Thailand, Philippines and Malaysia (Liao, 1990; Tucker, 2000). *Lutjanus* spp. culture in net cages was introduced in Malaysia in 1973 (Rahim, 1982; Ali & Ali, 1996). *Lutjanus argentimaculatus* and *L. johnii* are the most popular *Lutjanus* sp. cultured in Malaysia (Malaysia Fisheries Department, 2003). These *Lutjanus* sp. possess all the desirable biological attributes for culture, including rapid growth, hardiness, short reproductive cycle and good flavour (Tucker, 2000).

Lutjanus argentimaculatus are preferred species by all races in Malaysia. It was introduced to Peninsular Malaysia more than 20 years ago (Senoo, 2001b). Artificial seed production was successfully done by Sabah Fisheries Department in 1987 (Senoo, 2001b). *Lutjanus argentimaculatus* is sold as fresh and salted dried products in the markets (Mohsin & Ambak, 1996). This fish has a fast growth rate and the price for live fish is about RM18-28/kg (Senoo, 2001b). *Lutjanus johnii* has an excellent flesh and is sold fresh in the markets in Malaysia at a high price. Usually, people prefer it to be barbequed or cooked as soup or curry (Mohsin & Ambak, 1996).

1.3 WHITE BLOCTHED SNAPPER, *LUTJANUS RIVULATUS*

Lutjanus rivulatus (Photo 1.1) is another *Lutjanus* sp. that is newly targeted as an aquaculture fish (Senoo, 2001b; Senoo *et al.,* 2002). It has similar characteristics to *L. argentimaculatus* and *L. johnii* with great aquaculture potential.

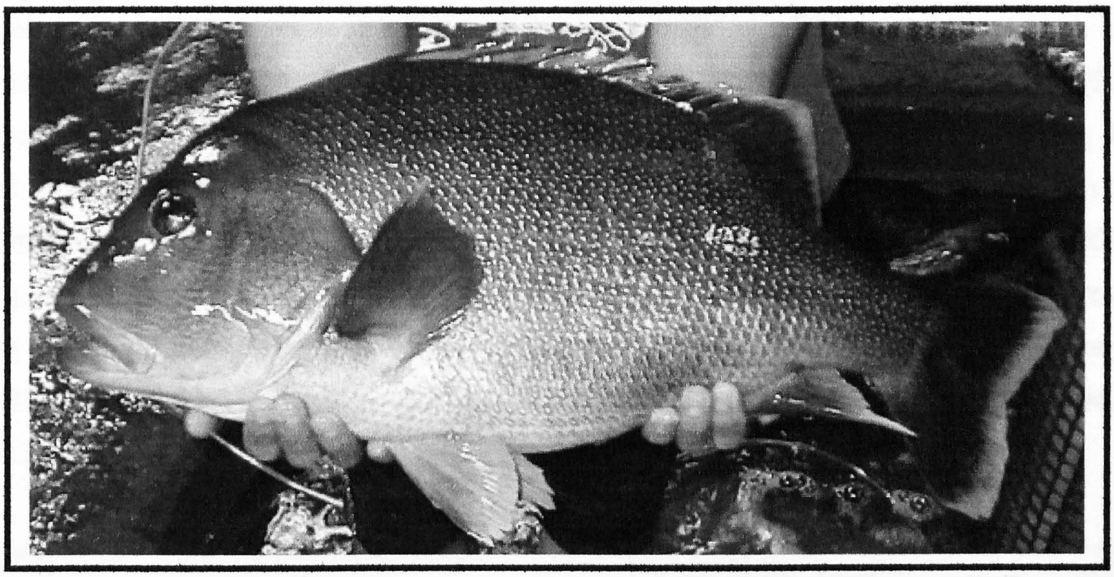

Photo 1.1: White blotched snapper, *Lutjanus rivulatus*, a mature female in Marine Hatchery Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS Hatchery), 7 kg in body weight, 65 cm in total length.

Lutjanus rivulatus (Cuvier & Valenciennes, 1828) belongs to the family Lutjanidae that has a wide distribution in Indo-Pacific, from Africa in the west to Tahiti in the east (Allen, 1985; Anderson, 1986; Senoo *et al.*, 2002) including Southeast Asia (Rau & Rau, 1980; Allen, 1985; Nguyen *et al.*, 1995; Monkolprasit *et al.*, 1997; Senoo *et al.*, 2002; Allen & Adrim, 2003); southern Japan (Masuda *et al.*, 1984; Senoo *et al.*, 2002) and Australia (Myers, 1999; Senoo *et al.*, 2002). The adult fish are normally found at a water depth of at least 100 m in offshore reefs (Allen, 2000). The juveniles can be found in coastal estuaries and freshwater streams (Allen, 2000; Allen, & Adrim, 2003).

Lutjanus rivulatus has several names. In Malaysia, it is commonly known as "Blubberlip Snapper" or "White Blotched Snapper" in English, "Hoi Tai Kai" in Chinese, which means "Sea Chicken", and "Ayam Laut" in Malay (Chin, 1998; Sadovy &Cornish, 2000; Senoo *et al.*, 2002). In Africa, it is called "Speckled Snapper" and in Tahiti "Haputu" (Allen, 1985; Anderson, 1986). The name in Japan is "Nami-fuedai" (Masuda *et al.*, 1984; Allen, 1985; Senoo *et al.*, 2002) and "Maori Seaperch" in Australia (Myers, 1999).

Lutjanus rivulatus is one of the expensive *Lutjanus* sp. because of its delicious taste (Chin, 1998). *Lutjanus rivulatus* is found in markets of Asian countries such as Thailand, Philippines and Indonesia (McManus *et al.*, 1992; Monkolprasit *et al.*, 1997; Allen & Adrim, 2003). In a survey in Kota Kinabalu, Kudat, Sandakan and Tawau, in Sabah, Malaysia, it was found that the price ranged from RM120-180/kg for live fish in seafood restaurants (Photo 1.2A) (Chin, 1998; own survey in May 2007; http://www.epu.jpm.my). In Sabah, Malaysia, *L. rivulatus* is an important commercial fish exported to countries such as Hong Kong and Singapore (Chin, 1998; Lee & Sadovy, 1998; Sabah Fisheries Department, 2003). The price is twice that of *L. argentimaculatus* (Photo 1.2B).

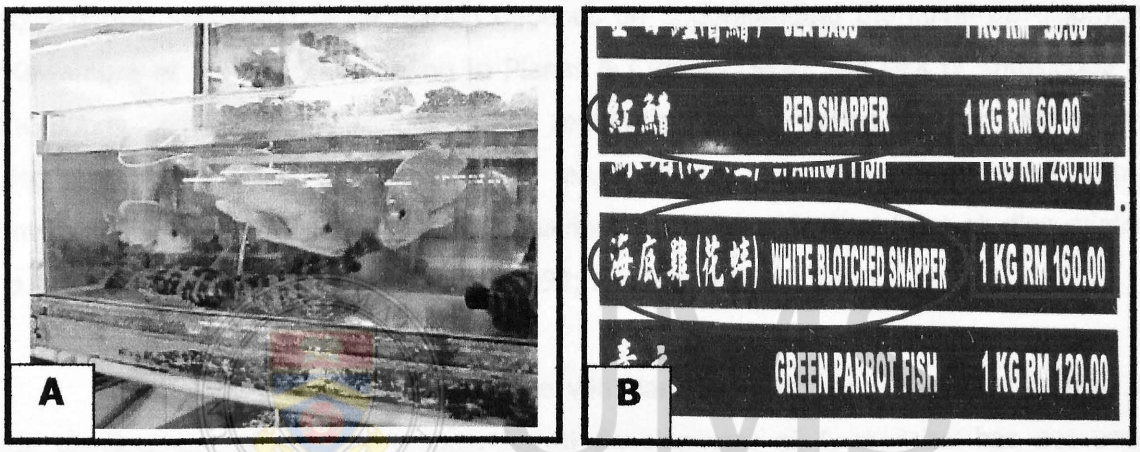

Photo 1.2: *Lutjanus rivulatus* in seafood restaurant has a commercial price. **A**, Fresh *L. rivulatus* display before cooked; **B**, the price cost RM160/kg for live fish, which is twice as expensive as *L. argentimaculatus* (own survey in May 2007).

With the high price and demand, *L. rivulatus* isw being fished using destructive methods such as poisoning (sodium cyanide, NaCN) and bombing (Chin, 1998; Senoo *et al.,* 2002). Consequently, over-fishing has caused an insufficient supply of fish from the wild (Senoo, *et al.*, 2002; 2003).

Many fish farmers are keen to develop *L. rivulatus* culture. However, there is no artificial production of *L. rivulatus* seeds (Senoo *et al.*, 2002; Sabah Fisheries Department, 2003). The seed production of *L. rivulatus* has not been successfully established yet and the biological information of *L. rivulatus* is limited (Senoo *et al.*, 2002).

1.4 PROBLEMS OF *L. RIVULATUS* CULTURE

Marine Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS hatchery) has been trying to produce *L. rivulatus* seeds. Broodfish were able to spawn naturally in the tank and the eggs were collected regularly. However, the hatched larvae could not survive beyond 10 days after hatching (dAH). Therefore, careful management is required in larval rearing of *L. rivulatus*.

In larval rearing, there are many factors related to larval mortality, such as deficiency nutrition and low water quality (Planas & Cunha, 1999). Fish larvae are being studied to describe their morphology, natural habitats and culture methods for aquaculture. Successful larval rearing depends on understanding the larval development (Kawamura *et al.*, 2003). According to Planas & Cunha (1999), the lack of knowledge of the optimal environmental conditions and feeding behaviour during the early life stages also are the causes of mortality in larval rearing. Sufficient knowledge about environmental conditions and feeding behaviour could be estimated from studies related to larval development (Planas & Cunha, 1999; Kawamura *et al.*, 2003).

Morphology and behaviour change with larval growth, and is closely related to the development of sensory organs. Larval development of *L. rivulatus* has not been studied yet. Therefore, study of larval development in *L. rivulatus* is very important to understand their early life and improve the larval rearing techniques.

1.5 OBJECTIVES

Since there is high mortality occurring during larval rearing of *L. rivulatus*, study of larval development had been carry out to understand the reasons for high mortality in the early larval stage of *L. rivulatus*. This study intends to describe the correlations among the development of morphology, sensory organs and behaviour in the early larval stage of *L. rivulatus*.

The following experiments were conducted to achieve the objective:

Experiment 1: Observation on Morphological Development with Behaviour Changes

I. To describe morphological development of the larvae