

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

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH



NGUANG SIEW ING

**THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE
OF MASTER OF SCIENCE**

Sila dapatkan
Video / CD / Kaset / Slaid / .. 330083
di bahagian Media

**BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2007**

UNIVERSITI MALAYSIA SABAH

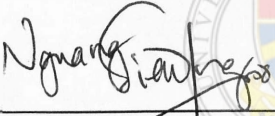
BORANG PENGESAHAN STATUS TESIS®

JUDUL : FEEDING PROBLEMS IN EARLY LARVAL STAGE OF WHITE
BLOTCHED SNAPPER, LUTJANUS RIVULATUS
IJAZAH : SARJANA SAINS (AKUAKULTUR)

SESI PENGAJIAN: 2003-2007

Saya, Nguang Siew Ing mengaku membenarkan tesis sarjana ini disimpan di perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

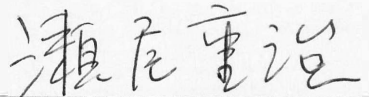
1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian saya.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. TIDAK TERHAD.


Penulis: NGUANG SIEW ING

Alamat:

Tarikh: 2008

Disahkan oleh
PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH
JAMIUN MICHEAL
PUSTAKAWAN
PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH
TANDATAN PUSTAKAWAN

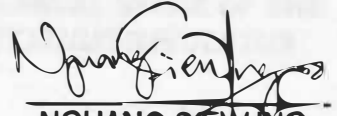

Penyelia: Prof. Dr. Shigeharu Senoo
Tarikh: 10/7/2008

CATATAN: ®Tesis dimaksudkan sebagai tesis Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau laporan Projek Sarjana Muda (LPSM).

DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

17 SEPTEMBER 2007


NGUANG STEW ING
PS 03-004-008

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH



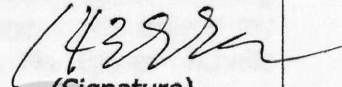
UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME : **NGUANG SIEW ING**
MATRIC NO. : **PS03-004-008**
TITLE : **FEEDING PROBLEMS IN EARLY LARVAL STAGE OF THE
WHITE BLOTCHED SNAPPER, *LUTJANUS RIVULATUS***
DEGREE : **MASTER OF SCIENCE**
VIVA DATE : **17 SEPTEMBER 2007**

DECLARED BY

1. **SUPERVISOR**
(Prof. Dr. Shigeharu Senoo)


(Signature)



UMS
UNIVERSITI MALAYSIA SABAH

ACKNOWLEDGEMENTS

I would like to express my sincere grateful acknowledgement to my supervisors, Professor Dr. Shigeharu Senoo, Borneo Marine Research Institute, for his invaluable suggestions, guidance and encouragement throughout the study period. A special acknowledgement to Professor Dr. Ridzwan Abdul Rahman, the Director of Centre for Research Management and Conference (the former Director of Borneo Marine Research Institute) for his support and encouragement to complete this research project successfully.

I thank Universiti Malaysia Sabah for the assistantship. I would like to express my gratitude to Professor Datuk Dr. Mohd. Noh Dalimin, Vice-chancellor, Universiti Malaysia Sabah and Professor Datin Dr. Maryati Mohamed, Acting Dean of Centre for Postgraduate Studies for their encouragement and assistance.

I wish to express heartily my indebtedness to Professor Dr. Saleem Mustafa, Director of Borneo Marine Research Institute, Dr. Sitti Raehanah Muhd. Shaleh, Mr. Motinius Guntalib, Mr. Norazmi Osman, Mr. Henry Lipan and all the staffs of Fish Hatchery, Borneo Marine Research Institute for their assistance and their co-operation. My sincere appreciation is also extended Dr. Yukinori Mukai for methodological teaching of histology experiment and usage of Scanning Electron Microscope. I also extend my thanks Ms. Sow Sok Hui, Ms. Mok Wen Jye, Ms. Ching Fui Fui, Ms. Esther Michelle Gunben, my friends, colleagues and well-wishers for their valuable inputs (directly or indirectly) for the successful completion of the thesis.

Last but not least, special note of thanks to my parents and sibling for their patience, encouragement, support and understanding in my absence.

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 dijalankan 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 ($80 \mu\text{m}$) dengan rahang atas yang bergerak, anus yang terbuka dengan usus yang bergerak, serta berenang secara melintang dan menunjukkan pergerakan 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 daripada spesies lain seperti Lates calcarifer, dan saiz mulut yang kecil telah menghalang larva-larva daripada memakan rotifer ($150\text{-}230 \mu\text{m}$). Berdasarkan keputusan yang didapati, adalah dicadangkan bahawa larva-larva tersebut mula diberikan makanan pada 42 jST, dengan saiznya kurang daripada $80 \mu\text{m}$ untuk mengelakkan larva daripada mati kelaparan.

CONTENTS

	PAGE
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	v
ABSTRAK	vi
CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF PHOTOS	xii
LIST OF ABBREVIATIONS	xvi
CHAPTER 1: INTRODUCTION	1
1.1 Aquaculture in Malaysia	1
1.2 <i>Lutjanus sp.</i> Culture in Malaysia	3
1.3 White Blotched Snapper, <i>Lutjanus rivulatus</i>	3
1.4 Problems of <i>L. rivulatus</i> Culture	6
1.5 Objectives	6
CHAPTER 2: LITERATURE REVIEW	8
2.1 Studies of Lutjanid	8
2.2 Background of <i>L. rivulatus</i>	15
2.3 First Feeding of Fish Larvae	16
2.4 Larval Rearing of <i>Lutjanus sp.</i>	18
2.5 Developments of Sensory Organs in Larval Period	20
2.5.1 Development of Eyes	21
2.5.2 Development of Free Neuromasts	22
2.5.3 Development of Inner Ears	25
2.5.4 Development of Olfactory Organs	26
2.5.5 Development of Taste Buds	26
2.6 Larval Fish Behaviour	27
2.6.1 Swimming Behaviour	27
2.6.2 Feeding Behaviour	29
2.6.3 Phototactic Behaviour	30
CHAPTER 3: MATERIALS AND METHODS	31

3.1	Preparation of the Rearing System for the Observation on Larval Development of <i>L. rivulatus</i>	31
3.1.1	Broodfish Management and Egg Collection	31
3.1.2	Larval Rearing	32
3.2	Observation for Morphological Development and Behaviour Changes	34
3.2.1	Larval Sampling and Measurement	34
3.2.2	Morphology and Behaviour Observation	36
3.3	Observation for Sensory Organs Development	37
3.3.1	Histological Observation	37
3.3.2	Scanning Electron Microscope (SEM) Observation	40
CHAPTER 4:	RESULTS	43
4.1	Morphological Development and Behaviour Changes	43
4.2	Development of Sensory Organs	56
4.2.1	Eye	56
4.2.2	Free Neuromast	59
4.2.3	Inner Ear	65
4.2.4	Olfactory Pits and Taste Buds	66
4.3	Correlation among the Developments of Morphology, Sensory Organs and Behaviour	69
CHAPTER 5:	DISCUSSION	71
5.1	Nutritional Transition Period of <i>L. rivulatus</i> Larvae	71
5.2	Mouth Size of <i>L. rivulatus</i> Larvae	74
5.3	Larval Size of <i>L. rivulatus</i> Larvae	75
5.4	Sensory Organs of <i>L. rivulatus</i> Larvae	76
5.4.1	Development of Eyes and Changes in Behaviour	76
5.4.2	Development of Free Neuromasts and Changes in Behaviour	77
5.4.3	Development of Inner Ear and Changes in Behaviour	78
5.4.4	Development of Olfactory Organ and Taste Buds and Changes in Behaviour	78
CHAPTER 6:	CONCLUSION	80
REFERENCES		82
APPENDIX		96
A:	Aquaculture species in Malaysia	96
B:	Histology Experiment (Dehydration and Clearing)	97
C:	Histology Experiment (Staining Process)	98
D:	Dehydration for SEM observation	99
E:	Larval Measurement of <i>L. rivulatus</i> (0-72 hAH)	100
F:	Larval Measurement of <i>L. rivulatus</i> (0-10 dAH)	101
G:	Survival Rate (0-10 dAH)	102
H:	Water Quality Record	103
I:	Growth of <i>L. rivulatus</i> larvae (0-72 hAH)	104
J:	Absorption of Oil Globule	105
K:	Publication	106

LIST OF TABLES

	PAGE
Table 2.1 Available Lutjanid in Malaysian water and their commercial importance (Allen, 1987; Mohsin & Ambak, 1996).	9
Table 2.2 The time of naturally spawning by representative Lutjanids (Doi & Singharaiwan, 1993; Tucker, 2000).	10
Table 2.3 Naturally spawned in captivity of representative Lutjanids (Doi & Singharaiwan, 1993; Tucker, 2000).	12
Table 2.4 Representative Lutjanids induce to ovulate by hormone treatment in the form of intramuscular injections and the fish were strip-spawned (Doi & Singhagraiwan, 1993; Watanabe <i>et al.</i> , 1998; Tucker, 2000).	12
Table 2.5 Reported age and size at maturity and spawning season of representative Lutjanids (Doi & Singharaiwan, 1993; Mohsin & Ambak, 1996; Tucker 2000).	13
Table 2.6 Egg production by representative Lutjanids (Mohsin & Amak, 1996; Tucker, 2000; Watanabe, 2001).	14
Table 2.7 <i>Lutjanus rivulatus</i> have several common names in different countries (Mohsin & Ambak, 1996).	15
Table 2.8 Sensory system provides information from the environment to larvae.	21
Table 4.1 Correlation among the development of the morphology, sensory organs and behaviour in the early larval stage of <i>L. rivulatus</i> .	69

LIST OF FIGURES

	PAGE
Figure 2.1 Morphological and sensory organ developments are closely related to behaviour changes in larval development (Senoo <i>et al.</i> , 2004).	18
Figure 2.2 Structure of free neuromasts of fish. A, A cupula that is jelly like rod and protruding from the body surface (Moyle & Cech, 1988); B, free neuromast in a 5.0 mm TL larva, scale bar, 2 μ m (Boglione <i>et al.</i> , 2003).	23
Figure 3.1 The sequence of the histological experiment was fixation, dehydration, clearing, embedding, cutting section and staining section, mounting and glass slides observation.	39
Figure 3.2 The sequence of the specimens prepared for SEM observation was fixation, dehydration, freeze-dried, coated with platinum and observation under SEM.	42
Figure 4.1 <i>Lutjanus rivulatus</i> larvae grow and remained same total length at 5-10 dAH. Vertical axis shows the total length of larvae (mm) and horizontal axis shows the age of larvae in days after hatching (dAH).	45
Figure 4.2 Yolk sac absorption of <i>L. rivulatus</i> larvae was fast and degenerated at 60 hAH. Vertical axis shows the yolk sac in volume (mm^3) and horizontal axis shows the age of larvae in hours after hatching (hAH).	45
Figure 4.3 Schematic diagram shows the swimming behaviour of newly hatched <i>L. rivulatus</i> larvae. Larvae suspended motionless in the water column but able to avoid water current from pipette.	47
Figure 4.4 Schematic diagram shows the swimming behaviour of 12 hAH <i>L. rivulatus</i> larvae. Larvae moved upward and suspended with head down in the water column.	48
Figure 4.5 Schematic diagram shows the swimming behaviour of 24 hAH <i>L. rivulatus</i> larvae. Larvae swam rapidly to water surface and suspended with head down in the water column.	49
Figure 4.6 Schematic diagram shows the swimming behavior of 36 hAH <i>L. rivulatus</i> larvae. Larvae stayed at bottom, swim from bottom and scattered at water with head down in the water column.	51
Figure 4.7 Schematic diagrams show the swimming behaviour of 42 hAH <i>L. rivulatus</i> larvae. Larvae showed the horizontal movement in the water column.	52

- Figure 4.8 Schematic diagram shows the nutritional transition period of *L. rivulatus* larvae. The larvae have developed for feeding at 42 hAH and the yolk sac was completely absorbed at 60 hAH, which means *L. rivulatus* larvae considered to have 18 hours of nutritional transition period. 54
- Figure 4.9 Changes of the survival in the early larval stage of *L. rivulatus*. High mortality occurred at 1-4 dAH. Vertical axis shows the amount of larvae in thousand ($\times 10^3$) and horizontal axis shows the age of larvae in days after hatching (dAH). 55
- Figure 4.10 Schematic diagrams show the distribution of free neuromasts (black spots) on early larval stage of *L. rivulatus*; A, newly hatched larva; B, 24 hAH larva; C, 36 hAH larva; D, 72 hAH larva. Scale bar, 500 μ m. 58



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF PHOTOS

	PAGE
Photo 1.1 White blotched snapper, <i>Lutjanus rivulatus</i> , 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.	4
Photo 1.2 <i>Lutjanus rivulatus</i> in seafood restaurant has a commercial price. A, Fresh <i>L. rivulatus</i> display before cooked; B, the price cost RM160/kg for live fish, which is twice as expensive as <i>L. argentimaculatus</i> (own survey in May 2007).	5
Photo 3.1 Broodfish culture tank and egg collection system. A, The broodfish were cultured in an indoor HDPE (High Density Poly-Ethylene) cylinder tank (3-m depth, 8-m diameter) for 12 months; B, the floating eggs flowed from broodfish tank into the egg collection net by water circulation (arrows show the water flow).	32
Photo 3.2 Egg collection and incubation. A, The eggs were harvested; B, the eggs were incubated in 1-tonne FRP (Fiberglass Reinforced Plastic) tank with 900 L seawater; C, egg development was observed under an optical-microscope (Nikon, model Eclipse E600, Japan); D, the larvae started to hatch 18 hours after spawning (hAS), scale bar, 100 µm.	33
Photo 3.3 <i>Lutjanus rivulatus</i> larval rearing. A, A total of 90,000 larvae were reared in 1-tonne FRP tank with microalgae, <i>Nannochloropsis</i> sp.; B, rotifer, <i>Brachionus</i> sp., was used as feed for the larvae.	34
Photo 3.4 Larval sampling and measurement. A, The larvae sampled into a 10-ml specimen bottle; B, the larvae were measured using profile projector (Mitutoyo, model PJ-3000, Japan).	35
Photo 3.5 The larval behaviour observations. A, The larvae aggregation was observed in 1-tonne FRP tank; B, the larval swimming behaviour was specifically observed in the 8-L aquarium.	36
Photo 3.6 Specimens embedded in paraffin. A, The specimens in alloy cast were transferred into molten paraffin wax in a paraffin oven device (Shandon, model Histocentre 2, UK); B, the specimens were embedded in paraffin wax in a paper holder on a paraffin-embedding device (Shandon, model Citadel 1000, UK).	37
Photo 3.7 Specimen cutting and staining section. A, The specimens in hard paraffin wax was pasted on wood block; B, the specimen in hard paraffin wax was cut up with a rotary microtome (Shandon, model AS325, UK); C, the specimen were stained with haematoxylin and	

eosin; D, the specimen slides was observed under an optical-microscope.

38

Photo 3.8 Specimen freeze-drying section. A, The specimens were freeze-dried on the freeze-drying device (JEOL, model JFD-310 Japan); B, the specimens bottle were covered with aluminum foil and placed on the specimen stage.

40

Photo 3.9 Specimens coated with platinum. A, The specimen stubs before and after being coated with platinum; B, the specimen stub was coated with platinum on Auto Fine Coater (JEOL, model JFC-1600 Japan).

41

Photo 3.10 Specimens observation. A, Specimen holder settled on the specimen stage in the chamber of SEM; B, specimens observed by using SEM (model JSM-5610, Japan) through computerized data.

41

Photo 4.1 Photomicrographs show the early larval stage of *L. rivulatus* from 0-18 hAH. A, Newly hatched larvae have a big yolk sac, unpigmented eyes, no mouth and no anus; B, the body of 6 hAH larva elongated; C, fin fold of 12 hAH larva developed in a shape; D, the yolk sac of 18hAH larva absorbed 95.8%, scale bar, 200 μm (Ff = Fin fold, Ie = Inner ear, L = Lens, Mp = Melanophores, Og = Oil globule, Ys = Yolk sac).

44

Photo 4.2 Photomicrographs show the free neuromasts and inner ear of a 6 hAH *L. rivulatus* larvae observed under microscope. A, A pair of free neuromasts with well-developed cupulae laid behind the lens and inner ear appeared as an otic vesicle with two otoliths, scale bar, 100 μm ; D, a cupula that is a jelly like rod and protruded from the body surface, scale bar, 100 μm (Cu = Cupula, Fn = Free neuromast, Ie = Inner ear, L = Lens, Og = Oil globule, O = Otolith).

46

Photo 4.3 Photomicrographs show the typical pattern on early larval stage of *L. rivulatus* from 24-72 hAH. A, The eyes of 24 hAH larvae slightly pigmented and yolk sac volume left 2%; B, the eyes of 36 hAH larvae deeply pigmented; C, the mouth and anus opened and 99% of the yolk sac volume absorbed; D, the yolk sac completely absorbed and degenerated at 72 hAH larvae, scale bar, 200 μm (A = Anus, Ey = Eye, Ie = Inner ear, It = Intestine, M = Mouth, Mp = Melanophores, Og = Oil globule, Ys = Yolk sac).

50

Photo 4.4 Photomicrographs show *L. rivulatus* larvae at 48 hAH. A, Appearance of larva after pressed by two slide glasses, scale bar, 500 μm ; B, *Nannochloropsis* sp. and cod oil juice were observed; C, no rotifer were observed in the larval stomach. Scale bar, 100 μm

	(Ey= Eyes, In = Intestine, M= Mouth, Tk= Trunk, Ys = Yolk sac).	53
Photo 4.5	Photomicrograph shows the 54 hAH larva was remained a tiny yolk sac volume in the body ($0.00070 \pm 0.0001 \text{ mm}^3$), scale bar, 500 μm (Ey= Eyes, In= Intestine, M= Mouth).	54
Photo 4.6	Photomicrographs show the horizontal section of the newly hatched <i>L. rivulatus</i> larvae. A, Body section of the newly hatched larvae; B, eye section of the newly hatched larvae; C, eye section of 18 hAH larvae. Scale bar, 50 μm (H= Head, L = Lens, R= Retinal, Tk = Trunk, YS = Yolk sac).	56
Photo 4.7	Photomicrographs show the horizontal section of eyes development of <i>L. rivulatus</i> larvae. Body section of 24 hAH larvae. Scale bar, 50 μm (Fn= free neuromast, Ie= Inner ear, L = Lens, R = Retinal, Pf= Pectoral fin, Tk= Trunk).	57
Photo 4.8	Photomicrographs show the horizontal section of eyes development of <i>L. rivulatus</i> larvae. A, eye section of 24 hAH larvae; B, eye section of 36 hAH larvae. Scale bar, 50 μm (G = ganglion, Hc = horizontal cells, In = Inner nuclear layer, Ip = inner plexiform layer, L = Lens, On = outer nuclear layer, Pe = Pigment epithelium, Vc = visual cell).	58
Photo 4.9	Photomicrographs show the horizontal section of eyes development of <i>L. rivulatus</i> larvae. A, Eyes section of 48 hAH larva; B, eyes section shows the nerve fiber at of 72 hAH larvae. Scale bar, 50 μm . (G = ganglion, Hc = horizontal cells, In = Inner nuclear layer, Ip = inner plexiform layer, Nf= Nerve fiber, On = outer nuclear layer, Pe = Pigment epithelium, Vc = visual cell).	58
Photo 4.10	Photomicrograph shows the horizontal section of the newly hatched <i>L. rivulatus</i> larvae, scale bar, 50 μm (Fn= Free neuromast, H= Head, L = Lens, R= Retinal, Tk = Trunk, YS = Yolk sac).	60
Photo 4.11	Scanning electron micrographs show the newly hatched <i>L. rivulatus</i> larvae. A, A pair of free neuromasts found on the head; B, appearance of the free neuromast. Scale bar, 100 μm (Fn= Free neuromast, H= Head, Tk= Trunk, Ys= Yolk sac).	61
Photo 4.12	Scanning electron micrographs show the ventral view of <i>L. rivulatus</i> larva. A, A pair of free neuromasts found above the eyes, rudiments of pectoral fin appeared and an incomplete mouth was observed at 24 hAH larvae; B, the free neuromasts and pectoral fins were developed and the mouth opened at 36 hAH larva. Scale bar, 50 μm (Ey= Eyes, Fn= Free neuromast, H= Head, M= Mouth, Pf= Pectoral fin, Tk= Trunk, Ys= Yolk sac).	62

- Photo 4.13 Scanning electron micrographs show the appearance of a 72 hAH *L. rivulatus* larvae, scale bar, 100 μm . A, A pair of free neuromasts was found on the mouth of larvae, scale bar, 5 μm ; B, appearance of free neuromasts on the head, scale bar, 10 μm (Ey= Eye, Fn= Free neuromast, H= Head, M= Mouth, Op= Olfactory pits, Pf= Pectoral fin, Tk= Trunk). 63
- Photo 4.14 Scanning electron micrographs show the appearance of free neuromasts of 5 dAH *L. rivulatus* larvae. A, The position of free neuromasts on the head and the trunk, scale bar, 200 μm ; B, free neuromast (Fn I) on the head, behind the eyes; C, first pair of free neuromast (Fn II) on the trunk; D, second pair of free neuromast (Fn III) above the end of intestine; E, third pair of free neuromast (Fn IV) at the middle of trunk. Scale bar, 2 μm (Fn= Free neuromast, Tk= Trunk). 64
- Photo 4.15 Photomicrographs show the inner ear of *L. rivulatus* larvae. A, Inner ear was an otic vesicle in the newly hatched larvae; B, inner ear developed at 36 hAH larvae. Scale bar, 100 μm (Ey= Eye, Fn= Free neuromast, Ie= inner ear, Op= Olfactory pit, Tk= Trunk, Ys= Yolk sac). 65
- Photo 4.16 Scanning electron micrographs show the newly hatched *L. rivulatus* larva. A, The circle shows the anterior of the head of larvae, scale bar, 200 μm ; B, no olfactory pits found on the newly hatched larvae, scale bar, 20 μm (Ff= Finfold, H= Head, Tk= Trunk, Tl= Tail, Ys= Yolk sac). 66
- Photo 4.17 Scanning electron micrographs show a 12 hAH *L. rivulatus* larvae. A, Olfactory pits were found above the mouth, scale bar, 50 μm ; B, olfactory epithelium was ciliated, scale bar, 5 μm (Ey= Eye, Fn= Free neuromast, Op= Olfactory pit, Tk= Trunk, Ys= Yolk sac). 67
- Photo 4.18 Photomicrograph shows the olfactory epithelium of 48 hAH *L. rivulatus* larvae were expanded and no taste buds were found, scale bar, 10 μm (Ey = Eye, H= Head, Op = Olfactory pit). 68

LIST OF ABBREVIATIONS

%	percentage
&	and
°C	degree Celsius
n	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	fibre-glass 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 hydrojeni (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



UMS
UNIVERSITI MALAYSIA SABAH

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, *Macrobrachium 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, natural 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.

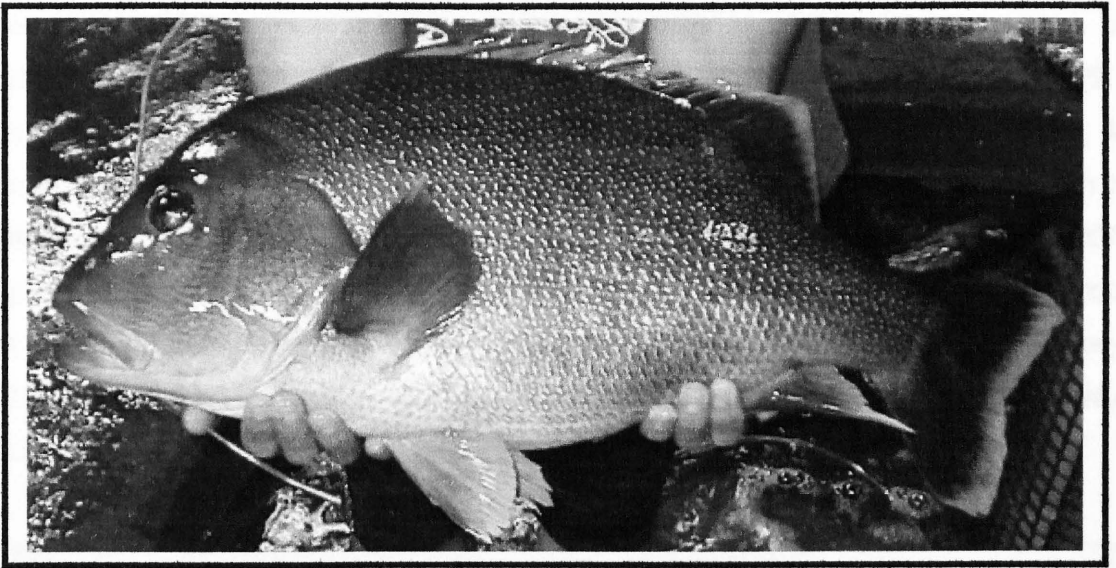


Photo 1.1: White blotched snapper, *Lutjanus rivulatus*, a mature female in Marine Hatchery Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS Hatchery), 7 kg in body weight, 65 cm in total length.

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).

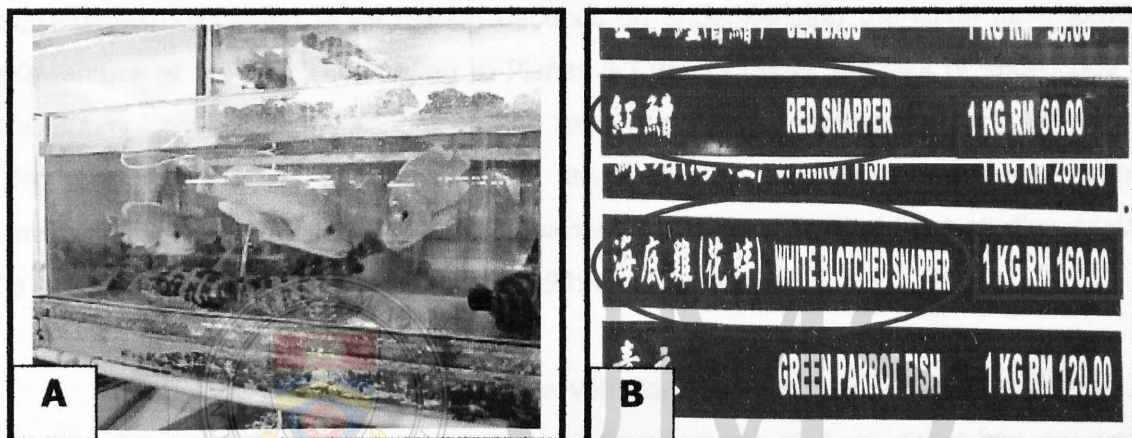


Photo 1.2: *Lutjanus rivulatus* in seafood restaurant has a commercial price. **A**, Fresh *L. rivulatus* display before cooked; **B**, the price cost RM160/kg for live fish, which is twice as expensive as *L. argentimaculatus* (own survey in May 2007).

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