

**DEVELOPMENT OF SENSORY ORGANS IN
THE LARVAE OF TIGER GROUPER,
*Epinephelus fuscoguttatus***



LIM LEONG SENG

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

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**BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2009**

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UNIVERSITI MALAYSIA SABAH

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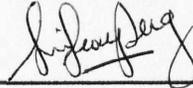
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19th May 2009



LIM LEONG SENG
PO2006-8577



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CERTIFICATION

NAME : **LIM LEONG SENG**

MATRIC NO. : **PO2006-8577**

TITLE : **DEVELOPMENT OF SENSORY ORGANS IN THE LARVAE OF TIGER GROUPER, *Epinephelus fuscoguttatus***

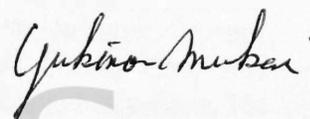
DEGREE : **MASTER OF SCIENCE (AQUACULTURE)**

VIVA DATE : **19 MAY 2009**

DECLARED BY

1. **SUPERVISOR**
Assoc. Prof. Dr. Yukinori Mukai

Signature



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ABSTRACT

DEVELOPMENT OF SENSORY ORGANS IN THE LARVAE OF TIGER GROUPER, *Epinephelus fuscoguttatus*

Development of sensory organs in tiger grouper, *Epinephelus fuscoguttatus* was examined by means of light- and scanning electron- microscopy, to provide useful information on their ecology and larval rearing. In newly hatched larvae, the eyes were not pigmented. The inner ears were oval-shaped vesicles with two otoliths. The larvae floated motionless in the water column. However, a pair of well-developed free neuromasts was found behind the eyes, and the larvae were capable to avoid an approaching transparent pipette. In three-day-old larvae, the eyes were morphologically completed without rod cells and they commenced feeding on rotifers. The three semicircular canals of the inner ears, which play a role in the balance regulation, were completed, and the larvae were capable to swim horizontally. In 20-day-old larvae, taste buds appeared in the buccal cavity, and they commenced feeding on *Artemia* nauplii. In 40-day-old larvae, rod cells appeared and the inner ears were morphologically completed. The larvae started to settle down to the bottom of the tank. In 50-day-old fish, canal neuromasts formed and taste buds were found on the lips and breathing valves. At this stage, the fish commenced feeding on minced fish and stay at the bottom of the tank. In 60-day-old fish, anterior and posterior olfactory pits were formed. Apparently, sensory organs of tiger grouper were well-developed when they settled to the bottom of the tank.



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ABSTRAK

*Perkembangan organ deria kerapu harimau, *Epinephelus fuscoguttatus* telah diperiksa dengan menggunakan mikroskop cahaya dan pengimbas elektron, untuk menyediakan maklumat yang berfaedah kepada ekologi dan pengkulturan larva. Untuk larva yang baru menetas, matanya tidak berpigmen. Telinga dalamannya berbentuk vesikel bujur serta terdapat dua otolit. Larva terapung di dalam air tanpa gerakan. Akan tetapi, sepasang neuromas bebas telah dijumpai di belakang mata dan larva mampu mengelakkan diri daripada pipet lutsinar yang mendekatinya. Larva yang berusia tiga hari mempunyai mata yang sempurna secara morfologi tanpa kehadiran sel rod dan mereka mula memakan rotifer. Tiga saluran semi-bulatan pada telinga dalam yang berfungsi untuk melaraskan keseimbangan badan telah terbentuk dan larva mampu berenang secara melintang. Larva yang berusia 20 hari mempunyai tunas rasa dalam ruangan mulut dan mereka mula memakan nupli *Artemia*. Larva yang berusia 40 hari mempunyai sel rod dalam retinanya dan telinga dalaman mereka telah sempurna secara morfologi. Larva mula untuk mendiami kawasan dasar tangki. Untuk ikan yang berusia 50 hari, saluran neuromas telah terbentuk dan tunas rasa juga telah terbentuk pada bibir dan inajap pernafasan. Pada masa ini, ikan junevil ini mula memakan ikan yang telah dicincang dan mendiami dasar tangki. Lubang olfaktori depan dan belakang telah terbentuk pada ikan yang berusia 60 hari. Ini menunjukkan bahawa organ deria kerapu harimau telah berkembang dengan baik sewaktu mereka mendiami dasar tangki.*



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TABLE OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	v
ABSTRAK	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xvi
CHAPTER 1: INTRODUCTION	
1.1 Fisheries and Aquaculture in Malaysia	1
1.2 Groupers	2
1.3 Groupers as the Target Species for Marine Finfish Culture	3
1.4 Status of Groupers Culture	4
1.5 Target Species: Tiger Grouper <i>Epinephelus fuscoguttatus</i>	4
1.6 Importance of Studying the Development of Sensory Organs and Changes of Behaviour	5
1.7 Objectives of Study	7
CHAPTER 2: LITERATURE REVIEWS	
2.1 Early Survival Strategy of Tiger Grouper in the Wild	8
2.2 Sensory Systems	8
2.2.1 Eyes	10
a. Structure of Eyes in Adult Fish	10
b. Development of Eyes	12
2.2.2 Inner Ears	
a. Structure of Inner Ears in Adult Fish	14
b. Development of Inner Ears	16
2.2.3 Lateral Line System	17
a. Free Neuromasts	18
b. Development of Lateral Line System	21
2.2.4 Olfactory Organ	22
a. Development of Olfactory Organ	27
2.2.5 Taste Buds	27

2.3	Larval Fish Behaviour	
2.3.1	Feeding	28
2.3.2	Predator Avoidance	29
2.3.3	Swimming	30
2.3.4	Phototaxis	31
2.3.5	Settlement	31

CHAPTER 3: MATERIALS AND METHODS

3.1	Egg Collection	32
3.2	Larval Rearing	32
3.3	Behavioral Observation	34
3.4	Morphological Observation	34
3.5	Histological Observation	34
3.6	Observation under Scanning Electron Microscope (SEM)	36

CHAPTER 4: RESULTS

4.1	The Growth and Morphological Development of Tiger Grouper Larvae	38
4.2	Changes of Behaviour in the Tiger Grouper Larvae	41
4.3	Development of Sensory Organs	
4.3.1	Eyes	44
4.3.2	Olfactory Organ	49
4.3.3	Taste Buds	54
4.3.4	Inner Ears	
a.	Development of Vestibular Organs (Pars Superior)	55
b.	Development of Auditory Organs (Pars Inferior)	56
4.3.5	Free Neuromasts	
a.	Distribution of Free Neuromasts	57
b.	Development of Free Neuromasts	50
c.	Sensory Cells Polarity	65

CHAPTER 5: DISCUSSION

5.1	Development of Eyes and Changes in Behaviour	68
5.2	Development of Olfactory Organ and Changes in Behaviour	69
5.3	Development of Taste Buds and Changes in Behaviour	72
5.4	Development of Inner Ears and Changes in Behaviour	73
5.5	Development of Free Neuromasts and Changes in Behaviour	74
5.6	Speculation on the Early Life History of Tiger Grouper in the Wild	76
5.7	Improvement of Larval Rearing Methods for Tiger Grouper	
5.7.1	Water Flow Field in the Rearing Tank	77
5.7.2	Light Condition of the Rearing Tank	78
5.7.3	Feeding Regimes	79

CHAPTER 6: CONCLUSIONS

81

REFERENCES

82

APPENDICES

99

LIST OF TABLES

	Page
Table 2.1	Function of sensory organs in fish. 10
Table 4.1	Number of free neuromasts on one side of the larvae of tiger grouper, <i>Epinephelus fuscoguttatus</i> . 57
Table 4.2	Major and minor axis measurement and number of sensory cells of free neuromasts on the unilateral trunk of the larvae of tiger grouper, <i>Epinephelus fuscoguttatus</i> by mean \pm S.D. 60
Table 4.3	Diagram of the relationship between the development of sensory organs and changes of behaviour in the tiger grouper, <i>Epinephelus fuscoguttatus</i> larvae. 67



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LIST OF FIGURES

	Page
Figure 1.1	A female tiger grouper, <i>Epinephelus fuscoguttatus</i> broodstock in UMS hatchery, 45.8 cm in total length and 3.1 kg in body weight. 5
Figure 2.1	Top panel shows the location of Komodo National Park in Indonesia (square box). The lower panel shows the area where the spawning aggregation site within the KNP (arrow head) (Source: Pet <i>et al.</i> , 2003). 9
Figure 2.2	Diagram of a section through the eye of a fish (Source: Bone, 1995). 11
Figure 2.3	Rod and cone photoreceptors neurons. The basic structure is illustrated for (a) rod and (b) cone cells. Outer segment membranes are the site of photo transduction. In rods the photosensitive membranes are in disks, separate from the external cell membrane; in cones the membranes are infoldings of the external membrane. The inner segments have many mitochondria (Source: Evans, 2004). 12
Figure 2.4	The paired ears of fish are membranous structures embedded in fluid-filled spaces within the cranium, on either side of the midbrain. The position of the two ears is shown for the cod in lateral (a) and dorsal (b) views (Source: Hawkins, 1993). 14
Figure 2.5	Drawing of the right ear of <i>Osteoglossum bicirrhosum</i> , the arawana (family Osteoglossidae), with medial on the left and lateral on the right. A-anterior semicircular canal; CC-common canal of semicircular system; H-horizontal semicircular canal; L-lagena; LN-lagenar branch of eighth cranial nerve; LO-lagenar otolith ; P-posterior semicircular canal; S-sacculus; SN-sacculus of eighth cranial nerve; SO-sacculus otolith; U-utricle; UO-utricle otoliths (Source: Popper <i>et al.</i> , 2004). 16
Figure 2.6	The lateral line system of the blind cave fish, <i>Astyanax mexicanus</i> (white dots). HMC, hyomandibular canal; IOC, infra-orbital canal; SOC, supra-orbital canal; TLL, trunk lateral line (Source: Bleckmann, 1993). 18

Figure 2.7	A. Diagram of a free neuromast of bony fishes. Characteristic features are the pear-shaped hair cell and a jelly like cupula. B. Schematic diagram of a vertebrate hair cell. Each hair cell consists of a single kinocilium and a bundle of stereocilia. In ordinary lateral line organs, they may display dual (afferent and efferent) innervations (Source: Bleckmann, 1993).	20
Figure 2.8	Part of a fish's body with free neuromasts, canal pores and a longitudinal section through a lateral line canal with canal organs (canal neuromasts) (Source: Bleckmann, 1993).	20
Figure 2.9	The olfactory organs in isoosmotic and cycloosmotic fish species illustrating the position of the olfactory epithelium (rosette) in relation to the anterior (inhalant) and posterior nostrils. Note the presence of accessory sacs in the cycloosmotic species and the funnel-like anterior nostril in the isoosmotic species (Source: Jobling, 1995).	23
Figure 2.10	Eight types of arrangements of lamellae in the olfactory organ of teleosts. An olfactory organ has (A) no lamella; (B) one longitudinal lamella; (C) one transverse lamella; (D) longitudinal lamellae arranged in parallel with one another; (E) lamellae arranged in a fan-shape; (F) lamellae radiating in all direction from a raphe; (G) lamellae arranged transversely or obliquely to an elongated raphe; (H) numerous lamellae mostly arranged transversely to a very long raphe (Source: Yamamoto, 1982).	23
Figure 2.11	Four types of distribution patterns of the sensory epithelium (shown dotted areas) in the lamellar face. The sensory epithelium is (I) continuous except for the margin of the lamella; (II) separated into some large areas by the non-sensory epithelium; (III) mixed irregularly with the non-sensory epithelium; (IV) dispersed in discrete islets (Source: Yamamoto, 1982).	24
Figure 2.12	Structure of the olfactory rosette showing the array of different cell type associated with the olfactory epithelium (Source: Jobling, 1995).	26
Figure 3.1	Feeding regime and water management during the larval rearing of tiger grouper, <i>Epinephelus fuscoguttatus</i> .	33
Figure 3.2	The procedure of histological studies	35
Figure 3.3	The procedure of preparing specimens for observation under SEM	37

Figure 4.1	Growth of the larval tiger grouper. Closed circle and vertical bar indicate mean total length and standard deviation, respectively.	38
Figure 4.2	Schematic diagrams of different developmental stages of tiger grouper, <i>Epinephelus fuscoguttatus</i> .	40
Figure 4.3	Schematic diagrams of behaviour in different developmental stages of tiger grouper <i>Epinephelus fuscoguttatus</i> , (a) newly-hatched; (b) one-day-old; (c) three-day-old; (d) 10-day-old and (e) 20-day-old.	42
Figure 4.4	Schematic diagrams of behaviour in 40-day-old and 50-day-old of tiger grouper, <i>Epinephelus fuscoguttatus</i> .	43
Figure 4.5	Eye diameter of tiger grouper, <i>Epinephelus fuscoguttatus</i> larvae. Closed symbols and vertical bars indicate mean diameter and standard deviation.	44
Figure 4.6	Eye diameter relative to total length of tiger grouper larvae.	44
Figure 4.7	Light micrographs of eyes in tiger grouper. (a) Newly hatched larva. Scale bar, 20 μm . (b) One-day-old larva. Br: brain (c) Two-day-old larva. Scale bar, 50 μm . (d) Three-day-old larva. Scale bar, 50 μm . GL: ganglion layer, IPL: inner plexiform layer, INL: inner nuclei layer, ONL: outer nuclei layer, VC: visual cell, PE: pigmented epithelium, ON: optic nerve. Thin arrows show the direction of nasal pit. Lens magnifications of each figure are shown.	45
Figure 4.8	Section of five-day-old larva from the direction of nasal to caudal. RNA =retinal nasal region; RBR = retinal bottom region; RTR = retinal temporal region. Scale bar, 100 μm . Lens magnification of the figure is shown.	46
Figure 4.9	Changes in width from pigmented epithelium to the outer nuclei layer in the retina of tiger grouper larvae.	46
Figure 4.10	Retinal sections of tiger grouper. (a) Three-day-old larva. (b) 40-day-old fish. Scale bar, 10 μm . (c) and (d) Magnification of visual cell and outer segment nuclei in (a) and (b). 1= pigmented epithelium; 2 = visual cells layer; 3 = outer nuclei layer; 4 = horizontal cell layer; 5 = inner nuclei layer. Lens magnifications of each figure are shown.	47

- Figure 4.11 Changes in the ratio of outer segment nuclei to visual cell number in retinal nasal, bottom and temporal regions of tiger grouper with growth. 48
- Figure 4.12 Scanning electron micrographs of the olfactory organ in the tiger grouper larvae. (a): Newly hatched larva. Arrow: nasal pit. Scale bar, 100 μm . (b) Nasal pit of newly hatched larva. Scale bar, 5 μm . (c) Three-day-old larva. Arrow: nasal pit. Scale bar, 100 μm . (d) Nasal pit of three-day-old larva. Scale bar, 5 μm . (e) Nasal pit of 10-day-old larva. Scale bar, 10 μm . (f) Nasal pit of 30-day-old larvae. Scale bar, 100 μm . (g) Nasal pit of 40-day-old fish. Scale bar, 100 μm . (h) Anterior and posterior pits of 60-day-old fish. Scale bar, 200 μm . Lens magnifications of each figure are shown. 50
- Figure 4.13 Light micrographs of the olfactory organs in tiger grouper larvae. (a) Olfactory organ of 20-day-old larva, without lamella. (b) Olfactory organ of 25-day-old larva, with an informing lamella. (c) Olfactory organ of 35-day-old larva, with a newly formed lamella. (d) Olfactory organ of 40-day-old larva, with two lamellae. Scale bars: 100 μm . Thin arrows show the caudal direction. Lens magnifications of each figure are shown. 51
- Figure 4.14 Scanning electron micrographs of the olfactory epithelium in the tiger grouper larvae. (a): Newly hatched larva. (b) One-day-old larva. CR, ciliated receptor cell. Scale bars, 1 μm . Lens magnifications of each figure are shown. 52
- Figure 4.15 Scanning electron micrographs of the olfactory epithelium in the tiger grouper larvae. (a): seven-day-old larva. (b) 10-day-old larva. CN, ciliated sensory cell; CR, ciliated receptor cell. Scale bars, 2 μm . Lens magnifications of each figure are shown. 53
- Figure 4.16 (a) 20-day-old tiger grouper. Arrow shows the taste buds in the buccal cavity. Scale bar, 100 μm . esp: esophagus. (b) Magnification of the taste bud in (a). Scale bar, 50 μm . (c) Schematic diagram of lower jaw of 50-day-old tiger grouper. (d) Scanning electron micrograph of lower jaw of 50-day-old tiger grouper. Arrows show taste buds. Scale bar, 100 μm . Lp: lips; Bv: breathing valve; Bc: buccal cavity; Jt: jaw teeth; Jg: jaw gum. (e) Magnification of taste bud in (d). Scale bar, 10 μm . Lens magnifications of each figure are shown. 54

- Figure 4.17 Otic vesicle of (a) newly hatched and (b) one-day-old larvae; thin arrows show otoliths; broad arrows show the thickened otic epithelium. (c)-(d): Continuous sections of inner ear of three-day-old larvae; aa: anterior ampulla; ha: horizontal ampulla; pa: posterior ampulla; pr: primodium of utricular macula. (e) Inner ear of 10-day-old larvae. u: utricular cavity; arrow shows utricular macula (um). Scale bars, 50 μ m. (f) Continuous sections cut parallel to body axis from dorsal to ventral direction, (c), (d), (e), from nasal to caudal direction, (b). Lens magnifications of each figure are shown. 55
- Figure 4.18 (a)-(f) Continuous section in axis from dorsal to ventral; (a)-(d) 25-day-old larvae; (e)-(f) 30-day-old larvae; aa: anterior ampulla; ha: horizontal ampulla; pa: posterior ampulla; u: utriculus cavity; thin arrow shows utricular macula; cc: calcified cartilage; mo: medulla oblongata; br: brain; s: sacculus cavity; L: lagenar cavity; broad arrow in (d) shows primodium of saccular macula; acc: anterior canal cavity; hcc: horizontal canal cavity; pcc: posterior canal cavity. Scale bars, 100 μ m. Lens magnifications of each figure are shown. 56
- Figure 4.19 The distribution of free neuromasts in different developmental stages of tiger grouper, *Epinephelus fuscoguttatus*. 58
- Figure 4.20 Scanning electron micrographs of free neuromasts in newly hatched larva of tiger grouper. (a) The position of free neuromasts; Trk: trunk; Hd: head; YS: yolk sac; arrow: free neuromasts. Scale bar 100 μ m. (b) Magnify of free neuromasts. Scale bar, 10 μ m. Lens magnifications of each figure are shown. 59
- Figure 4.21 Development of sensory cells number by mean \pm S.D in the free neuromasts on the unilateral trunk of tiger grouper, *Epinephelus fuscoguttatus*. 60
- Figure 4.22 Development of major and minor axis by mean \pm S.D of the free neuromasts on the unilateral trunk of tiger grouper, *Epinephelus fuscoguttatus*. 61
- Figure 4.23 Development of ratio (major/minor axis) by mean \pm S.D of the free neuromasts on the unilateral trunk of tiger grouper, *Epinephelus fuscoguttatus*. 61

- Figure 4.24** The development of free neuromasts in tiger grouper with growth. (a) Newly appeared free neuromasts on the trunk of 1-day-old larvae. Scale bar, 2 μm (b) Free neuromasts on the trunk of 10-day-old larvae. (c) Free neuromasts on the trunk 20-day-old larvae. (d) Free neuromasts on the trunk of 40-day-old fish. Scale bars, 5 μm . a: anterior, p: posterior, d: dorsal, v: ventral. Lens magnifications of each figure are shown. 62
- Figure 4.25** Head portion of 40-day-old tiger grouper. Black arrows show canal pores. Circles in the picture show free neuromasts on the skin. White arrows show the presumptive free neuromasts of canal organ. Scale bar, 500 μm . Picture 1-3 show the presumption free neuromasts of canal organ. Scale bars, 20 μm . Picture 4-6 show the free neuromasts on the skin surface. Scale bars, 10 μm . Lens magnifications of each figure are shown. 63
- Figure 4.26** Scanning electron micrographs on the lateral line position in 50-day-old of tiger grouper. (a) Trunk portion. (b) Peduncle portion. Arrows show the canal pores. Scale bars, 500 μm . Square box in figure (a) shows the ctenoid scale (elder) while in figure (b) shows the spiny scale (younger). Lens magnifications of each figure are shown. 64
- Figure 4.27** Diagrams of the orientation of sensory polarity in tiger grouper. (a) Free neuromasts on the trunk of 10-day-old larvae. K: Kinocilium; S: Stereocilium. Scale bar, 1 μm . Lens magnification of the figure is shown. (b) Orientation of sensory polarity of the free neuromasts. A: Anterior; P: Posterior. 65
- Figure 4.28** Diagrams of free neuromasts arrangement and the orientation of sensory cell polarity in 20-day-old larvae of tiger grouper. Dots with bars indicate the orientation of neuromasts in terms of sensory cells polarity. (a) Free neuromasts on the unilateral side of body. Scale bar, 1.0 mm. (b), (c) Distribution of free neuromasts on the head; (b), dorsal view; (c), ventral view. Scale bars, 200 μm . 66

LIST OF APPENDICES

	Page
Appendix A Larval measurement of tiger grouper, <i>Epinephelus fuscoguttatus</i>	99
Appendix B Eye diameter (μm), total Length (μm), and ratio (ED/TL)	101
Appendix C Measurement of retina	103
Appendix D Measurement of free neuromasts major axis, minor axis, ratio (major/minor axis), sensory cells	104
Appendix E Water parameters in the rearing tank	105
Appendix F Sequence of Haematoxylin and Eosin counter-stain method	108
Appendix G Preparation of the Karnovsky's solution	109



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CHAPTER 1

INTRODUCTION

1.1 Fisheries and Aquaculture in Malaysia

In Malaysia, the total fisheries production in 1998 and 2004 was 1.35 and 1.54 million metric ton. These were valued at RM 453 millions and RM 551 million respectively. Fisheries created employment for eighty-nine thousand fishermen and twenty-two thousand fish culturists in year 2004 (Annual Fisheries Statistics, 2004).

Malaysian fisheries comprise two sub-sectors, namely marine capture fisheries and aquaculture (Annual Fisheries Statistics, 1998; 2004) with the former being the main contributor to the total fish production in the country. In 2004, 87% of the total fish production, at 1.33 millions tonnes with the value of RM 424 million, was derived from marine capture fisheries.

Marine capture fisheries include coastal and deep sea fisheries. Research has indicated that coastal fisheries have been optimally exploited, leaving expansion limited only to deep sea fisheries (Annual Fisheries Statistics, 2004). However, deep sea fisheries are still lacking in terms of large fishing vessels and skilled manpower. The operations of deep sea fishing vessels to a certain extent are still reliant on foreign work force although the number of foreign fishermen registered had reduced by 6.17% from 30,008 people in 2003 to 28,154 people in 2004 (Annual Fisheries Statistics, 2004). Various measures have been implemented by the Malaysian government to ensure the expansion and development of deep sea fisheries; it is the government's belief however that the real potential in increased fish production still remains within the aquaculture sector (Annual Fisheries Statistics, 2003).

The growth of the aquaculture sector in Malaysia has vast potential as the country has extensive inland and coastal areas for both fresh water and marine aquaculture development (Ang, 1990; Annual Fisheries Statistics, 2003). Being a

tropical country, aquaculture activities are viable throughout the year with few limitations of seasons (Galid, 2003). The stable politics and economic situation in Malaysia have encouraged and attracted long-term investments into aquaculture (Sugiyama *et al.*, 2004). In addition, as a multi-racial country, Malaysian aquaculture products can meet the demand without any boundaries of race, religion or ethnic group (Ang, 1990; Galid, 2003). Although the contribution from the aquaculture sector is small, the production had increased from 9.9% of the total production in 1998 to 13.2% in 2004. By 2010, a 662,000 metric ton volume of fish production is projected from the aquaculture sector.

1.2 Groupers

Groupers are classified in 15 genera of the sub-family Epinephelinae (Heemstra and Randall, 1993; Tucker, 1999), which comprises at least half the approximate 449 species in the family Serranidae (Tucker, 1999). The 15 genera are *Aethaloperca*, *Alphestes*, *Anyperodon*, *Cephalopholis*, *Cromileptes*, *Dermatolepis*, *Epinephelus*, *Gonioplectrus*, *Gracila*, *Mycteroperca*, *Parathias*, *Plectropomus*, *Saloptia*, *Triso*, and *Variola* (Heemstra and Randall, 1993). Grouper species are identified by their colour pattern and (or) a suite of morphological characters including body shape, configuration and size of the fins, the shape and relative size of the head and various parts of the head and body, and the number of fin rays, scales and gill rakers (Heemstra and Randall, 1993).

Groupers can be found in tropical and sub-tropical waters of all oceans and are commonly known as reef fishes as they comprise the dominant group on coral reefs (Herwerden *et al.*, 2002). As the major predators of the coral reef ecosystem, groupers are typically carnivorous and feed on a variety of fishes, larger crustaceans, and cephalopods. Most groupers are ambush predators; they hide amongst the corals and rocks and catch their unwary prey with a quick rush and snap of their jaws (Heemstra and Randall, 1993). Groupers are usually found in shallow water and some species can also be found in estuaries or on rocky reefs. Groupers are generally associated with rocky bottom, although juveniles are found in sea grass bed and adults of several species prefer sandy or silt areas (Heemstra and Randall, 1993; Tucker, 1999). Groupers mature relatively late in their life with

most attaining maturity within 2 to 6 years. They are protogynous hermaphrodites where they initially mature as female then later as males. Others may change only if there is a shortage of males (Heemstra and Randall, 1993).

1.3 Groupers as the Target Species for Marine Finfish Culture

Groupers are amongst the most popular species in the live reef food fish (LRFF) industry in Asia-Pacific region (SEAFDEC, 2001). Due to their culinary attributes and scarcity (Aquaculture Center SEAFDEC, 2001), groupers command high prices and are in demand, especially in the LRFF markets of Hong Kong, China. In Hong Kong, consumption of imported and live reef fish assumes an important cultural and social role particularly for business dinners and banquets as well as special festivals or occasions (Lau and Parry, 1999). In 2000, the approximately 10,000 tonnes of groupers imported into LRFF market of Hong Kong had a combined total retail value of around US\$ 350 million (Sadovy *et al.*, 2003).

Retail price of groupers ranges from US\$5 to US\$180 per kilogram (kg), depending on species, taste, texture, availability and time of year. During festival periods, the prices would significantly increase (Sadovy *et al.*, 2003). Among the species, the humpback grouper (*Cromileptes altivelis*), giant grouper (*Epinephelus lanceolatus*), leopard coral grouper (*Plectropomus leopardus*), and spotted coral grouper (*Plectropomus maculatus*) are categorized as the highest value species (Lau and Parry, 1999; Sadovy *et al.*, 2003). Other popular species include orange-spotted grouper (*Epinephelus coiodes*), tiger grouper (*Epinephelus fuscoguttatus*) and camouflage grouper (*Epinephelus polyphekadion*) (Sadovy *et al.*, 2003).

In aspects of aquaculture, groupers are generally fast growing, hardy, suitable for intensive culture and with excellent characteristics for processing (SEAFDEC, 2001). Due to its high price and demand, groupers have become the most popular species for marine culture, especially in China, Vietnam, Malaysia, Thailand and Indonesia (Sadovy *et al.*, 2003).

1.4 Status of Grouper Culture

Although grouper culture is widespread in Asia and the Pacific, its continued development is constrained by the limited availability of seeds. Fish farmers rely heavily on wild-caught seeds for stocking and grow-out (SEAFDEC, 2001; Mous *et al.*, 2006). This demand for wild seeds has led to unsustainable and illegal seed collection practices such as the use of cyanide (CN) to capture large numbers of seed with relatively less investment in time and effort (Lau and Parry, 1999; SEAFDEC, 2001; Sadovy *et al.*, 2003). Moreover, nomadic fishery, which remains in one area for a short period and subsequently move on only when the target fish becomes scarce, may further decrease the groupers' population in a particular area due to the species' spawning aggregations (Morris *et al.*, 2000; Sadovy *et al.*, 2003). These factors have led to over-fishing of the wild grouper. In short, the existing supply of wild-caught seeds cannot sustain the demand of the expanding grouper culture industry.

Many Southeast Asia and Pacific countries focus on supplying groupers through artificial mass production of seeds. However, larval rearing of groupers is complicated and mass mortality is regularly reported (Tucker, 1999; Rimmer, 2000; Queensland Government Department of Primary Industries and Fisheries, 2003-2004). Grouper larvae are small and fragile with small reserves of endogenous nutrition and low feeding rate (Ordonio-Aguilar *et al.*, 1995). The combination of these factors is considered to be a fundamental cause of the high mortality and delayed development observed during larval rearing (Kohno *et al.*, 1997). Under these circumstances, groupers have become a challenging species for aquaculture scientists to study and investigate aspects of grouper larval rearing to improve the survival rate as well as consistency of production in the hatchery phase.

1.5 Target Species: Tiger Grouper (*Epinephelus fuscoguttatus*)

The tiger grouper, or brown-marbled grouper (*Epinephelus fuscoguttatus*) (Figure 1.1), is also known as kerapu harimau or kerapu hitam in Malaysia, flowery cod in Australia, kerapu macan in Indonesia and lapu-lapu in Philippine (SEAFDEC, 2001; Reef Fish Aquaculture R&D Project Annual Report, 2003-2004). It is one of the most valuable species of groupers in terms of export, especially to countries with

significantly large Chinese populations, including Hong Kong (Lau and Parry, 1999), Taiwan Province of China, Singapore, Malaysia, Singapore and Thailand (Ottolenghi *et al.*, 2004) where groupers are considered a delicacy especially during festivals. In local Malaysian seafood restaurants, live tiger grouper can be priced up to RM100 per kilogram while in Hong Kong, the selling price could be as high as RM300 per kilogram.

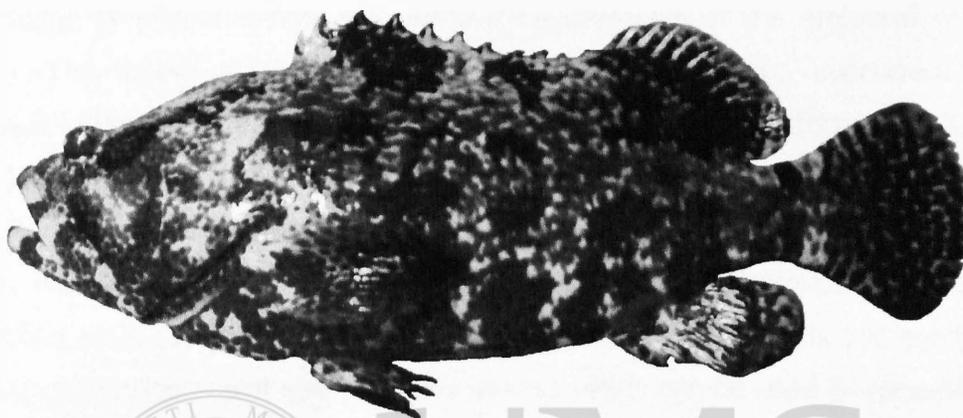


Figure 1.1: A female tiger grouper, *Epinephelus fuscoguttatus* broodstock in UMS hatchery, 45.8 cm in total length and 3.1 kg in body weight.

Tiger grouper has become one of the most sought-after groupers. From 1999 to 2002, tiger grouper imported into Hong Kong, China had increased by 50%. In contrast, import of the orange-spotted grouper and camouflage grouper declined by 35% and 55% respectively during the same period (Sadovy *et al.*, 2003). This constant high demand has led to over-fishing of the wild tiger grouper to the extent that it is now categorized in the red lists of the International Union of Conservation of Natural Resources (IUCN) as a nearly threatened species (Cornish, 2004). Artificial mass production of tiger grouper seeds meanwhile is severely impeded by difficulties of larval rearing.

1.6 Importance of Studying the Development of Sensory Organs and Changes of Behaviour

The sensory organs of the fish larvae are utilized to obtain information from the environment and generate appropriate behavioral response for their survival. Changes of larval behaviour are therefore closely related to the development of

sensory organs (Ishida, 1987). During ontogeny, fish may differ in size and morphology, and switch habitat at the late larval stage which results in total changes in their feeding niches and behaviour (Leis, 1991). Ontogeny of sensory organs and behaviour are thus important for different developmental phases of fish larvae, to feeding and avoidance of predators at sea (Blaxter, 1986).

The basic breakthrough in aquatic organism cultivation includes the knowledge of environmental and nutritive requirements of the organism (Kinne, 1977). The timing of changes in environment and nutrition, concurrent with changes in growth of the organism are also essential requisite information (Kinne, 1977). However, current available literature on groupers is inadequate in providing useful information on successful mass production of groupers in hatchery. Despite earlier research on the larval development of brown marbled groupers carried out by Kohno *et al.* (1990a; 1990b; 1993), ontogeny of sensory organs and behaviour in interval developmental stages of this species which can be used to speculate of its early life history still remain undocumented.

While *in situ* observation can be performed to discover the early life history of tiger grouper at sea, the practice is not recommended as this method has its limitations (Leis and McCormick, 2002). Fertilized groupers eggs are transparent, small (0.70 to 1.20 mm in diameter) and colourless (Heemstra and Randall, 1993). It is therefore extremely difficult to continuously track the eggs in the open sea. Added to this is the fact that most species of grouper larvae are morphologically similar (Kendall, 1984; Leis, 1987) and are difficult to be species-distinguished.

It is due to this situation then that understanding the development of sensory organs and ontogeny of behaviour under laboratory conditions is more viable to explain or predict the species' specific adaptations in the different developmental stages and provide knowledge for adapting rearing techniques to the ecological needs of tiger grouper (Kawamura *et al.*, 2003) which is the significant of this study.