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

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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.

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 injap 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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 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 coralgrouper (*Plectropomus leopardus)*, and spotted coralgrouper (*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)* (Savody *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.

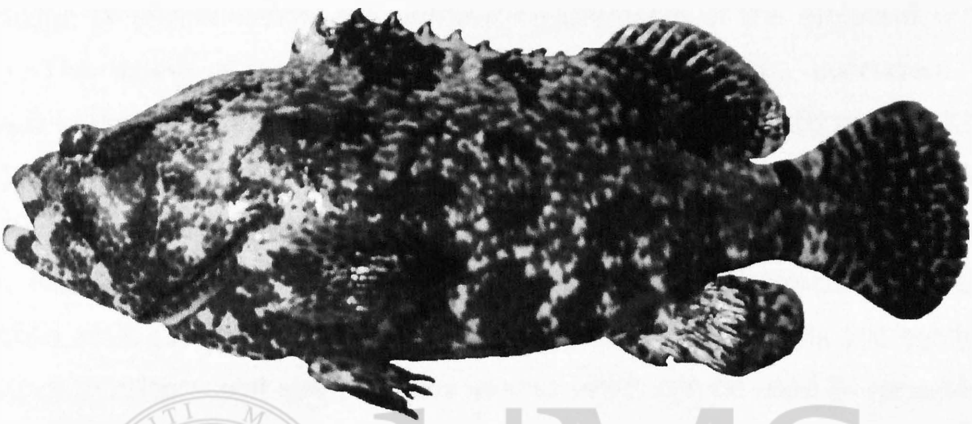

Figure 1.1: A female tiger grouper, *Epinephelus fuscoguttatus* broodstock in UMS hatchery, 45.8 cm in total length and 3.1 kg in body weight.

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.