

**LARVAL DEVELOPMENT OF GROUPEL
HYBRIDS *Epinephelus coioides* X *E.
fuscoguttatus* AND
E. coioides X *E. lanceolatus***

IVAN KOH CHONG CHU



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**BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
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DECLARATION

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Ivan Koh Chong Chu
15 April 2009

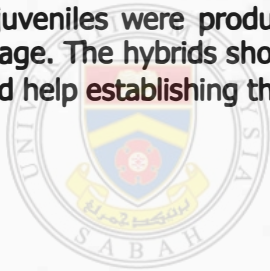


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ABSTRACT

LARVAL DEVELOPMENT OF GROUPER HYBRIDS, *Epinephelus coioides* X *E. fuscoguttatus* AND *E. coioides* X *E. lanceolatus*

This study was conducted to investigate the possibility of grouper hybridization for increasing potential in aquaculture. 2 combinations were produced, which were orange-spotted grouper *E. coioides* x tiger grouper *E. fuscoguttatus* (OGTG) and orange-spotted grouper *E. coioides* x giant grouper *E. lanceolatus* (OGGG). The egg and larval development of the above new hybrids were observed under artificial conditions. Newly ovulated eggs from a female *E. coioides* (7.5 kg in body weight) were measured at $806 \pm 20 \mu\text{m}$ (mean \pm SD) in diameter and weighed 3,505 eggs/g. After fertilization with sperm obtained from a male *E. fuscoguttatus* (11.0 kg in body weight) and a male *E. lanceolatus* (35.0 kg in body weight), the eggs diameter measured $833 \pm 22 \mu\text{m}$ (OGTG) and $833 \pm 22 \mu\text{m}$ (OGGG). The eggs of both hybrids hatched at 17 h 30 min (17:30 h) (OGTG) and 17:15 h (OGGG) after fertilization. Early larval development was similar in both hybrids till metamorphosis. Larvae of both hybrids commenced first feeding at 3 days after hatched (d AH) when larvae were morphologically prepared. Hybrid larvae showed typical early *Epinephelus* type pigmentation and differentiation of dorsal and pelvic-fin spines was observed prior to 10 d AH and thereafter elongated. 750 (OGTG) and 235 (OGGG) tails with mean total length of $22.8 \pm 3.6 \text{ mm}$ and $28.4 \pm 2.5 \text{ mm}$ of 50 d AH juveniles were produced. Both hybrids showed normal development till juvenile stage. The hybrids showed potential for aquaculture and the results of this study could help establishing the basis of seed production of the hybrids.



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ABSTRAK

Kajian ini dijalankan untuk menyiasat kemungkinan penghasilan hibrid kerapu untuk peningkatan potensi dalam akuakultur. 2 kombinasi dihasilkan, iaitu kerapu *E. coioides* x kerapu harimau *E. fuscoguttatus* (OGTG) dan kerapu *E. coioides* x keratang *E. lanceolatus* (OGGG). Perkembangan telur dan larva 2 ikan hibrid baru itu diperhatikan di bawah keadaan tiruan. Telur yang baru diovulasikan oleh *E. coioides* betina (berat badan 7.5 kg) dan diukurkan diameternya ialah $806 \pm 20 \mu\text{m}$ (purata+SD) dan beratnya 3505 telur/g. Selepas persenyawaan dengan sperma yang diperolehi daripada seekor *E. fuscoguttatus* jantan (berat badan 11.0 kg) dan seekor *E. lanceolatus* jantan (35.0 kg), diameter telur bertambah menjadi $833 \pm 22 \mu\text{m}$ (OGTG) dan $833 \pm \mu\text{m}$ (OGGG). Telur menetas pada 17 jam 30 min (17:30 h) dan 17:15 h selepas persenyawaan. Perkembangan peringkat awal larva bagi hibrid adalah hampir serupa bagi kedua-dua hibrid hingga metamorfosis. Kedua-dua larva ikan mula makan pada hari ketiga selepas penetasan (d AH) apabila larva sedia dari segi morfologi. Larva hibrid menunjukkan pigmentasi tipikal *Epinephelus* dan pembezaan sirip duri dorsal dan pelvic diperhatikan sebelum 10 d AH dan selepas itu memanjang. Larva mula berpindah habitat daripada pelagik kepada bentik pada 40 d AH. 750 (OGTG) dan 235 (OGGG) ekor dengan purata panjang keseluruhan $22.8 \pm 3.6 \text{ mm}$ dan $28.4 \pm 2.5 \text{ mm}$ punya 50 d AH juvenil telah dihasilkan. Kedua-dua hibrid menunjukkan perkembangan normal sehingga peringkat juvenil. Hibrid menunjukkan potensi untuk akuakultur dan keputusan kajian ini boleh membantu menghasilkan asas bagi penghasilan bibit kacukan.



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LIST OF ABBREVIATIONS

%	percent
°C	degrees centigrade
µm	micrometer
Ac	<i>Anthocidaris crassispina</i>
AF	after fertilization
AH	after hatching
AI	after injection
APF	artificial powder feed
BH	body height
BL	body length
BMRI	Borneo Marine Research Institute
BC	body circumference
BW	body weight
BWd	body width
cm	centimeter
d	day(s)
DO	dissolved oxygen
DSL	dorsal spine length
<i>E.</i>	<i>Epinephelus</i>
ED	eye diameter
ELC	Estimated larvae count
F1	first generation
g	gram
<i>H</i>	Heterosis
h	hour(s)
HCG	human chorionic gonadotropin
HL	head length
IU	International Unit
kg	kilogram
kL	kiloliter
L	liter

m	meter
mg	miligram
min	minute(s)
mL	mllllter
NTP	nutritional transition period
OgD	oil globule diameter
OGGG	<i>Epinephelus coioides</i> x <i>Epinephelus lanceolatus</i>
OGTG	<i>Epinephelus coioides</i> x <i>Epinephelus fuscoguttatus</i>
OgV	oil globule volume
ppt	parts per thousand
PSL	pelvic spine length
RNA	Ribonucleic Acid
SGR	Specific growth rate
SI	<i>Strongylocentrotus intermedius</i>
SL	standard length
Sn	<i>Strongylocentrotus nudus</i>
sp.	Species
TGGG	<i>Epinephelus fuscoguttatus</i> x <i>Epinephelus lanceolatus</i>
TL	total length
UJL	upper jaw length
UMS	Universiti Malaysia Sabah
US\$	United States Dollar
YsH	yolk sac height
YsL	yolk sac length
YsV	yolk sac volume

CHAPTER 1

INTRODUCTION

1.1 Fisheries and Aquaculture Status in Malaysia

Fish has always been a main source of food for the people in the world. In 2004, up to 140.5 million tonnes of fisheries product was captured globally. Of this total, food fish production amounted to 106 million tonnes of food fish in 2004, providing an apparent per capita supply of 16.6 kg (live weight equivalent), which is the highest on record. (The State of World Fisheries and Aquaculture, 2006). However, the world's population has been increasing more quickly than the total food fish supply. The growth rate of the world's population has been increasing exponentially rather than geometrically while capture fisheries production with the exception of China have remained relatively stable over the last few years (The State of World Fisheries and Aquaculture, 2006). The situation of Malaysia's fisheries sectors are similar to that of the global fisheries status, as production of marine capture fisheries has stagnated. The capture fisheries have been fully exploited and prospects of further expansion for increased yield seems to be quite limited (Mustafa and Rahman, 2000; Annual Fisheries Statistics, 2005).

Malaysia is a country in which seafood demand is high and continues to rise. (Mustafa and Rahman, 2000). In most Southeast Asian countries, seafood is an important source of protein as well as an important culinary delicacy, and Malaysia is of no exception. With a multi-racial population, seafood products are widely accepted and has no boundaries of race, religion or ethnic group (Ang, 1990; Galid, 2003). The population has increased to 25.3 million in 2005, and with it a parallel increase in demand of seafood for consumption. Although various measures have been taken by Malaysian government to ensure the development of sustainable increase in production of capture fisheries to it is acknowledged that the real potential for expansion of its fisheries remains with aquaculture (Annual Fisheries Statistics, 2002).

Aquaculture is defined as farming of aquatic animals and plants useful to humans. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators; and the organisms must also be harvested by an individual or corporate body which has owned them throughout their rearing period (Aquaculture production statistics, 2004). In the recent years, aquaculture has become the fastest growing food production sector in many countries, contributing substantially to national development, food supply, food security, poverty alleviation, income generation and employment (Subasinghe, 2000).

Malaysia has a great potential in developing the aquaculture sector due to its many advantages geo-morphologically. It is blessed with vast potential inland and coastal areas, allowing for the development of both freshwater and marine aquaculture (Ang, 1990; Annual Fisheries Statistics, 2002). Peninsular Malaysia, Sabah and Sarawak are mostly bounded by sea, and has a coastline of 4809 km (Mustafa *et al.*, 1997). Malaysia is a tropical country that has climatological conditions favouring aquaculture all year round. The mean annual temperature range is 25 – 28°C, mean value of humidity is 82 - 86°C and average total annual rainfall is about 200 cm (Yusoff *et al.*, 1997). The water temperature also remains consistently high throughout the year (Mustafa and Rahman, 2000). These conditions allow the practice of aquaculture activities to be conducted all year round (Galid, 2003). The stable political conditions and economic growth in Malaysia also have encouraged and attracted long-term investment into aquaculture (Sugiyama *et al.*, 2004). These advantages make Malaysia an ideal place for the development of aquaculture.

Aquaculture in Malaysia started in the early twentieth century, with the culture of Chinese carps in the mining pools. In 1984, the Government of Malaysia formulated the national Agriculture Policy for the development of agriculture, which included fisheries and aquaculture which benefited the freshwater culture in the country (Ang, 1990). The contribution from the aquaculture sector has been small, however the production has been increasing, from 9.9% of the total production in 1998 to 13.2% in 2004. It is acknowledged that Malaysia has a great potential to

develop aquaculture and that this sector is expected to be the most important sector to increase fish supply in Malaysia (Annual Fisheries Statistics, 2003). In recent years, the marine brackish water and marine sectors have been increasing in importance. Out of a total of 21,114 fish farmers/culturist involved in the aquaculture industry in 2003, only 21.0% were involved in the marine sectors. However, the bulk of the aquaculture production was contributed by the marine brackish water and marine sectors with up to 74.63% or 146,926 tonnes of the total aquaculture production in 2003. In marine fish culture, the major species cultured from brackish water pond were snappers and groupers which contributed to 51.54% to this sector (Annual Fisheries Statistics, 2003).

Various efforts have been undertaken by the government to develop aquaculture in recent years. The Department of Fisheries has identified suitable land areas and water bodies deemed suitable for aquaculture development, as well as actively encouraged the involvement of the corporate sector in integrated aquaculture ventures. This is in a way tied up with the intention of the Department to establish large scale aquaculture zones, with the Department of Fisheries providing the needed infrastructure. Suitable parcels of land of various sizes, would then be allotted to potential farmers or investors. Research and development on new culture technologies, disease prevention and control, seed production and feed formulation would hopefully lead to greater aquaculture production (Annual Fisheries Statistics, 2003).

1.2 Groupers as Food Fish

Groupers are one of the most popular food fishes in the live reef food fish industry in Pan Pacific Asia (Rimmer, 2004). Their scarcity and good culinary taste results in groupers being highly valued food fishes especially in the live markets of Hong Kong and Southern China fetching prices up to US\$70/kg (wholesale) (McGilvray and Chan, 2001). Buyers often have to pay a premium for live cultured groupers and even more for wild-caught live ones (Leong, 1998). In 1997, the volume of live fish traded in Southeast Asia was estimated at about 53,000 MT, including approximately 30,000 MT of groupers (Johannes & Riepen, 1995).

The ever increasing market demand has resulted in overfishing of the wild grouper stocks. Due to the lure of fast profits, fishermen employ unsustainable and illegal seed collection practices such as the use of cyanide to capture large numbers of seed with relatively less investment in time and effort (Lau and Parry, 1999; Aquaculture Department Southeast Asian Fisheries Development Center, 2001; Sadovy *et al.*, 2003). Besides that, the nomadic fishery, which tends to remain in one area for a short period and only move on when the target fish has becoming hard to find, may further decrease the groupers' population in a particular area as most of the groupers, perform spawning aggregations (Morris *et al.*, 2000; Sadovy *et al.*, 2003). Because of declining catches of grouper in the world ocean, aquaculture has been a popular method of increasing fish production. Main considerations for culture are high economic value, fast growth, hardiness and suitability for intensive culture. Since various groupers possess these characteristics for culture; thus they have become a popular marine species for culture (Leong 1998; Aquaculture Department Southeast Asian Fisheries Development Center, 2001).

1.3 Grouper Culture

Grouper culture has been carried out since 1970's, with the introduction of grouper net-cage culture in Singapore, Malaysia, Hong Kong, Thailand and Taiwan (Leong, 1998). Since then, many Southeast Asian and Pacific countries have focused on supplying this apparently lucrative trade through aquaculture (Rimmer, 2004). However, its continuous development is constrained by the limited availability of seeds. The culture of groupers is still heavily dependent on the capture and grow out of wild caught juvenile fish with up to 70-85% of cultured groupers grown out from wild captured fry (Sadovy *et al.*, 2003; Mous *et al.*, 2006).

Many researches in different countries made attempts to produce groupers under artificial conditions but results have proved dissatisfactory (Hussain and Higuchi, 1980; Lim, 1993; Duray *et al.*, 1997; Toledo, 1999). Larval rearing of groupers is difficult and mass mortality is regularly reported (Tucker, 1999; Rimmer *et al.*, 2000). Grouper larvae are small and fragile with small reserves of endogenous nutrition and low feeding rate (Ordonio-Agullar *et al.*, 1995). This