CHEMICAL SENSITIVITY OF JUVENILE BROWN-MARBLED GROUPER, *Epinephelus fuscoguttatus* TO BETAINE AND AMINO ACIDS, AND THEIR PREFERENCE FOR AQUAFEED DEVELOPMENT

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BORNEO MARINE RESEARCH INSTITUTE
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2015

DECLARATION

I hereby declare that the materials in this thesis are my own, except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Grouper aquaculture is expanding but most grouper farmers are challenged by high cost of feed input due to the dependency on the expensive fish meal. Soybean meal (SBM) has high potential to replace fish meal but at high inclusion levels, diets palatability is affected. The use of suitable feeding stimulant (via gustation) or attractant (via olfaction) in non-fish meal based diets might be able to increase feed intake. Amino acids and betaine are commonly used as feeding stimulant and attractant in aquaculture feeds. Unfortunately, little is known about the specific response of grouper to these substances. The present study aimed to investigate the chemical responses in the grouper *(Epinephelus fuscoguttatus)* to feeding stimulant and attractant and evaluate its potential to promote the intake on SBM-based diets through an electrophysiology (Experiment 1), two behavioural (Experiments 2 & 3) and a feeding experiments (Experiment 4). In experiment 1, cysteine was determined as the most sensitive amino acid to the olfaction of grouper, followed by lysine and arginine at the concentration of 10^{-3} M through electro-olfactogram analysis. The threshold level of cysteine was also determined at 10^{-5} M. Experiment 2 was carried out to confirm the results of Experiment 1 through behavioural study. Cysteine yielded the highest frequency in both the swimming and food searching activities, indicating its suitability as food attractant. In this experiment, it was realized that olfaction may not play a very critical role in cultured grouper because they are visual feeder and food are always provided by the farmers. Besides, the decision in food ingestion is mediated by gustation. Consequently, identification of feeding stimulant instead of attractant is more appropriate for the cultured grouper. Therefore, Experiment 3 was conducted to study the taste effect of betaine and the preference for amino acids in tiger grouper using video recording and agar gel pellet as the delivery medium. It was determined that betaine functions best as a feed enhancer in grouper. Betaine itself has low palatability but it enhanced the flavour of the amino acids mixture (also low in palatability) and improved the intake of the agar gel pellet by the fish. Interestingly, only small amount of betaine was required to enhance the flavour of the amino acids mixture (betaine: amino acids mixture 1:4). Among all the amino acids tested, only amino acid A was properly ingested by the fish. The fish acceptance for the amino acid A did not change with growth. In fact, the taste sensitivity for amino acid A improved with the size of fish. In experiment 4, an 8-week feeding trial was conducted to evaluate the potential of betaine and mixture of the selected amino acids to promote intake of SBM-based diets in the grouper. Although the best performance was achieved by the fish fed control diet, supplementation of betaine and the amino acids mixture improved the feed intake and growth in fish fed SBM-based diets. The optimum supplementation level of betaine was also determined at 1.0% based on the higher feed intake and fish growth in this treatment. In conclusion, narrow chemical sensitivity to spectrum of amino acids in the grouper is observed. The findings of the study suggest that the issue with low palatability in SBM-based diets in grouper aquaculture can be solved by the use of species-specific attractant and stimulant.

ABSTRAK

KEPEKAAN KIMIA DAN KECENDERUNGAN JUVENIL KERAPU HARIMAU, Epinephelus fuscoguttatus TERHADAP BETAINE DAN ASID AMINO UNTUK PEMBANGUNAN MAKANAN AKUAKULTUR

Akuakultur kerapu sedang berkembang tetapi kebanyakan penternak menghadapi cabaran kos makanan yang tinggi disebabkan kebergantungan terhadap tepung ikan yang mahal. Tepung kacang soya (SBM) mempunyai potensi yang tinggi untuk menggantikan tepung ikan tetapi pada tahap penggantian yang tinggi ia boleh mengurangkan penerimaan ikan terhadap makanan tersebut. Penggunaan perangsang (melalui deria rasa) atau penyedap (melalui deria bau) makanan ke dalam diet yang bukan berasaskan tepung ikan berkemungkinan untuk meningkatkan pengambilan makanan. Asid amino dan betaine merupakan perangsang dan penyedap makanan yang biasa digunakan dalam makanan akuakultur. Malangnya, tidak banyak yang diketahui berkenaaan respon spesifik kerapu terhadap bahan-bahan ini. Kajian ini dijalankan untuk menyiasat respon kimia kerapu harimau (Epinephelus fuscoguttatus) terhadap bahan perangsang dan penyedap dan menilai potensi bahan tersebut untuk menggalakan pengambilan makanan berasaskan SBM. Dalam eksperimen 1, sistein telah ditentukan sebagai asid amino yang paling sensitif terhadap deria bau kerapu, diikuti oleh lisin dan arginin dalam kepekatan 10^{-3}M melalui analisa elektro-olfaktogram. Ambang kepekatan sistein untuk membangkitkan gerak balas daripada deria bau juga telah ditentukan pada 10^{-5}M. Eksperimen 2 dijalankan untuk mengesahkan keputusan yang diperolehi dalam Eksperimen 1 melalui kajian perlakuan. Sistein menghasilkan kekerapan yang paling tinggi dalam kedua-dua aktiviti berenang dan mencari makanan. Dalam eksperimen ini, disedari bahawa deria bau mungkin tidak memainkan peranan yang terlalu penting kepada kerapu yang diternak untuk mencari makanan kerana kerapu mengesan makanan melalui penglihatan dan makanan sentiasa dibekalkan oleh penternak. Selain itu, keputusan akhir untuk menelan makanan adalah dibantu oleh deria rasa. Oleh itu, Eksperimen 3 dijalankan untuk mengkaji kesan rasa betaine dan kecenderungan terhadap asid amino pada kerapu melalui rakaman video dan penggunaan pelet agar gel sebagai medium perantara. Betaine berfungsi dengan baik sebagai penambah perisa makanan untuk kerapu. Tahap kesedapan betaine adalah rendah tetapi ia meningkatkan rasa campuran asid amino (juga rendah tahap kesedapan) dan pengambilan pelet agar gel oleh ikan. Menariknya, hanya sedikit kandungan betaine memadai untuk menambah-baik rasa campuran asid amino (betaine: campuran asid amino 1:4). Antara semua asid amino yang diuji, hanya asid amino A telah dimakan dengan sempurna oleh ikan. Keutamaan ikan kepada asid amino A tidak berubah dengan tumbesaran. Di samping itu, kepekaan rasa terhadap asid amino A meningkat dengan saiz ikan. Dalam Eksperimen 4, satu percubaan pemberian makanan selama 8 minggu telah dijalankan untuk menilai potensi betaine dan campuran asid amino terpilih untuk menggalakkan pengambilan makanan berasaskan tepung kacang soya oleh ikan kerapu. Walaupun prestasi terbaik dicapai oleh ikan yang diberi diet kawalan, penambahan betaine dan campuran asid amino boleh meningkatkan pengambilan makanan dan tumbesaran ikan yang diberi diet berasaskan tepung kacang soya. Kandungan betaine yang optimum juga telah ditentukan pada 1.0% berdasarkan kepada pengambilan makanan dan tumbesaran ikan yang lebih tinggi di dalam rawatan ini. Sebagai kesimpulan, spektrum sensitiviti kerapu terhadap asid amino adalah sempit. Keputusan kajian ini mencadangkan penggunaan perangsang dan penyedap dapat mengatasi masalah penerimaan ikan terhadap diet berasaskan tepung kacang soya di dalam akuakultur kerapu.

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LIST OF ABBREVIATIONS

AAM	Amino acids mixture
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BET	Betaine
BM	Blood meal
BW	Body weight
CF	Condition factor
EAA	Essential amino acids
EEG	Electro-encephalogram
EGG	Electro-gustogram
EOG	Electro-olfactogram
ERE	Energy retention efficiency
FAO	Food of Agriculture of United Nation
FCR	Feed conversion ratio
FE	Feed efficiency
FI	Feed intake
FM	Fishmeal
FRP	Fiberglass reinforced plastic
FSBM	Fermented soybean meal
FTM	Feather meal
HSI	Hepatosomatic index
IFR	Intraperitoneal fat ratio
IUCN	International Union for Conservation of Nature
IW	Initial weight
LRFF	Live Reef Food Fish
MBM	Meat bone meal
MMBM	Processed meat meal and blood meal mixture
NPU	Net protein utilization
NRE	Nitrogen retention efficiency
NTR	Nerve twig recording
PAG	Pure agar gel
PBM	Poultry by-product meal
PE	Protein efficiency
PER	Protein efficiency ratio
PMS	Processed meat solubles
POM	Poultry offal meal
PR	Protein retention
SBM	Soybean meal
SEAFDEC	Southeast Asian Fisheries Development Center
SGR	Specific growth rate
SPSS	Statistical Package for the Social Sciences
SR	Survival rate
TAP	Terrestrial animal-based protein
TNW	Total nitrogen waste outputs
VSI	Visceral somatic index
WG	Weight gain

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
cm	Centimeter
e.g.	Examples
et al.	And others
etc.	Et cetera; and so on
g kg^{-1}	Gram/ kilogram
g	Gram
kg	Kilogram
L min^{-1}	Liter/minute
L	Liter
M	Molar
mg kg^{-1}	Milligram/ kilogram
mg	Milligram
ml	Milliliter
SD	Standard deviation
sp.	Species
US$	United States Dollar

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Status Quo of Grouper Culture

Groupers are classified under the sub-family Epinephelinae of the family Serranidae. They can be found in tropical and sub-tropical waters of all oceans (Heemstra and Randall, 1993). Groupers are amongst the most popular species in the live reef food fish (LRFF) industry in Asia-Pacific region. Due to their culinary attributes and scarcity, groupers command high prices and are in demand, especially in the LRFF markets of Hong Kong, China (SEAFDEC, 2001). Annually, approximately 15,000 – 20,000 tonnes of groupers have been imported into the LRFF market of Hong Kong with a combined total retail value of around US$ 350 million (US CTI Program., 2013). Retail price of groupers ranges from US$5 to US$180 per kilogram (kg), depending on species, taste, texture, availability and time of year. For example, significant increase of grouper price was reported during festival season (Sadovy *et al.*, 2003). Among the species, the humpback grouper (*Cromileptes altivelis*), giant grouper (*Epinephelus lanceolatus*), leopard coral grouper (*Plectropomus leopardus*), spotted coral grouper (*Plectropomus maculatus*), orange-spotted grouper (*Epinephelus coioides*), brown-marbled grouper (*Epinephelus fuscoguttatus*), camouflage grouper (*Epinephelus polyphekadion*) and the hybrid (*E. fuscoguttatus* ♀× *E. lanceolatus* ♂) are popular for cage aquaculture, especially in China, Vietnam, Malaysia, Thailand, Indonesia, and Taiwan (Lau and Parry, 1999; Sadovy *et al.*, 2003; Ottolenghi *et al.*, 2004; US CTI Program, 2013). According to the FishStat Plus provided by the Food and Agriculture Organization (FAO) of the United Nation, the aquaculture production of groupers from Southeast Asia has increased since 2000. In 2010, the production reached 20000 metric ton which was about 2 times the production in 2006 (see Appendix A). Nonetheless, the rapid expansion of grouper culture is challenged by several issues which include the feeding practice.

1.1.1 Feeding Challenges in Grouper Culture

Most grouper farmers are challenged by high cost of feed production (Bombeo- Tuburan *et al.*, 2001; Pomeroy *et al.*, 2006; Afero *et al.*, 2010). Groupers are strict carnivorous species which require high dietary protein levels (Williams, 2009; Shapawi *et al.*, 2014). The industry is currently relying on low value fish for feeding. However, this is not a longterm sustainable approach (Williams, 2009). For example, the supply of low value fish is inconsistent, given the immense demand for this product (Sim *et al.*, 2005). Commercial dry formulated diets for groupers, mostly based on fish meal (FM) as the protein sources, are available. Nonetheless, the continuous increase of FM price (Tacon and Metian, 2008) has resulted in increased price for formulated diets (Usman *et al.*, 2007; Rachmansyah *et al.*, 2009). In order to maintain consistent feed supply and competitive production cost, alternative protein sources are critically needed.

1.1.2 Replacement of Fish Meal in Grouper Diets and its Constraints

Studies have been conducted to exploit the use of protein from terrestrial animals and plants as replacements of FM in the diets for juvenile groupers with promising findings (Lim *et al.*, 2014). If the diet were formulated correctly, farm-made pelleted feeds with alternative ingredients can provide better growth performance, more efficient feed utilization, and higher survival (Shapawi *et al.*, 2011). However, the acceptable substitution levels of these proteins in the diets are considerably low, particularly for the plant proteins. One of the factors that contribute to this limitation is the poor palatability of the diets (Luo *et al.*, 2004; Shapawi *et al.*, 2013a, b; Shiu *et al.*, In Press). In fact, incorporating high content of the most commonly use plant protein, soybean meal (SBM) in diets also reduced the feed intake of many marine carnivorous species, such as European sea bass (*Dicentrarchus labrax*) (Dias *et al.*, 1997), Asian sea bass (*Lates calcarifer*) (Boonyaratpalin *et al.*, 1998), red snapper (*Lutjanus campechanus*) (Davis *et al.*, 2005), Japanese flounder (*Paralichthys olivaceus*) (Deng *et al.*, 2006), and tiger puffer (*Takifugu rubripes*) (Kikuchi and Furuta, 2009). Nevertheless, specific stimulants or attractants for some of these fish species have been identified and proven to be effective in promoting their feed intake on diets with alternative protein sources (Dias *et al.*, 1997; Deng *et al.*, 2006; Kikuchi and Furuta, 2009; Papatryphon and Soares, 2000a, b). Wang *et al.* (2008) reported that supplementation of squid viscera meal into diets with a blend of rendered animal protein ingredients cannot stimulate feed intake of juvenile *E. coioides*. Apart from this, there is no study conducted to find potential feeding stimulants for groupers. Therefore, investigation on the suitable feeding stimulants to be supplemented in the terrestrial animals or plants proteins-based diets for juvenile groupers is needed.

1.2 Feeding Stimulant, Feed Enhancer, and Attractant in Fish Culture

Feeding stimulant is defined as a substance which has high ingestion rate by the fish. Meanwhile, feed enhancer is referred to a substance which by itself is taste-indifferent but will promote fish intake by enhancing the taste of other chemical substances, such as amino acids. When a substance demonstrates high ability to trigger the feeding desire in fish, it is termed as an attractant. The feeding stimulant and feed enhancer are detected by fish through their gustatory sense while the attractant is detected via the olfactory sense. Therefore, the applications of feeding stimulant and feed enhancer can be done through supplementation in feed while the attractant by introducing into the fish rearing water. Majority of the chemical substances used as the feeding stimulant, feed enhancer and attractant in fish feed are the amino acids and betaine (Kasumyan and Døving, 2003).

1.2.1 Amino Acids and Betaine as Feeding Stimulant, Feed Enhancer, and Attractant

Amino acids and betaine have been proven to function as feeding stimulant, feed enhancer or attractant to promote feed intake in fish, either on the fish meal-based diets or those with alternative protein sources (*e.g.* Takeda *et al*., 1984; Takaoka *et al*., 1995; Dias *et al*., 1997; Fredette *et al*., 2000; Papatryphon and Soares, 2000a, b, 2001; Xue and Cui, 2001; Erteken and Nezaki, 2002; Choi *et al*., 2004; Deng *et al*., 2006; Shankar *et al*., 2008; Tiril *et al*., 2008; Trushenski *et al*., 2011; Tusche *et al*., 2011; Zakipour *et al*., 2012; Fattahi *et al*., 2013; Hill *et al*., 2013; Plaipetch *et al*., 2014). However, there are also exceptional cases when the amino acids and betaine did not function well to promote the feed intake in fish (*e.g.* Dabrowski and Kaushik, 1985; Hughes, 1991, 1993; Kubitza *et al*., 1997; Normandes *et al*., 2006). Such variations could be mainly due to differences in the taste preferences in fish which is reported to be species specific (Kasumyan and Døving, 2003). In addition, olfaction plays important roles in the feeding behaviour of fish (Hara, 2006). In order to identify the suitable amino acids and determine if betaine can be used as the feeding stimulant, feed enhancer or attractant for grouper, information on the stimulatory effectiveness of amino acids and betaine to both olfaction and gustation of grouper, and their behavioral responses to these chemical substances are required. However, such information is not available up-to date although there are several reports on the morphology of sense organs in grouper larvae (Boglione *et al*., 1999; Mukai *et al*., 2006; Lim and Mukai, 2014).

1.3 Olfaction and Gustation in Fish

Olfaction and gustation are the two major chemical senses in fish, and they are facilitated by the olfactory organ and the taste buds (both intra- and extra-oral types), respectively. The information received through olfaction is delivered to the brain via the olfactory nerve (cranial nerve I) while that received through gustation is delivered via the facial (VII), glossopharyngeal (IX), and vagus (X) nerves. The olfactory nerve is connected to the olfactory lobe while the facial, glossopharyngeal and vagus nerves are connected to the telencephalic region of the brain (including the facial and vagal lobes) (Hara, 2011). For understanding the stimulatory effectiveness of chemical substances to the olfaction and gustation in order to identify the suitable attractant and feeding stimulant for the fish, electrophysiology and behavioural experiments are the standard practices to be carried out.

1.3.1 Electrophysiology on Fish Chemical Senses and Behavioural Experiments to Examine Fish Response to Chemical Substances

Electrophysiology is the science that studies the flow of ions in biological tissues. To be precise, it refers to the electrical recording techniques that enable the measurement of this flow. The classical electrophysiology techniques include placing electrodes into various preparations of biological tissue to measures the changes in voltage or electric current. The pioneer work of electrophysiology on fish chemical senses has been reported by Konishi and Zotterman (1961) on carp (*Cyprinus carpio*). After then, many electrophysiological studies have been conducted on both olfaction and gustation of fish, but mainly on the catfishes, cyprinids and salmonids (*e.g.* Hara, 1973; Carpio, 1978; Funakoshi *et al.*, 1981; Byrd and Carpio, 1982; Marui *et al.*, 1983a, b; Carpio and Byrd, 1984; Johnsen *et al.*, 1988, 1990; Michel *et al.*, 1993; Hara *et al.*, 1994; Kitada and Hara, 1994; Michel and Lubomudrov, 1995; Michel and Derbidge, 1997; Hara *et al.*, 1999; Kohbara and Carpio, 2001; Yacoob *et al.*, 2001; Yacoob *et al.*, 2002; Yamashita *et al.*, 2006; Dolensek and Valentincic, 2010). Although electrophysiology is an effective way for researchers to understand the stimulatory effectiveness of the chemical substances to the fish, the experimental results did not imply that whether the particular substance is a favor or a deterrent for the fish. Therefore, behavioural experiment is a must especially for the feeding stimulant and attractant identification, and confirming the fish preference for the selected chemical substances (*e.g.* Sutterlin and Sutterlin, 1970; Carr and Chaney, 1976; Carr, 1976; Carr *et al.*, 1977; Hidaka *et al.*, 1978; Mackie and Adron, 1978; Mackie and Mitchell, 1983; Mearns, 1985, 1986; Mearns *et al.*, 1987; Carr and Derby, 1986;

Jones, 1989; Stradmeyer, 1989; Kasumyan and Morsi, 1996; Oikawa and March, 1997; Bórquez and Cerqueira, 1998; Kasumyan, 1999; Valentincic *et al.*, 1999; Kasumyan and Prokopova, 2001; Hara, 2006; Jafari Shamushaki *et al.*, 2008; Goli *et al.*, 2013).

1.4 Brown-Marbled Grouper, *Epinephelus fuscoguttatus*

The brown-marbled grouper or tiger grouper, *E. fuscoguttatus* 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). It is one of the most valuable species of groupers in terms of export value, especially to countries with significantly large Chinese populations, such as Hong Kong, Taiwan Province of China, Singapore, Malaysia, Singapore and Thailand (Lau and Parry, 1999; Ottolenghi *et al.*, 2004) where groupers are considered a delicacy especially during festivals. The constant high demand has led to over- fishing of the wild *E. fuscoguttatus* 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). Nonetheless, the culture of *E. fuscoguttatus* larvae is now widely practiced in hatcheries because the species is easily grown into broodstocks with high fecundity, and the larvae show fast growth and high survival (Lim, 1993). Availability of *E. fuscoguttatus* in the hatchery has facilitated studies on its biology at different life stages (*e.g.* Kohno *et al.*, 1993; Ching *et al.*, 2012; Muhammadar *et al.*, 2012; Mukai *et al.*, 2012a, b; Oedjoe *et al.*, 2012a, b; Salari *et al.*, 2012; Cheng *et al.*, 2013; Firdaus-Nawi *et al.*, 2013; Tan *et al.*, 2013; Lim and Mukai, 2014; Shapawi *et al.*, 2014). Although Lim and Mukai (2014) reported the morphogenesis of sense organs in larvae of *E. fuscoguttatus*, knowledge on the abilities of chemical senses in grouper species is still unknown.

1.5 Significance of Study

Alternative protein sources have been exploited to replace the fish meal used in grouper diets. High inclusion levels of these proteins can reduce the diets palatability but such problem can be solved by the supplementation of suitable dietary feeding stimulant, feed enhancer or attractant, such as amino acids and betaine. However, it is unknown that which amino acids and if betaine are suitable for groupers as the abilities of chemical senses in fish are species-specific. Therefore, there is a need to study the chemical sensitivity of grouper to amino acids and betaine, select the suitable one and evaluate their potential as the feeding stimulant, feed enhancer or attractant to promote intake of diets with alternative protein source.

1.6 General Objectives

The main aim of the present study is to identify the suitable amino acids, and also to determine if betaine can function as the feeding stimulant, feed enhancer or attractant to promote the intake of soybean meal-based diet in the juvenile *E. fuscoguttatus*. Four specific objectives are as below:

a) To determine the stimulatory effectiveness of amino acids and betaine to the olfactory sense of grouper using electrophysiology
b) To examine the behavioural response of grouper to amino acids in relation to olfaction
c) To determine the taste effect of betaine and the preference for amino acids in the grouper behaviourally
d) To evaluate the potential of betaine and amino acids supplementations to promote intake of soybean meal-based diet in the grouper

CHAPTER 2

LITERATURE REVIEW

2.1 Feeding Management in Grouper Culture

The efforts to breed groupers in captivity have been started since 1970s (Lim, 1993). Following the successful breeding for many species of grouper (*e.g.* Chen *et al.*, 1977; Fukuhara, 1989; Tucker *et al.*, 1991; Lim, 1993; Alava *et al.*, 1993), the protocols to culture groupers from larval to juvenile stages were also developed intensively (see Rimmer *et al.*, 2004; Liao and Leaño, 2008; Sugama *et al.*, 2012). Feeding management is one of the key factors to successful grouper culture as the proper feeding practices can enhance the fish health, growth and survival, and increase fish production (Chou *et al.*, 2008). The feeding management and protocols in grouper culture generally comprise two main phases, which are those for the 1) grouper larvae and early juvenile, and the 2) grow-out fish. Information on these feeding management and protocols was further described in the following sections.

2.1.1 Feeding Regimes for Grouper Larvae and Early Juvenile

There are several major aspects to be concerned in the feeding management for grouper larvae and early juvenile to ensure the fish were fed properly. These aspects were as listed in below:

a. Food Types

The mouth gap size of grouper larvae is very small, and the gap size increased with the larval growth (Marte, 2003). Along the larval rearing period, therefore, suitable size of food should be provided to the larvae at different developmental stages. In common, groupers larvae started exogenous feeding after 2 - 3 days from hatching, and they were fed with rotifers (60 – 180 µm) in hatcheries (Knuckey *et al.*, 2004; Sugama *et al.*, 2012). Microalgae such as *Nannochloropsis* sp. were also introduced to the rearing tank as live food (Sugama *et al.*, 2012), and to maintain quality of the rearing water environment by preventing the blooming of pathogenic bacteria (Sharifah and Eguchi, 2011). Following the fish growth, larger size of zooplanktons such as brine shrimp (*Artemia*) nauplii and copepods were used as the foods (Doi *et al.*, 1997; Toledo *et al.*, 1997, 1999), and the operation of weaning the fish to microdiets also can be started (Sugama *et al.*, 2012; García-Ortega *et al.*, 2013). Although such standard feeding protocol has already been established (Sugama *et al.*, 2012; Ma *et al.*, 2013), many studies have been conducted to discover new types of live food to improve the feeding efficiencies of grouper larvae. For examples, Eusebio *et al.* (2010) reported that mysids (*Mesopodopsis orientalis*) can be used to replace Artemia nauplii as live food for grouper larvae, from the nutritional aspect. Reyes *et al.* (2011) showed that the free-living nematode (*Panagrellus redivivus*) can be used as live food for grouper larvae at first feeding. Chen *et al.* (2012) also reported the potential of *Picochlorum* to replace *Nannochloropsis* for grouper larval rearing.

b. Environmental Factors

Environmental factors are important to be concerned in order to enhance the feeding efficiencies of the grouper larvae and early juveniles. Marine finfish larvae including grouper species (Mukai *et al.*, 2012a, b; Lim and Mukai, 2014) are commonly known to be visual feeders; therefore, ambient lighting condition is essential for the grouper larvae and early juveniles to detect food. Indeed, Yoseda *et al.* (2008) reported that the optimum light condition for the feeding of larval leopard coral grouper (*Plectropomus leopardus*) was 3000 lux. Mukai *et al.* (2012b) also reported the minimum requirement in light intensity for the early juvenile of brown-marbled grouper (*E. fuscoguttatus*) to feed was 10 lux.

Other than lighting conditions, water flow field in the larval rearing tank is also proven important to improve the efficiency of first feeding in grouper larvae by distributing the zooplanktons evenly inside the tank (Sakakura *et al.*, 2007).

2.1.2 Feeding Regimes for Grouper at Grow-Out Stage

The feeding practices and management for juvenile groupers at grow-out stage have been well reviewed by Williams (2009). In general, how to reduce the high feed cost is the main concern in the feeding regimes for grouper grow-out culture. As mentioned earlier in Section 1.1.1., the conventional feeding practice with low value fish is not a sustainable approach yet commercially available formulated diets are expensive due to the reliance on fish meal as the main dietary protein source. Therefore, substitution of fish meal in the grouper practical diets is the way to reduce the feed production cost.