EFFECT OF VARYING DIETARY PROTEIN LEVELS IN SNAKEHEAD BROODSTOCK, *Channa striata* (Bloch, 1793) ON EGG, LARVAL QUALITY, DIGESTIVE ENZYME ACTIVITY AND ONTOGENIC DEVELOPMENT OF THE DIGESTIVE TRACT

By

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ABBREVIATION

FAO	Fisheries and Agricultural Organization
NOR	Nucleolar organizer
DAH	Day after hatch
FAA	Free amino acid
DPH	Day prior hatch
IUBMB	International union of biochemistry and molecular biology
CCK	Cholecystokinin
BAL	Bile salt activated lipase
HCL	Hydrochloric acid
AOAC	Association of official analytical chemists
H_2SO_4	Sulphuric acid
K_2SO_4	Potassium sulphate
$CuSO_4$	Copper sulphate
NaOH	Sodium hydroxide
HPLC	High performance liquid chromatography
AABA	Alpha amino butyric acid
HEWL	Hen egg white lysozyme
IAA	Indispensable amino acid
DAA	Dispensable amino acid
GSI	Gonadosomatic index
EDTA	Ethylenediaminetetraacetic acid
PVA	Polyvinyl alcohol
TCA	Trichloroacetic acid
BSA	Bovine serum albumin
DDH2O	Double distilled water
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid

KESAN PEMAKANAN PROTEIN YANG BERBEZA DALAM INDUK IKAN HARUAN, *Channa striata* (Bloch, 1793) PADA KUALITI TELUR, LARVA, AKTIVITI ENZIM PENCERNAAN DAN PERKEMBANGAN ONTOGENI SALURAN PENCERNAAN

Abstrak

Kajian ini telah dijalankan untuk menentukan kualiti telur dan larva dari induk ikan haruan yang telah diberikan diet yang mengandungi pelbagai paras protein. Juvenil ikan haruan (min berat 54.5 ± 2.6 g) telah dipilih secara rawak dan disimpan di dalam tangki kanvas, 4m x 1m x 1m (panjang x lebar x kedalaman) pada kepadatan 60 ekor ikan secara tiga replikasi untuk setiap rawatan. Empat diet eksperimen yang mengandungi paras protein yang berbeza (350, 400, 450 dan 500 g kg^{-1}) telah diformulasi dan diberi makan dua kali sehari, pada jam 10:00 pagi dan 17:00 petang selama 6 bulan sehingga pembenihan. Selepas enam bulan pada diet eksperimen, induk jantan dan betina telah dipilih secara rawak untuk pembiakan pada nisbah 1: 1. Dua siri eksperimen telah dijalankan untuk mencapai objektif kajian ini. Pertama, kualiti telur dan larva telah dinilai berdasarkan biometrik telur dan larva, lisosom telur, toleransi larva kepada kebuluran, toleransi kepada tekanan suhu dan pH dan kandungan asid amino. Saiz telur dan larva serta toleransi larva kepada kebuluran adalah lebih tinggi ($P < 0.05$) pada diet mengandungi 400 g kg^{-1} dan ke atas paras protein. Aktiviti lisosom menunjukkan perbezaan tertinggi secara signifikan ($P < 0.05$) dalam telur ikan yang diperolehi daripada induk diberi makan diet mengandungi 450 g kg^{-1} paras protein. Larva daripada ikan haruan yang diberi diet mengandungi 450 g kg^{-1} protein menunjukkan kemandirian yang terbaik pada suhu 28°C dan pH 7. Keseluruhan profil asid amino dalam telur dan larva menunjukkan kandungan yang lebih tinggi untuk asid amino diperlukan (IAA) dan

asid amino tidak diperlukan (DAA) di 450 g kg^{-1} kumpulan protein berbanding diet protein yang lain. Dalam eksperimen kedua, perkembangan larva telah dinilai berdasarkan morphometrik, ontogeni dan aktiviti enzim pencernaan dalam larva. Secara amnya, hubungan yang linear dapat diperhatikan dalam perkembangan morphometric larva dari segi mulut, kepala dan mata. Larva yang diperolehi daripada induk haruan yang diberi diet protein yang tertinggi, 450 dan 500 g kg^{-1} menunjukkan saiz mulut, kepala dan mata yang lebih besar berbanding diet protein yang lain. Seterusnya, perkembangan ontogeni larva mendedahkan perbezaan antara kumpulan protein dari segi perkembangan organ pada 5, 7 dan 21 DAH. Perkembangan saluran pencernaan yang lengkap dalam larva haruan dicapai pada 21 DAH untuk kumpulan protein 400 g kg^{-1} dan ke atas. Dari segi pemerhatian, ia boleh dijelaskan bahawa aktiviti enzim pencernaan dipengaruhi oleh peekembangan saluran pencernaan larva dan juga pengambilan diet protein oleh induk haruan. Berdasarkan keputusan yang diperolehi dari pada eksperimen ini, pengambilan diet yang mengandungi protein 450 g kg^{-1} oleh induk haruan adalah disyorkan untuk meningkatkan kualiti telur dan larva, kemandirian hidup larva, profil asid amino, aktiviti lisosom dalam telur, morphometrik dan perkembangan ontogeni. Kesimpulannya, kandungan protein sebanyak 450 g kg^{-1} dalam diet induk haruan dapat meningkatkan kualiti telur dan larva serta perkembangan larva pada peringkat awal kehidupan.

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Abstract

The present study was carried out to determine the quality of eggs and larvae from *Channa striata* broodstock that were fed different levels of dietary protein. *C. striata* juveniles (mean weight 54.5 ± 2.6 g) were randomly selected and stocked in 4m x 1m x 1m (length x width x depth) canvas tanks at a density of 60 fishes per tank in triplicates for each treatment. Four experimental diets containing varying protein levels (350, 400, 450 and 500 g kg^{-1}) were formulated and fed twice daily to satiation, at 10:00 a.m. and 17:00 p.m. for 6 months until spawning. After six months on the experimental diets, matured male and female brood fish were randomly selected for artificial spawning at the ratio of 1: 1. A series of two experiments were conducted to achieve the objectives of this current study. First, the quality of eggs and larvae were assessed based on biometric of egg and larvae, egg lysozyme, tolerance of larvae to starvation, tolerance to stress triggers such as temperature and pH and amino acid content. The size of the eggs and larvae as well as the tolerance of larvae to starvation was significantly higher ($P < 0.05$) at 400 g kg^{-1} and above dietary protein. A significantly highest ($P < 0.05$) lysozyme activity was noticed in the eggs obtained from the brood fish fed 450 g kg^{-1} protein diet over the remaining treatments. The survival of *C. striata* larvae was best at 28°C and pH 7, with highest larval survival obtained from the brood fish fed the, 450 g kg^{-1} dietary protein. The overall amino acid profile of eggs and larvae showed a significantly higher indispensable amino acids (IAA) and dispensable amino acids (DAA) at 450 g kg^{-1} protein group over all the treatments. In the second experiment, the development of larvae was assessed based on the morphometric and ontogenic development as well as the digestive enzyme activity. Generally, a positive relationship was observed in the morphometric development of the larvae in terms of mouth, head and eye. The larvae obtained from the brood fish fed the high dietary protein, 450 and 500 g kg^{-1} showed a wider mouth, head and eye diameter over the remaining treatments. Further, the study of the ontogenic development of larvae revealed differences in terms of organ development among the treatments at 5, 7 and 21 DAH. The complete development of gastrointestinal tract in *C. striata* larvae was achieved by 21 DAH for 400 g kg^{-1} and above dietary protein treatments. From the current observation, it can be elucidated that the digestive enzyme activities are influenced by the development of the digestive tract of larvae as well as the intake of dietary protein by the brood fish. Based on the result obtained from the current finding, the intake of dietary protein at 450 g kg^{-1} by the *C. striata* broodstock is recommended to improve the egg and larval quality, survival of larvae, amino acid profile, lysozyme concentration in eggs, morphometric and ontogenic development. In conclusion, the inclusion of protein at 450 g kg^{-1} in the diet of *C. striata* broodstock can improve the egg and larval quality as well as the development of larvae at the early stage of life.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In the last few decades, aquaculture has seen tremendous growth from a production of one million tonnes in early 1950 to a 70.5 million tonnes production by 2013 (FAO, 2014). This unpredicted growth of aquaculture was driven by the need to minimize the adverse effect from capture fisheries. In addition, the aquaculture industry has developed rapidly to reduce the pressure in capture fisheries to meet the growing global demand for fish. Aquaculture is the cultivation of marine or freshwater animals, particularly fish, shellfish and certain invertebrates under controlled conditions.

The freshwater aquaculture industry is dominated by fish, with numbers reaching 41.9 million metric tons in 2012 out of the total global production of 158 million metric tons (FAO, 2014). *Channa striata* has gained the attention of many researchers at present and has been chosen as foremost species among some of the freshwater fish species that has been widely cultivated through aquaculture. The aquaculture production of *C. striata* was 480 tonnes in the early 1950 and this number increased 31 times more to reach 14944 tonnes in 2012 (FAO, 2013). This species has high economic importance and widely cultured in Vietnam, Thailand, Philippines, India and Pakistan (Courtenay & Williams, 2004).

Channa striata, (Bloch, 1973) is a freshwater fish that belongs to the Channidae family and it is commonly known as snakehead. This fish is a strictly carnivorous, obligate air-breathing, freshwater fish species which are indigenous to many tropical countries (Wee, 1982). According to Mat Jais (2007), *C. striata* can adapt and inhabit aquatic environments with a pH ranged from 4.3-7.9, a temperature of 20.7-26.4°C, water turbidity of 2-268 ppm and dissolved oxygen at about 1.20-6.10 ppm. Ponds, small lakes, agricultural canals, small rivers, water catchments and rice fields become habitats for *C. striata* due to its wide range of adaptability (Sarowar et al., 2010). Snakeheads can survive without water for a number of months, using their air-breathing mechanism to absorb dissolved oxygen through their moist skin (Smith, 1945).

A genetic variability study conducted by Mat Jais (2007) successfully proved that this fish has been in Malaysia water bodies for more than 600 000 years. The demand for snakehead has been increasing over the years as its delicate boneless meat contains wound healing properties that are widely used in postoperative medicine particularly to reduce pain and discomfort. The mucous of *C. striata* has a role as anti-microbial, anti-inflammatories, anti-nociceptives, in cell proliferation as well as in the induction of platelet aggregation. These qualities also make it an ideal candidate for pharmacology studies (Mat Jais, 2007). *Channa striata* is reported to have high protein content, (78.32%) and low lipid level, (2.08%) (Mat Jais, 2007). The healing properties in the snakehead has been attributed to the high content on glutamic acid, aspartic acid and lysine ranging from 9.7 to 21.7% and the fatty acids, arachidonic acid and decosahexanoic acid, ranging 19.02% and 15.80% (Zuraini et al., 2006). All these traits highlighted this particular species as a superior model for aquaculture.

However, to date the nutrition and feeding management of *C. striata* remains under reported and has necessitated studies to investigate the effect of varying nutrients in *C. striata* at different stages of life. The egg and larval stage are two foremost crucial stages in the life cycle of all the farmed species, as they are completely dependent on the broodstock for their nutrition (Brooks et al., 1997). The quality of the brood fish in captivity can be influenced by several factors including conditions in captivity, fish age, feeding duration and broodstock nutrition (Nguyen et al., 2012). Among all of these factors, broodstock nutrition plays a significant influence on the quality of eggs and larvae. Therefore the formulation of appropriate broodstock diet is gaining attention among aquaculturists (Norambuena et al., 2012). To date, studies on *Channa striata* nutrition have focused on the nutrient requirement of the larvae (War et al., 2011), fry (Sarowar et al., 2010) and fingerlings (Aliyu-Paiko et al., 2010; Srivastava et al., 2012). However, among the nutrients necessary, there is no literature available on the protein requirement in *C. striata* brood fish, for the production of good quality eggs and larvae.

1.2 Problem Statements

Presently, the broodstock nutrition and poor reproductive development are major issues hindering the sustainable production of *Channa striata* seed in the hatchery. The feeding management in hatcheries have improved with the use of trash fish or in combination with commercial diets but, these diets is lack of sufficient nutrients for the fish. The use of inadequate diets with limited nutrients leads to poor growth and affects the health status of the brood fish.

Production of good quality eggs and larvae is a serious bottleneck in the sustainable development of *C. striata* in aquaculture. This is because; the production of good quality eggs completely relies on the maturation of oocytes (Brooks et al., 1997) in the ovary. This progressive development of oocytes in the fish can be hindered by many factors such as inadequate nutrition, genetics, physiological stress and environmental factors (Brooks et al., 1997). However, this situation can be overcome by the nutritional manipulation of the brood fish, which can result in improved quality of eggs and larvae. Therefore, the current study was conducted to investigate the quality of eggs and larvae obtained from a diet of varying protein levels, bearing in mind that *C. striata* is strictly carnivorous fish and requires higher levels of protein for a healthy life cycle.

1.3 Research Objectives

The main objective of this study is to determine the optimal dietary protein in *Channa striata* broodstock to produce quality eggs and larvae.

Specific objectives:

1. To determine the quality of eggs and larvae obtained from broodstock fed different dietary protein intake by growth, immune response and the composition of amino acid.

2. To determine the effects of different dietary protein intake in snakehead broodstock on larval capacity to endure the stress such as starvation, extreme temperature and pH.

3. To evaluate the effect of different dietary protein intake in snakehead broodstock to the morphometry, ontogenic development of the digestive tract, digestive enzyme activity in fish larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture

Aquaculture plays a vital role to fulfil the world demand to support both the sea and inland water capture fisheries which are considered as completely exploited. The data published by FAO (2014) stated that the total capture fisheries and aquaculture production supplied for food consumption was 128 million tonnes in 2010 and increased to 131 million tonnes in 2011. The rapid increase of nearly 3 million tonnes in one year showed the significant role of aquaculture in daily human consumption. Aquaculture is expanding in all aspects, mainly, in terms of species, areas as well as intensification of aquaculture products to consumer needs. Roughly, about 600 species are cultured in captivity worldwide for the high demand for global production with improved farming systems and technological sophistication in freshwater, brackish and marine water.

Sustainable development of aquaculture relies heavily on nutrition and feeding practices. A diet of cultured fish that is economically viable and nutritionally balanced is critical in aquaculture as fish feed consumes 40-50% of the entire production costs (Craig & Helfrich, 2009). According to Hasan (2000), good quality and quantity of nutrients provided will enhance the growth, reproduction and health of cultured species in aquaculture. In aquaculture, fishes are one of the more favoured choices of aquatic animals as it is an important source of protein for human consumption (Finegold, 2009). Fish is the most affordable source of protein that is rich in iron, zinc, magnesium, iodine, phosphorus and vitamin A. The output from aquaculture is not only responsible for fulfilling global demand, but also provides a means as a low-cost nutritional food source for the masses. According to Finegold

(2009), fish has been called as 'rich food for poor people' due to its unique characteristics to promote health, combating disease and reduce child mortality.

Besides feeding cost, another most critical issue that often becomes a challenge to the aquaculturist is the selection of suitable species for culture. The local availability, easy adaptation to the culture conditions, higher nutritive value and ultimate market value are among the important characteristics considered in the species selection for aquaculture.

Wide adaptation and naturally hardy are the two main points that have attracted attention among fish farmers to choose the snakehead and other air breathing fish to culture without much investment (Hossain et al., 2008). Among other freshwater species that have been selected for culture on a commercial scale, *C. striata* plays a significant role as important cultivable indigenous finfish in Asia (War et al., 2011). According to a report published by FAO (2013), the global production of *C. striata* aquaculture was recorded as about 14944 tonnes in 2012. In fact, this total had increased triple times more from 5448 tonnes in 2003. However, the global capture production had broken the record with 62155 tonnes in 2003 and increased to 76793 in 2012. Previous reports on this fish stated that it is widely cultured in Vietnam, Thailand, Philippines, India and Pakistan (Courtenay & Williams, 2004; FAO, 2013).

2.2 *Channa striata*

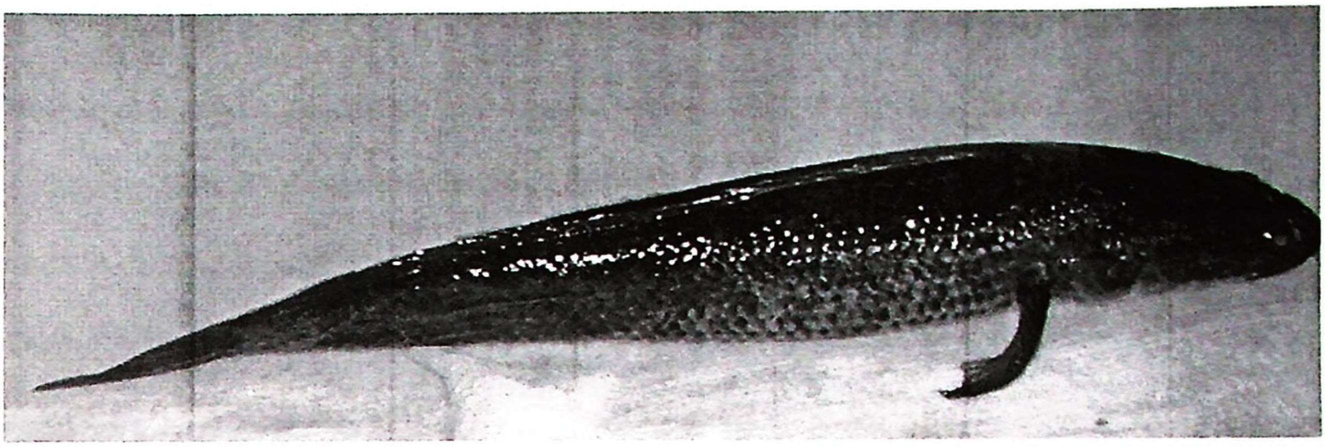

Plate 2.1: The snakehead fish, *Channa striata* (Original source, taken during the study period)

Among freshwater species, *Channa striata* or commonly known as snakehead murrel is widely selected for culture due to its special characteristics as rapid growth, high consumer acceptance, medicinal values, delicious taste, great adaptation to the environment and good market worldwide. There are about 28-30 species of Channa reported around the world with seven species in Peninsular Malaysia (Lee & Ng, 1994; War et al., 2011). According to Courtenay & Williams, (2004), *C. striata* is the most important and widely cultured fish among other *Channa* spp such as *C. argus, C. maculata, C. asiatica, C. micropeltes, C. marulius, C. punctata* and *Parachanna obscura*. In Asia, *Channa* spp widely selected as the most favoured food, especially in Southeast Asia and India, also chosen for the aquarium trade in Japan, Europe and North America. Besides this, *Channa* spp also hugely contribute as a biological control to overcome mosquito larvae, unwanted pest problems and overproduction of tilapia in culture ponds due to the fish cannibalistic characteristics (Courtenay & Williams, 2004).

2.3 Distribution and Morphology of *Channa striata*

Channa striata is a carnivorous and freshwater fish that belongs to order Perciformes and Channidae family (Courtenay & Williams, 2004). *Channa striata* commonly known as a snakehead due to the special presence of scales on its head, its snake-like movement and the eyes that are fixed on the dorso-lateral point at the anterior part of its flat head (Courtenay & Williams, 2004). This species commonly inhabit in lakes, ponds, reservoirs, canals, drain, rice fields, streams, polluted lower zone and even in pH>5 black water (Lee & Ng, 1994). This fish can tolerate a wide range of temperature (11-40°C), pH (4.25-7.90), dissolved oxygen (1.20-6.10 ppm) and water turbidity (2-268 ppm) (Mat Jais, 2007). In addition, Varma (1979) stated that *C. striata* is able to survive up to 90% at pH 3.10 and 100% in pH 4.25-9.40.

Snakehead fish is a carnivore that feeds on fish, snakes, tadpoles, frogs, snails, prawns, shrimps and worms (Lee & Ng, 1994). Interesting fact by Dasgupta (2000) stated that *C. striata* is the most carnivorous species among *C. punctatus, C. marulius* and *C. orientalis*, evident by the presence of large buccal cavity, longer esophagus, larger stomach and shorter intestine that are the characteristics of carnivorous species. For an adult snakehead, the range of pelvic or anal length is 3.5-8.8cm, inter orbital width is 5.1-8.9cm, post orbital head length is 20.2-34.3cm, caudal length is 11.9-23.3cm, head length is 28.6-37.2cm and the preopercular length is ranging from 6.2-26.4cm (Lee & Ng, 1994).

The body colour of *C. striata* varies at different life stages. The adult snakehead fish will be black or dark grey in colour, while the larvae to fry will be a mixture of black and orange. Amornsakun et al (2011) reported that the female snakehead fish will be sexually mature when they reach 26.45 ± 3.07 cm in total length and 167.4 ± 48.09 g in body weight. The male and female snakehead fish are easily distinguishable when they are sexually matured. Bulging and soft abdomen is the physical change occurring in the female fish when they are sexually matured while male fish have flat abdomen. Sexually matured snakeheads can breed two times or more in a year if the environment favours. Interestingly, upon spawning, aggressive behaviour is noticeable in male *C. striata* to protect the fertilized eggs. In addition, this carnivorous fish also tend to show parental behaviour to their larvae until they reach fingerling stage (Marimuthu et al., 2001).

Although *C. striata* is a native fish species in some Asian countries, this fish has been introduced to Madagascar, Philippines, China, Mauritius and Fiji (Courtenay & Williams, 2004). Some studies of molecular genetics revealed the evolution of channid fishes. A study conducted by Naorem and Bhagirath (2006) stated that nucleolar organizer (NOR) that is a part of a chromosome associated with nucleolus after the nuclear division and the differentiation of constitutive heterochromatin (C-band) are among the factors that are responsible for the evolution of *Channa* species.

2.4 Fish Nutrition

Fish nutrition is a fundamental aspect in aquaculture that need a through consideration in feed preparation as it involves the ability of transforming dietary nutrient into molecules absorbed and deposited in tissue and muscle (Liu, 2011). Fish requires high quality and a balanced diet, particularly when reared in high densities to ensure rapid growth and for a healthy life cycle (Craig & Helfrich, 2009).

Macronutrient and micronutrient are two components of nutrient which provide energy from an absorbed end product when the nutrients are oxidized and metabolized (Cho, 1983). Protein, lipid and carbohydrate function as macronutrients for energy, growth and tissue building while micronutrients such as vitamin and mineral salts are useful as cofactors in enzymatic reactions. For a fish, nutrients existed in the pellets are digested in the gut by enzymes right after ingestion of the food. Brush border of the microvillus in intestinal wall functions as a major tool to absorb nutrients digested by the enzymes and transport them all around the body through the bloodstream for maximum utilization. When all the recommended nutrients are at the appropriate level, fish growth will be typically fast and can maintain a healthy condition.

2.5 Broodstock nutrition

Broodstock nutrition has received much attention with respect to its effect on the quality of eggs (Izquierdo et al., 2001; Bobe & Labbe, 2010), in turn resulting in optimum larval production. During the last two decades, consideration has been given to the nutrient level provided in the fish broodstocks diet as this plays a vital role in fecundity and oocyte maturity in incessant spawners with short vitellogenesis (Izquierdo et al., 2001). It has also been stated that broodstock diets do influence the oocyte size, chemical composition of egg, egg hatchability, larval survival and fish growth (Cerdá et al., 1994). Therefore, high quality broodstock is necessary to ensure the sustainability production of aquaculture. Although the production and quality of the seed is directly influenced by a number of factors such as brood fish age, spawning cycle, over ripening process, captivity condition, maternal hormones and genetics, hence manipulation of the nutrition in the diet of the broodstock has been an effective strategy to enhance seed production (Izquierdo et al., 2001; Nguyen et al., 2012). Broodstock management with a nutritious diet and suitable environmental condition in captivity is very much important to prevent reproductive dysfunction and to ensure the successful development of aquaculture.

2.5.1 General nutrient requirement for broodstock

Fish requires energy for various life processes, including growth, reproduction and other activities. The asset of nutrients that is released during the metabolic oxidation of protein, lipid and carbohydrates provides energy to the fish to maintain a healthy life cycle. The energy requirement of a fish is species specific. For instance, studies have reported that carnivorous fish requires more protein and lipid for growth compared to the herbivorous (Tacon, 1995; Hasan, 2000). Besides growth and maintenance of daily activity, the energy from the metabolism of essential nutrients is largely used for gonad development in broodstock. Several studies have reported the role of various nutrients in the development of gonad such as protein (Watanabe et al., 1985; Gunasekera et al., 1995; Muchlisin, 2004), fatty acids (Zohar et al., 1995; Izquierdo et al., 2011), carbohydrate (Hemre et al., 1995) and vitamins (Palace & Werner, 2006).

2.5.2 Protein requirement for broodstock

Protein is known as the foremost nutrient in both animal and plant species for the multi role as enzyme, hormones, receptors, antibodies, genetic materials and transport proteins. Amino acids are the building blocks of protein made from positively charged amino $-NH_2$ and negatively charged carboxyl $-COOH$ group (Campbell & Farrell, 2006). In fish, protein digestion begins after the ingestion of the food and when the food enters the stomach. Protein digestion occurs with the secretion of acidic protease, pepsin, and continues in the intestines with the secretion of alkaline proteases trypsin and chemotrypsin by the pancreas. The end products obtained from the protein digestion are free amino acids, which are then transported via the bloodstream to different organs in the body.

Protein represents the largest portion of feeding costs among all the crucial nutrients that are needed in formulating a balanced fish diet. The protein ingredient in the diet can be modified based on the specific requirement of the species either using plant or animal protein. Recently, there is a blooming interest in animal protein alternatives, either by partially or completely replacing the fish meal with plant protein sources. For instance, soybean meal, corn gluten meal, cassava meal, cottonseed meal and lupins are some of the important plant protein sources which can replace the expensive animal protein effectively to reduce the feeding cost (Lim et al., 2008). The respective ingredients that replace the animal protein, especially fish meal should be nutritionally balanced and well digestible besides being favourable economically. Many studies have shown that fish meal can be replaced with alternative protein sources without causing any harmful effects on fish growth, behaviour and feed effectiveness. For example, Mambrini et al (1999) stated that the fish meal can be replaced with soy protein up to 50% without affecting the performance in rainbow trout.

Fish mainly requires 30% to 50% of protein from the diet for optimal utilization (De Silve & Anderson, 1995). Some studies have been conducted on marine and freshwater fish species to evaluate their optimum level of dietary protein requirement. For instance, studies on dietary protein of Nile tilapia female broodstock revealed that 35% crude protein is an optimum level to produce better quality of eggs and larvae (Gunasekera et al., 1996). Another comparative study conducted by Santiago et al (1985) on Nile tilapia showed that the brood fish fed with 40% protein diet tend to produce larger quantities of fry compared to the brood fish fed 20% dietary protein. Continuously, bagrid catfish (*Mystus nemurus*) fed at 35% dietary protein supported growth and better egg quality, though the protein content of the egg is higher from the fish fed at 40% dietary protein (Abidin et al., 2006). Melo et al (2012) also reported that the best reproductive performance was observed by 27% of dietary protein in the female brood of the silver catfish (*Rhamdia quelen*).

Previously, a study conducted on *C. striata* fry by Muntaziana et al (2013) stated that this carnivorous fish showed higher protein content in muscle with the consumption of bloodworms which contained 68.93% of protein. Hence, the uptake of trash fish with 77.2% of protein greatly influences the specific growth rate and weight gain of *C. striata* fry. This result indicates that a higher level of dietary protein is required to support better growth, though the 68.9% protein in the live feed is adequate enough to increase the protein deposition in the muscle. Additionally, Mohanty and Samantaray (1996) also reported that *C. striata* fry fed at 55% dietary protein with fish meal dominant diet showed a better weight gain, specific growth rate and daily tissue protein deposition. Another study revealed that the supplementation of diet with 49.72% protein and 13.54% lipid promotes a better growth in *C. striata* fry (Srivastava et al., 2012).

Several studies were also conducted on other carnivorous fish to understand their optimum level of protein requirement such as red drum (40-45%; Serrano et al., 1992), mutton snapper (45%; Watanabe et al., 2001), mangrove red snapper (42.5-50%; Catacutan et al., 2001), spotted rose snapper (40-45%; García-ortega, 2009) and bullsye puffer (50%; García-ortega, 2009). The literature has shown that the protein requirement in carnivorous fish is higher than herbivorous and omnivorous group (German et al., 2004). However, higher consumption of protein above the tolerance level may negatively affect on the growth, health status and reproductive performance in fish, as well as the economic status of the farm (Abidin et al., 2006). Therefore, the knowledge of the optimum need of protein is vital for the improvement of the species cultivated.

2.6 The important role of amino acid in fish

Amino acids are the fundamental metabolites in the entire living organism due to its multirole as fuel molecules, appetite inducer, larval metamorphosis inducer, environmental stress resistance, immunity and growth (Li et al., 2008). Among all the 20 amino acids, alanine, aspartate, glutamate, glycine, serine and tyrosine are grouped as non essential or dispensable amino acids, which can be synthesized by the fish itself and the rest are categorized as essential or indispensable which must be supplied in the diet as the fish are not able to synthesize on its own (Li et al., 2008). In fish, the utilization rate of the amino acids should be greater than the synthesis rate to ensure the complete assimilation of protein in the diet (Li et al., 2008). It has been reported that, the amino acid profile of a species is greatly influenced by the efficient absorption of amino acids, the rate of protein synthesis and utilization of amino acid for energy purposes (Conceição et al., 2003). The necessity of amino acids can be influenced by species, captivity condition, diet, as well as different life stages of fish (Conceição et al., 2003).

Yolk in the fertilized eggs functions as the key note during the hatching process and as an energy source until the hatched larvae start their exogenous feeding (Watanabe & Kiron, 1994). This yolk consists of a bulk of nutrients, metabolites and signalling molecules (Finn & Fyhn, 2010) that are derived from the broodstock and accumulated in the ovary during vitellogenesis. The requirement of amino acids during the formation of eggs in fish varies between marine and freshwater species (Finn & Fyhn, 2010). In marine teleost, free amino acids are utilized for oocyte hydration and egg acquisition during oocyte maturation, while in freshwater fish, the assimilation of amino acids is essential for fertilization of eggs and development of organs (Finn & Fyhn, 2010). A similar finding by Wright and Fyhn (2001) reported the synthesis of free amino acids for specific yolk protein in the eggs of marine teleost during the oocyte maturation in the brood fish. However, in freshwater fish species, the free amino acids function as a fundamental energy source during the endogenous feeding of the larvae (Rønnestad et al., 1992).

Besides, its major role as an energy source and growth, amino acids also play a significant role in immunity. A study conducted by Li et al (2007) revealed the relationship between amino acids and the immune system. An efficient network of chemical communication includes lysozyme, antigen, immunoglobulins, and cytokine synthesis (Calder, 2006) ensures a proper regulation in both innate and acquired immunity in fish (Tort et al., 2003). These complex systems rely extremely on the adequate supply of amino acids for proteins and polypeptide syntheses. Certain amino acids such as arginine, methionine, cysteine, glutamic acid, glycine, tyrosine, alanine and proline function essentially to improve the immune system in fish (Li et al., 2007). Therefore, the deficiency of particular amino acids can cause major effect, such as autoimmune dysfunction in fish, as each amino acid play a vital role in immune responses.

2.7 Influence of dietary protein on egg and larval quality

The phase of nutrition in fish larvae is largely diverse and can be divided into four types, which are endogenous, absorption, mixed feeding and exogenous (Jaroszewska & Dabrowski, 2011). The transition period between endogenous to exogenous feeding is crucial in newly hatched larvae. The endogenous reserves, such as yolk sac, provide nutrients needed by the larvae for most of the species approximately 3 to 5 days after hatching. Although many nutrients are supplied through the maternal yolk, proteins that are present as lipoprotein and phosphoprotein (Brooks et al., 1997) function as a major constituent for the development of larvae. Many studies have been conducted on various fish species to expose the relationship between the influence of dietary protein and egg as a consequence impact on larval production.

A study conducted by Watanabe et al (1984) on red sea bream female brood resulted in 50% fish meal replacement with cuttlefish meal, displayed a better quality of egg viability, although there was no significant difference found in the number of eggs produced by the females. Furthermore, the same author reported another study on Japanese sea bream where one third lower numbering eggs was obtained from the brood fish fed lower than 45% dietary protein (Watanabe et al., 1984). Further, the modification of brood fish diet performed by Watanabe and Kiron (1995) for the red sea bream brood by adding krill, a small crustacean resulted in two fold increase of buoyant egg numbers, higher hatching rate and less abnormal larvae development.

The broodstock nutrition contributes to a major effect on egg quality, which in turn affects the quality of larvae. A study conducted by Binu Varghese et al (2009) reported a positive relationship between the intake of dietary protein and the length of the larvae in true sebae clownfish, (*Amphiprion sebae*). In agreement, Gunasekera et al (1996b) reported a significant influence on the larval length, head width and rate of yolk utilization in nile tilapia, (*Oreochromis niloticus*) larvae from the higher dietary protein fed brood fish.

2.8 Egg and larval quality assessment

Bromage et al (1992) claimed that the eggs, which exhibit low mortality during fertilization, hatching, beginning of exogenous feeding, and those that produce healthier larvae, are defined as good quality eggs. Several parameters can be used to determine the quality of eggs such as egg diameter, egg weight, biochemical composition, fecundity, abnormal blastomeres, egg buoyancy, fertilization success and oil droplet diameter (Kjorsvik et al., 1990; Nguyen et al., 2012).

During the embryogenesis of egg, certain changes in environmental parameters or the alteration of living condition can influence the hatching process and significantly affect the morphological development of an embryo (Semmens & Swearer, 2011). In fact, the newly hatched larvae which are exposed to those challenges might face mortality when they are dependent exclusively on their yolk sac. However, to ensure the sustainable development of species, some fish larvae have the capability to adapt or tolerate to any challenges up to a certain level. Many factors have been identified to influence the tolerance level of a species. Therefore, nowadays, many studies have been conducted to evaluate the quality of larvae to understand the capability to withstand the stress during early life by measuring length and weight of the larvae, survival rate, endurance to starvation, swimming speed, behaviour of larvae, histochemistry of the larval mucous cell and larval organogenesis (Villaluz & Unggui, 1983; Pittman et al., 1990; Green & Fisher, 2004; El-Gamal, 2009; Semmens & Swearer, 2011).

2.8.1 Effect of temperature on egg and larvae

All organisms, including fish have a lethal limit when they are exposed to environmental fluctuations (Green & Fisher, 2004). The performance of fish, particularly at their early stage of life towards the changes in water bodies is vital to understand their physiological and behavioural capabilities (Green & Fisher, 2004). The exposure of egg in various environmental fluctuations can determine its quality as the nutrient rich yolk functions as a barrier against potential hazardous environmental influences such as temperature, pH and salinity (Brooks et al., 1997).

For instance, a study conducted by El-Gamal (2009) on common carp, (*Cyprinus carpio*) discovered that 77% of egg hatchability occurred at 27°C and reduced to 59% when the temperature increased to 30°C. Additionally, the same study also reported that no hatchability of eggs occurred at an extremely low or high temperature, neither 20°C nor 38°C. A similar study conducted by Green and Fisher (2004) showed a slower swimming speed in cinnamon clownfish, (*Amphiprion melanopus*) when the larvae were reared at 25°C compared to those at 28°C. Further, Semmens and Swearer (2011) mentioned a positive relationship between the lengths of common galaxias, (*Galaxias maculatus*) larvae with the rearing temperature, in which shorter larval length was obvious at 10°C compared to those larvae reared at 15°C and 17°C. Previous literatures have reported that temperature alterations do affect the extended incubation period in fish embryos and delay the hatching process (Kawahara et al., 1997; Semmens & Swearer, 2011). At higher temperature, the rapid utilization of yolk in larvae resulted in a depleted reserves, thus reducing the survival rate during the extended incubation period.

2.8.2 Effect of pH on egg and larvae

Environmental pH was classified as another physiochemical parameter that critically influences the physiology and metabolism of aquatic life (Zaniboni-Filho et al., 2008). The exposure of eggs in unfavourable surroundings with higher or lower hydrogen ion than the favourable point may delay the hatching process. Previous studies have been conducted to discover the relationship between pH and the effects to the development of the egg. A study conducted by Nchedo et al (2012) reported a longer incubation period (20 hours) for the eggs that were exposed to acidic pH 4.5 and alkaline pH 9.5 whereas 17 hours of incubation period was recorded for pH 6.5-8.5. In addition, it was reported that no hatching occurred when the pH was achieved at 4 and 10, a condition with extreme acidic and alkaline. In fact, the hatching rate of the egg increased as the level of pH increases from 4 to 8 and reduces at an extreme alkaline pH, 9.5.

The extreme pH alteration in water bodies either extremely acidic or alkaline condition could be lethal for fish within a few hours (Copatti et al., 2011). At low pH, an imbalance of ionic equilibrium can cause circulatory system failure and will lead to the massive death of cultured species (Van Dijk et al., 1993). Continuously, at extreme acidic condition, fish can be killed by asphyxia with the enhanced mucous production and the degeneration of gill tissue (Zaniboni-Filho et al., 2008). Whereas, the higher alkalinity level may cause the inhibition of ammonia and carbon dioxide excretions which results increases of water toxicity (Copatti et al., 2011). Nchedo et al (2012) discovered that African sharptooth catfish, (*Clarias gariepinus*) larvae showed depressed activity at low and high pH, that is pH 4 and pH 10 respectively. In addition, higher survival and active performance of the larvae were discovered to be, at pH 7.5-8.5 which was also evident that larvae were intolerant to extreme pH condition. Another study on silver catfish (*Rhamdia quelen*) larvae exposed to pH 5.5 and 6 showed the slower growth of larvae in terms of length and weight compared to the larvae exposed to alkaline pH 8 and 8.5. The larvae exposed to alkaline pH showed a significant effect of greater length, weight and higher survival rate (Lopes et al., 2001).

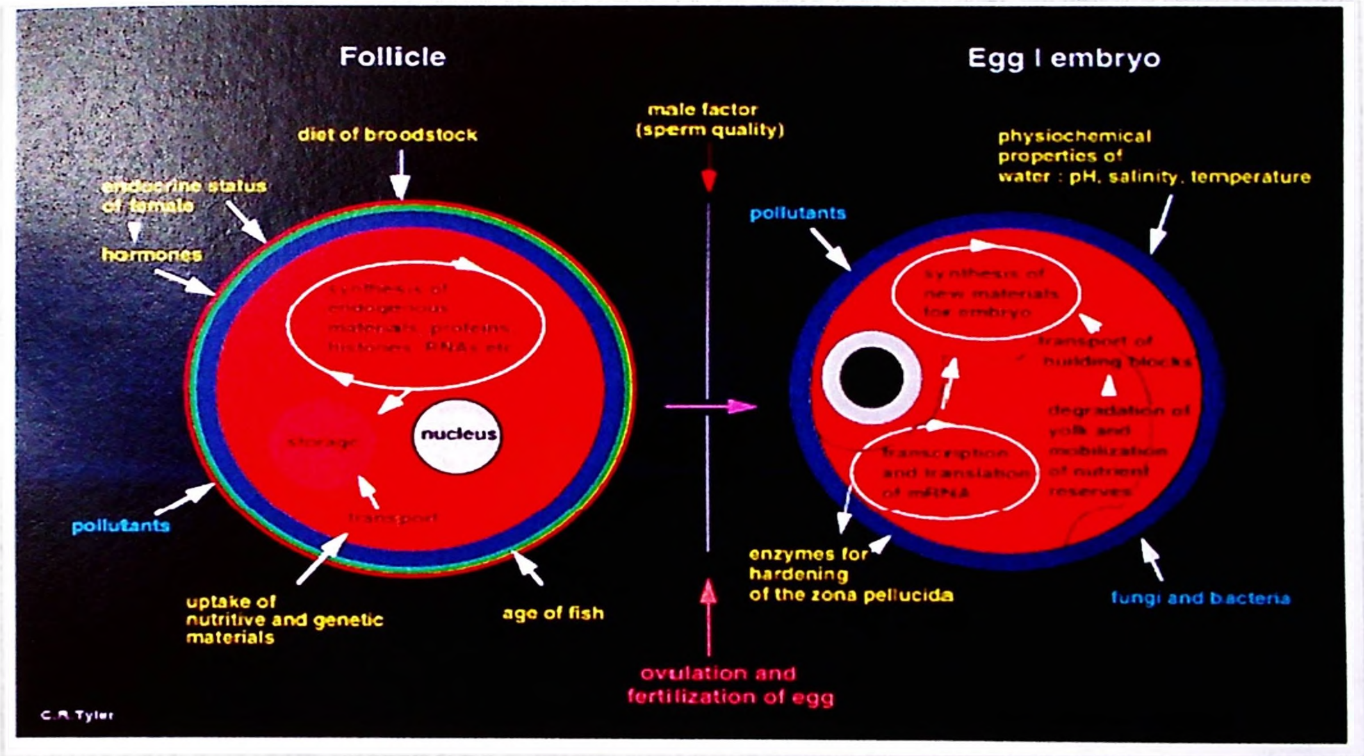

Figure 2.1: Environmental and genetic factor that affects egg quality (Fig 3, page 396, Brooks et al., 1997)

2.9 Ontogenic development of digestive tract in fish larvae

The ontogenic development of fish larvae has been the most interesting subject of many scientific studies in recent years. In commercial hatcheries, the weaning process of larvae becomes as an important part and blooming curiosity among researchers (Zambonino-Infante et al., 2008). Therefore, many studies have been conducted to gain better knowledge on larviculture and to find a solution for a serious bottleneck in aquaculture. The ontogenic development of the digestive tract and the secretion of enzymes is a complex process, especially in adult fish and less known in fish larvae. The digestive mechanism in fish is species dependent and varies at different life stages, thus adequate knowledge is vital in order to supply appropriate diets throughout the rearing duration (Drossou et al., 2006).

The transition period from endogenous to exogenous feeding is a critical period, which can affect the survival of fish larvae (Aristizabal, 2005). The growth of larvae is strictly depends on proper nutrient provided by the diet once the exogenous feeding has been established. Food deprivation of the larvae after the completion of yolk reserves can cause the abnormal behaviour, growth retardation, degeneration of alimentary tract, reductions in food utilization efficiency and abnormal feeding (Lazo et al., 2010). This is because, the feeding behaviour in fish larvae functions as the key to the entire digestive system. The initiation of exogenous feeding begins with some important anatomical features such as vision systems (eyes and chemosensory organs), prey capture (mouth, fin and muscle fibers), ingestion and digestion (digestive system).

Mouth functions as an essential tool to optimize the feeding behaviour and hugely responsible to initiate the development of the digestive tract in fish larvae. The development and size of mouth in newly hatched larvae becomes a major limiting factor for feeding and ingestion (Irwin et al., 2002) at the beginning of exogenous feeding. Previous literatures have mentioned the relationship between the developments of chemosensory organs with the growth of larvae. The development of fish mouth is hugely dependent on the prey size, variety of diet and the amount of feed consumed, which significantly influence the survival and growth of larvae. El-

Hag et al (2012) reported the morphological and functional development of mouth, in particular the upper and lower jaw in river catfish, (*Mystus nemurus*) larvae which develop rapidly, coinciding with exogenous feeding. In addition, Aristizabal (2005) stated the development of jaw and functional mouth appeared between 3 to 4 DAH in red porgy, (*Pagrus pagrus*) larvae and 4 DAH in mahseer, (*Tor tambroides*) larvae (Ramezani-Fard et al., 2011).

Progressive development of the larvae with new cells and tissues can be related to the secretion of enzymes, which is very important for digestion. The development of organs and digestive tract in fish larvae are sensitive to some internal and external factors. The genetic factors, broodstock nutrition, metabolism of fish, life history, reproductive strategy and vision system are some examples of the internal factors, whereas the external factors include environmental parameters, water bodies, feeding behaviour and nutritional factor in the diet (Datta-Munshi & Dutta, 1996; Lazo et al., 2011).

The requirement of food completely varies with the development of the digestive system, as the fish progressively develop from the larval stage to adult (Govoni et al., 1986). The structural and functional system of an adult fish tends to be more complex than that of the larvae that exist with the simple alimentary canal at the beginning of exogenous feeding. Fish larvae begin their life with the simple digestive tract with a straight tube of incipient gut that remain unchanged during yolk absorption (endogenous) and then gradually develop as the larvae begin their exogenous feeding.

Larvae undergo a rapid change leading to the differentiation of several organs, namely buccopharynx, esophagus, stomach, intestine, pancreas and liver during the transition period from larvae to fry. Previous studies on gilthead sea bream (*Sparus aurata*) (Sarasquete et al., 1995), California halibut (*Paralichthys californicus*) (Gisbert et al., 2004), yellowtail kingfish (*Seriola lalandi*) (Chen et al., 2006), common pandora (*Pagellus erythrinus*) (Micale et al., 2006), redbanded seabream larvae (*Pagrus auriga*) (Sanchez-Amaya et al., 2007) showed a differentiation of digestive tract corresponding with first time feeding into buccopharynx, esophagus, intestine and rectum between day 3 to 5 after hatch. The formation of stomach is very diverse and completely varies between species. The formation of stomach in California halibut larvae (Gisbert et al., 2004) was reported to develop between 27 to 30 dph and in white seabass (Galaviz et al., 2011) around day 12 after being hatched. In fish, the stomach region basically can be divided into three parts, namely cardiac, fundic and pyloric. Cardiac is an anterior portion with the presence of cubical epithelial cells, whereas pyloric is the posterior portion with an arrangement of epithelial cells in columnar shape. The stomach development in fish larvae also can be influenced by the intake of food, in particular the prey ingestion. It was reported that the stomach size of a carnivorous species tends to be bigger than herbivorous and omnivorous due to the type of food intake (Lazo et al., 2011). The formation of gastric glands in the stomach functions as a tool to increase the activity of digestive enzymes, mainly acidic protease (Baglole et al., 1997). However, the digestion of protein in fish larvae may be possible with the help of the pancreas or intestine in the situation of lack stomach development (Bolasina et al., 2006; Drossou et al., 2006).

2.9.1 Influence of protein on fish ontogenic development

The nutritional factor in the diet and the amount of feed consumed can influence the secretion of digestive enzymes in larvae (Govoni et al., 1986). Among