# EFFECT OF VARYING DIETARY LIPID LEVELS IN SNAKEHEAD BROODSTOCK, *Channa striata* ON EGG, LARVAL QUALITY AND ONTOGENIC DEVELOPMENT OF DIGESTIVE TRACT

**by**

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**Thesis submitted in fulfillment of the requirements**
**for the Degree of**
**Master of Science**

**July 2017**

# ACKNOWLEDGEMENT

Gratitude is what I can't able to expressed enough to all the people revolving around me when I pursue my study. Without the people that supported me throughout this period, I would have come to this day.

I would like to send my special appreciation to my supervisors Prof. Roshada Hashim and Porf. Alex Chong, for the guidance, advices and caring throughout this study, without them I couldn't finish my studies at all. And also thanks to my fellow lab mates and friends that I've known during the time of my studies, Aunty Anna, Jana, Kabir, Ali, Amelia, Nahid and Annette for their constant support and helps they provided in the labs and fish house.

And last but not least, my greatest support from my beloved family members in particularly my mother and my sister, who supported me all the way during my studies. Also I would like to show my greatest gratitude to my late father who is the one that encourage me to go for the field of what I'm studying today, the passion we both share together. Unfortunately he was unable to witness the full journey of my study, but he will forever remain in my heart.

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| | |
|---|---|
| ARA | Arachidonic acid |
| DHA | Docosahexaenoic acid |
| EFA | Essential fatty acid |
| EPA | Eicosapentaenoic acid |
| FA | Fatty acid |
| FAME | Fatty acid methyl acid |
| FAO | Food & Agricultural Organization |
| GC | Gas chromatography |
| MUFA | Monounsaturated fatty acid |
| n-3 FA | Omega 3 series fatty acids |
| n-6 FA | Omega 6 series fatty acids |
| PUFA | Polyunsaturated fatty acid |
| SD | Standard deviation |

**KESAN PEMAKANAN LIPID YANG BERBEZA DALAM INDUK IKAN HARUAN, *Channa striata*, PADA KUALITI TELUR, LARVA DAN PERKEMBANGAN ONTOGENI SALURAN PENCERNAAN**

## ABSTRAK

Haruan atau dikenali sebagai *Channa striata*, merupakan ikan air tawar yang terkenal dengan nilai-nilai nutraseutikal untuk penyembuhan luka dalam kalangan penduduk tempatan dan juga di Asia Tenggara. Pada masa ini, koleksi benih dari ikan liar menurun disebabkan lebihan penuaian dan pencemaran alam sekitar. Pembiakan tiruan adalah pilihan yang boleh diamalkan oleh ladang-ladang komersial untuk memenuhi permintaan yang tinggi. Walau bagaimanapun, secara tradisinya ikan baja biasanya diberi makan kepada ikan haruan induk dan mengakibatkan pengeluaran benih yang berkualiti rendah. Pengurusan pemakanan induk yang baik dalam kurungan adalah penting. Satu kajian telah dijalankan untuk menentukan tahap lipid optimum untuk *Channa striata* induk yang akan menghasilkan larva yang berkualiti tinggi. Kajian ini telah dibahagikan kepada dua bahagian, i) untuk menilai kualiti fizikal dan kimia larva; ii) untuk memerhati pembangunan ontogenik dalam saluran pencernaan dan aktiviti enzim sebagai tindak balas terhadap diet yang berbeza. Tiga kumpulan *Channa striata* dewasa (berat purata 176.76 ± 23.53 g) telah diberi makanan untuk tempoh tiga bulan dengan diet eksperimen yang mengandungi 100, 140 dan 180 g lipid $kg^{-1}$ diet. Saiz telur (1.4 ± 0.01 mm) dan saiz larva (15.5 ± 0.39 mm) yang ketara tertinggi di 15DAH diperolehi daripada induk diberi diet yang mengandungi 180 g $kg^{-1}$ lipid. Walau bagaimanapun, dengan 20DAH tiada perbezaan ketara dalam berat badan dan kepanjangan larva diperhatikan dari induk yang diberikan diet yang mengandungi 140 g

$kg^{-1}$ dan 180 g $kg^{-1}$ lipid. Kadar penetasan telur *Channa striata* adalah paling tinggi (59.75 ± 6.01%) dalam kumpulan larva dari 180 g $kg^{-1}$ induk pada 25 °C suhu air, manakala pada suhu air yang lebih tinggi di antara 30 °C dan 35 °C menyebabkan kadar penetasan melebihi 90% untuk telur dari induk yang diberikan 140 g $kg^{-1}$ dan 180 g $kg^{-1}$ lipid dalam diet. Penetasan telur dan kadar kelangsungan hidup larva adalah lebih tinggi dari induk diberi diet 140 g $kg^{-1}$ dalam keadaan air berasid (pH 4); manakala keadaan air alkali (pH 9) adalah dari kumpulan 180 g $kg^{-1}$ diet. EPA adalah tertinggi dalam telur sehingga 3DAH larva manakala tahap DHA hanya dalam larva dari 0DAH sehingga 3DAH dan bukan dalam telur, adalah lebih tinggi dari induk yang diberi 140 g $kg^{-1}$ lipid diet. Namum semua aktiviti enzim dikesan dari 0DAH tiada trend yang ketara telah ditemui di kalangan larva dari kumpulan induk yang berbeza. Pengambilan lipid dalam induk tidak mempengaruhi pembangunan ontogenik memandangkan saluran penghadaman larva untuk semua rawatan dibentuk dengan serentak. Berdasarkan dari keputusan yang diperolehi berkemungkinan bahawa pengambilan makanan sekurang-kurangnya 140 g $kg^{-1}$ lipid dalam diet untuk induk adalah mencukupi untuk mendapatkan kualiti yang baik benih *Channa striata*.

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## ABSTRACT

Snakehead or *Channa striata*, a freshwater fish well known for its nutraceutical values for wound healing is popular amongst the locals and also in Southeast Asia. Currently collection of seeds from the wild is declining due to over harvesting and environmental pollution. Artificial breeding is a feasible option practiced by commercial farms to meet to its high demand. However, traditionally trash fish is usually fed to snakehead broodstock which result in low quality seed production. Therefore good management of broodstock nutrition in captivity is significant. A study was carried out to determine the optimum lipid level for *Channa striata* broodstock that will produce high quality larvae. The study was divided into two parts, i) to assess the physical and chemical qualities of the larvae; ii) to observe the ontogenic development of digestive tract and enzyme activities in response to the different diets. Triplicate groups of *Channa striata* adults (average weight 176.76 ± 23.53 g) were fed for a period of three months with experimental diets containing 100, 140 and 180 g lipid $kg^{-1}$ diet, respectively. Significantly highest eggs (1.4 ± 0.01 mm) and larval (15.5 ± 0.39 mm) size at 15DAH were obtained from broodstock fed with 180 g $kg^{-1}$ lipid diet. However, by 20DAH no significant difference in weight and length of larvae were observed from broodstock fed with 140 g $kg^{-1}$ and 180 g $kg^{-1}$ diets. Hatching rate of *Channa striata* eggs was significantly highest (59.75 ± 6.01%) from 180 g $kg^{-1}$ fed broodstock at 25°C water temperature, while higher water temperature of 30°C and 35°C resulted in hatching rate exceeding 90% for the 140 g $kg^{-1}$ and 180 g $kg^{-1}$ diets. Egg hatching and larval survival rates were significantly highest from broodstock fed with 140 g $kg^{-1}$ in acidic (pH 4) condition; while alkaline (pH 9) condition was from 180 g $kg^{-1}$ group. EPA was significantly highest in eggs until 3DAH larvae while only DHA levels in larvae from 0DAH to 3DAH, and not the eggs, were significantly highest in the 140 g $kg^{-1}$ treatment. All enzyme activities were detected from 0DAH however no notable trend was found among the different dietary treatments. Dietary lipid intake in the broodstock did not influence the ontogenic development since the digestive tract of larvae for all treatments developed simultaneously. Based from the results obtained is likely that a minimum dietary intake of 140 g lipid $kg^{-1}$ diet for broodstock was sufficient to obtain good quality *Channa striata* seed.

# CHAPTER 1

# INTRODUCTION

## 1.1 Freshwater Aquaculture

Generally, fishes are one of the important food sources to about 2.9 billion people, which contribute almost 20 percent to total animal protein intake (FAO, 2012). With the global population vastly growing over the years, fish supplies demand has outpaced the production of capture fisheries. Since then, the aquaculture industry has become a key player and developed rapidly. This is to ensure that there are adequate food supplies to the world population by minimizing the disruption or shortage of food supplies issue.

Aquaculture industry plays a crucial role as a way of increasing production for food security and export revenue. Based on FAO Fisheries and Aquaculture Statistics for 2012, Asia had accounted for about 90 percent of worldwide aquaculture output; while China alone had produced over 60 percent of global aquaculture production. In the recent years, aquaculture production has been rapidly increased in Southeast Asia, with the total output of fewer than 2 million tonnes in 1990 to more than 7 million tonnes in 2005 (Hishamunda *et al.*, 2009).

From 2003 to 2012, freshwater aquaculture industry solely managed to increase almost 50% of production from 22.4 million metric tonnes to 41.9 million metric tonnes (FAO, 2014). Among the top 20 cultured freshwater finfish listed in 2010 were grass carps (*Ctenopharyngodon idella*), Nile tilapia (*Oreochromis niloticus*), iridescent shark

(*Pangasius hypophthalmus*), rohu (*Labeo rohita* ) and snakehead (*Channa striata*). (FAO, 2014).

## 1.2 Cultivation of Snakehead, *Channa striata*

Snakehead (*Channa striata*) is one of the freshwater fish species that are commonly cultured in Africa and Asia (Wee, 1982). Characteristics such as able to survive in a harsh environment with low dissolved oxygen and high ammonia content (Ng and Lim, 1990) is one of the advantages of cultivating snakehead. Furthermore, the firm white flesh with agreeable flavour has made this species commercially viable (Qin and Fast, 1998). From “police fish” in polyculture to one of the most cultivated species in the freshwater aquaculture, snakehead has developed into a major player in this industry (Qin and Fast, 2003). Over the years, the cultivation of snakehead had been supported by many Asian countries’ fisheries and aquaculture industries (Ling, 1977). According to Amornsakun *et al.* (2011), the snakehead is considered as a first-grade fish in South East Asia and commercially important species for inland fisheries. With the increasing demand of the species in the market, widespread pond culture of snakehead has resulted in high demand for fry and fingerlings too (Ali, 1999).

In 2008, production from captured *C. striata* in South East Asia region namely Indonesia, Philippines and Thailand had contributed about 30,120, 10,000 and 6,630 metric tons, respectively (FAO, 2008). With the rapid increase of 17% of total production from the year 2000 to 2010, snakehead has become among the top 20 species of freshwater fish cultivated the most in the Asian region (Funge-Smith *et al.*, 2012). The total production of snakehead in Asian region reached a total of 421,275 tonnes in

2010, which was produced in top 5Asian countries such as China, Indonesia, Thailand, Cambodia and Bangladesh (Funge-Smith *et al.*, 2012).

Presently, most of the commercially cultured snakeheads depend on seeds from the wild. However, snakehead seed supplies from the wild are slowly declining with most of their breeding grounds have been destroyed due to the increment of human activities (Muntaziana *et al.*, 2012). Unfortunately, due to the degradation of the species habitats, sources of seeds from the wild are declining too and will no longer be able to cope with the increasing demand for snakehead supplies. Therefore, progressively more hatchery techniques were practiced in commercial farms to continuously provide larvae for growing out to marketable sizes.

## 1.3 Problem Statement

The increasing demand for snakehead in the market has put a towed on farmers as getting seeds from the wild is very difficult. Pollution of the natural habitat of snakehead and overharvesting of seeds have affected the sustainability of the seed production in the wild. In fish hatchery system, the main concern is to maximize the number of products with the highest seeds and fingerlings quality from the broodstock (Marimuthu and Haniffa, 2006). Besides that, the prerequisites for domestication and the sustainability establishment of aquaculture industry are the capacities to control reproductive processes in captivity and to acquire high-quality seed for grow-out to the marketable product (Mylonas *et al.*, 2010). However, one of the problems faced by farmers to produce sustainable stock for hatchery is low productivity due to poor broodstock nutrition and management.

Broodstock nutrition is important for fish reproduction and good development of larvae as proper nutrition is the key in aiding the development of a good reproductive system of the broodstock. The improvement of broodstock nutrition and feeding management has been proven in improving the egg and sperm quality and the seed production (Izquierdo *et al.*, 2001). However, different fish species require different nutritional composition, depending on the feeding habits and habitats of the fish.

To date, broodstock nutrition and dietary requirements for snakehead are poorly studied compared to another area of research in aquaculture (Marimuthu *et al.*, 2009). Not only that, the development in embryonic and larval stages of *C. striata* larvae is considered very sensitive when it comes to sufficient nutrition from the broodstock. Since the establishment of snakehead culture, farmers rely mostly on trial and error approaches and there is no ecological rearing requirements have been systematically evaluated (Qin and Fast, 2003). Hence, more researches and studies are required to investigate on how broodstock diets can affect the quality of the eggs and larvae produced.

### 1.4 Aims and objectives

This research was designed to investigate the effect of different dietary lipid levels for broodstocks on their offspring quality. The objectives of this study were shown as follow:

1) To determine the effects of different dietary lipid levels on the *C. striata* larvae quality.
2) To determine the suitable level of lipid for good development of *C. striata* larvae.
3) To observe enzyme activities and digestive tract development of *C. striata* from different lipid levels.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Snakehead, (*Channa striata*)

Snakehead (*Channa striata)* or locally named as the Haruan is an obligatory air-breathing carnivorous freshwater fish from the family of Channidae. Being an air-breathing fish has its advantages as snakehead is easy to culture and able to survive without water (Hughes and Mushi, 1973). Snakeheads are indigenous species found in the Asian countries such as India, Pakistan, Bangladesh, Myanmar, Thailand and Malaysia. This species is the most famous among the *Channa sp.* (Courtenay and Williams, 2004). Snakeheads can be found in freshwater ponds, streams or usually in stagnant muddy waters in India (Talwar and Jhingran, 1992); whereas in Malaysia are rivers, lakes, swamps and rice paddies (Mohsin and Ambak, 1983). Snakeheads are popular in Malaysia as this species possesses medicinal values, which heals wound due to the effect of its fatty acids composition (Mat Jais *et al.*, 1997). It can be said that a snakehead fillet contains the high content of arachidonic acid (AA), a precursor of prostaglandins, which may initiate blood clotting and responsible for tissue growth (Mat Jais *et al.*, 1994).

Snakehead is a carnivorous fish consuming mainly fish, frogs, snakes, insects, earthworm and tadpoles (Dasgupta, 2000; Rahman *et al.*, 2012), which exhibits high levels of cannibalism at the juvenile stage (Wee, 1982; Ng and Lim, 1990). This species has enlarged canine teeth that make it ideal for gripping, killing and tearing (Ng and Lim, 1990). They are also known to swallow their prey wholly due to their nature as the gapelimited predators (Diana et al, 1985; Qin and fast, 1996). However, the cannibalism feeding behaviour of the fish was reported to be affected by the availability of alternative food, which can be reduced at the juvenile stage by size grading and ad libitum feeding (Qin and Fast, 1996).

Snakeheads can grow up to a total length of 90 cm (Bardach *et al.*, 1972) and can reach maturity as small as 25.5 cm in total length and 150 g (Ali, 1999). There are 6 stages of ovarian development in this species with developing oocytes, which allows the fish to produce seeds for all year-round (Ali, 1999).

Plate 2.1 Snakehead, *Channa striata* broodstock used in the study (Source: From the experiment)

### 2.1.1 Larval development

Embryonic and larval developments are vital in a life cycle of fish in order to produce good and healthy fish. The life cycle of a snakehead from egg to juvenile to adult is shown in Figure 2.1. During spawning, *C. striata* eggs (Plate 2.1) will be fertilized and change into non-adhesive straw yellow coloured eggs (Marimuthu and Haniffa, 2007). The fertilized eggs undergo embryonic development within 10 hours after spawning and differentiation of embryo occur within a few hours starting from the neural stage to hatching (Marimuthu and Haniffa, 2007).

After hatching (Plate 2.2), a transparent and faintly brown in colour larvae will be formed with a length of 3.4 ± 0.2 mm (Marimuthu and Haniffa, 2007) and increases few millimeters longer at every few hours. Until the 15$^{th}$ day after hatching, different parts of the body will be formed accordingly. On the 20$^{th}$ day post hatch, a fry will be formed that has an average length of 40.8 mm and post-anal length of 20.7 mm (Marimuthu and Haniffa, 2007).

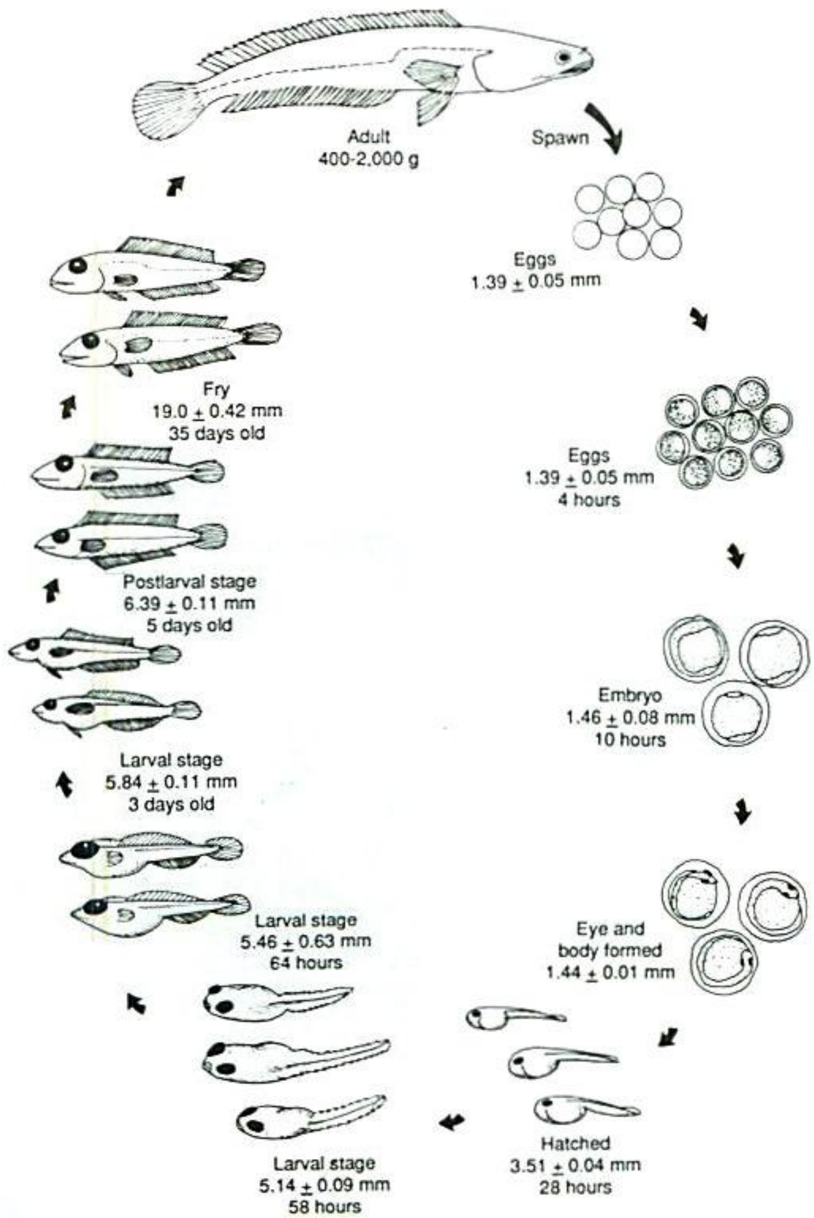


Figure 2.1 Life cycle of *C. striata* from spawning of egg until adult phase (Yaakub and Ali, 1992)

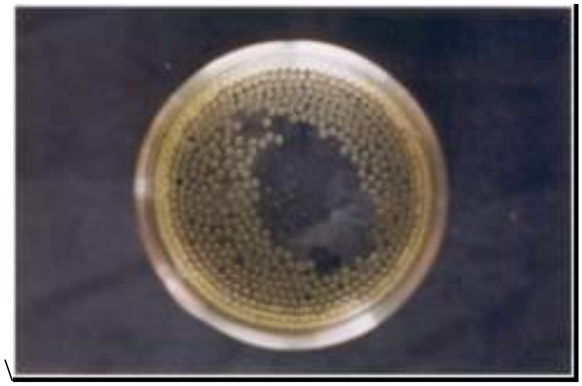

Plate 2.2 Fertilized eggs of *C. striata* in straw yellow colour (Marimuthu and Haniffa, 2007)

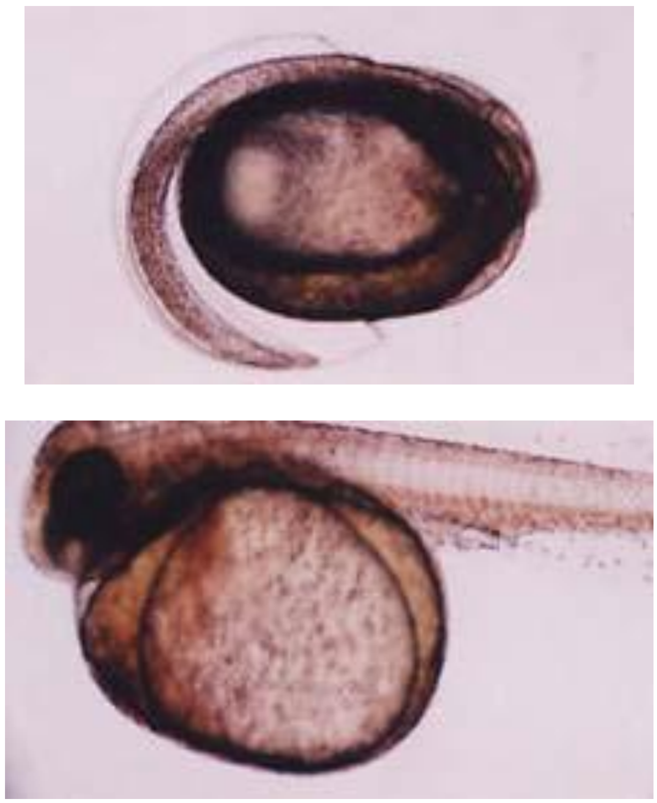

Plate 2.3 Newly hatch *C. striata* larvae with yolk sac and 36 hours post hatch larvae with yolk sac and eyes visible (Marimuthu and Haniffa, 2007)

### 2.1.2 Status of snakehead culture and feed in commercial farms

The culture of snakehead is popular among fish farmers in Asia as it is considered as one of the valuable species (Wee, 1981). In Asia, there are 33 native species of snakehead and among them, 11 species are commercially produced in aquaculture and are also important in the biodiversity conservation (Rahman & Awal, 2016). Most commercial snakehead farms rely on the capture of wild fry usually during the peak availability, which is during the wet season from May to October for growing out to marketable size (Diana *et al.,* 1985). In Thailand, snakeheads are usually monoculture; while in India, they were cultured with other fish such as *Anabas* sp. (Coche & Edwards, 1990). In Bangladesh, snakehead (including *C. striata* with other species of the *Channa* genus) contributed about 4.2% of the total cultured fish produced (Mollah *et al.*, 2009). Meanwhile, in Vietnam, *C. striata* and *C. micropletes* are the two species commonly being cultured to provide staple food to the people (Hien *et al.*, 2015).

In commercial snakehead culture practices, trash fish and cattle blood mixed with the rice bran, wheat flour or spent grains that are usually fed to the fish (Boonyaratpalin *et al.*, 1985; Victorand Akpocha, 1992). The use of traditional method of feeding snakeheads with small-size fish as their food sources in Southeast Asia, especially Vietnam and Cambodia has become a major concern as it is an unsustainable practice, which can cause the risk of developing diseases to the stocks due to the poor quality of the feeds (Hien *et al*., 2016). Meanwhile, in Thailand, larvae are fed with finely chopped trash fish until they grew into fingerlings size. After that, they are fed with the combination of trash fish and rice bran (Wee, 1981).

## 2.2 Broodstock and larval nutrition

Nutrition is one of the vital essences when it comes to the broodstock reproduction and larval development. A perfect combination of balanced nutrients in broodstock diet will improve the reproductive system and larval growth in many aspects. Among the major nutrients that mostly discussed in the previous studies were protein, lipid and carbohydrate. A balanced combination of the 3 main macronutrients can enhance the optimum growth of larvae.

The advancement of broodstock nutrition has shown to improve eggs and sperm quality with certain essential dietary nutrient can further influence the gonadal and fecundity development to enhance the continuous spawning in a short period (Izquierdo *et al.*, 2001). Many studies have shown that the parameters used for egg quality assessments are fecundity, hatching and larval survival. These are mainly affected by a nutritional deficiency in the broodstock diets (Fernández-Palacios *et al.*, 1995). According to Izquierdo *et al.* (2001), reduction of the fecundity in some marine fish species are either caused by the influence of a nutrient imbalance on the brain-pituitary-gonad endocrine system or the lack of biochemical component availability in egg formation.

The carnivorous fish like *C. striata* requires relatively high protein and lipid in the diet in order to meet the fish's minimum nutrition. In a recent broodstock nutrition study done by Ghaedi *et al.* (2014), snakehead broodstock fed with 180 g $kg^{-1}$ dietary lipid had achieved the optimum reproductive performance, which showed the highest fatty acid composition in its liver and female ovary. According to Aliyu-Paiko *et al.*

(2010), *C. striata* fry requires at least 450 g $kg^{-1}$ of protein and 65 g $kg^{-1}$ of lipid in theie diets to enhance their growth performance and survival.

### 2.2.1 Dietary lipid requirements

Among the dietary nutrients, lipid and fatty acid composition are identified to be one of the major dietary factors that determine successful reproduction and survival of offspring (Izquierdo *et al.*, 2001). Lipids consist of various classes, which constitute to different types of fatty acids. Generally, polar lipids are richer in n-3 PUFA and Arachidonic acid (AA); whereas neutral lipids are rich in monounsaturated fatty acids (MUFA) (Murray, 1996). Lipid plays several critical roles in fish nutrition and it has been studied widely in various aspects. For decades, lipid and fatty acids have been studied for its beneficial properties in aquaculture feeds (Craig and Helfrich, 2002). One of the examples of protein sparing effect of lipid is the increasing in the dietary lipid with a constant amount of protein. It has significantly increased the viscerosomatic index, hepatosomatic index and daily gain energy in juvenile cobia (Wang *et al.*, 2005). Being effective in a non-protein energy source that releases more energy per unit weight (De Silva and Anderson, 1995), it is easily digested and metabolized comparing to carbohydrates. Hence, lipid can be considered as a better source of energy compared to protein.

In several studies, one of the major roles has been proven successful in reserving energy among the fishes and fish embryos (Bell and Tocher, 1989; Rainuzzo *et al.*, 1997; Watanabe, 1993; Furuita *et al.*, 1996). Lipid or fatty acid generates metabolic energy in the form of ATP via mitochondrial $\beta$-oxidation that supplies an abundance of metabolic energy. That is the reason why this type of energy is more favoured in fish (Sargent *et al.*, 1989). Throughout the embryonic development, lipid will continuously provide metabolic energy (Sargent, 1995) as larvae are dependent upon the endogenous energy reserves (Rainuzzo *et al.*, 1997).

Besides providing energy resources, lipids also provide appropriate nutrients to broodstock to improve their fecundity and hatching rate. According to Izquierdo *et al*. (2001), the determination of the reproductive success and also the survival rate of offspring depends on the content of lipid and composition of fatty acid, which has identified as the major dietary factors in broodstock diets. Duray *et al.* (1994) also highlighted from his studies that in rabbitfish broodstock, fecundity and hatching rate have increased with the elevation of dietary lipid levels in the diets. Despite the increment of phospholipid does not affect the mortality of pike perch larvae, the dietary phospholipid increment can affect such phenomena. In fact, it can induce the weight gain of the larvae significantly (Hamza *et al.*, 2008).

### 2.2.2 Fatty acid requirements

Lipid requirements will differ among not only in fish species and fish feeding habits, but also marine and freshwater fish. Unlike other animals, fish larvae require certain fatty acids such as highly unsaturated fatty acids (HUFA) i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to assist in normal development of their growth, which is the main reason why these fatty acids are called essential fatty acids (Rainuzzo *et al.*, 1997). In general, lipid utilization in freshwater fish eggs can occur during the whole developmental period, including embryogenesis (Sargent *et al.*, 2002). Unlike marine fish, freshwater fish can manufacture longer chain of n-3 HUFA, EPA and

DHA from the enzyme desaturation system of linolenic acid (Craig and Helfrich, 2002). However, freshwater fish needs essential fatty acids, namely α-linoleic acid (18:2n-6) and linolenic acid (18:3n-3) in their diets, in which the fatty acids will further desaturate and elongate to form the physiologically essential polyunsaturated fatty acid (PUFA) (Tocher *et al.*, 2003; Craig and Helfrich, 2002). Polyunsaturated fatty acid (PUFA) composition may vary among freshwater and marine fish and even among other species (Abd Rahman *et al.*, 1995). Unfortunately, this fatty acid usually cannot be produced by the fish and need to be added into their diet. As Teshimas (1985) described, there are different requirements of EFA in freshwater fish. Freshwater fish such as rainbow trout require n-3 PUFA such as C18:3n3; tilapia requires n-6 PUFA; and carp require both n-3 and n-6 PUFA (Teshima, 1985). In freshwater fish, the requirements for EFA are not as straightforward as it seems, considering the feeding habits of the fish are much needed to be taken into account (Sargent *et al.*, 2002).

PUFA usually affects directly or through broodstock metabolite, maturation and steroidogenesis (Izquierdo *et al*., 2001). In cobia, studies have shown that a higher amount of n-3 HUFA of more than 1.86% in total dry weight is needed for better fertilization succession (Nguyen *et al.*, 2010). In other studies, with increasing of dietary n-3 HUFA, fecundity in gilthead seabream has been reported to be significantly increased (Fernández-Palacios *et al.*, 1995). In many fish species, deficiencies in highly unsaturated fatty acid (HUFA) in broodstock will affect fecundity, fertilization rate and hatching rate (Rainuzzo *et al.*, 1997). This is also mentioned by Mourente and Odriozola (1990) for the case of gilthead seabream eggs in such a way that if the n-3 HUFA is deficient in the broodstock diets. It can be said that the muscle lipid reserves are utilised for the maturation of ovaries (Lie *et al.*, 1993). In snakehead broodfish, the muscle fatty acid is increasing significantly with the dietary lipid diets with a higher fecundity in the mature oocyte (Ghaedi *et al.*, 2014).

HUFA components, especially DHA are essential for larval development (Watanabe, 1993). EFA requirements are higher during the larval development than the broodstock or juvenile stage (Lazo *et al.*, 2011). This mainly due to the higher metabolic activity and growth rate of larvae and also the rapid development of specific EFA-dense tissues such as brain, sensory organs and gills (Lazo *et al.*, 2011). Besides the characteristics of providing energy throughout the life stages of fish, essential fatty acids also aid in membrane structure of fish. The fish bilayers cell membrane constitutes phosphoglycerides such as 20:5n3 (EPA), 22:6n3 (DHA) and other fatty acids as the principal fatty acids (Sargent *et al.*, 2002). Sargent *et al.* (2002) described that the abundance of DHA (C22:6n-3) in the neural tissue has a particular implication in the fish nutrition, especially during the larval stage. An adequate supply of DHA in larval nutrition will support and develop the normal functions of neural and visual (Sargent *et al.*, 2002). As also described by Mourente (2003), during early stages of larvae, an adequate supply of n-3 HUFA, especially DHA is crucial for normal development of the neural system and also to prevent behavioural abnormalities. It is also believed that the structure of DHA enables a fish to adapt and resist the temperature and pressure changes (Rabinovich and Ripatti, 1991). In the n-6 HUFA, arachidonic acid (ARA, C20:4n6) is considered as important as the other n-6 members. In one of the studies on the effects of ARA, this fatty acid has reported increase with the weight of Japanese eel as the levels of ARA increased (Bae *et al.*, 2010). On top of that, the insufficient of DHA (C22:6n3) in the larval diets can cause larvae to experience the negative impact due to impair neural that can retard the visual development (Sargent *et al.*, 1999).

Essential fatty acids are important for broodstock reproductive system and producing good quality larvae. However, an excess amount of fatty acids might cause adverse effects. In several studies, the excess of the EPA and DHA could lead to negative effects. Even though the role of lipid in the early stage of embryonic and larval development is vital, the high amount of eicosapentaenoic acid (EPA) in relation to docosahexaenoic acid (DHA) may also cause structural composition imbalance in phospholipids and could affect the normal growth and quality of the larvae (Rainuzzo *et al*, 1997). Growth and survival can be suppressed when the ratio of EPA and DHA level is 1% to 5%. This phenomenon will induce poor development of sea bass larvae (Villeneuve *et al.*, 2005; Zambonino-Infante and Cahu, 2010). According to Santiago and Reyes (1993) and Corraze *et al.* (1993), fatty acid composition in diets for broodstock can affect the reproductive performance of the females, which can alter the fatty acid composition of the eggs (Hardy *et al.*, 1990; Yuneva *et al.*, 1990) and its quality. As suggested by Henrotte *et al.* (2010), high levels of EPA could cause disturbances in the embryo and larval development. Other studies also reported that high dietary levels of ARA (between 0.42% to 0.60% dw) will cause a negative effect towards the fertilization succession in cobia (Nguyen *et al.*, 2010). Similar results are also observed in the Japanese flounder fed with higher levels of ARA in the broodstock diets, whereby higher levels of ARA had inhibited the bioconversion of EPA, causing a low percentage of egg production, hatching rate, larval survival and normality of larvae (Furuita *et al.*, 2003).

## 2.3 Development of ontogeny and enzyme activities

The ontogenic development and digestive activities of fish larvae provide crucial information to understand better of the larval growth mechanism. Between the period from hatching until exogenous feeding, larvae will undergo a series of metamorphosis in the digestive, sensory, muscular, circulatory and respiratory system in order to convert from readiness to exogenous feeding (Sanderson and Kupferberg, 1999). Generally, in most of the fish species, the alimentary canal in fish larvae morphologically and histologically less elaborate than those found in adult fishes (Govoni *et al.*, 1986). The fundamental knowledge of digestive physiology and organization during the larval development will thus be used as an indicator to ascertain the developmental status of the digestion and nutritional status of larvae (Dabrowski, 1982; Ueberschar, 1993). A better understanding of the physiology and developmental mechanisms of fish larvae is vital for the development of formulated diets for larvae (Chen, *et al.*, 2006; Zheng *et al.*, 2010).

### 2.3.1 Ontogenic Development of Digestive Tract

Generally, the digestive system of fish in warm water is developed earlier than cold water species, larvae (Portella and Dabrowski, 2009). The digestive system morphogenesis and differentiation in fish species will depend on the type of egg cleavage (Zambonino Infante et.al, 2008). There are four regions of the digestive tract system, which are the headgut, foregut, midgut and hindgut (Harder, 1975). Wilson and Castro (2010) described the headgut consists of mouth and pharynx; foregut comprises oesophagus and stomach; midgut or known as intestine and hindgut is the last section that consists of the rectum. At hatching, Lazo *et al.* (2011) described that in most species, the alimentary canal appears to be an undifferentiated straight tube that is lying dorsally to the yolk sac. Few freshwater fishes such as yellowtail catfish, *Pelteobagrus fulvidraco* (Yang *et al.*, 2010), butter catfish, *Ompok bimaculatus* (Pradhan *et al.*, 2012) and mud loach have been used in the previous studies. *Misgurnuis anguillicaudatus* (Zhang *et al.*, 2014) used a straight tube of the digestive tract that was dorsally attached to the yolk sac at hatching. After hatching, larvae are depending on the yolk sac for endogenous feeding until the yolk sac is completely absorbed.

Larvae will undergo further rapid development, which will lead to the differentiation of several regions and organs of the digestive system, namely buccopharynx, oesophagus, intestine, pancreas and liver. Meanwhile, the morphogenesis of stomach depends on species (Lazo *et al.*, 2011). In the digestive tract, there are 4 basic layers, which are mucosa, submucosa, muscularis and serosa. However, the characteristics of the layers may change depending on species (Lazo *et al.*, 2011). Generally, at the onset of exogenous feeding, buccopharynx, oesophagus, stomach, intestine and anus are distinctively differentiated and functional in larvae (Rønnestad *et al.*, 2013). However, the differentiation and development timing of digestive tract regions depends on the fish species. Each fish species general life history and reproductive guilds are few factors that affect the timing of organ development and physiological functions (Balon, 1975).

Generally, in most fish, the oesophagus is not differentiated upon hatching and morphogenesis only take place at the stage of development just before the onset of exogenous feeding (Lazo *et al.*, 2011). Differentiated oesophagus will be short, wide and straight with the appearance of goblet cells, which serve as the mucus secreting function

(Lazo *et al.*, 2011). In most freshwater fish, the oesophagus is lined by a multi-layered squamous epithelium with large numbers of goblet cells (Lazo *et al.*, 2011). In Siberian sturgeon when the yolk sac has been completely adsorbed, the elongated oesophagus was differentiated into two distinct regions, which are abundant in goblet cells and food transport region which are rich in ciliated cells (Gisbert *et al.*, 1998). In the studies on bay snook, the oesophagus comprises a simple columnar epithelium with a basophilic basal membrane lined at the entire oesophagus while the goblet cells increase as the larvae increase with age (Treviño *et al.*, 2011).

Another alimentary canal region that is widely studied in the histological morphology of fish larvae is the buccopharyngeal cavity. The pharynx is lined by simple monostratified squamous epithelium at the mouth opening (Zambonino Infante et.al, 2008). In Siberian sturgeon, at the onset of exogenous feeding, the alimentary canal of the larvae is well developed with an organised buccopharynx, which is lined by a pseudostratified epithelium with the formation of teeth and taste buds (Gisbert *et al.*, 1998). Besides that, teeth are often associated as part of the morphology of the buccopharyngeal cavity. Same as differentiation of other digestive tract organs, the development of teeth varies among species. It is common in some species that teeth only develop at a later stage of the larval phase, which occurs at the onset of exogenous feeding (Gisbert *et al.*, 1998; Pradhan *et al.*, 2012; Treviño *et al.*, 2011) or sometimes after exogenous feeding has been initiated (Chen *et al.*, 2006; Mai *et al.*, 2005; Yang *et al.*, 2010). The formation of teeth is mostly used for grasping and ingesting their prey wholly rather than masticating the prey, which normally can be seen in adult fishes (Govoni *et al.*, 1986; Zambonino Infante *et al.*, 2008). However, there are some species that are lacking teeth formation in the larval stage as reported in the mud loach, whereby the larvae's feeding habit involves the action of suction the whole prey rather than biting, seizing or cutting (Zhang *et al.*, 2014).

On the other hand, one of the organs in the digestive tract that is first to be differentiated among many fish species is the intestine, which is the longest portion of the digestive tract that occupies most of the abdominal cavity (Lazo *et al.*, 2011). This is the region where the chemical digestion of food and adsorption mainly occurs (Wilson and Castro, 2011). The intestine of newly hatch larvae is lined by a simple columnar epithelium with a striated border of microvilli projecting from the apical surface of cells (Zambonino Infante *et al.*, 2008). During endogenous feeding, the formation of the intestinal valve or ileo-rectal valve will divide the posterior and anterior of the intestine (Lazo *et al.*, 2011; Zambonino Infante *et al.*, 2008). Studies of Bay snook have shown that its intestine is the first to be differentiated into 9 post-hatch and the oesophagus is the last part to be differentiated that is after the 21 days of post-hatch (Treviño *et al.*, 2011). On the other hand, as early as within the 3 days of post-hatch, mud loach larvae's digestive tract undergoes a rapid development from a straight tube to 3 main segments, which are buccophyarynx, oesophagus and intestine (Zhang *et al.*, 2014). The dynamic characteristics of the intestinal mucosa will act as the main site of digestion and absorption of nutrients (Lazo *et al.*, 2011). As the larvae continue to develop, the digestive tract increases its absorption capacity by elongating and folding of the mucosa (Rønnestad *et al.*, 2013). Subsequently, the intestine will coil and form a loop to accommodate the increasing size of the organ in the visceral cavity (Zambonino Infante *et al.*, 2008).

Besides those mentioned regions in the digestive tract, another organ, which is as important as the others is the stomach. The stomach is the latest organ of the digestive system to be differentiated although it is distinguished soon after hatching (Zambonino Infante *et al.*, 2008). However, the differentiation of stomach may vary among the marine and freshwater fishes, including interspecies too. Treviño *et al.* (2011) reported that in Bay snook, the stomach is formed not long after the formation of the intestine with a cluster of undifferentiated cuboidal cells which will eventually further developed into gastric glands. The complete development of stomach will expand in size and well-differentiated that occupies most of the abdominal cavity with additional mucosal folds and gastric glands (Treviño *et al.*, 2011). As the larvae convert to juvenile, the stomach epithelium becomes structurally differentiated with formation of glands and gastric digestion that has already been established (Rønnestad *et al.*, 2013). Portella and Dabrowski (2009) suggested that the appearance of the gastric gland is an indicator for the completion of stomach differentiation and it is an important threshold in the transformation from larvae to the juvenile that allows the fish to digest more complex and variable diets. The region of the foregut, including stomach, is the place that the chemical digestion of food started (Wilson and Castro, 2011).

*C. striata* larvae are just like the other finfishes which revealed the almost similar development of digestive system during the development based on several studies. The digestive tract of the snakehead larvae was reported to be in a straight tube that positioned dorsally to the yolk sac with the absence of the other digestive organs at the 0 days after hatching (Paray *et al.*, 2015; Bugar *et al.*, 2017). Intestine and mouth are visible as early as the 2 days after hatching and the larvae could feed exogenously by the

3rd day of the post-hatch as reported by Paray *et al*. (2015). As the larvae slowly develop, differentiation of the organs start to occur and reach a complete metamorphosis by the day of 25th after hatching (Bugar *et al.*, 2017).

### 2.3.2 Enzyme activities

Digestive tract in most of the fish species contains enzymes that will utilise for metabolisms such as digestion, absorption and assimilation of food, which comprises proteins, lipids and glycogen (Kolkovski, 2001). However, the enzyme activity in fish larvae is much lower compared to the activity found in an adult fish as some regions of the digestive organs are still underdeveloped. The presence of digestive enzymes secretion is the best indicators for the development of each region of the organs from the digestive tract as the different type of organs secrete enzyme with various functions at different pH levels. The pancreas is one of the organs which responsible for secreting pancreatic enzymes that aid in the intestinal digestion and hormones (Lazo *et al.*, 2011). The stomach is where protein digestion occurs. It consists of gastric juices which contain pepsin and HCl (Zambonino Infante *et al.*, 2008). Another organ that involved in the digestion is the gallbladder, which often associated with the liver. This part of the digestive system secretes bile produced by the liver to assist in emulsification of lipids in foods and also increases intestinal pH (Lazo *et al.*, 2011).

There are 3 main basic enzymes which are protease, lipase and amylase that metabolised nutrients such as proteins, lipids and carbohydrates, respectively. The assessment of the development of different digestive enzyme can help to predict the ability of fish species to utilise the different nutrients in the food (Furne *et al.*, 2005).

The digestive system morphogenesis of fish will reflect on the adaptability of specific diets and requirement of nutrition (Buddington, 1985). Fish that is coming from different feed preferences will secrete different levels of digestive enzymes in order to digest the content of their food.

Pancreatic enzymes such as amylase are reported to be detected as early as before the opening of the mouth. The authors suggested that this type of enzyme might not be activated by food digestion (Zambonino Infante and Cahu, 2001). In Indian Carp, there is a significant role of carbohydrate digestion at the early development in the fish as amylase can be detected during the first feeding and there is a temporary decrease of the enzyme during the development which might be related to the transition of the fish digestive system for suitable feeding habits (Chakrabarti and Rathore, 2010; Rathore *et al.*, 2005). On the other hand, the protease enzymes such as alkaline protease can be detected at the beginning of the 3 days post-hatch in larvae while acidic protease is only significantly higher after the 25 days of post-hatch (Chong *et al.*, 2002). A similar pattern is reported in the carp as protease activities can be detected as early as the 4 days but it remains low after hatching and the total proteolytic enzymes do not increase until the larva reaches the 24 days after hatching. The authors suggested that these changes might due to the modifications of digestion towards the adult mode of digestion (Rathore *et al.*, 2005). Lipase activities can be detected at an early stage of carp larvae and only increase as the larvae developed at the later stage. This indicates that the larvae have a better digestion and utilization of dietary lipids during these periods (Rathore *et al.*, 2005). After the hatching of butter catfish, lipase activities are present in between the development stage of the larvae. There are fluctuations of lipase enzyme activities as