EFFECTS OF SELECTIVE DIETARY PREBIOTICS AND PROBIOTICS ON GROWTH AND HEALTH STATUS OF SNAKEHEAD (*Channa striata*) FINGERLINGS

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by

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LIST OF ABBREVIATION

β	Beta
K	Kappa
AOAC	Associationof official analytical chemists
ANOVA	Analysis of variance
BLAST	Basic local alignment search tool
CMC	Carboxy methyl cellulose
CFU	Colony forming unit
ESR	Erythrocyte sedimentation rate
EUS	Epizootic Ulcerative Syndrom
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FCR	Food conversion ratio
FM	Fish meal
FOS	Fructooligosaccharides
GE	Gross energy
GI	Gastrointestinal
GOS	Glacto-oligosaccharides
Hb	Haemoglobin
HCL	Hydrochloric acid
Ig	Immunoglobulin
IMO	Isomalto-oligosaccharides
IPF	Intraperitoneal fat

ISAPP	International Scientific Association of Probiotics and Prebiotics
LBA	*Lactobacillus acidophilus*
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MOS	Mannan-oligosaccharides
MV	Microvilli
NFE	Nitrogen free extract
NFSC	Northwest Fisheries Science Center
NF κB	Nucleor factor kappa-B cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Packed cell volume
PER	Protein efficiency rate
PEG	Polyethylene glycol
PVA	Polyvenyl alcohol
RBC	Red blood cell
SGR	Specific growth rate
SPSS	Statistical package for social science
SOS	Soy-oligosaccharides
SR	Survival rate
TEM	Transforming electron microscope
TGF β1	Transforming growth factor beta 1
TOS	Transgalactosylated-oligosaccharides

T-RFLP	Terminal Restriction Fragment Length Polymorphism
VSI	Viscerosomatic index
qPCR	Quanitified Polymerase Chain Reaction
WBC	White blood cell
XOS	Xylo-oligosaccharides

KESAN PEMAKANAN PREBIOTIK DAN PROBIOTIK TERPILIH KE ATAS PERTUMBUHAN DAN STATUS KESIHATAN ANAK IKAN HARUAN (*Channa striata*)

ABSTRAK

Pembenihan ikan haruan yang dilakukan secara berterusan telah memberikan beberapa masalah misalnya kemerosotan kualiti air dan wabak penyakit terhadap ikan. Sejak kebelakangan ini, penyakit yang dihadapi oleh ikan telah di atasi melalui penggunaan antibiotik yang mengakibatkan mikro yang resistan kepada mikrob, pengurangan mikrobiota dalam ekosistem gastrik (GI) termasuk pengumpulan sisa antibiotic di dalam otot ikan dan menyebabkan ia tidak sesuai untuk dimakan oleh manusia. Untuk mengatasi masalah ini, satu pendekatan yang menggunakan pendekatan pemberian pemakanan baru menggunakan prebiotic dan probiotic telah dikaji. Kajian ini dijalankan untuk menilai kesan pengambilan makanan tambahan prebiotik dan probiotic ke atas tumbesaran anak ikan *Channa striata* untuk mengurangkan masalah di dalam sistem akuakultur dengan cara berterusan. Eksperimen ini melibatkan pemberian permakanan yang mengandungi β-glucan, Galakto-oligosakarida (GOS), Mannan-oligosakarida (MOS), yis hidup (*Saccharomyces cerevisiae*) dan serbuk LBA (*Lactobacillus acidophilus*) untuk tempoh 16 minggu (Fasa 1) diikuti dengan pemakanan yang tidak menggunakan bahan tambahan selama 8 minggu (Fasa 2). Kajian ini telah dibahagikan kepada dua fasa untuk menentukan keupayaan benih *C. striata* untuk mengekalkan manfaat yang diperolehi selepas pengambilan makanan tambahan ini dalam tempoh yang ditetapkan. Kumpulan 800 ikan (22.40g±0.06) secara duplikat diberi enam olahan

yang berbeza, iaitu 3 jenis prebiotics- 0.2% β-glucan, 1% GOS, 0.5% MOS dan 2 probiotik - 1% yis hidup, 0.01% serbuk LBA dan diet kawalan (tanpa makanan tambahan). Semua diet ini mengandungi 40% protein dan 12% lipid. Ikan yang digunakan dalam kajian ini diberi makan sebanyak tiga kali sehari. Selepas 16 minggu diberi makan makanan tambahan yang mengandungi prebiotic dan probiotic, perubahan dalam tumbesaran ikan, penghadaman protein, aktiviti penghadaman enzim, gut microflora, penghadaman protein relatif, aktiviti enzim penghadaman, usus mikroflora, hematologi dan parameter darah imunologi, ketahanan penyakit terhadap *Aeromonas hydrophila* dan ekspresi terhadap gen peraturan imun dengan ketara ($P < 0.05$) berbanding dengan makanan kawalan. Diet makanan yang ditambah dengan probiotic menghasilkan keputusan yang terbaik secara signifikan berbanding dengan 3 diet makanan yang menggunakan prebiotic yang mana hasil yang baik adalah daripada makanan yang dicampur dengan *L. acidophilus*. Walaupun ikan diberi makan dengan diet pemakanan β-glucan menunjukkan prestasi yang lebih baik untuk semua parameter yang dipantau selepas 8 minggu makan berbanding diet GOS dan MOS, namun tiada perbezaan ketara diperhatikan pada minggu ke-16. Dalam Fasa 2, tumbesaran ikan berterusan sehingga minggu ke-5 dan minggu ke-6, masing-masing untuk *S. cerevisiae* dan *L. Acidophilus* dan sehingga 4 minggu untuk prebiotik ditambah diet sebelum dikurangkan prebiotik tersebut. Keputusan yang diperoleh dalam analisis usus mikroflora yang menggunakan kaedah T-RFLP menunjukkan bahawa komuniti bakteria lebih banyak dalam diet permakanan (38) berbanding diet pemakanan dengan LBA yang menunjukkan keputusan tertinggi (49) daripada bakteria phylotypes. Ungkapan imun kawal selia dua gen (TGF β1 dan NF κ B) adalah dimasukkan dalam semua diet tambahan. Keputusan yang diperolehi daripada kajian ini menunjukkan bahawa makanan tambahan dengan *L. acidophilus*

(P<0.05) bukan sahaja menunjukkan prestasi pertumbuhan dan kesihatan yang terbaik kepada benih *C. striata* tetapi kelebihan ini dikekalkan dalam tempoh yang lebih lama berbanding diet makanan yang mengandungi *S. cerevisiae* dan prebiotik lain.

EFFECTS OF SELECTIVE DIETARY PREBIOTICS AND PROBIOTICS ON GROWTH AND HEALTH STATUS OF SNAKEHEAD (*Channa striata*) FINGERLINGS

ABSTRACT

Intensive culture of snakehead has resulted in problems such as deterioration of water quality and the outbreak of diseases. Currently, fish disease is managed through the use of antibiotics which has led to antimicrobial resistant pathogens, reduction in beneficial microbiota in the gastrointestinal (GI) ecosystem, including the accumulation of residual antibiotics in fish muscle making it unsuitable for human consumption. To overcome these problems a new feeding approach using prebiotics and probiotics is explored. The present research was conducted to evaluate the effect of feeding *Channa* striata fingerlings with different prebiotics and probiotics as well as duration of feeding on growth and health performance. The experimental design involved feeding experimental fish with β-glucan, Galacto-oligosaccharides (GOS), Mannan-oligosaccharides (MOS), live yeast (*Saccharomyces cerevisiae*) and LBA (*Lactobacillus acidophilus*) powder respectively, for a total of 16 weeks (Phase 1) followed by feeding of a control unsupplemented feed for 8 weeks (Phase 2). Duplicate groups of 800 fish (22.40 g$\pm$ 0.06) were raised on six different treatments respectively, three prebiotics - 0.2% β-glucan, 1% GOS, 0.5% MOS, and two probiotics - 1% live yeast, 0.01% LBA and a control (unsupplemented) diet. All diets contained 40% protein and 12% lipid. Fish were fed to satiation three times daily. After 16 weeks of feeding, prebiotics and probiotics supplemented diets improved growth performance, relative protein digestibility, digestive enzymes activities, gut microflora, haematological and immunological blood parameters, disease resistance against *Aeromonas hydrophila* and the expression of immune regulatory genes significantly ($P<0.05$) compared to the control diet. Among the supplemented diets feeding with probiotics resulted in better performance compared to the three prebiotics tested, with highest performance in fish fed with *L. acidophilus*. Although fish fed the β-glucan supplemented diet showed better performance for all the parameters monitored after 8 weeks of feeding compared to GOS and MOS supplemented diets, no significant differences were observed by the 16th week of feeding. In Phase 2, fish growth continued until the 5th and 6th week, for *S. cerevisiae* and *L. acidophilus*, respectively and up to 4 weeks for the prebiotics supplemented diets before decreasing. The results of gut microflora analysis using T-RFLP method revealed that bacterial community richness and evenness were enhanced regardless of dietary supplements compared to the control diet (38) while LBA resulting in the highest number (49) of bacterial phylotypes. The expression of immune regulatory two genes (TGF β1 and NF κB) were up-regulated in all supplemented diets. The results obtained from the present study showed that supplementation with *L. acidophilus* significantly ($P<0.05$) supports not only best growth and health performance of *C. striata* fingerlings but this advantage is retained over a longer period compared to feeding with diets containing *S. cerevisiae* and the other prebiotics.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The snakehead, *Channa striata* (Bloch, 1793), belongs to Channidae family, is a carnivores, obligatory air-breather freshwater fish. It is known as snakehead murrel, chevron snakehead, or striped snakehead, widely distributed in Asia, mostly in south-east Asian countries. It is the valuable food fish in Asia (Wee 1982), as it contains higher protein (16.2g in 100g) compare to similar other freshwater fishes (Annasari *et al*., 2012) like gold fish, eel etc. The fish has a high market value due to the high quality of flesh, low fat, less intramuscular spines and medicinal qualities (Haniffa and Marimuthu, 2004) particularly it's extracts like fins, scales are a good source of albumin for the people who have a deficiency of albumin. Albumin extracted from the snakehead is also used for injuries, burns as well as used in post operative stage. Traditionally it is used to accelerate healing process (Annasari *et al*., 2012). Therefore snakehead murrel has recently gained more attention from the aquaculture researchers and scientists; and the production yields have increased from 16 tons in 1998-2000 to 42 tons in 2010-12 (FAO, 2014).

The boost population growth in the world has increased the demand of the fish as it is the ample source of protein. To mitigate this demand, fish production are increased in both capture and culture sector. Presently the production trend in aquaculture are become higher than the capture fisheries. The statistical data represented that the production of culture fish increased from 49.9 metric ton (capture fisheries 90.8 metric ton) in 2007 ton to 66.6 metric ton (capture fisheries 91.3 metric tons) in 2012 (FAO, 2014). The fastest growing of world aquaculture is expanding into new directions, intensifying and diversifying. The persistent goal of new world aquaculture is maximizing the efficacy of fish production optimizing the profitability (Bondad *et al.*, 2005). Therefore, both commercial and artisal aquaculture farmers make attention more in fish production through adopting the new technologies like super-intensive, intensive and semi-intensive which make this sector as risk. The farmers can not able to follow the standard hygienic procedure for the aquaculture production. As a result, water quality is deteriorating which causes for out-breaking the disease. Farmers use the antibiotics to get rid of the disease These antibiotics develop the antimicrobial resistant pathogens, inhibit or kill the beneficial microbiota in the gastrointestinal (GI) ecosystem, and finally making antibiotic residue into fish body that accumulated in fish product to be harmful for human consumption (FAO, 2005). For this, the export importer countries tended to ban to export fish. It was already happened on 2006 by declaration of ban by the the European Union for exporting the fish from this sources. Infact, after imposing the ban of fish export, the world economy fall into a disrupt situation. To recover such problem, the researchers made more attention to explore new strategies in sustainable feeding and health management of aquaculture (Balcâzar *et al.*, 2006). These included evaluating the new dietary supplementation strategies in which various health and growth-promoting compound as dietary prebiotics, probiotics, symbiotics, phytobiotics and other functional dietary supplements (Denev, 2008).

Feed supplementation with dietary prebiotics and probiotics are present interest to adopt new aquaculture strategies to enhance growth performance and health status leading to increase the fish production (Diana 1997; Abdelghany and Ahmed, 2002) through reducing chronic fish disease in a sustainable way. Dietary probitics and prebiotics are proven as bioactive components (Kapka *et al.*, 2012) of functional foods that are providing not only nutrients, but also microorganisms, oligosaccharides and polysaccharides. These are usually indigestible in the living organisms' alimentary tract, but have been proven a positive effect on growth performance, nutrient digestibility and gut bacterial profile. Dietary prebiotics and probiotics are also considered as the antibiotics substitutes'. Prebiotics is a non-digestive feed ingredient (Gibson and Roberfroid 1995) that benefits fish by selectively stimulating growth (Grisdale *et al., 2008*), metabolism of health-promoting bacteria like lactobacillus, bifidobacteria, in the intestinal tract, while probiotics are live bacteria or cyanobacteria, microalgae, fungi etc. (Fuller, 1989) having beneficially affects the host growth by improving its intestinal (microbial) balance (Al-Dohail *et al.,* 2009, Dhanaraj *et al.,* 2010, Talpur *et al*., 2014). Environment-friendly aquaculture is another present interest in the aquaculture nutrition science (Denev, 2008).

The present aquaculture nutrition research focus on dietary prebiotics and probiotics as these are the alternative solution of antibiotics; and prominent functional feed supplements that have a unique attribute to increase the expression or change in the composition of short-chain fatty acids to colonocytes, to increase the fecal weight, to increase expression of the binding proteins or active carriers associated with the mineral absorption, to increase oligosaccharide exhibiting low β-glucuronidase and nitroreductase activity, and to enhance immunity and modulation of mucin production (Arturo *et al*.,2010). Therefore inclusion of prebiotics and probiotics functional feed supplements in fish diet may enhance not only the fish growth with reducing mortality percentage, but could also up or down regulation of immune regulatory genes. The innate immune regulatory system, also known as non-specific immune system and first line defence, is a subsystem of the overall immune regulatory system that comprises the cells and mechanisms that defend the host from infection by other organisms in a non specific manner. This means that the cells of the innate system recognize and respond to pathogens in a generic ways. There was an outstanding progress which has been obtained in isolating and characterizing immunological genes from fish (Feng *et al*., 2009). Presently, the aquaculture nutritionists have focused more attention on cytokine (pro- and anti-inflammatory genes particularly transforming growth factor beta 1 or TGF-β1 and nuclear factor kappa-light-chain-enhancer of activated B-cell or NF-κB) in fishes (Awad *et al*.,2011).

The present research was carried out to evaluate the effect of three prebiotics (β-glucan, GOS and MOS) and two probiotics (live yeast or *Saccharomyces cerevisiae* and LBA or *Lactobacillus acidophilus*) on growth performance and health status; and the capacity of *Channa striata* fingerlings to retain the benefits derived after the intake of these supplements with time. The research was designed with special attention to present need and involvement of molecular techniques particularly terminal restriction fragment length polymorphism (T-RFLP) methods to analyze the gut microflora, and the expression of immune regulatory genes using real time qPCR. The involvement of such modern techniques make the present research more rational and the need to examine the effect of different prebiotics and probiotics as well as to define the effectiveness duration of each supplementation.

1.2 Problem Statement

The increasing intensification and commercialization of aquaculture systems has accelarated the outbreak of diseases that are responsible for huge fish losses (Bondad-Reantaso *et al.*, 2005). In common with other intensive aquaculture practices, snakehead culture has also resulted in problems associated with the deterioration of water quality and diseases outbreak (FAO, 2012). The fish, *Channa striata* are bottom living species (Sahoo *et al*., 2012) and because of its habitat in bottom regions of boggy waters, where the bacterial population is usually 10–20 times higher than in the water column (Lewis and Bender, 1961), it is more susceptible to infection. *C. striata* is recognised as one of the most vulnerable species to epizootic ulcerative syndrome (EUS) showing severe ulcerations and mortality (Sahoo *et al*., 2012). The other disease associated with the snakehead are mostly by parasite particularly protozoa and worms.

Presently, snakehead cage aquaculture is adopted by the farmers of Thailand, Cambodia, Vietnam, Peninsula Malaysia and Indonesia use bamboo made cages for rearing the *Channa striata* fingerlings in the swamp water (Dina, 2013). In snakehead cage aquaculture, the farmers stock high density of snakehead fingerlings in one cage which require high amounts of feed resulting the high organic load in the cage environment. Similar to intensive culture system, the high organic matter leads to deteriorate the water quality which leads to the out-break diseases (Sinh and Pomeroy, 2010)..

Furthermore, these aquaculture practices do not accelerate the growth of *Channa striata* as it is a biologically slow growing fish. Farmers use the sub-therapeutic antibiotics as growth-promoting agents, which was banned by the European Union in 2006 (Denev *et al*., 2009) due to the growing incidence of antimicrobial resistant pathogens which reduce the beneficial microbiota in the gastrointestinal (GI) ecosystem, and the accumulation of residual antibiotics in fish muscle making it unsuitable for human consumption (FAO, 2005).

In addition, the decrease in seed collection of *Channa striata* from the wild source has prompted an increase in the number of commercial seed producing companies. Farmers collect the hatching seeds from those companies who use the wild source broods. Sometimes, these broods carry diseases (known as parental diseases) which transmit to the hatchlings eggs. These parental diseases are very difficult to treat using different meditational treatements (Sinh and Pomeroy, 2010). Moreover, the addition of such approaches increase the cost of snakehead production. To overcome these problems, aquaculture nutritionists are exploring alternative approaches for feed administration such as including prebiotics and probiotics in the diet.

The present research addresses the above issues by determining the most suitable probiotic and prebiotic and investigating the duration of effectiveness of the supplements in retaining the benefits acquired.

1.3 Research Objectives

This present research was designed to evaluate the effect of duration of feeding with selected prebiotics (β-glucan, GOS, MOS) and probiotics (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*) on growth performance and health status; and the capacity of *Channa striata* fingerlings to retain the benefits derived after the intake of these supplements with time. Specifically, the research objectives were:

1) To measure the effect of supplementation diets with dietary prebiotics and probiotics on fish growth, nutrient digestibility, digestive enzymes activity, blood parameters and diversity of gut microflora.

2) To determine the effect of the experimental feeds on the capability of the fish immune system to figure off infections caused by pathogens

3) To investigate the response of fish innate immune system towards the corresponding feed supplements

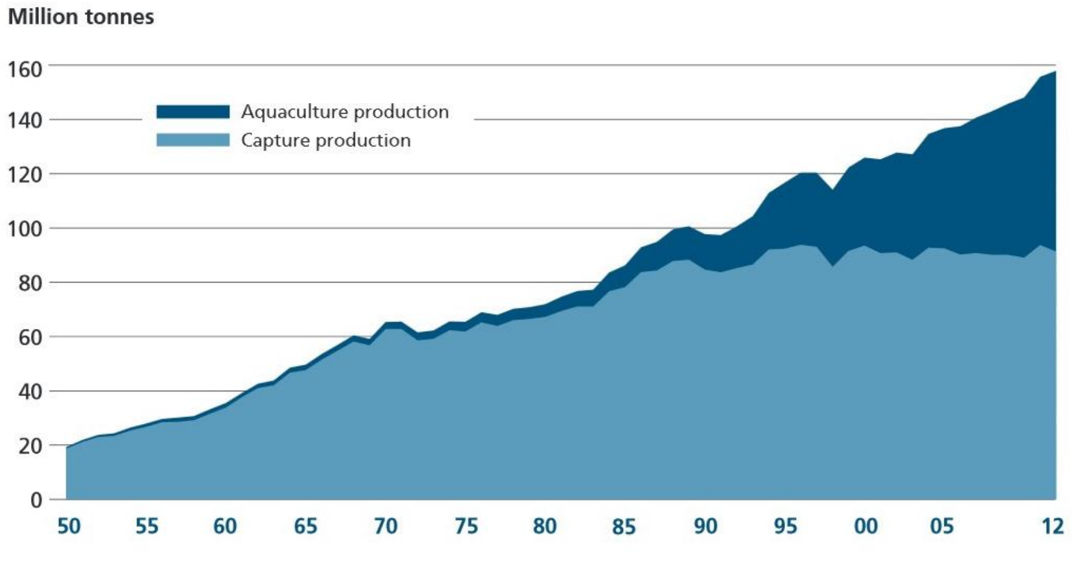
4) To determine the capacity of *C. striata* fingerlings to retain the benefits derived after the intake of these supplements with time

CHAPTER 2

LITERATURE REVIEW

2.1 Global Aquaculture and Challenges

The principal challenge of global aquaculture tends to mitigate the supply and demand of fish and fisheries products in paralleled with the outbreaking population growth in the world. The FAO (2014) reported that the total aquaculture production including the aquactic plants for 2012 was 90.4 million ton, where only fish and fisheries aquaculture production was 66.6 million tons with a farm-gate value US$106.38. This inland aquaculture production was accounted for almost 50% contribution in total fisheries production (Figure 2.1). The State of World Fisheries and Aquaculture (FAO, 2014) reported that the world food fish aquaculture production increased at an average annual rate of 6.2% in the period of 2000–2012 (9.5 percent in 1990–2000) from 32.4 million to 66.6 million tonnes. During this



Source: The state of World Fisheries and Aquaculture, FAO (2014)

Figure 2.1: A graph showing the present fisheries production both capture and culture

period, the growth was relatively faster in Africa (11.7%), followed by Latin America and the Caribbean (10%)., and the Asia (8.2%, excluding China). It was reported that China alone produced 43.5 million tonnes of food fish and 13.5 million tonnes of aquatic algae on 2012 (FAO, 2014) where the annual growth rate in China, the largest aquaculture producer, averaged 5.5% in 2000–2012. Nevertheless, comparision to the projected population by 2030, an additional 40 million tons of fish and fisheries production will be requires to maintain the present per capita consumption. Therefore, this sector are going to face some challenges, which are already adopted. Presently, aquaculture is thought to be the fastest growing food producing sector, and is perceived as having the greatest potential to meet the growing demand for aquatic food. Analyzing the future challenge in the fisheries sector, Food and Agriculture Organization (FAO) has scrutinized the following challenges which include:

1) The present aquaculture is growing with special attention for maintaining the food security, mitigating the unemployement, involving to develop the national economy including recreation. The success rate of aquaculture varies with the geographic location, market access and the affordable technology through taking some specific interventions which allow the maintain the production in a sustainable way;

2) The baseline data collection method is needed to be strengthening by evolving the scientific and social assessment concerning management and development option. It includes a) making consutation with the data users particularly extension workers and managers, so that they can perform their work perfectly; b) introducing the need base appropriate data collection method as well as data management system; c) ensuring the national commitment for the production of

fish and fisheries without any conflicts as well as to ensure from the national management body for sharing the data; d) involving the relevent organizations like FAO and non-FAO regional fisheries stakeholders and other appropriate institutions and organizations which are the part and percel of the regional fisheries production both in capture and culture;

3) The intensification of present aquaculture needs to get support from all sectors particularly the improvement between the government and private sectors. The is the most difficult part or challenges in present intensified aquaculture.

4) The most important challenge is to ensure to participate all relevant stakeholder and communities to make decision. This is specially for community based aquaculture management and co-management practices of common aquaculture pool;

5) Need to improve easy access, dissemination the good quality information timely using appropriate formats, in support of responsible aquaculture, and it's trade

6) The fishing gears are widely used in developing countries. The rules adopted for using the fishing gear are still needed to improve and impose during harvesting;

7) The fish trade is needed to promote with a view to avoiding disputes and imposition of sanctions; minimizing the impact on international fish trade on those groups most vulnerable to food insecurity;

8) The integration of the fisheries resources management is needed to develop in a sustainable way;

9) Need to adopt new technology, ensuring seed, feed (free of antibiotics) and fertilizer in terms of quantities and qualities;

10) Need to minimize the production loss through improvement in fish health management using need feeding strategies based on the culture fish;

11) Need to maximizing the source of feed ingredients with minimum cost and to minimize the severe completion of aquaculture resources use;

12) Need to maintain the good water quality for target aquaculture fishes;

13) Need to adopt integrate aquaculture management supporting with other farming activities creating an integrated new approach for low income target beneficiaries;

14) Need to take necessary action for improving the environmental management of aquaculture particularly the fish growth and health in terms of climate change;

15) Need to ensure to follow the intenational rules and regulation during operation of inland aquaculture that make the assurance of food safety to the final consumers.

In order to mitigate these challenges, the aquaculture sector must develop the capacity to build and run effective quality assurance systems to comply with increasing stringent international standards of international markets as well as extending these to the domestic markets. Similarly, it should promote efforts to improve selective feeding technologies to make economical utilization of fishes.

2.2 Taxonomy and Distribution of Snakehead (*Channa striata*)

The snakehead murrel (Plate 2.1) is reported as a species of snakehead fish belongs to the freshwater perciform (called the Percomorphi or Acanthopteri, are the largest order of vertebrates) fish, family Channidae, native to parts of Africa and Asia. The detail scientific classification is given below:

Plate 2.1: The snakehead (*Channa striata*) fingerling used in this study

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Infraclass: Teleostei

Superorder: Acanthopterygii

Order: Percoformes (Bleeker 1859)

Suborder: Channoidei

Family: Channidae (Fowler, 1934)

Genus: *Channa*

Species: *Channa striata* (Bloch, 1793)

The fish is known as Chevron snakehead or striped snakehead or banded snakehead or common snakehead which are reported as the common names of *C. striata*. The local name of this fish varies with the localities. Different localities have different local names. The names are soali (Pakistan); murrel (India); haal, shawl, shol (Assam, India); shol (West Bengal, India); shol (Bangladesh); morrul, morl, soura (Bihar, India); sowl, dhoali, carrodh (Punjab, India); dolla (Jammu, India); sola (Orissa, India); korramennu, korramatta (Andhra Pradesh, India); sowrah, veralu, kaunan (Kerala, India); poolikuchi, koochinamarl (Karnataka, India); sohr, dekhu

(Mararashtra, India); hal path maha, lulla (Sinhalese, Sri Lanka); viral (Tamil, Sri Lanka); pla chon or pla chorn (Thailand); trey phtuok (juveniles) and trey raws (adults; Cambodia); ikan aruan, haruan, ruan, tomam paya (Malaysia); gabus (Java); delak, gabus, telak (Kalimantan), cá ló (Vietnam); dalag, dalak (Tagalog or Moro, Philippines); bakule or bulig (young; Tagalog or Moro, Philippines); pongee (Hawaii).

2.2.1 Native distribution

Channa striata is a freshwater fish having a wide-range of native distribution in the world (Plate 2.2). Numerous studies have been reported that the fish is as a native fish of Pakistan (Indus River basin; Mirza, 1975), India, southern Nepal (Koshi, Gandaki, and Karnali River basins; Shrestha, 1990), Sri Lanka (Mendis and Fernando, 1962; Fernando and Indrassna, 1969; Pethyagoda, 1991); Bangladesh, Myanmar, Thailand, Cambodia, southern China, Malay Archipelago including Malaysia, Sumatra, Borneo (Pethiyagoda, 1991; Rainboth, 1996; Jayaram, 1999); Sabah (Inger and Kong, 1962); western Java (Giltay, 1933; Roberts, 1993); Vietnam, Laos (Kottelat, 2001a,b).

2.2.2 Introduced distribution

The snakehead, *Channa striata*, has been considered as the most widely introduced species (Plate 2.2) of snakehead. Although the fish was first introduced into Hawaii before 1900 and Madagascar in 1978 (Jordan and Evermann, 1903; Cobb, 1905; Smith, 1907; Tinker, 1944; Brock, 1952, 1960; Raminosoa, 1987; Reinthal and Stiassny, 1991), the misidentification with *Channa maculate* done by a group of scientists of American Museum of Natural History (AMNH) led not to make sure the first introduction at those waterbodies. The identification of *Channa*

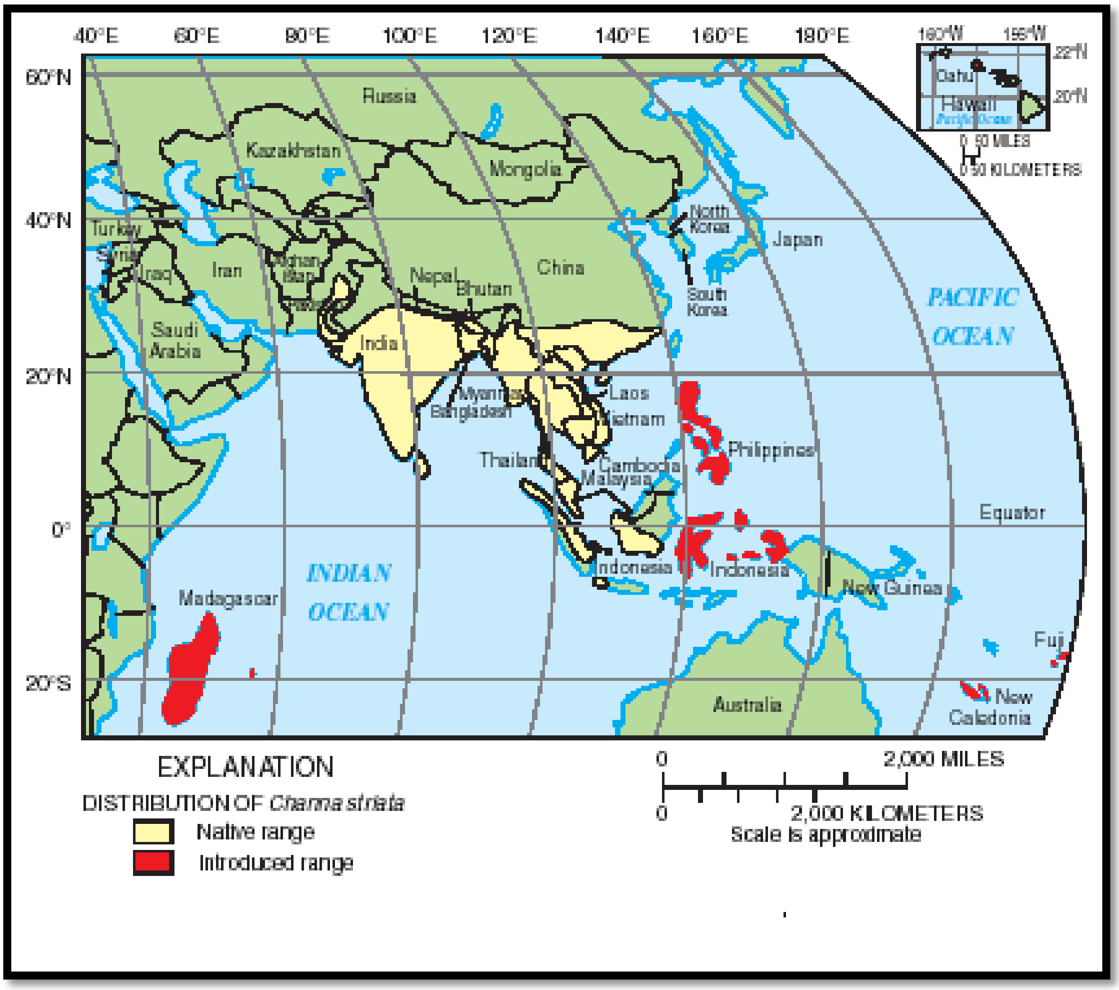


Plate 2.2: Distribution (native and introduced range) of *Channa striata*

striata is confirmed by another group of scientists in US Bureau of Fisheries (Welcomme, 1981; Kotellat *et al*., 1993; Lever, 1996). Based on the different literatures, the fish was introduced in Philippines, Vogelkop Peninsula, Papua, Indonesia during 1970s or 1980s (Seale, 1908; Herre, 1924, 1934; Conlu, 1986; Allen, 1991). In Fiji (Maciolek, 1984; Eldredge, 1994); in Mauritius (Parameswaran and Goorah, 1981; Welcomme, 1988; Lever, 1996); in New Caledonia (Maciolek, 1984); in Guam (Maciolek, 1984; Eldredge, 1994). Herre (1924) recorded the source of introduction into Hawaii as southern China. Kottelat *et al*., (1993) reported some populations in China to have been introduced but there were no specific locations. The introduction of *Channa striata* into the Philippines probably happened in the early to mid1800s, indicated by two synonyms (*Ophiocephalus vagus* and

Ophiocephalus philippinus) stated from the Philippines by Peters (1868). Although Jayaram (1999) found Borneo in the native range of this species, Roberts (1989) made arguments against this findings and the scientist hinted that its presence might have resulted from introductions in western Borneo (Plate 2.2).

Yamamoto and Tagawa (2000) identified the *Channa striata* introduced in the Hawaii and Madagasker before 1900 as *Channa maculate*, and it was blotched snakehead, reported as environmental threat invasive fish species (Courtenay *et al*., 2004).

2.3 Biology of *Channa striata*

The adults of *Channa striata* lives usually in ponds, streams and rivers, preferring stagnant and muddy water of plains (Menon, 1999). The fish is also found mainly in swamps, but also occurs in the lowland rivers. It is more available in relatively deep (1-2 m) and very common in freshwater plains (Tirant, 1929; Vidthayanon, 2002). The fish occurs also in medium to large rivers, brooks, flooded fields and stagnant waters including sluggish flowing canals (Taki, 1978) as well as ox-bow. The fish has a special mechanism for being survive in the dry season by burrowing in bottom mud of lakes, canals and swamps as long as skin and air-breathing apparatus which remain wet (Davidson, 1975) and subsists on the stored fat (Rahman, 1989). The fish *Channa striata* is carnivores, but the present study found this fish as a passive carnivores, it means when it feel hungry it attacks to other fishes living surround its' environment. It feeds on shrimp, prawn, crustaceans (Allen, 1991), fish, frogs, snakes, insects, earthworms, tadpoles (Rahman, 1989).

Channa striata is the species living in single or solitary except during spawning seasons (Lee and Ng, 1991) they are living together. They are spawning surround the year and laying a few hundred to more than 1,000 amber colored eggs (Parameswaran and Murugesan, 1976a; Talwar and Jhingran, 1992). The peak spawning coincides with peak rainfall (Parameswaran and Murugesan, 1976a). Howell (1913) observed that the eggs are non-adhesive, not over than 1.25 mm in size and hatched within 1 to 3 days. The females of *Channa striata* mature usually about 30 cm in length at about 2 years of age (Talwar and Jhingran, 1992; Ali, 1999). The parents of this fish clear a shallow depression by biting off aquatic vegetation (Ling, 1977). Nevertheless, *Channa striata* can able to spawn in the absence of vegetation (Alikunhi, 1953). Eggs usually float to the surface after fertilization (Lee and Ng, 1991). The most interesting part is the parents of *Channa striata* guard the pelagic eggs (Lowe-McConnell, 1987) and it is usually seen in Philippines and possibly throughout the native range of the species. Nevertheless, as it is by nature of passive carnibalism fish, therefore when the parents feel hungry during guarding, they usually fed the young fish after hatching and it was observed by Herre (1924). The ripe females present throughout the year in ricefields in Perak, northwestern Malaysia (Ali, 1999). The peak spawning in southwestern Sri Lanka occurs between May and September, with a secondary spawning October through December (Kilambi, 1986). Jhingran (1984) cited fecundity as 3,000-30,000 ova. Lee and Ng (1991) had collected fry without seeing parents nearby and the eggs hatch in 3 days in Malaysia, the fry developing a deep orange color which persists until the young reaching a length of 15 mm when only an orange lateral stripe exists. The orange color is lost when it becomes 40 mm in length , but there is a “pseudo-ocellus” appears on the posterior lobe of the dorsal fin. This characteristic usually lost in adulthood (Mookerjee *et al*., 1948).

2.4 Present status of *Channa striata*

The fish, *Channa striata*, is an important food fish (Wee, 1982) for many countries trade. It is a high priced fish when it is caught freshly. *Channa striata* is the most widely distributed and economically important member of the genus. It attains a length of 60 to 75 cm; common size 30 to 40 cm. Various reports revealed that the fish has been misidentified in places where this species has been reported as introduced (Madagascar and Hawaii in particular), and the introduced snakehead is *C. maculata* (Courtenay *et al*.,2004). Until identification of introduced "*C. striata*" is verified, its reputation as the most widely cultured snakehead.

Channa striata is one of the most valuable fish in Asian people; the fish is mostly common staple food fishes in Thailand, Indochina and Malaysia (Davidson, 1975), probable due to its firm, white and almost boneless tasty flesh and also easy to operate making commercially viable to culture (Qin and Fast, 1998). Because of having air-breathing attribute, the fish can be sold alive in the market with higher price compared to dead fish because people like to consume fresh fish for better taste.

Channa striata is presently considered as a 'police fish' in poly-culture technique. It has been branded as an undesirable intruder to other fish culture systems due to its piscivorous behaviour. Therefore the fish has however amazingly developed into a foremost species in aquaculture nowadays (Chen, 1976; Qin and Fast, 2003). In addition the fish has an economical importance in both culture and capture fisheries throughout southern and southeastern Asia (Vidthayanon, 2002). During the culturing of snakehead, the farmers are noticed to facing cannibalism and the huge size variation problem (Wee, 1982; Diana *et al*., 1985), which was also reported in a survey report made by Boonyaratpalin *et al*. (1985). The poor survival was reported because of having the cannibalism behaviour during the initial period when the size variation occurred. When they feel hungry and having inadequate of food, the juvenile snakehead can eat their siblings which were smaller size (Diana *et al*., 1985; Qin and Fast (1996). The regular size grading and feeding the fish *ad libitum* can able to reduce such cannibalism. Various report suggested that the suitable stocking density for snakehead for grow out in tanks can be increased to more than 30 m^{-2} when food is not limited (Rahman *et al*., 2012). The environmental high temperature can help the fish gaining more weight and the greater size disparity.Nonetheless, temperature could not affect the cannibalistic behavior among snakeheads (Qin and Fast, 1998).

Presently, the fish *Channa striata* is used to control the fast breeding of tilapia. The treatement sex-reverse of nile tilapia may create some antibiotic residual problem in the human body. Therefore, in order to control the use of sex-reverse antibiotics (Yang *et* al., 2004) the aquaculture researchers has recommended to use *Channa striata* as it is a predator fish helps to be the biological control at ratios of (1:80), (1:40), (1:20) and (1:10) with nile tilapia mix culture. During the harvesting in mixed culture, these predatory snakeheads not only acted as biological control of tilapia but also contributed to economic gain since it had high market value.

2.5 Economical Importance of *Channa striata*

2.5.1 Introduced Region

Although the mis-identification has been occurred during introduction of *Channa striata* in Hawaii (Maciolek, 1984), but it is utilized as a food resources. At that moment, several thousand metric tons of frozen snakeheads are reported as being imported annually for food purposes into mainland USA.

After proper identification of *Channa striata*, the US Department of Agriculture Small Business Innovation Research Program funded a Phase II project to the Hawaii Fish Company of Waialua, Hawaii, $230,000 for 24 months, to develop commercial culture of *Channa striata*. This project had three phases: Phase I consisted of the establishment of feasibility of rearing striped snakeheads in captivity, spawning, and studies on rearing juveniles on artificial diets whereas in Phase II was targeted to production of larvae and juveniles through induced spawning. The additional studies on feeding, and cost-effective grow-out performance to marketable size was conducted in Phase II. Phase III was designed to result in a commercial effort to produce farm-raised snakeheads for Hawaii, mainland U.S., and Canada. This species often appears in aquarist-oriented websites and has been sometimes listed for sale by commercial aquarium websites. Interest in its use as an aquarium fish seems to be limited due to the size it attains and its aggressive nature toward other fishes.

2.5.2 Native Region

Because of having its economical importance, the fish is reported as being cultivated in Pakistan, India and Sri Lanka. The fish is also used as food fishes in these regions (Fernando and IndrassnaInrassna, 1969). The fish is also cultured in Vietnam (Pantulu, 1976; Bard, 1991), Thailand, Java (Hofstede *et al.*, 1953), and the Philippines (Guerrero, 2000). It is one of the expensive fish of these region (Bard 1991). The fish is also as "a popular food fish in Malaysia" remarking that ricefields have provided the largest source of this fish (Ali, 1999). The fish are utilized for medicinal purposes, particularly in Indonesia and Malaysia (Ng and Lim, 1990 and Lee and Ng, 1991). The fish was used to prepare a postnatal diet and during recuperation from illnesses or surgery (Lee and Ng, 1991). The oils from the *Channa striata* are used in Malaysia, to greatly reduce scarring.

In Malaysia, the cream is commercially extracted from *Channa striata* tissues which contains a high levels of arachidonic acid, a precursor of prostaglandin, essential amino acids (particularly glycine), and polyunsaturated fatty acids which are necessary to promote prostaglandin synthesis (Baie and Sheikh, 2000a and b). The fish is good for the treatment of wounded and burned patient. The fish contains an antimicrobial quaternary ammonium compound which increase the tensile strength (Baie and Sheikh, 2000a). Lee and Ng (1991) indicated that the flesh of these larger snakeheads is rejuvenating following illnesses, prepared by being double-boiled with herbs, and only the soup is consumed. Nevertheless, for the soup to be effective in recovery, it is firmly believed that the fish must be killed just before cooking, dispatched with careful but firm blows to the head with a mallet. Herre (1924) reported much the same for the Philippines. Conceivably, this could be a reason that obtaining live snakeheads in live-food fish markets is considered important to some persons of southeast Asian descent living in the United States.

2.6 Global aquaculture production of *Channa striata*

The fish, *Channa striata*, has an economical importance in both culture and capture fisheries throughout the world. The fish is widely cultured in greater Asia mainly because having it's high protein content (Annasari *et al*., 2012), low fat and minimal intramuscular spines and medicinal qualities, (Haniffa and Marimuthu, 2004) used traditionally to treat injuries and burns. Hence, in recent years the snakehead aquaculture industry has expanded and production yields have increased (Figure 2.2) from 16 tons in 1998-2000 to 42 tons in 2010-12 (FAO, 2014).

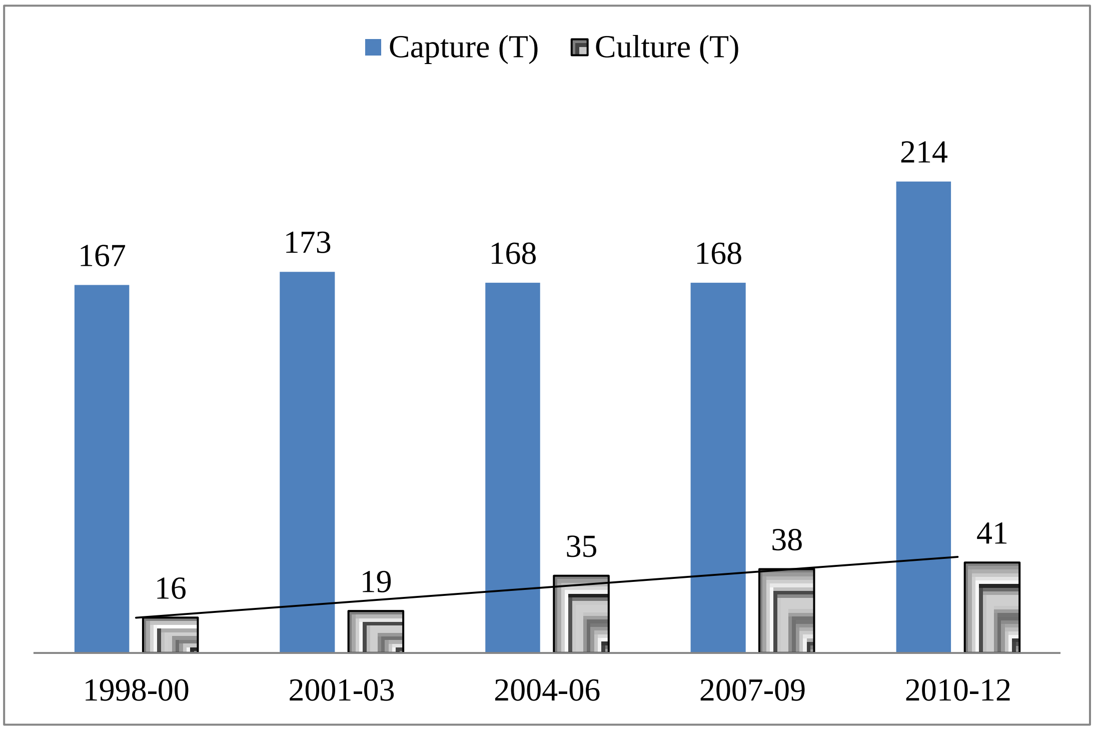


Figure 2.2: Global production (both capture and culture) of *Channa striata* in MT (FAO, 2012)

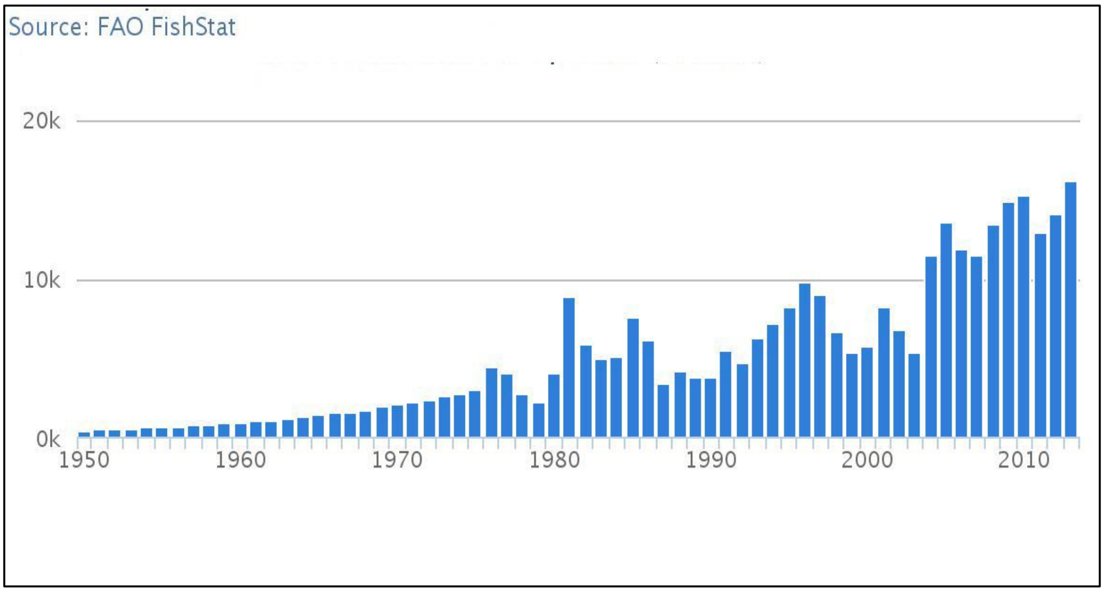


Figure 2.3: Global aquaculture production of *Channa striata* in kg

Figure 2.3 represents the global aquaculture production of *Channa striata* in killogram. It indicates that before 1970, the production from aquaculture was very low; it became familiar to culture after 1980. Besides the misidentification of introduction of *Channa striata* may lead the reduction of culture production. After correction of identification in 2002, the US Department of Agriculture started the aquaculture production of *Channa striata* commercially. Therefore, the global production from aquaculture of *Channa striata* became high almost near to reach 20,000 kg (FAO, 2014) at the end 2012.

2.7 Feeding Mechanism and Digestive System of Carnivores Fish

As *Channa striata* is a carnivores fish of teleost group, the feeding mechanism of the teleost fish might be well enough as literature rev iew. The teleost

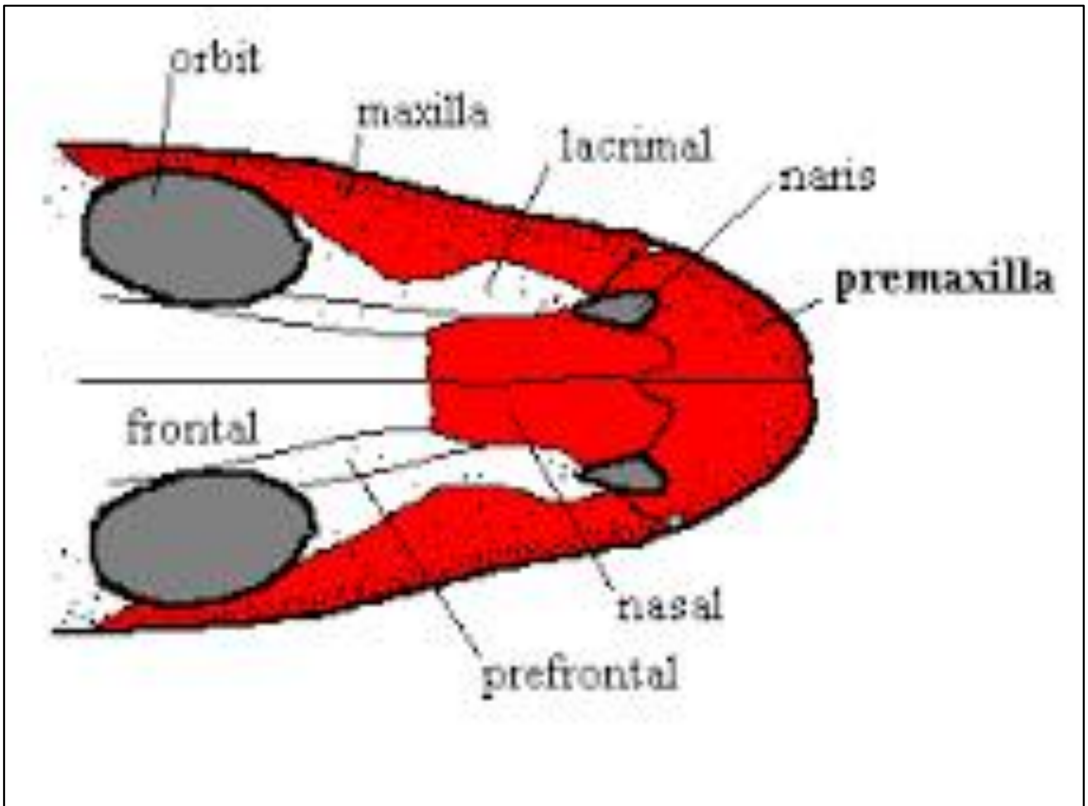


Plate 2.3: Dorsal view of primitive premaxilla

fish is notable from that of more primitive halecostomes by the separation of the premaxilla (Plate 2.3) into a mobile lateral toothed portion and a medial portion which becomes associated with the ethmoid complex (Patterson, 1973). Most of predaceous teleosts {e.g., Hoplias, Salmo) the premaxilla has become secondarily firmly attached to the neurocranium, but the primimeval condition for teleosts as exemplified by Pholidophorus, Leptolepis, or ichthyodectiforms, is a small mobile premaxilla (Patterson, 1977; Patterson and Rosen, 1977).

Whilst there have been major modifications within the Teleostei in the overall shape of the jaw and its component elements, only three major types of change have occurred in the pattern of interconnections in the structural network, of the head. The first specialization involves a shift in introduction of the mandibulohyoid ligament to the interoperculum. The interoperculohyoid ligament characterizes the feeding mechanism of eurypterygian fishes (=Aulopiformes + Myctophiformes + Paracanthopterygii + Acanthopterygii; Rosen, 1973) and effectively shifts the action of the hyoid and opercula coupling onto the interoperculum. Only the interoperculomandibular ligament transmits posterodorsal hyoid and opercular movement to the mandible in the Eurypterygii, while other teleosts retain the primitive two-coupling system of halecostomes.

The second major structural specialization within teleosts is the development of an elongate ascending process on the premaxilla and modification of maxillary and premaxillary articular surfaces and ligaments, all associated with protrusion of the upper jaw toward the prey during feeding.

Finally, a number of changes in the jaw adductor musculature have occurred. Primitive teleosts are characterized by the presence of a geniohyoideus muscle extending anteroposteriorly between the mandibular symphysis and the ceratohyal and epihyal. The geniohyoideus muscle of teleosts represents a fused intermandibularis posterior and interhyoideus of primitive actinopterygians (Winterbottom, 1974). Teleosts have lost the branchiomandibularis of primitive actinopterygians (Lauder, 1980a; Wiley, 1979), as well as the suborbital adductor component. Only a single non-branched lateral adductor muscle is present in primitive members of the Osteoglossomorpha, Elopomorpha, and Clupeomorpha, whereas in many euteleostean lineages both lateral and medial subdivisions of the main adductor mass (Winterbottom, 1974) are present. Of particular importance for the evolution of protrusile mechanisms in teleosts is the independent evolution in many lineages of one or more adductor divisions with insertions on the maxilla. Stomiiforms, myctophiforms, some paracanthopterygians, and some primitive acanthopterygian fishes possess a medial subdivision of the main adductor mass. which inserts on the maxilla (Fink and Weitzman, 1982; Rosen, 1973). A well-developed intramandibular adductor division is present in most teleosts.

During feeding, the acanthopterygians suborder fishes have an ability to extend the premaxilla and maxilla toward the prey, which is the most widely discussed features of the teleost feeding mechanism (Alexander, 1967; Eaton, 1935; Gregory, 1933; Lauder and Liem, 1981; Liem, 1970, 1979, 1980; Nyberg, 1971; Pietsch, 1978; Schaeffer and Rosen, 1961; van Dobben, 1937). It is now clear demonstrated that there is a number of different mechanisms protrusion involving non-homologous articular surfaces possessed by acanthopterygians sub-order fishes. In 1967, Alexander stated a mechanical explanation for premaxillary protrusion, which seems to apply to some primitive acanthopterygians sub order fishes. According to his explanation that depression of the lower jaw causes rotation of the maxilla along its long axis and tightening the adductor mandibulae. The phenomenon is responsible for the premaxillary process of the maxilla pressing against the articular process of the premaxilla which forces the premaxilla to protrude anteriorly. There is needed two prerequisites for motivating this mechanism, which are (1) maxillary twisting; (2) apposition of the premaxillarv articular surface with the premaxillary condyle of the maxilla. The movements of the suspensory apparatus