GROWTH AND REPRODUCTIVE PERFORMANCE OF FEMALE GUPPY (*POECILIA RETICULATA*) IN RESPONSE TO DIETARY FATTY ACIDS

Thesis submitted in partial fulfillment

of the requirements

for the degree of

Ph. D. (Inland Aquaculture)

by

H.M.P.KITHSIRI, M. Phil.

(IAC-211)

CENTRAL INSTITUTE OF FISHERIES EDUCATION
(Deemed University)
Indian Council of Agricultural Research
Versova, Mumbai – 400 061

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Dedicated to,
My parents, my wife Lali, sons Tharindu and Ravindu

केन्द्रीय मात्स्यिकी शिक्षा संस्थान
(समतुल्य विश्वविद्यालय) भारतीय कृषि अनुसंधान परिषद
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(Deemed University) Indian Council of Agricultural Research

Dated: 3rd April 2007

CERTIFICATE

Certified that the thesis entitled "**GROWTH AND REPRODUCTIVE PERFORMANCE OF FEMALE GUPPY, (*POECILIA RETICULATA*) IN RESPONSE TO DIETARY FATTY ACIDS**" is a record of independent bonafide research work carried out by Mr. H.M.P.Kithsiri during the period of study from October 2004 to March 2007 under our supervision and guidance for the degree of **Doctor of Philosophy (Inland Aquaculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar titles.

Major Advisor/Chairman

(G. Venkateshwalru)
Senior Scientist
Div. of Fish Nutrition and Biochemistry

Advisory Committee

(Dilip Kumar)
(Director, CIFE)

(A. K. Pal)
Principal Scientist
Div. of Fish Nutrition & Biochemistry

(N. P. Sahu)
Senior Scientist
Div. of Fish Nutrition & Biochemistry

(S.G.S.Zaidi)
Technical Officer
Div. of Aquatic Animal Health Management

ीज युनिवर्सिटी रोड, सात बंगला, वरसोवा, मुंबई - 400 061
eries University Road, Seven Bungalows,
sova, Mumbai - 400 061
✆ : (कार्यालय/ Office) : 022-2636 1446/7/8, 26361632/26320395
तार / Tele. : फिशईन्स्ट/FISHINIST, फैक्स : 91-22-26361573,
ई-पत्र / E-mail : fishinst@bom3.vsnl.net.in • cife@vsnl.com
: www.fisheries university.org • http : //cife.bom.nic.in

DECLARATION

I hereby declare that the thesis entitled **"GROWTH AND REPRODUCTIVE PERFORMANCE OF FEMALE GUPPY, (*POECILIA RETICULATA*) IN RESPONSE TO DIETARY FATTY ACIDS"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

(H.M.P.Kithsiri)
Ph.D. Student
Central Institute of Fisheries Education

Date:
Place: Mumbai

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(H.M.P.Kithsiri)

सारांश

दक्षिण एशिया में गप्पी (पोइसिलिया रेटिकुलेटा) के पालन में इस्तेमाल आहार का मूल्यांकन क्रमानुसार दो आहार परीक्षण के द्वारा किया गया तथा इस बात की जांच की गई कि विभिन्न प्रकार के वसीय अम्ल का मादा गुप्पी के वृद्धि एवं प्रजनन प्रदर्शनों के स्तर पर क्या प्रभाव पड़ता है । इस हेतु तीन प्रकार के आहार 1, 2 एवं 3 जिनमें प्रोटीन की 18.26%, 29.27% एवं 43.60% मात्रा एवं लिपिड की 4.17%, 4.55% एवं 9.47% मात्रा का क्रमशः प्रयोग किया गया । आहार 3 जिसमें प्रोटीन तथा लिपिड बताए गए स्तर तक पाया गया तथा उल्लेखनीय उच्च विशिष्ट दर ($P < 0.05$), गर्भाशय वजन, पूर्ण गर्भाधान, फ्राई की संख्या एवं उनकी जीवितता उच्च विशिष्ट दर पर पाई गई । मादा गप्पी के आहार के वसीय अम्ल प्रोफाइल एवं मसल्स से स्त्रावित लिपिड, अंडा एवं इम्ब्रयो GC-MS द्वारा नियत था । आहार 3 में EPA, DHA, n-3, PUFA, n-3 HUFA एवं n-3/n-6 का औसत उल्लेखनीय रुप से अधिक था । इस आहार से गप्पी के मसल्स एवं ओवरी यह संकेत करता है कि यह वसीय अम्ल उच्चतम वृद्धि, प्रजनन प्रदर्शन एवं फ्राई जीवितता में अहम भूमिका निभाता है । दूसरे प्रयोग में, चार आइसोकैलोरोफिक , आइसो लिपीडिक, आइसो-प्रोटिक आहारों की संरचना में नारियल तेल, सूर्यमखी तेल, लिनसीड तेल एवं काड लीवर तेल का इस्तेमाल कर क्रमशः आहार - CO, आहार - SO, आहार - LO एवं आहार - FO तैयार किया गया । आहार - FO जिसमें उल्लेखनीय रुप से सबसे अधिक n-3 HUFA था, (P 0.05) विशिष्ट वृद्धि पर, ओवरी वजन, जननग्रंथि (गेनाडोसोमैटिक इनडैक्स), पूर्ण गर्भाधान फ्राई उत्पादन एवं उनके जीवितता को दर्शाता है । दूसरा सबसे उच्चतम वृद्धि एवं प्रजनक प्रदर्शन मत्स्य आहार - CO में देखा गया जो संभवतः गप्पी के मसल्स एवं अंडे में n-3 HUFA के चयनित धारणक्षमता के कारण हो सकता है । n-6 पाथवे (18:3, 20:3, 20:4) के एवं n-3 पाथवे (18:4, 20:4, 20:5, 22:5, 22:6) के गप्पी के मसल्स में क्रमशः आहार - SO एवं आहार-LO के कारण उल्लेखनीय मात्रा में पाया गया तथा यह संकेत करता है कि इसकी धारिता असांद्रित है । प्रतिगमन (रीग्रेशन) विश्लेषण यह उद्घाटित करता है कि मत्स्य टिशू (ऊतक) एवं एग लिपीड की संरचना में वसीय अम्ल है जो उन सबके आहारीय लिपीड को दर्शाती है । प्रमुख अवयवों के विश्लेषण से सभी स्तरों पर वसीय अम्ल संरचना के डेटा मैट्रिक्स प्रदर्शन का पता चलता है । इसके साथ यह भी उद्घाटित होता है कि नमूने एवं जिम्मेदार अंतरों के बीच सहसंबंध है जो स्कोर प्लॉटस, लोडिंग प्लाट्स एवं बाई-प्लाट्स के रुप में है । वर्तमान अध्ययन यह प्रदर्शित करता है कि असंतुलित पौष्टिकता के आहार का इस्तेमाल गप्पी संवर्धन में अल्प प्रजनन प्रदर्शन का एक कारण है । तत्काल मछली के n-3 HUFA की उपलब्धता या तो आहारीय स्रोत के रुप में या बायोसेंथेसिस रुप में गुप्पी के प्रजनन प्रदर्शन एवं वृद्धि को बढ़ाता है ।

ABSTRACT

Two feeding trials in the sequential order were conducted to evaluate the feeds used in the guppy (*Poecilia reticulata*) farming in South Asia, and to investigate the effects of varying dietary fatty acid levels on growth and reproductive performance of female guppy. The feeds namely Diet-1, 2 and 3 contained 18.26%, 29.27% and 43.60% of protein and 4.17%, 4.55% and 9.47% of lipid respectively. The Diet-3 which was found to contain the recommended levels of protein and lipid had shown significantly ($p<0.05$) the highest specific growth rate, ovary weight, absolute fecundity, number of fry and their survival. The fatty acid profiles of the diets, and lipid extracted from muscle, egg and embryo of female guppy were determined by the GC-MS. The EPA, DHA, n-3 PUFA, n-3 HUFA and n-3/n-6 ratios were significantly higher in Diet-3 and the muscle and ovary of the guppy fed this diet indicating the significant role of these fatty acids to achieve the highest growth, reproduction performance and fry survival. In the second experiment, four iso-calorific, iso-lipidic and iso-proteic diets were formulated using coconut oil, sunflower oil, linseed oil, and cod liver oil as lipid sources for preparing Diet-CO, Diet-SO, Diet-LO and Diet-FO respectively. The Diet-FO which had significantly the highest n-3 HUFA, showed significantly ($p<0.05$) the highest specific growth rate, ovary weight, gonadosomatic index, absolute fecundity, fry production and their survival. The second highest growth and reproductive performance was observed in the fish fed Diet-CO which may be due to the selective retention of n-3 HUFA in the muscle and egg of guppy resulting from preferential oxidation of short chain fatty acids. The significant amounts of metabolites of n-6 pathway (18:3, 20:3, 20:4) and n-3 pathway (18:4, 20:4, 20:5, 22:5, 22:6) were found in the muscle of guppy fed Diet-SO and Diet-LO respectively, indicating its capacity to desaturate and elongase the essential fatty acids into HUFA. Regression analyses revealed that the fatty acid composition of fish tissue and egg lipids reflected those of the dietary lipids. The principal component analysis, performed on the data matrix of fatty acid composition in all stages, revealed the correlation among samples and the responsible variables in the form of "score" plots, "loading" plots and bi-plots. The present study demonstrated that the use of feeds with inadequate nutrients could be one of the reasons for poor reproductive performance observed in the guppy farming. Further, the availability of n-3 HUFA either in the form of dietary source or biosynthesis from the dietary precursors enhanced the growth and reproductive performance of guppy.

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Abbreviations

AA	Arachidonic Acid
ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
CMC	Carboxy Methyl Cellulose
DE	Digestible Energy
DHA	Docosahexaenoic Acid
DM	Dry Matter
DPA	Docosapentaenoic Acid
EE	Ether Extract
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
FCR	Food Conversion Ratio
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GSI	Gonadosomatic Index
HUFA	Highly Unsaturated Fatty Acids
LA	Linoleic Acid
mg	Milligram
ml	Milliliter
mm	Millimeter
MUFA	Monounsaturated Fatty Acids
NL	Neutral Lipids
PCA	Principal Component Analysis
PL	Polar Lipids
PUFA	Polyunsaturated Fatty Acids
SAFA	Saturated Fatty Acids
SE	Standard Error
SGR	Specific Growth Rate
TAG	Triacylglycerols
TC	Total Carbohydrate
wt	Weight

Introduction

1. INTRODUCTION

Fish keeping in captivity is an age-old practice. Today, culture of ornamental fish is a rewarding industry and fish keeping is a popular hobby. Owing to popular demand and pressure on the wild resources, farming of ornamental fish especially the tropical live-bearers (guppies, swordtails. platies and mollies) is now an established industry in several Asian countries. The percentage contributed by the Asian countries to the global ornamental fish trade is 68 and the USA is the largest market of ornamental fishes followed by the European Union and Japan. In the ornamental fish trade, about 90% of the species are from aquaculture while about 10% are collected from the wild. Of the nearly 1500 different species of ornamental fish that are imported yearly, the quantities are dominated by only few species. The freshwater species dominating the market are the guppy, neon tetra, platy, swordtail, molly, angelfish, goldfish, zebra danio and discus. Four of the top 10 species are live-bearing (viviparous) tooth carps belonging to the family Poeciliidae (Singh, 2005).

The guppy (*Poecilia reticulata* Peters, 1860), native to fresh and brackish waters of northeastern South America and adjacent islands of Caribbean, is one of the most popular ornamental fish in the world. The word reticulata refers to the overlapping scales that form a lace-like pattern on the body of the guppy. The guppy is commonly called the rainbow fish because of the numerous colour patterns. The guppy is also called as millions fish because it is a prolific breeder leading to large number of varieties as a result of hybridization. The male *P. reticulata* have bright colours and are, therefore, a lot more attractive than females.

Generally, guppy produces 10-80 numbers of fry at a time of spawning. The number of fry produced mainly depends on the broodstock nutrition, size of the fish, health condition of the fish, and the water quality parameters. The production cost can be minimized through increasing the production with minimum facility. Thus, nutritionally balanced broodstock diets play a big role in the increase of production with minimum

facilities. The commercial level guppy farming industry has been facing several problems such as low brood size, low quality and survival of fry, broodstock replacement period is low and length of the brood interval is high. One of the reasons for these may be due to the use of nutritionally unbalanced diet for the broodstock. According to the literature, there is a lack of information on the nutritional requirements of ornamental fish. Most of the nutritional studies have been carried out on the protein requirements of fish and there is a lack of literature on the effect of dietary lipid sources on the growth and breeding performance of ornamental fish.

Ornamental fish culture is one of the fastest growing farming activities in the world during last two decades. Some of the reasons for the popularity and commercial culture of Poeciliids in most of the Asian countries are: suitable climatic conditions, and availability of several fringe varieties with diverse colours. The farming of Poeciliids is usually carried out in indoor cement tanks, out door earthen ponds or net cages. Feeding of broodstock in Asian farms still relies on the live feed such as blood worm, *Tubifex,* coupled with daily prepared paste consisting mixture of fish meal and skimmed milk powder (Fernando *et al.*, 1991). Besides risk of harmful pathogens, these feeding practices may not provide adequate nutrient levels required by broodstock fish.

A full and comprehensive understanding of the reproduction mechanisms such as gonadal maturation, fertilization success and larval quality is far from complete as these coordinated processes are very complex. Broodstock nutrition studies offer to provide knowledge by determining the responsible factors for improvement of reproductive performance by maternal dietary intake. In teleosts, numerous nutrition related factors such as feed ration, nutrient levels and compositions have been shown to influence various reproductive parameters such as gonadal development, egg quantity and quality, spawning success, hatchability and larval quality (Izquierdo *et al.*, 2001; Watanabe and Vassallo–Agius, 2003). Provisions of lipids to oocytes, followed by storage and accumulation in yolk and subsequent utilization by developing embryos are essential processes in reproduction and development (Brooks *et al.*, 1997).

In many cultured species, unpredictable and variable reproductive performance is an important limiting factor for the successful mass production of juveniles. An improvement in broodstock nutrition and feeding has been shown to improve not only egg and sperm quality but also seed production. Gonadal development and fecundity are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenesis periods (Izquierdo *et al.*, 2001). The production of aquaculture species can be economical only when its qualitative and quantitative feed requirements are known and the formulation of nutritionally balanced low cost diets are possible (Tacon *et al.*, 1983, Hashim *et al.*, 1992, James *et al.*, 1993).

Growth, gonadal development and reproduction of fish are influenced by many factors. In particular, reproductive performance is highly affected by the nutritional status of fish, which is known to condition several reproductive traits, such as age at maturity, fecundity, egg size, chemical composition of egg and also embryonic developments (Shepherd and Bromage, 1998; Eskelinen, 1989; Carillo *et al.*, 2000). In freshwater fish, embryonic development depends on the energetic reserves of the yolk sac. Among dietary components, a primary role is played by energy. In fact, net energy requirements for maintenance are to be satisfied before growth and reproduction (Shepherd and Bromage, 1998).

Feed composition, quality and quantity and ration size are among the most important nutritional factors (Sampath and Pandean, 1984, James *et al.*, 1993, Jobling, 1998). Thus during the last two decades, more attention has been paid to the levels of different nutrients in broodstock diets. In the wild, fish eat a range of foods and their diet is dependent upon the availability of food in that particular environment. The available food types also change with the change of season. In a closed environment, when the aquarium fishes are maintained, in addition to different live feeds, formulated feeds are also given to obtain better breeding and maintenance. In nature, the guppy feed on small invertebrates, aquatic insect larvae, algae and other plant materials to fulfill their dietary nutrient requirements (Dussault and Kramer, 1981).

It has been reported that the fertility in the guppy was reduced under conditions of food scarcity (Hester, 1964).

Lipid and fatty acid composition of broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring. Studies in the last two decades involving in the variety of farmed fish species have identified lipid, and in particular Highly unsaturated fatty acids (HUFA), as key nutrients affecting broodstock reproductive performances (Bell and Sargent, 2003; Watanabe and Vassallo–Agius, 2003). The HUFA play an integral role in regulating levels of eicosanoids, which in turn control selected stages of reproduction, such as steroidogenesis and ovulation. Although a number of studies have indicated the ability of freshwater fish species to utilize diets with low levels of HUFA, very little is known about HUFA requirements and utilization during freshwater fish reproduction (Izquierdo *et al.*, 2001).

Most of the ornamental fish farmers believe that the live-bearer fish have the ability to use any kind of feed for their growth and reproduction. Therefore, the broodstocks are provided with diets, which contained varying dietary nutrient levels. Studies undertaken so far on the nutrients requirements of guppy are mainly based on the dietary protein requirements in growth and breeding performance. However, In some research papers it has been indicated that dietary omega-3 fatty acids play a major role on breeding performance of fish and some reports indicated that omega-6 fatty acids play a major role in breeding performance and hence there is an uncertainty of the type of fatty acids require for the breeding performance of fish.

Several studies have highlighted the importance of both quantity and quality of dietary lipid on reproductive performance of broodstock (Bell *et al.*, 1997; Fernandez-Palacios *et al.*, 1995). Many studies have highlighted that dietary fatty acids play a big role on the growth and reproductive performance of fish. However, as per the available literature no work has been reported on the effect of dietary fatty acids on the reproductive performance of guppy. Thus, it is essential to carry out a comprehensive

study and find out whether there is a significant effect of dietary lipid sources on the growth and reproductive performance of guppy. Hence, the current study was undertaken to investigate on the growth and reproductive performance of guppy with the following objectives.

Objectives of the Study:

1. To evaluate the diets currently being used in the guppy fish farming on growth and reproduction of female guppy.

2. To study variation in the fatty acid profile of female guppy during maturation in response to different dietary lipids.

3. To study composition, accumulation and utilization of egg fatty acids in guppy.

4. To investigate the effects of varying dietary fatty acid levels on the growth, gonadal development, fecundity and fry survival.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Status of guppy in ornamental fish trade

A conservative estimate of the annual wholesale value of the world trade in ornamental fish puts it at more than US$ 1 billion. Some 1.5 billion fish are traded yearly with a retail value of at least US$ 6 billion. The entire industry, including accessories, is said to be worth about US$ 14 billion. Some 30-35 species of fish dominate the market; for example, in the USA, 30 species accounts for 60% of imports with two species (the guppy and neon tetra) alone comprising 40% of the total. The ornamental fish market can be divided into four sectors; tropical fresh water species (which, at 80-90%, is the largest sector), tropical marine and brackish water species, cold (fresh) water species (mainly gold fish and koi), and cold (marine and brackish) water species. All together, about 1500 species are traded with about 750 coming from fresh water. About 90% of the species are from aquaculture while about 10% are collected from the wild (Singh, 2005). The freshwater fish dominating the market are guppy (*Poecilia reticulata*), Neon tetra (*Paracheirodon innesi*), Platy (*Xiphophorus maculates*), Swordtails (*Xiphophorus helleri* and *X. variatus*), Molly (*Poecilia sphenops*), Angels (*Pterophylum scalare*), Gold fish (*Carassius auratus*), Zebra danio (*Danio rerio*) and Discus (*Symphysodon aequifasciatus*) (Singh, 2005).

2.2 Contribution of Asian countries to global ornamental fish trade

World exports of ornamental fish rose from US$ 44.5 million in 1982 to a peak of US$ 204.8 million in 1996. Exports dropped to US$ 159.2 million in 1998 and this was attributed to the financial crisis the world went through during that period. Exports have since been on the rise with the figure for 2002 topping US$ 189.5 million. Out of the figure of US$ 189.5 million of total exports in 2002, Asian countries had a major share of 60%. Singapore was the world's largest exporter contributing 22% of total exports (mainly guppies), followed by Malaysia (9%), the Czech Republic (7%),

and Indonesia (6.7%). Other important players were China (Hong Kong) (5%), USA and Japan (4% each), and Peru, the Philippines and Sri Lanka (3% each) (Singh, 2005).

The trend in world imports followed closely the exports, with imports rising from US$ 50 million 1982 to a peak of around US$ 330 million in 1994-1996. Imports then fell to US$ 262 million in 1998, attributed again to the world financial crisis mentioned earlier, and have been hovering in that range. The main importing countries are USA (accounting for 16.9% of all imports), Japan (10.9%), Germany (10.4%), United Kingdom (10.11%), and France (8.8%). Other important destinations for ornamental fish are Singapore, Italy, Belgium and Netherlands, China and Canada (Singh, 2005). Singapore is the major exporter of tropical aquarium fish in the world and live bearing fish of the family Poecilliidae accounts for about 30% of the total ornamental fish exported from Singapore. About 25-30 color patterns and finage varieties of the guppy, molly, sail fin molly (*Poecilia latipinna*), platies and swordtail are commercially cultured in Singapore for export market. Some of the reasons for the popularity and commercial culture of poeciliids in tropical countries such as Singapore and Sri Lanka are suitable climatic conditions, availability of more than twenty colors and fringe varieties in each group, short generation intervals (3 months (guppy), 4 months (molly) and 5- 6months (platy and sword tail)), and the hardiness of fish (NAQDA, 2005).

Among the estimated number of 1.2 million aquarist in Japan, the most popular species is guppy and it represents 28% of the total Japanese ornamental fish market. About 15% of the total quantity of ornamental fish exported from Singapore consists of guppies. It is the most dominant species contributing around 60% to the freshwater ornamental fish export from Sri Lanka, followed by swordtails, angels, platies and tetras (NAQDA, 2005).

2.3 Studies on culture of live-bearers

The farming of Poeciliids is carried out in indoor cement tanks, out door earthen ponds or net cages (Fernando and Phang 1994). The breeding, nursery, grow-out, stocking and conditioning aquaria are mainly concrete aquaria and net cages. Concrete tanks are used as breeding, nursery and conditioning and concrete and net cages are used for grow-out. Type of aquaria and size depend on the size of the farm, facility available. Percentage area required for breeding, nursery grow-out and conditioning are 3-5%, 8-12%, 50-55%, and 1-3% respectively. There should be 1-2 water storage tanks for each farm and the volume of the tanks depend on the size of the breeding and culture facility. The capacity of the water stock tanks should be 20%-30% of the total culture area. The length, width and the depth of the tank should be 300cm, 300cm and 45cm respectively. Water level should be maintained at 30-35cm (Kithsiri *et al.*, 2005).

Management of guppy fry

Only fry of the same variety from different breeding tanks are to be pooled and placed together in the same nursery tanks. Stocking density of fry in nursery tanks should be maintained at 140-300 fry/m^3 for 2-3 weeks. Fry should be fed with live feed till 1-2 weeks and then they can be fed with formulated feed. Fry are harvested for sexing after 17-21 days (Kithsiri *et al.*, 2005).

Grow -out management of guppy

After sexing juvenile males and females are raised separately in net cages or cement ponds. The stocking density is 7-10/ sq.ft. Fish should be fed at least twice daily and unconsumed feed should be siphoned out daily. Harvesting can be done after 45 days depending on the management conditions. Fish are sorted according to

their colour pattern and graded in to small (2.0-2.5 cm), medium (2.5-3.5 cm) and large (>3.5 cm) (Kithsiri *et al.*, 2005).

2.4 Water quality management in ornamental fish farming

The most important water quality parameters in aquaculture are water temperature, water pH, dissolved oxygen, total ammonia and nitrite. All livebearer species tolerate a wide range of water quality parameters, but different species have slightly different optimal ranges. Guppy is found in nature in waters where the temperature range is 23-30°C (Liley and Seghers, 1975). Like many ectotherm creatures, fish are strongly influenced by temperature, affecting their reproduction, growth rate and survival. The upper lethal temperature reported for guppy is 32°C (Gibson, 1954). A difference between male and female temperature preference was found (Johnson and Cross, 1980). It is important to note that extreme temperature drops can cause stress on the fish and result in illness. They are also adaptable to wide range of pH level from 6.5 to 8.5. The dissolved oxygen level should be above 5 mg/l and the guppy can be cultured and bred in the water hardness range of 25 to 250 mg/l. The total ammonia content should be below 0.1 mg/l and unionized ammonia level should be below 0.025 mg/l and the nitrite levels should be below 0.3 mg/l. Filtration or aeration should be efficient enough to keep ammonia and nitrate levels down to a healthy level to accommodate the amount of guppies and feedings for the tank. Water changes should be 10% to 25% weekly. Water conditioners should used to neutralize any chlorine, chloramines, or other metals that maybe in the tap water (Kithsiri *et al.*, 2005).

2.5 Feed and feeding management in ornamental fish

Feed is the most potent exogenous factor that affects growth and other physiological mechanisms in organisms. Feed quantity considerably affects fish reproduction (Tyler and Dunn, 1976; Townshend and Wooton, 1984). Supplementary feeding is typically practiced in aquaculture to enhance growth of organisms to marketable size within a short period. The limited feeding negatively affects survival,

food intake and growth, whereas excess feed not only pollute the environment by leaching nutrients but also increase the production cost (Sampath, 1984). The development of manufactured feed could be considered as one of the contributing factors to the tremendous growth of this hobby's widespread popularity over the past 50 years (Earle, 1995). The increased acceptability of and reliance upon manufactured feed for ornamental fish have focused the attention on the nutritional requirements of these animals. Most information on the quantitative and qualitative nutrient requirements of ornamental fish kept in public and home aquaria is derived principally from research carried out by the aquaculture industry since the 1970s. These results do have limitations in their applicability to ornamental fish, because it is based on a small number of species of fish raised for food, which are often kept under totally different conditions from those of fish kept as ornamentals in public or home aquaria (Pannevis, 1993; Earle, 1995).

One of the main problems is the diversity of species kept in home and public aquaria and how to provide them with adequate diets (Pannevis and Earle, 1995; Macartney, 1996). With the exception of a small number of tropical freshwater carnivorous fish species (Cichlidae), and goldfish and koi carp, ornamental fish are seldom kept in a single-species environment (Macartney, 1996). It is impractical to feed very specific diets to individuals in an aquarium environment (Pannevis, 1993; Pannevis and Earle, 1995). The diet must be suitable for all tank inhabitants, which may include herbivorous, omnivorous, and carnivorous. Not only will these fishes have different nutrient requirements, but also the digestibility of various components of the diet will differ depending on the natural diet and intestinal morphology. Furthermore, the physical characteristics of the diet and the feeding regime must satisfy the different lifestyles and feeding habits, such as surface, middle, and bottom feeders, and diurnal variations in feeding among these groups.

Physical characteristics of the diet also play an important role when species of various weights are fed on the same diet. Food particles need to be small enough for the smaller species to ingest, but large enough to be identified and eaten by

the larger species (Macartney, 1996). The general nutritional classification of ornamental fish is based on biotic (i.e. physiology, life stage or feeding behaviour) and abiotic (i.e. environmental temperature, salinity) factors. Temperature (temperate, tropical, arctic), environmental salinity (sea water, brackish water, fresh water) and water hardness (soft water, hard water), are the important abiotic nutritional classifications. The most important biotic nutritional classifications are those of herbivorous, omnivorous or carnivorous ornamental fish. The combination of the main classification groups results in more than 18 distinguishable nutritional groups of ornamental fish. Ornamental fish can exhibit a very consistent preference for one diet over another when the two diets are fed simultaneously (Pannevis, 1993). As ornamental fish are poikilotherms, their maintenance energy requirements adapt to changes in water temperature.

Feeding practices, however, are of poor standard due to over-reliance on live feeds such as *Tubifex* worms and freshly prepared wet feeds (Fernando *et al.*, 1991). Development of proper formulated diets to avoid potential problems dealing such diets is therefore needed (Chong *et al.*, 2004). It has been observed that the feeding practices may not provide adequate nutrient levels required by broodstock fish (Fernando *et al.*, 1991). The critical requirements of a satisfactory diet for aquarium fish are different, and actually more demanding, than those for commercial food fish. Generally, a diet for aquarium feeding should have the following properties: (1) nutritionally balanced; (2) palatable; (3) resistant to crumbling; (4) water stable; (5) buoyant, and (6) enhance pigmentation in ornamental fish (Boonyaratpalin and Lovelli, 1977).

Ornamental fish have traditionally been fed live feed, which is often nutritionally deficient, and can act as the transmitter of diseases (parasitic, bacterial and viral) if it is not stored properly (Pannevis, 1993; Earle, 1995). However, large commercial producers of aquarium fish emphasize the importance of regular supplementation of formulated feeds with live feed, as the inclusion of live feed improves growth (Fernando *et al.*, 1991). It was demonstrated by Kruger *et al.* (2001)

that a daily supplementation of *Daphnia* spp. as live feed to swordtail broodstock maintained on an artificial flake diet resulted in a significant increase in fecundity as a result of more rapid growth, a higher number of embryos, and an improved feed conversion ratio, while supplementation of diets with *Artemia* increased growth of juvenile angelfish (*Pterophylum scalare*) (Degani, 1993). Some species, such as the ruby barb (*Puntius nigrofasciatus*), a voracious and indiscriminate feeder, prefers live feed to artificial feeds (Weerasooriya *et al.*, 1999). In freshwater ornamental fish culture, *Moina* used to be the most common live feed organism for feeding young fish in the industry (Lim *et al.*, 2001).

However, as *Moina* is cultured in water enriched with organic manure, an increasing number of ornamental fish farmers have shifted from the use of potentially contaminated *Moina* to *Artemia* nauplii for feeding their fish (Lim *et al.*, 2001). Decapsulated *Artemia* cysts were found by Lim *et al.* (2002b) as a more hygienic and off-the-shelf alternative to *Artemia* nauplii for growth of adult guppies and fry from guppies, platies (*Xiphophorus maculates*), swordtails, mollies (*Poecilia sphenops*) and black neon tetras (*Hyphessobrycon herbertaxelrodi*). Large-scale discus (*Symphysodon aequifasciata*) breeders rely mostly on live food such as Tubifex, bloodworms and *Artemia* nauplii to feed the growing fry (Chong *et al.*, 2000). The rotifer, *Brachionus calyciflorus*, compared to egg yolk, could be used to improve growth and survival of juvenile dwarf gourami (*Colisa lalia*) and brown discus. The use of rotifers resulted in that discus larvae could be reared in absence of the dependence of body slime of parents as nutrient source during the first 2 weeks of exogenous feeding (Lim and Wong, 1997).

Since guppy can be bred easily in captivity, the farmers do not pay much attention to provide the fish with nutritionally well balanced diet. Due to the use of feeds with different nutrient levels in guppy farming industry, guppies are provided with nutritionally unbalanced feeds. This will result of low brood size and low survival of fry. It has also been observed that malnutrition and deterioration of genetic quality can lead to deformities of fry in guppy. Nutritionally unbalanced diets may cause short broodstock

replacement periods and lengthened brood interval. Ultimately this will lead to the production of poor quality fry with low survival rate.

An increased food amount caused a higher growth rate in the guppy. Feeding fish in the aquarium is not so difficult, and there is now a wide range of prepared feed available in the market. But the cost is so high and it is not economical to use those feeds for commercial level ornamental fish culture. On the other hand the fecundity, the number of fry produced for one female guppy vary from 20-80 numbers and the number produced is mainly depend on the quality of the ingredients and the water quality of the culture and breeding tanks. In commercial level fish breeding, the feed requirements of fish are mainly depend on artificially formulated feed.

Sheenan *et al.* (2005) studied the effects of feeding guppy fry a diet offered as either powder or fakes on their growth and survival were tested over a period of 8 weeks. The results showed that the growth of both male and female fish was considerably enhanced when the diet was presented in the form of a finely ground powder compared with a flake form. The ability of the fish to consume the food in a rapid manner, preventing leaching of vital nutrients from the feed before being engulfed by the fish probably led to the better growth results exhibited by the fish given the powdered food.

Food restriction itself can seriously affect spawning success. In guppy reduced number of offspring and reduced large size oocyte counts were associated with conditions of feed scarcity (Hester, 1964). A reduction in feeding rate has been reported to cause an inhibition of gonadal maturation in several fish species, including guppy (Hester, 1964), goldfish (*Carassius auratus*, Sasayama and Takahashi, 1972), European seabass (*Dicentrachus labrax*, Cerda *et al.*, 1994), and male Atlantic salmon (*Salmo salar*, Berglund, 1995).

Preparation of fish feed

Nutrients essential to fish are the same as those required by most other animals. These include water, proteins (amino acids), lipids (fats, oils, fatty acids), carbohydrates (sugars, starch), vitamins and minerals. In addition, pigments (carotenoids) are commonly added to the diet of salmonid and ornamental "aquarium" fishes to enhance their flesh and skin coloration, respectively. In their natural environment fish have developed a wide variety of feeding specializations (behavioral, morphological, and physiological) to acquire essential nutrients and utilize varied food sources. Based on their primary diet fish are classified as carnivorous (consuming largely animal material), herbivorous (consuming primarily plant and algae), or omnivorous (having a diet based on both plant and animal materials). However, regardless of their feeding classification, in captivity fish can be taught to readily accept various prepared foods, which contain the necessary nutrients (Royes and Chapman, 2003).

Increased understanding of the nutritional requirements for various fish species and technological advances in feed manufacturing, have allowed the development and use of manufactured or artificial diets (formulated feeds) to supplement or to replace natural feeds in the aquaculture industry. Abundant supplies of feedstuffs are available, and farmers and hobbyists are now able to prepare their own fish feeds from locally available ingredients.

Proteins and Amino Acids. Fish meal, soybean meal, fish hydrosylate, skim milk powder, legumes, and wheat gluten are excellent sources of protein. Additionally, the building blocks of proteins (free amino acids) such as lysine and methionine are commercially available to supplement the diet.

Utilizing raw fish as a main ingredient in fish feeds has long been recognized to be harmful to the health and growth of fish due primarily to the presence of the anti-nutrient, thiaminase. Thiaminase, an enzyme that destroys thiamine (vitamin B_1), one of the essential water-soluble vitamins, is mostly found in freshwater fish and is

destroyed by heat (i.e., cooking). Other concerns related to using raw fish in diets include the spread of infectious diseases such as mycobacterium and botulism. In preparing diets, preferential use of marine fish is suggested to minimize thiaminase activity, and raw fish could be steamed or poached.

Lipids. Oils from marine fish, such as menhaden, and vegetable oils from canola, sunflower and linseed, are common sources of lipids in fish feeds.

Carbohydrates. Cooked carbohydrates, from flours of corn, wheat are relatively inexpensive sources of energy that may spare protein (which is more expensive) from being used as an energy source.

Vitamins and Minerals. The variety and amount of vitamins and minerals are so complex that they are usually prepared synthetically and are available commercially as a balanced and pre-measured mixture known as a vitamin or mineral premix. This premix is added to the diet in generous amounts to ensure that adequate levels of vitamins and minerals are supplied to meet dietary requirements (De Silva and Anderson, 1995).

Pigments. A variety of natural and synthetic pigments or carotenoids are available to enhance coloration in the skin of freshwater and marine ornamental fish. The pigments most frequently used supply the colors red and yellow. The synthetically produced pigment, astaxanthin, is the most commonly used additive (100-400 mg/kg). Cyanobacteria (blue-green algae such as *Spirulina*), dried shrimp meal, shrimp and palm oils, and extracts from marigold, red peppers and Phaffia yeast are excellent natural sources of pigments (Yanong, 1999).

Binding Agents. Another important ingredient in fish diets is a binding agent to provide stability to the pellet and reduce leaching of nutrients into the water. Beef heart has traditionally been used both as a source of protein and as an effective binder in farm-made feeds. Carbohydrates (starch, cellulose, pectin) and various other polysaccharides, such as extracts or derivatives from animals (gelatin), plants (gum

arabic, locust bean), and seaweeds (agar, carageenin, and other alginates) are also popular binding agents (De Silva and Anderson, 1995; Royes and Chapman, 2003).

Preservatives. Preservatives, such as antimicrobials and antioxidants, are often added to extend the shelf-life of fish diets and reduce the rancidity of the fats. Vitamin E is an effective, but expensive, antioxidant that can be used in laboratory prepared formulations. Commonly available commercial antioxidants are butylated hydroxyanisole (BHA), or butylated hydroxytoluene (BHT), and ethoxyquin. BHA and BHT are added at 0.005% of dry weight of the diet or no more than 0.02% of the fat content in the diet, while ethoxyquin is added at 150 mg/kg of the diet. Sodium and potassium salts of propionic, benzoic or sorbic acids, are commonly available antimicrobials added at less than 0.1% in the manufacture of fish feeds (De Silva and Anderson, 1995).

Attractants. Other common additives incorporated into fish feeds are chemoattractants and flavorings, such as fish hydrosylates and condensed fish solubles (typically added at 5% of the diet). The amino acids glycine and alanine, and the chemical betaine are also known to stimulate strong feeding behavior in fish. Basically, attractants enhance feed palatability and its intake (De Silva and Anderson, 1995).

Other Feedstuffs. Fiber and ash (minerals) are a group of mixed materials found in most feedstuffs. In experimental diets, fiber is used as a filler, and ash as a source of calcium and phosphorus. In practical diets, both should be no higher than 8-12% of the formulation. A high fiber and ash content reduces the digestibility of other ingredients in the diet resulting in poor growth of the fish (De Silva and Anderson, 1995).

Other common feedstuffs used in ornamental fish diets include live, frozen or dried algae, brine shrimp, rotifers or other zooplankton. The addition of fish or squid meal will enhance the nutritional value of the diet and increase its acceptance by the fish. Fresh leafy or cooked green vegetables are often used. Although vegetables are composed mainly of water, they contain some ash, carbohydrates and certain vitamins nutrients (Royes and Chapman, 2003).

2.6 Breeding of live-bearers

The guppy is considered omnivorous and viviparous which is a very popular ornamental fish species due to variety of body colours, fin patterns and also can be bred within wide range of environmental conditions. The live-bearers are known to breed easily (Ling *et al.*, 2006). Live-bearers will reproduce when subjected to wide-ranging water quality parameters as long as adequate feeds are available and appropriate temperatures are maintained. Commercial production of live-bearers has been principally performed in cement tanks or earthen ponds where broodfish are stocked and allowed to spawn freely for 6-7 months. The reproductive capacity of female guppies is known to be affected by photoperiod (Turner, 1937), water temperature (Dildine, 1936b), food (Hester, 1964; Silliman, 1968), copulation frequency (Breder and Coates, 1932), living space (Rose, 1959; Rose and Rose, 1965) and population density (Waren, 1973; Dahlgren, 1979). Guppies retain their fertilized eggs within the follicle throughout gestation (Turner, 1937; Lambert, 1965). Female guppy can retain active sperms in spermatophores in their ovaries and oviduct walls for a period of up to 8 months and can be pregnant without copulation (Lambert, 1965) as cited by Dzikowski *et al.*, 2001). Fry can be sexed at 1-2 weeks of age. In general males are more colourful than the females. Anal fin of males modified to gonopodium, which is used to transfer sperm to females. When the males' anal fin becomes pointed, it is definitely time to remove the females (this may occur at 3 to 6 weeks of age).

Guppy males exhibit a gonopodium resulting from the modification of several anal fin rays into a kind of erectile gutter which allows discharging sperm clusters, or spermatozeugmata, into the female genital papilla (Rosen and Gordon, 1953). Spermatozoa get through the oviduct into the ovarian cavity where they can retain their fertilizing ability for several months. Storage sites are either a seminal receptacle formed by an extension of the antero-dorsal region of the ovarian cavity, where spermatozoa are inserted into specialized cells presenting an astonishing ultra structural analogy with testis sertoli cells (or knob-shaped micro pockets expanding

from the ovarian cavity to the follicle surface which are probably the sites of sperm entry at fertilization time. Oocyte maturation is not followed by ovulation, but by intrafollicular fertilization with spermatozoa released from storage sites. Embryo development also occurs inside the follicles, up to parturition of fully developed fry (Kobayashi and Iwamatsu, 2002). The culture of guppy and swordtail are concentrated in Singapore, Malaysia, Indonesia, Thailand, India, Sri Lanka and China in earthen ponds or floating net cages (Chong *et al.*, 2004). In Sri Lanka guppy is mainly bred and cultured in cement tanks (Kithsiri *et al.*, 2005).

Criteria for breeding of live-bearers

The following aspects should be taken care for the breeding of live-bearers

1. Healthy brooders should be selected.
2. They should be specially prepared for breeding purposes.
3. Provision for enough space in the tanks for breeding.
4. Water temperature should be maintained within the acceptable range for breeding.
5. Other water quality parameters should be within acceptable range.
6. There should be enough light.
7. Tanks used for breeding must be clean.
8. Tanks should be provided with suitable hiding places for fry such as aquatic plants, polythene strips.
9. Tanks must be covered to prevent entry of predators of brooders and fry.
10. They should be provided with nutritionally well-balanced feeds.

During the breeding period of live-bearers, breeding tanks are arranged in three different ways to avoid new swimming fry from parental cannibalism.

1. Tank is provided (1/4th of the tank) with aquatic plants (*Hydrilla* or *Valisneria*) or polythene strips arranged in bundles to offer shelter for fry.

2. Brooders are kept in a breeder box or nylon mesh cage inside the breeding tank and the newly born fry will escape from the cage and come in to the tank. Mesh size should be small enough to retain the brooders.

3. Brooders are kept in the tank and the tank is provided with the nylon net cage or breeder box, which has the smaller mesh size, enable small fry to go in to the net cage. Mesh size should be small enough to avoid brooders going in to the net cage.

The breeding conditions required for live-bearers are given in Table 1.

Table 1. Breeding conditions required for live-bearers

	Male/Female ratio	**Age (months)**	**SD/fish/m^3**	**No. of fry produce at a time of spawning**	**Brood interval**	**No. of broods**
Guppy	1:2 1:3 1:4 1:5	4-6	115-180	10-80	26-28 days	4-6
Platy	1:3 1:4 1:5	4-5	110-135	20-80	3-4 weeks	6-8
Molly	1:3 1:5 2:3	4-5	100-120	20-80	4-6 weeks	6-10
Swordtail	1:4 1:5	6-8	100-120	20-100	4-6 weeks	8-10

SD=stocking density

2.7 Studies on broodstock nutrition

Despite intense research interest, broodstock nutrition remains one of the most poorly understood areas of finfish nutrition. To a large extent, this has been due to the necessity of suitable indoor and outdoor facilities for maintaining large groups of adult fish and the consequent higher cost of running and conducting extended broodstock feeding trials. However, as in human and livestock nutrition, it is clear that the dietary nutrient requirements of broodstock will be different from those of rapidly growing juvenile animals. Moreover, as in other animals, it is also clear that many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are directly related to the feeding regime (including nutrient level and duration) of the broodstock. Interactions between nutrients and reproductive processes, however, remains poorly understood. Studies on broodstock nutrition are relatively expensive to conduct and limited to a few species (Brooks *et al.*, 1997; Izquierdo *et al.*, 2001).

Teleost reproduction involves a suite of complex behavioural, physiological, and biochemical processes. Specifically, female fish mobilize and transfer substantial quantities of nutrients for ovarian development at both previtellogenic and vitellogenic period (Patino and Sullivan, 2002). Accordingly, female fish require considerable quantities of dietary nutrients to support this maturation cycle as well as the endogenous nutrition phase of their offsprings. The nutritional quality of the dietary components provided to broodstock greatly affects reproduction and subsequent offspring viability (Craik and Harvey, 1984; Fernandaz-Palacios *et al.*, 1995). Detailed knowledge of the nutrients required by broodstock to improve reproductive out put and offspring viability is limited (Bromage, 1995; Izquierdo *et al.*, 2001). Numerous studies have demonstrated that reproductive performance and egg quality are influenced by nutrients like protein, lipid, minerals, vitamins and ration size in fish such as gilthead seabream, *Sparus aurata* (Mourente and Odriozola 1990; Ferna´ndez- Palacios *et al.*, 1995), sea bass, *Dicentrarchus labrax* (Cerda´ *et al.*, 1994), red seabream, *Pagrus major* (Watanabe *et al.*, 1985), rainbow trout, *Oncorhynchus mykiss* (Washburn *et al.*,

1990; Choubert and Blanc 1993; Blom and Dabrowski 1996; Choubert *et al.*, 1998; Pereira *et al.*,1998), Atlantic salmon, *Salmo salar* (Eskelinen 1989; Christiansen and Torrissen 1997), Coho salmon, *Oncorhynchus kisutch* (Hardy *et al.*, 1984), tilapia, *Oreochromis niloticus* (De Silva and Radampola 1990; Cumaratunga and Mallika 1991; Santiago and Reyes 1993; Gunasekera *et al.*,1995, 1996a,b, 1997; Gunasekera and Lam 1997; Siddiqui *et al.*, 1998) and common carp, *Cyprinus carpio* (Manissery *et al.*, 2001).

Broodstock nutrition is one of the most important factors limiting fish fry production and larval quality (Izquierdo *et al.*, 2001). One of the parameters, fecundity, has been used to determine reproductive performance, which is also affected by a nutritional deficiency in broodstock diets. Fecundity is the total number of eggs produced by each fish expressed either in terms of eggs/spawn or eggs/ body weight. Studies have shown that reproductive performances of these live breeders are influenced by nutrition (Dzikowski *et al.*, 2001; Kruger *et al.*, 2001). An improvement in broodstock nutrition and feeding has been shown to improve not only egg quality but also seed production. Several methods have been developed to assess the egg quality of fish (Kjorsvik *et al.*, 1990; Fernandez-Palacios *et al.*, 1995

In freshwater fish, embryonic development depends on the energetic reserves of the yolk sac. Previous research with Salmonids demonstrated that changes in yolk composition, through diet and feeding levels, could also affect fry survival. Among dietary components a primary role is played by energy. In fact net energy requirements for maintenance are to be satisfied before growth and reproduction (Knox *et al.*, 1988). Quality of diet ingredients is very important with regard to nutritional composition and palatability. The demand for better quality fish feeds is constantly increasing in this sector. In many cultured species, unpredictable and variable reproductive performance is an important limiting factor for the successful mass production of juveniles. The production of aquaculture species can be economical only when its qualitative and quantitative feed requirements are known, making the

formulation of nutritionally balanced low cost diet possible (Tacon *et al.*, 1983, Hashim *et al*, 1992, James *et al.*, 1993).

Gonadal development and fecundity are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenesis periods (Izquierdo *et al.*, 2001). Due to the differences in biological processes, the nutritional requirements of broodstock may be different from growing juvenile animals. However, a full and comprehensive understanding of the reproductive mechanism such as gonadal maturation, fertilization success and larval quality is far from complete as these coordinated processes are very complex. Broodstock nutrition studies offer to provide knowledge by determining if reproductive performance of a particular fish species can be improved by maternal dietary intake (Chong *et al.*, 2004).

Growth, gonadal development and reproduction of fish are influenced by many factors. These are either endogenous; genotype, age and size of brood stock, ovarian characteristics, egg size and gamete age (Craik and Harvey, 1984; Knox *et al.*, 1988; Lahnsteiner *et al.*, 1999; Carillo *et al.*, 2000) or exogegenous: bacterial colonization on egg surface, egg management, brood stock feeding (Bromage and Roberts, 1995). In particular, reproductive performance is highly affected by the nutritional status of fish, which is known to condition several reproductive traits, such as age at maturity, fecundity, egg size, chemical composition of egg and also embroyanic developments (Shepherd and Bromage, 1998; Eskelinen, 1989; Carillo *et al.*, 2000). In fresh water fish, embryonic development depends on the energetic reserves of the yolk sac. Among dietary components a primary role is played by energy. In fact, net energy requirements for maintenance are to be satisfied before growth and reproduction (Cho *et al.*, 1999). Female fish need adequate proteins, lipids, vitamins and minerals for egg development and spawning/breeding. Protein is required for formation of follicle in the embryo. The absence of any of these nutrients can reduce larval survival (James and Sampath, 2002).

The research has shown that there are four main constituents in the diets of fish, proteins, carbohydrates, lipids (fats) and vitamins, which are important for both growth (anabolism) and as an energy source (catabolism) (Moyle and Cech, 1982). Carbohydrates are found primarily in plants and carnivorous fish have problems digesting it. Lipids, on the other hand, are found in both plant and animal tissues and are completely digestible (Moyle and Cech, 1982). Many herbivorous fish have symbiotic bacteria in their guts, which digest the carbohydrates and liberate its energy to the fish. Lipids provide much more energy than do carbohydrates and they also supply fatty acids, which are used for the construction of energy reserves in fish. Predaceous fish normally have very high growth rates due to their diet of live fish, which are naturally high in lipids (Moyle and Cech, 1982). The importance of protein and lipids as energy sources becomes quite apparent during periods of starvation. The quantities of both proteins and lipids are significantly reduced in starved fish (Moyle and Cech, 1982). Female fish need adequate proteins, lipids, vitamins and minerals for egg development and spawning/breeding. Yolk is composed of phospholipids, proteins, and an amalgam of minerals (phosphorus).

The majority of research on the nutritional requirements of fish has, not surprisingly, been performed mainly on commercially important fish such as trout and salmon. There are reports on the influence of nutrition on growth and reproduction in ornamental fish (Degani, 1991; Degani and Gui, 1992; Degani and Yehuda, 1996, James and Sampath, 2004), but no report has been published on the impact of live food, pelleted feed and mixed diet on growth, gonadal development and reproductive performance in tropical fishes. Most of the nutritional studies on fish have been carried out on food fish. There has been little work on the nutrition of tropical ornamental fish (Shim and Chua, 1986).

Numerous studies have been carried out on scientific sound specifically related to nutrition of species used in the ornamental fish trade. The basic general principles of fish nutrition and nutrition of food fishes are well described in numerous

publications (NRC, 1993; Hepher, 1988; Lovell, 1989; Steffens, 1989; De Silva and Anderson, 1995; Halver and Hardy, 2002).

In teleosts, nutrients such as protein, fatty acids, vitamin E, ascorbic acids and carotenoids have been implicated to play influencing roles affecting various reproductive related processes such as gonadal maturation, gamate quality and spawning performances (Izquierdo *et al.*, 2001; Watanabe and Vassallo-Agius, 2003). Like all the fishes, ornamental fishes require protein, lipid, carbohydrates, vitamin and minerals. Proteins and lipids provide the necessary material with which a fish builds up muscle, cells and tissue. Carbohydrates provide instant energy, while vitamins and minerals help to buildup a fish's health and strengthening. It has been reported that all the fishes require crude protein level in a range between 30-45%, crude lipid 4-8% and carbohydrates 30-50%. Based on this, the suggested nutritional requirements for ornamental fishes are: the young ones can be fed 40-50% protein, 4-6% lipid and 40-50% carbohydrates. The adult or brood fishes can be fed with 30-35% protein, 6-8% of lipid and 40-50% of carbohydrates. In addition to this self-prepared vitamins and minerals 1% each can be added to the feed. Correct feeding of fish is very important to get high survival rate, growth and healthy broodstock.

In aquaculture, production of fish to the marketable size with in a short period is of almost importance (Bulkley, 1997). Nutritionally rich live feed can also be used for feeding especially larval stages. Some time there is a shortage of the live food used in the large-scale commercial culture and spawning of ornamental fishes. Dry feed for formulations have been tried on the substitutes for live food for edible and ornamental fishes (Khan and Jafri, 1994; Lochman and Phillips 1994, James and Sampath, 2002). During the last two decades, more attention has been paid to the levels of different nutrients in broodstock diets. In nature, the guppy feed on small invertebrates, aquatic insect larvae, algae and other plant materials (Dussault and Kramer, 1981). In the wild, fish eat a range of foods and their diet is dependent upon the availability of food in that particular environment. The food types also change with change of season. When aquarium fishes are maintained in a closed environment, in

addition to different live feeds, formulated feeds are also given for obtaining better breeding and maintenance. Therefore, nutritionally well balanced diets should be used for feeding of guppy to, obtain better growth and reproduction.

2.7.1 Protein

Proteins are large, complex molecules made up of various amino acids that are essential components in the structure and functioning of all living organisms (NRC, 1983). Proteins are very important in the growth of fish and research has shown that if certain proteins are lacking, then growth will be stunted. In the wild, omnivorous fish normally feed on abundant live organisms, rich in proteins, which provide a valuable energy source (Moyle and Cech, 1982). However, many commercial foods lack abundant protein since it is expensive and the fish use a lot of energy to break down large, complex proteins. As a result, carbohydrates and lipids are substituted as energy sources (Moyle and Cech, 1982).

Proteins are the major organic material in fish tissue, making up about 65 to 75% of the total on a dry-weight basis. The protein is digested or hydrolyzed and releases free amino acids, which are absorbed from the intestinal track and distributed by the blood to the organs and the tissues (Wilson, 2002). The first need regarding protein requirements of fish is to supply the indispensable amino acid requirement of the animal, and secondly to supply dispensable amino acids or sufficient amino nitrogen to enable their synthesis (Macartney, 1996). The quantity and composition of dietary protein are known to affect fish reproduction (Shim *et al.*, 1989). Protein is usually the most expensive major constituent in a diet. Information on the optimum level of dietary protein required would thus be useful in formulating an economical and well-balanced feed for the guppy (Shim and Chua, 1986).

Dietary protein levels play a major role in growth and reproductive performance of fish (Watanabe *et al.*, 1984; Furuita *et al.*, 2002). The protein requirements of these juvenile omnivorous (guppy, goldfish, tinfoil barb), carnivorous

(discus) and herbivorous (readheaded cichlids) ornamental fish species are in accordance with requirements reported for food fishes (NRC, 1993). Requirement in the above is to be understood as a dietary percentage of protein needed for optimal growth, rather than a true requirement, that is the amount of needed per animal per day (Guillaume, 1997). Requirements stated in table 2 were derived through dose–response studies over periods of 8–12 weeks, and are only applicable to similar conditions under which it was evaluated. According to Shim *et al.* (1989) female dwarf gouramis receiving a diet with 25–45% protein had the highest fecundity. However, the relative ontogenetic stage of the fish being fed can significantly affect the protein level required in their food. A high requirement level for protein (53%) was found for goldfish (*Carassius auratus*) larvae, in comparison to 29% for juvenile fish (Table 2.) Further, proteins act as a source of amino acids and reservoir of materials used during biosynthetic activities essential for early stages of embryogenesis (Metcoff, 1986). It has been pointed out that there is an optimal protein level for reproductive success and that this level is related to the growth of the concerned species (De Silva and Anderson 1995). However, very few studies have evaluated the effects of dietary protein level on the reproductive performance of fish (Dahlgren, 1980; Watanabe *et al.*, 1984, 1985; De Silva and Radampola, 1990; Gunasekera *et al.*, 1995, 1996a; Gunasekera and Lam, 1997; Siddiqui *et al.*, 1998; Manissery *et al.*, 2001). Several workers have maintained that diets for broodstock should be tailor-made to ensure good egg quality as different fish species have different dietary requirements (Brooks *et al.*, 1997).

Fish eat to satisfy their energy requirement, and thus protein and energy in the diet should be balanced (Macartney, 1996). Although the fish use energy efficiently as an energy source, excessive dietary intake may restrict protein consumption and subsequent growth (NRC, 1977). Kruger *et al.* (2001) stated that it would appear that a diet with at least 45% protein at a 6% lipid level is needed for the best specific growth rate and feed conversion of growing (6-8 weeks of age) swordtails. Ornamental fish in captivity need to utilize their dietary protein with the utmost efficiency, as the breakdown products of protein metabolism (mainly ammonia) will directly pollute their living environment (Pannevis, 1993; Ng *et al*, 1993; Earle, 1995;

Macartney, 1996; Raj and Jesily, 1996). Protein is required for formation of follicle in the embryo. The deficiency of any of these nutrients can reduce larval survival (James and Sampath 2004). Dietary protein requirements varied from around 30% for growing omnivorous goldfish (*Carassius auratus*) to 50% for the carnivorous discus (*Symphysodon aequifaciata*). Chong *et al.* (2004) found that the 30% dietary protein should be provided in the broodstock diet of the swordtails to achieve better reproductive performance. Because of the increasing cost of high quality fish meal, requirements for aqua feeds that increase, and to help reducing fishing pressure which is currently causing declining stocks of fish that are turned in to meal. Priority is given internationally to the search for alternatives for fishmeal in animal feeds as a source of protein. The protein requirements for growing some ornamental fish under captive conditions are presented in Table 2.

Table 2. Protein requirements of some ornamental fish species

Species	Initial size (g)	Protein source	Parameters	Estimated requirements (%)	Reference
1.*Poecilia reticulata*	0.1	Fish meal, casein	WG, FCR, GD	30-40	Shim and Chua, (1986)
2.*Xiphophorus helleri*	1.1-1.2	Fish meal, casein	SGR, FCR, GD	30	Chong *et al*, (2004)
3.*Carassius auratus*	0.2 0.008	Fish meal, casein Fish meal, casein	WG, FCR, PER SGR,WG,FCR, GD	29% 53%	Lochmann and Phillips, (1994) Fiogbé and Kestemont (1995)
4. *Barbodes altus*	0.81	Casein	WG	41.7	Elangovan and Shim, (1997)
5.*Symphysodon aequifasciata*	4.45-4.65	Fish meal, casein	SGR	44.9-50.1	Chong *et al.*, (2000)
6.*Cichlasoma syaspilum*	0.28	Fish meal	SGR	40.81	Olivera Novoa *et al.*, (1996)

WG=weight gain, FCR=feed conversion ratio, GD=gonadal development

PER= protein efficiency ratio, SGR= specific growth rate

It has been reported by the many authors that dietary protein and lipid play important roles in the growth and reproductive performance of female guppy. Dahlgren (1980) found that the female guppy fed 31% protein levels showed the highest reproductive performance. Shim and Chua (1986) suggested that the 30-40% dietary protein should be the optimal level for feeding guppy and the female fish fed 30%-40% dietary protein levels and 9-10.5% lipid levels gained the highest mean body weight, ovary weight, % GSI and number of yolky oozytes. Ling *et al.* (2006) proposed the dietary protein and lipid requirements for female swordtails for optimized growth and reproductive performance to be at 30% and 12% respectively. Chong *et al.* (2004) found that a minimum of 30% protein should be included in the diet of female swordtail broodstock.

2.7.2 Lipids

Lipid and fatty acid composition of broodstock diets have been identified as major dietary factors that determine successful reproduction and survival of offspring (Watanabe *et al.*, 1984; Furuita *et al.*, 2002). Dietary lipids play an important role in fish nutrition for provision of both essential fatty acids (EFA) and energy (Sargent *et al.*, 1999). Several studied have highlighted the importance of both quantity and quality of dietary lipid on reproductive performances of broodstock (Bell *et al.*, 1997; Fernandez-Palacios *et al.*, 1997; Rainuzzo *et al.*, 1997; Mazorra *et al.*, 2003). Furthermore, there have been reports on improvements in feed conversion ratio (FCR) and also nitrogen and phosphorus retention in fish when diets containing higher lipid levels were provided (Hillestad *et al.*, 1998; Hemre and Sandnes, 1999). High lipid concentration in feed pellets also contributes to stability of feeds in water (Chaiyapechara *et al.*, 2003). However, excessive lipid in diets can lead to decrease in feed consumption, which in turn will reduce intake of other nutrients. Numerous studies have highlighted the interactive importance of dietary lipid levels on fish growth performances (Miller *et al.*, 2005; Usman *et al.*, 2005; Ozorio *et al.*, 2006). Although fish have a low energy demand, and is thus susceptible to deposition of excessive lipid (Earle, 1995). Lipids do have a role as carriers for fat-soluble vitamins and sterols are important in the structure

of biological membranes at both the cellular and sub cellular levels, are components of hormones and precursors for synthesis of various functional metabolites such as prostaglandins, and are also important in the flavour and textural properties of the feed consumed by fish (NRC, 1983). The current trend in aquaculture is to feed high-rich diets Traditionally, studies on lipid absorption and metabolism in farm animals have focused on the role of dietary lipid as source of energy and on the process of fat deposition ((Usman *et al.*, 2005).

Research into broodstock nutrition has led to the recognition that lipids, and their constituent fatty acids, have a major influence on reproduction, egg viability and the survival of offspring (Wiegand, 1996a; Izquierdo *et al.*, 2001; Sargent *et al*, 2002). Lipids, and their fatty acids, have a number of biological functions including as structural components in cell membranes, as precursors for chemical messengers, and as substrates for catabolism. The lipids obtained from the egg yolk fulfill all these roles during development until the larvae start to feed, and lipids obtained from exogenous sources take over these functions. The lipoprotein yolk complex contains both polar lipids (PLs) and neutral lipids (NLs), whereas egg oil droplets are normally composed of NLs, such as triacylglycerols (TAGs) and wax esters (Wiegand, 1996a; Sargent *et al.* 2002). Although the lipid content and lipid class composition vary with species, fish eggs usually contain quite high proportions of PLs. PLs often represent 50-90% of the egg lipids in the small, pelagic eggs produced by many marine species (Tocher and Sargent, 1984; Fraser *et al.*, 1988; Falk-Petersen *et al.*, 1989; Finn *et al.*, 1995; Silversand *et al.*, 1996; Wiegand, 1996a; Sargent *et al.*, 2002). On the other hand, species with eggs that have long incubation times produce larger eggs with higher lipid contents and higher proportions of NLs (Cowey *et al.*, 1985; Henderson and Tocher, 1987; Ashton *et al.*, 1993; Jobling *et al.*, 1995; Sargent *et al.* 2002). Feed composition. quality and quantity and ration size are among the most important (Sampath and Pandean 1984, James *et al.*, 1993, Jobling, 1998).

The elevation of dietary lipid levels from 12% to 18% in broodstock diets for rabbit fish (*Siganus guttatus*) resulted in an increase in fecundity and hatching

(Duray *et al.*, 1994), although this effect could also be related to a gradual increase in the dietary essential fatty acid content. Indeed, one of the major nutritional factors that have been found to significantly affect reproductive performance in fish is the dietary essential fatty acid content (Watanabe *et al.*, 1984).

Essentiality of fatty acids depends upon its chemical structure, specifically the position of the first double bond proximal to the terminal methyl carbon in the fatty acid chain. Fatty acids are designated by the use of three numbers: the first indicates the number of carbon atoms, the second the number of double bonds, and the third, the position of the first double bond in relation to the terminal methyl carbon. A possible explanation for the difference in fatty acid requirement is that the omega-3 structure permits a greater degree of unsaturation, which is necessary in the membrane phospholipids to maintain flexibility and permeability characteristics in the fish at low temperatures. Several studies have shown that adipose as well as membrane lipids in fish are affected by temperature.

Dietary lipids: source of essential fatty acids

As well as providing energy, dietary lipid is also important as the source of essential fatty acids (EFA). The precise nature of the EFA and their absolute dietary requirements vary with species. However, it is also apparent that the quantitative requirement for EFA may vary with the total dietary lipid level, and this may also vary with the stage of development (Izquierdo, 1996). Feeding very high levels of unsaturated lipid can increase the prooxidant stress on fish consuming the diets. Thus, as the lipid content of the diet increases, the dietary n-3 HUFA levels also increase and the resulting increased unsaturation index of the diet must be balanced by increasing dietary antioxidant content, especially vitamin E (tocopherol). It has been shown in tilapia that the vitamin E requirement increased as the level of dietary lipid increased (Halver and Hardy, 2002). The fatty acid composition of fish tissue lipids usually reflect those of the dietary lipids (Hendersion and Tocher, 1987; Sargent *et al.*, 2002; Bell 1998; Higgs and Dong, 2000; Jobling, 1998) even though there is potential for

modification and metabolism of fatty acids sequestered from the diet (Hendersion, 1996; Sargent *et al.*, 2002; Bell, 1989).

Use of fish oil and vegetable oils as lipid source for fish feed

The steady global expansion of aquaculture industry over the last three decades has placed a heavy dependence on capture fisheries as a source of fish meal and fish oil for aqua-feeds (Tacon, 2004), two ingredients of limited supply and relatively high price. Consequently, great emphasis has been directed towards the identification of alternative, economically sustainable feed ingredients, with plant oil source chiefly showing the most promising results, providing that essential fatty acid requirements are met (Izquierdo *et al.*, 2003). Marine oils are the preferred source of lipids in the feeds for fish larvae (Sargent, 1995), including cyprinids (Charlon *et al.*, 1986; Dabrowski and Poszyczyfiski, 1988). Marine oils are nutritionally superior to vegetable oil due to their high content of long chain PUFA especially EPA and DHA. Developing neutral tissues have a high requirement for DHA (Sargent *et al.*, 1992), and resistance to stress may be enhanced by provision of EPA in the diet (Sargent, 1995). The disadvantages of marine oils as a source of dietary lipid for fish larvae are cost and vulnerability to oxidation (Pozernick and Wiegand, 1997). Sunflower oil and linseed oil also used as lipid sources for preparation of fish feed due to the high percentage of 18:2 n-6 and 18:3 n-3 present in those oils respectively.

Effect of dietary lipids on fish tissue lipid composition

It has been known that there is a strong relationship between the dietary lipid levels and the levels of lipid in the carcass of fish (Cowey, 1993). Deposition of excess lipid in the carcass will clearly be a major serious problem in those species that tend to store lipid in the flesh, although it appears that flesh lipid levels are increased in most species when fed high fat diets. Thus, feeding diets with high lipid levels has been shown to increase flesh lipid levels in fresh water fish (Halver and Hardy, 2002).

Fatty acids as source of energy

A major role of fatty acids in all organisms is to generate metabolic energy in the form of ATP via mitochondrial β-oxidation, a process which has been well established in fish (Sargent *et al*, 2002). Lipids, especially fatty acids, are the favoured source of metabolic energy in fish. Fatty acids not only are the major source of metabolic energy in fish for growth from the egg to the adult (Tocher *et al.*, 1985), but also the major source of metabolic energy for reproduction (Henderson, 1995; Sargent *et al.*, 2002). The fish oil is the major lipid component by far of current fish feeds, the fatty acids that are potential sources of metabolic energy in fish feeds include 16:0 and 18:1 n-9; 20:1 n-9 and 22:1 n-11, which are particularly abundant in the so-called northern fish oils and n-3 PUFA 20:5 n-3 and 22:6 n-3. It is certain that 16:0, 18:1 n-9; 20:1 n-9 and 22:1 n-11 are heavily catabolized to generate metabolic energy in fish because they are well consumed in large amounts during the growth of farmed fish species (Henderson, 1996).

In general, fresh water fish from warm or temperate environments have a requirement of n-6 fatty acids. Some warmwater fish, such as tilapias, which are indigenous to tropical regions, require principally n-6 fatty acids. Some warm water fish such as channel catfish and carp seem to favour a combination of both n-6 and n-3 fatty acids. Determining requirements for fatty acids is difficult for fish because the metabolic requirement is very small and fatty acids stored in the body or even carried over from the egg yolk can influence performance of the experimental fish (Halver and Hardy, 2002).

The specificity of fatty acid oxidation in fish is important in determining the fatty acid composition of triacylglycerols deposited in fish adipose tissue. This composition, in turn, is important for the well-being of the fish, particularly its successful reproduction (Halver and Hardy, 2002). Besides having important roles in generating energy for heat production in warm-blooded fish and for swimming in fish in general,

fatty acids also have a major role in providing energy for reproduction and particularly egg production (Henderson, 1996).

Fatty acids as structural components of membranes

The phosphoglycerides that constitute cell membrane bilayers in fish generally contain 16:0, 18:1 n-9, 20:5 n-3 and 22:6 n-6 as their principal fatty acids. Additionally, 22:6 n-3 is generally present at about twice the level of 20:5 n-3 in fish phospholipids (Halver and Hardy, 2002). Although tissue fatty acid profiles can be modified by altering the sources of fat, highly unsaturated fatty acids (HUFA) such as arachidonic acid (AA), EPA and DHA are important structural and physiological components of cell membranes and are thought to play important roles in permeability, enzyme activity and other functions in polar lipids of membranes (Bell *et al.*, 1986; Lee, 2001). Given that DHA is naturally found at very high levels in neural tissues, it is thought to play a specialized role in neural membrane structure and function (Bell *et al.*, 2001).

Mammals generally need more n-6 PUFA than n-3 PUFA, while several fish species need more n-3 PUFA than n-6 PUFA (Sargent *et al.*, 1999). Different fish species may even have different requirements for fatty acids within the n-3 family. Essential fatty acids are served as a precursor for the production of metabolic eicosonoid (20-carbon compounds). The eicosonoids are the components of the bio-membrane phospholipids, and are mobilized from this site to be metabolized into other products. Arachidonic acid is the major precursor of eicosanoids in fish cells (Bell *et al.*, 1994). Eicosanoids derived from n-6 fatty acids are of the AA (20:4 n-6) series, and those derived from n-3 fatty acids belong to the EPA (20:5 n-3) family. Biomembranes in fish must be in a fluid state to function properly at various temperatures. Membrane fluidity is depending on the fatty acid composition of the membrane phospholipids: the HUFAs such as EPA and DHA are more fluid than more saturated fatty acids at low temperatures. As water temperature changes, the amount of phospholipids in the biomembrane does not change but the fatty acid composition changes. As water

temperature decreases, the ratio of n-3 PUFAs to more saturated fatty acids has been found to increase in the liver of rainbow trout (Sellner and Hazel, 1982).

Fatty acids and immune function

Lipids play several roles in immune responses in animal in that some fatty acids are precursors of leukotrienes. Eicosanoids are important in the development in the immune system (Mustafa and Srivastrava, 1989). Macrophages and neutrophiles which have immunostimulatory functions are produced using 20-carbon compounds. The usual fatty acid precursor for leucotrienes in warmblodded animals is linoleic acid, which through Arachidonic acid leads to the production of leucotriene B_4 a potent immune stimulator. When warm-blooded animals are given n-3 fatty acids, such as EPA or DHA, they will produce leucotriene B_5 which is antagonistic to the activity of leucotriene B_4. Studies by Fracalossi *et al.* (1994) showed that channel catfish fed diets containing fish oil or linseed oil, both high in n-3 unsaturated fatty acids, were less resistant to experimental infection with the bacterial pathogen *Edwardsiella ictaluri* than fish fed diets with corn oil, which is high in n-6 fatty acids.

Fatty acid deficiency signs reported for various fishes are reduced growth, dermal anomalies (such as fin rot), elevated muscle water (edema), and increased susceptibility to bacterial infection, increased permeability of cellular and sub cellular (mitochondrial) membranes, and reduced reproductive performance. Deficient fish exhibited a fainting or shock syndrome (Lovell, 1989).

Dietary fatty acids vis-à-vis egg quality

Certain dietary nutrients also exert a marked effect on fertilization. Dietary EPA and AA levels show correlation with fertilization rates in gilthead bream broodstock (Fernandez-Palacios *et al.*, 1995). Studies on broodstock nutrition revealed that several nutrients are essential for the normal development of the embryo, and their optimum level in broodstock diets improves egg morphology and hatching rates. The percentage

of morphologically normal eggs (as a parameter to determine egg viability) has been found to increase with an increase in the n-3 HUFA levels in broodstock diets and an incorporation of these fatty acids in to the eggs (Fernandez-Palacios *et al.*, 1995). Improved egg quality has been associated with higher total n-3 fatty acids content in European seabass fed à pelleted diet enriched with high quality fish oil (Navas *et al.*, 1996).

Increased n-3 HUFA (particularly DHA) levels in broodstock diets have been reported to significant enhance the weight of fish larvae and their resistance to osmotic shock (Aby-Ayad *et al.*, 1997). In a similar way, increasing n-3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk sack re-absorption. The exact amount of EFAs that a species needs has proved to be very difficult to determine (Bezard *et al.*, 1994), because it depends on the quality of the fat source, the ratio of n-6 to n-3 fatty acids in the feed, the stage of animal development, and the *in vivo* fatty acid metabolism.

Fecundity in gill head bream (*Sparus aurata*) was found to significantly increase with an increase in dietary n-3 HUFA (poly unsaturated fatty acids with 20 or more carbon atoms) levels up to 1.6% (Fernandez-Palacios *et al.*, 1995). However, studies on the reproductive performance of the Nile tilapia (*Orechromis niloticus*), as indicated by the number of females that spawn, spawning frequency, number of fry per spawning and total fry production over a 24-week period, show that their performance was much higher in fish fed a basal diet supplemented with soybean oil (high in n-6 fatty acids, essential for this fish species (Watanabe *et al.*, 1984) and relative low in fish fed a 5% cod live oil supplemented diet (high in n-3 fatty acids) (Santiago and Reyes, 1993).

Fish fed the diet containing cod liver oil showed the highest weight gain (Santiago and Reyes, 1993). In sparids, the fatty acid composition of the female gonad is greatly affected by the dietary fatty acid content, which in turn significantly influences egg quality in a short period of time (Harel *et al.*, 1992). Thus, gilthead seabream, the

fatty acid composition of the eggs is directly affected by the n-3 HUFA content of the brood stock diet. Both the n-3 fatty acid and n-3 HUFA content of gilthead bream eggs increased with an increase in n-3 HUFA dietary levels, mainly due to the increase of 18:3 n-3, 18:4 n-3 and 20:5 n-3 (EPA) contents in the eggs (Fernandez-Palacios *et al.*, 1995).

In addition some species have the ability to elongate and desaturate C18 fatty acids to higher molecular weight n-3 HUFA, while other species do not have such ability (Ibeas *et al.*, 1994, 1996). It has been reported that HUFA content of brood fish feed significantly affects fecundity, fertility, hatching and viability of fish eggs, egg quality and larval growth (Mourente and Odriozola, 1990, Fernandez-Palacios *et al.*, 1995; Izquierdo *et al.*, 2001). Selective retention of DHA has also been found during embryogenesis (Izquierdo, 1996) and during starvation (Tandler *et al.*, 1989) denoting the importance this fatty acid for the developing embryo and larvae. Poly unsaturated fatty acids can also regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes (Moore, 1995), including the production of steroid hormones and gonadal development such as ovulation. Certain dietary nutrients also exert a marked effect on fertilization. Dietary EPA and AA levels showed correlation with fertilization rates in gilthead bream broodstock (Fernandez-Palacios *et al.*, 1995).

The nutritional quality of broodstock diets influences the chemical components of eggs of various freshwater and marine species. Egg lipids, which play an important role as an energy reserve in developing fish embryos, are therefore also influenced by the availability of lipids in the broodstock diet. Most of the work in fish nutrition has shown that n-3 fatty acids are essential in broodstock diets, especially n-3 highly unsaturated fatty acids (HUFA), which are effective in preventing various larval deformations. Among the n-3 fatty acids, linolenic acid (18:3 n-3) alone, comprising 1% of the diet, is sufficient to allow normal growth, egg development and survival of offspring in rainbow trout. In fish digestion and absorption of SAFA and MUFA is inferior to that of PUFA (Bell *et al.*, 2002). Maternal nutrition is the only source of fatty acids

until the beginning of exogenous feeding. Watanabe *et al.* (1984) observed that lipid sources in broodstock diets affect egg quality, their hatching rate, also demonstrating that a dietary fatty acid deficiency could cause a decrease in gamete numbers and a high mortality of embryos. The specific fatty acid compositions of fresh water fish eggs varies with species and are affected to some extent by diet (Henderson and Tocher, 1987). However, in the freshwater common carp it has been demonstrated that dietary levels of dietary DHA significantly affected egg hatchability (Shimma *et al.*, 1977), as has been demonstrated in many species of food fishes (Rainuzzo *et al.*, 1997). Some fish species readily incorporate dietary unsaturated fatty acids in to eggs, even during the course of the spawning season. Highly unsaturated fatty acids (HUFA) with 20 or more carbon atoms affect, directly or through their metabolites, fish maturation and steriodogenesis.

Researches have shown that the freshwater fish commonly contain lower portions of PUFA than marine fish (Henderson and Tocher, 1987). In a similar way, increasing n-3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk sack re-absorption. The percentage of morphologically normal eggs (as a parameter to determine egg viability) has been found to increase with an increase in the n-3 HUFA levels in broodstock diets and an incorporation of these fatty acids in to the eggs (Fernandez-Palacios *et al.*, 1995). Improved egg quality has been associated with higher total n-3 fatty acids content in European seabass fed a pelleted diet enriched with high quality fish oil (Navas *et al.*, 1996). Apart from the dietary EFA deficiencies causing detrimental effects in fish, their excess has also been reported to have a negative effect on reproduction performance of fish. For example, high level of n-3 HUFA reduced the total number of eggs produced by gilthead bream broodstock despite an increase in egg n-3 HUFA concentration (Fernandez-Palacios *et al.*, 1995).

A positive correlation was observed between the levels of n-3 HUFA in the diet and the eggs with the EPA concentration being more readily affected by dietary n-3 HUFA than DHA. Rainbow trout (*Oncorhynchus mykiss*) fed an n-3 deficient diet during

the last 3 months of vitellogenes is produced a moderate affect on the incorporation of DHA into egg lipid whereas EPA concentration decreased by 50% (Fremont *et al.*, 1984). Almansa *et al.* (1999) demonstrated in marine fish that a very long period of feeding with a low quantity of n-3 PUFA can alter egg quality, fatty acid composition of eggs and fry growth, causing anomalies in the development of the nervous tissues (Brodtkorb *et al.,* 1997). In particular ecosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) seem to be necessary for the development of eyes and brain of the early stages of fish. Studies of cultured fish have also shown that the inadequate levels of n-3 fatty acids affect the number and viability of eggs (Yu *et al.*, 1979).

In aquaculture nutrition DHA, EPA and AA are widely studied in order to determine the optimal dietary requirement levels of broodstock, juvenile and larvae of cultured species. These HUFA are broadly responsible for a generalized role in maintenance of the cell membrane integrity. More specifically, those HUFA also serve as precursors for eicosanoids, an important group of hormones responsible for a whole range of physiological activities including development, immunity and reproduction (Hardy and Halver, 2002). Eicosanoids are important in the control of ovulation and probably involved in the embryogenesis, hatching and early larval development (Mustafa and Srivastrava, 1989). Compared to requirements of fatty acids determined with freshwater and marine food fishes (NRC, 1993), research on fatty acids requirements of ornamental fish, specifically the need for broodstock to achieve optimal larvae quality, is lacking. In fresh water fish essential fatty acids requirements can be met by supplying 18:3 n-3 and/or 18:2 n-6, although better growth performance can be achieved by supplying the "bioactive" forms of the n-3 HUFA namely EPA and DHA (Yone, 1978; Kanazawa, 1985).

Fish in general require fatty acids of longer chain length and a higher degree of unsaturation than mammals (James and Jassens, 2003). Fatty acids with low melting points are needed at the lower body temperature to support cell membrane flexibility at low water temperatures (Earle, 1995). Compared to requirements of fatty

acids determined with freshwater and marine food fishes (NRC, 1993), research on fatty acids requirements of ornamental fish, specifically the need for broodstock to achieve optimal larvae quality, is lacking. In some research papers it has been indicated that dietary omega 3 fatty acids play a major role on breeding performance of fish and some reports indicated that omega 6 fatty acids play a major role in breeding performance and hence there is a uncertainty of the type of fatty acids require for the breeding performance of fish. As per the available literature no work has been reported on the effect of dietary fatty acid requirements on the reproductive performance of guppy.

As well as being a major energy source, dietary lipids are also a source of fatty acids required for the synthesis of new cellular lipid for growth and reproduction and for turnover of existing lipid. Therefore, lipid requirements can be broken down into three main categories, gross lipid requirement in terms of energy provision and qualitative and quantitative EFA requirements (Halver and Hardy, 2002). All vertebrate species have absolute dietary requirements for certain PUFA. If a dietary deficiency occurs, the animal stops growing and reproducing, develops varies pathologies and eventually dies (Sargent *et al.*, 1989). The PUFA in question are termed "essential fatty acids" (EFA) and they include members of both the n-6 and the n-3 series typified by linoleic and α-linolenic acid. All vertebrate species probably require both n-6 and n-3 PUFA, i.e., both n-6 and n-3 PUFA are EFA for vertebrates, and the biologically active forms of EFA are generally the C20 and C22 metabolites of 18:2 n-6 and 18:3 n-3, viz., 20:4 n-6+22:5 n-6 and 20:5 n-3 + 22:6 n-3, which are often termed HUFA. The accurate definition of EFA requirements for a given fish species involves determining not only the absolute requirements of each series of PUFA, but also the optimal balance between the two series (Sargent *et al.*, 1999; Izquierdo, 1996).

As with marine fish eggs, the lipid content and lipid class composition of fresh water fish eggs varies between species, but in general freshwater fish eggs have lipid contents in the range 2.5-10% of the wet weight (Henderson and Tocher, 1987. The eggs with higher lipid contents have higher levels of neutral lipids, stored almost

invariably in the form of oil globules or droplets, in addition to the phospholipid-rich yolk lipid (Henderson and Tocher, 1987; Weigand, 1996a). However, some lipid-rich egg can have globules that are predominantly wax esters, as in gourami (*Trichogaster cosby*). Fatty acid profiles of fish eggs are often characterized by n-3 highly unsaturated fatty acids (HUFAs), especially DHA and EPA, the MUFA 18:1 n-9 and the SAFA 16:0 (Kairanta and Linko 1984; Tocher and Sargent 1984; Henderson and Tocher, 1987; Anderson *et al.*, 1990; Wiegand, 1996a; Sargent *et al.*, 2002).

Utilization of lipids during embryogenesis

There is less information available on lipid and fatty acid utilization during embryonic and early larval development in fresh water fish. However based on the available data it appears that the patterns of utilization are similar to those in marine fish. Therefore, in general, lipid utilization in freshwater fish eggs can occur during the whole development period including embryogenesis. As with marine fish and irrespective of lipid class utilized, all types of fatty acids, saturated, monounsaturated, and PUFA, can be metabolized for provision of energy during development of fresh water eggs (Henderson and Tocher, 1987).

Fresh water species can be one of the three main types, those that require mainly n-3 PUFA such as salmonids and whitefish, species that require mainly n-6 PUFA such as tilapia, and species that require significant amount of both such as channel catfish (*lictalurus punctatus*) and carps (common and grass) (Sargent *et al.*, 2002).

2.7.3 Carbohydrates

No dietary requirement for carbohydrates has been demonstrated in fish. However, carbohydrates present a cheap energy source that would "spare" the catabolism of other components such as protein and lipids to energy. Warm water fish can use much greater amounts of dietary carbohydrate than cold water and marine

species (NRC, 1993). Most herbivorous fish, such as goldfish and koi carp, use the microflora in their hindgut to digest complex carbohydrates (Pannevis, 1993; Earle, 1995). Carbohydrate digestibility can vary from 70% in goldfish to as low as 50% for moonlight gourami (*Trichogaster microlepis*) (Pannevis, 1993).

2.7.4 Minerals

Minerals are inorganic elements required by fish for tissue formation and various functions in metabolism and regulation (NRC, 1977). Ornamental fish can absorb some water-soluble minerals from the water, complicating studies in determining dietary mineral requirements (Shim and Ho, 1989). Of all the minerals required by fish, phosphorus is one of the most important because it is essential in growth, bone mineralization and lipid and carbohydrate metabolism, and is needed in the diet due to low content in natural water. Furthermore, the pollution of water by excess phosphorus excreted appeared highly critical, as this may lead to eutrophication. In accordance to results obtained with food fishes (NRC, 1993), dietary calcium was found to be non-correlated to fish growth in guppies (Shim and Ho, 1989). Requirements of ornamental fish during the growth phase for some minerals are presented in Table 3. Similar as with food fishes, depressed appetite, scoliosis and lordosis have been reported in guppies fed phosphorus deficient diets (Shim and Ho, 1989).

Table 3. Mineral requirements of *Poecilia reticulata*

Mineral	**Initial size (g)**	**Parameters**	**Dietary requirements (%)**	**Reference**
Phosphorus	0.24	Weight gain, mineralization	0.53-1.23	Shim and Ho, (1989)
Iron	4 weeks	Prevention of hypochromic, mycrocytic anaemia[a]	0.008	Shim and Ong, (1992)
Magnesium	0.17	Weight gain	0.054	Shim and Ng, (1988)
Zinc	0.25	Weight gain, feed conversion	0.01	Shim and Lee, (1993)

[a] A condition characterized by a reduced blood cell count, hemoglobin content, haematocrit and erythrocyte value

2.7.5 Vitamins

Vitamins are organic compounds required in relative small quantities in the function of most forms of life, but which some organisms are unable to synthesize (NRC, 1983). Fracalossi *et al.* (1998) reported that the lowest level of ascorbic acid tested in their study (25 mg kg–1 diet) was sufficient to prevent growth reduction and ascorbic acid deficiency signs (deformed opercula and jaws, hemorrhage in the eyes and fins, lordosis) in juveniles (29.2 g) of an Amazonian ornamental fish, the Oscar (*Astronotus ocellatus*). Oscars without ascorbic acid supplementation took 25 weeks to start presenting clinical ascorbic acid deficiency signs. Blom *et al.* (2000) proposed a conservative dietary ascorbic acid requirement of 360 mg kg–1 diet necessary to maintain maximum tissue storage of this vitamin in angelfish juveniles. Stress-resistance, evaluated as resistance to osmotic shock in pre-aerated water containing 35

ppt sodium chloride, was significantly higher in guppies fed a moist formulated diet supplemented with ascorbic acid at either 1000 or 2000 mg kg–1 diet compared to fish fed a control diet without any supplementation (Lim *et al.*, 2002b). Water-soluble vitamins are most vulnerable to nutrient leaching. A large percentage of vitamin C, vitamin B_{12}, choline, and panthothenic acid are lost in water within 30 s of feeding some commercial flake diets (Pannevis and Earle, 1995).

2.8 Fatty acid metabolism of fish

2.8.1 *De novo* synthesis of fatty acids

All known organisms, including fish can biosynthesize the saturated fatty acids like 16:0 and 18:0 *de novo* by the conventional pathways catalyzed by the cytosolic fatty acid synthatase (Sargent *et al.*, 1989). All organisms including fish are capable of desaturating 16:0 and 18:0 by the microsomal fatty acid Δ9 desaturase to yield palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9) respectively. Fatty acid Δ9 desaturase has been particularly well characterized and its gene was cloned in carp, where the enzyme involved in enhancing monounsaturated fatty acid production in response to a lower environmental temperature to maintain the membrane fluidity (Tiku *et al.*, 1996).

Nutritional studies suggest that 18:3 n-3 and/ or 18:2 n-6 could satisfy the essential fatty acid requirement of freshwater fish whereas the n-3 highly unsaturated fatty acid (HUFA) i.e., 20:5 n-3 and 22:6 n-3 were required to satisfy the essential fatty acid requirement of marine fish. No vertebrate species can synthesize polyunsaturated fatty acids (PUFA) *de novo*, as they lack the fatty acid desaturase enzymes required for the production of linoleate (18:2 n-6) and linolenate (18:3 n-3) from oleic acid (18:1 n-9) (Wallis *et al.*, 2002). The fatty acid composition of fish lipids is determined by the ability of fish to desaturate and elongate de novo synthesized and dietary fatty acids (Henderson, 1996).

However, many vertebrates can convert dietary 18:2 n-6 and 18:3 n-3 to long chain highly unsaturated fatty acids (HUFA) such as arachidonic acid (20:4 n-6, AA), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (22:6 n-3, DHA) via a pathway involving a series of microsomal fatty acid desaturation and elongation steps (Cook, 1996). The production of EPA requires Δ6 and Δ5 desaturases, and the production of DHA from EPA requires a further desaturation originally thought to be effected by a Δ4 desaturase acting on a C22 fatty acid intermediate, although evidence now suggests it may actually be affected by a Δ6 desaturase acting on a C24 intermediate (Sprecher, 2000; De Antueno *et al.*, 2001; D'Andrea *et al.*, 2002). The extent to which fish species can convert C18 PUFA to C20/22 HUFA varies, associated with their complement of fatty acid desaturase and elongase enzymes. Freshwater fish are capable of producing DHA from 18:3 n-3 (Buzzi *et al.*, 1996; Bell *et al.*, 2001) and so must express all the desaturase and elongase activities necessary for this biosynthetic pathway (Sargent *et al.*, 2002).

Henderson *et al.* (1995) have shown that mature pike *Esox lucius*, an extreme carnivore consuming largely smaller fish does not convert either 18:2 n-6 or 18:3 n-3 to their respective HUFA to any significant extent whereas another carnivorous fish red Pirhana, *Serrasalmos nateri* reared on mosquito larvae (have 18:2 n-6 as their major HUFA and deficient in C20 and C22 HUFA) readily convert 18:2 to 20:4 n-6 and 18:3 to 20:5 n-3 and probably also 22:6 n-3. Moreover, studies on another herbivorous fish silver dollar Pirhana, *Mylassoma aureum*, reared on oat flakes can able to convert 18:2 n-6 to 20:4 n-6 and 18:3 n-3 to 20:5 n-3 and 22:6 n-3 (Henderson *et al.*, 1995). Therefore, a strict carnivore such as mature pike that consumes fish has very little or no ability to convert C18 PUFA to C20 and C22 HUFA but a strict carnivore such as juvenile Pirhana that consumes insects has this ability. However, it is possible that early developing stages that consume relatively small food items such as insects and zooplankton may readily convert C18 PUFA to HUFA, whereas more mature stages that become piscivorous may lost this ability.

In contrast, marine fish are unable to produce DHA from 18:3 n-3 at a physiologically significant rate (Owen *et al.*, 1975; Sargent *et al.*, 2002) due to apparent deficiencies in one or more steps in the pathway (Ghioni *et al.*, 1999). The PUFA in the marine food web are dominated by n-3 HUFA originating from marine algae that have 20:5 n-3 and 22:6 n-3 in abundance with 18:3 n-3 and 18:2 n-6 less prominent (Sargent, 1995). Carnivorous marine fish consumes smaller fish that are rich in EPA and DHA that are derived from the phytoplankton via zooplankton and consequently have no need to convert their very limited dietary intake of 18:2 n-6 and 18:3 n-3 to 20:4 n-6, 20:5 n-3 and 22:6 n-3. It appears that the capacity for this conversion has effectively been lost during evolution. A majority of studies suggest that marine fish species cannot convert any substantial amount of 18:3 n-3 to 20:5 n-3 and 22:6 n-3 (Mourente and Tocher, 1993; Salhi *et al.*, 1999). The abundance of EPA and DHA in marine algae ensures that even herbivorous marine fish can receive a sufficient amount of HUFA in its natural diet and has little or no need to convert C18 PUFA to C20 and C22 HUFA.

In recent years, attempt has been made in characterizing fatty acid desaturases involved in HUFA synthesis (Tocher *et al.*, 1998). Full-length cDNAs for Δ6 desaturases isolated from the filamentous fungus *Mortierella alpina* (Huang *et al.*, 1999), the nematode *Caenorhabditis elegans* (Napier *et al.*, 1998), rat (Aki *et al.*, 1999), mouse and human (Cho *et al.*, 1999), the freshwater zebrafish *Danio rerio* (Hastings *et al.*, 2001) using extant sequence information, revealed its high similarity to mammalian Δ6 desaturase genes. Zebra fish desaturase gene was found to be unique in encoding an enzyme having both Δ6 and Δ5 desaturase activities. Fish desaturase clones also showed measurable but very low levels of Δ5 activity. Unlike the zebra fish desaturase, this showed very significant Δ5 desaturase activity at around 70% of the Δ6 activity (Hastings *et al.*, 2001). Trout produced radio labeled EPA and DHA from 14C-labelled 18:3 n-3 given in a food pellet (Owen *et al.*, 1975). Almost 22% of added [1-14C] 18:3 n-3 was converted to Δ5-desaturated products and that culture of the cells in EFAs deficient medium doubled the recovery of radioactivity as Δ5-desaturated products (Tocher and Dick, 1999).

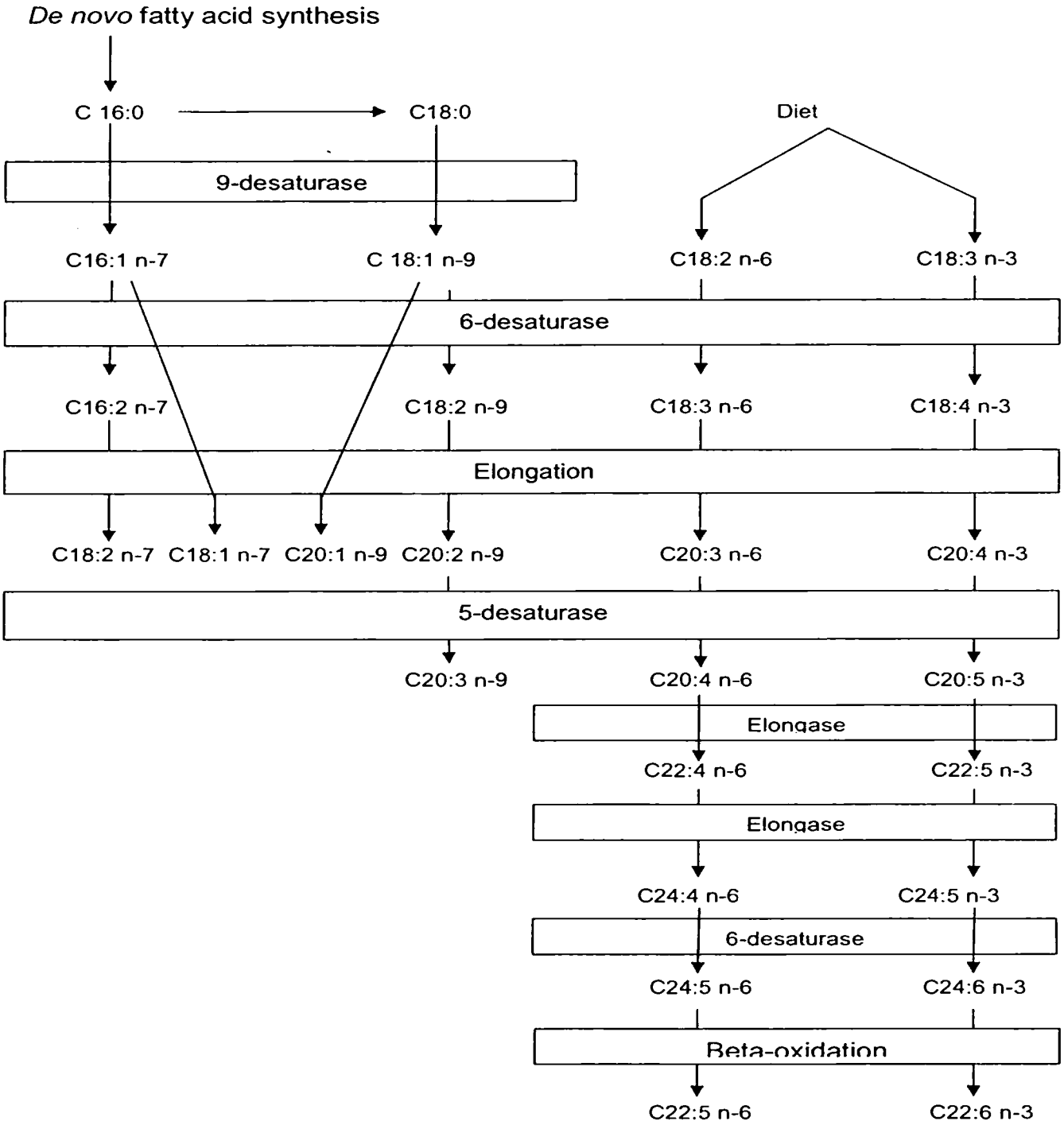


Figure 1. The elongation and desaturation pathway of fatty acids

2.8.2 Dietary modulation of fatty acid profiles in fish

The accumulation of essential nutrients such as essential fatty acids are dependent on the nutrient reserve in the mother animal, and consequently on the dietary input of broodstock in the period preceding gonadogenesis (Blom and Dabrowski, 1996). In this regard broodstock nutrition deserves special attention in order to guarantee optimal survival and development of the larvae during the period of endogenous feeding, and even start feeding when there might only be a marginal uptake of essential nutrients (Lavens *et al.*, 1999).

Tocher *et al.* (2001) observed that feeding the vegetable oil diet increased the levels of LA, 20:3 n-6, total n-6 PUFA, ALA, 18:4 n-3, 20:3 n-3 and 20:4 n-3, and decreased DHA and the n-6/n-3 ratio in salmon and brown trout. By contrast, in charr fed the vegetable oil diet, there was no increase in ALA, 18:4 n-3, 20:3 n-3 or 20:4 n-3 in liver polar lipids and the level of DHA was not decreased. In addition, there was only a modest increase in the levels of LA and total n-6 PUFA, and so the n-6/n-3 ratio was only slightly decreased. The percentage of AA, which was not increased in salmon and brown trout fed vegetable oil, was increased in charr fed the vegetable oil diet.

Bell *et al.* (2001) conducted a study to evaluate the suitability of Rapeseed oil (RO) as a replacement for fish oil (FO) in diets of Atlantic salmon. Duplicate groups of Atlantic salmon post-smolts were fed five practical-type diets in which the added lipid was 100% fish oil FO+ 0% rapeseed oil (0% RO), 90% FO + 10% RO (10% RO), 75% FO + 25% RO (25%RO), 50% FO + 50% RO (50% RO) or 100% RO, for a period of 17 week. Fatty acid compositions of muscle lipid correlated with RO inclusion in that the proportions of 18:1 n-9, 18:2 n-6 and 18:3 n-3 all increased with increasing dietary RO. The concentrations of EPA and DHA in muscle lipid were significantly reduced along with total saturated fatty acids, with increasing dietary RO. Hepatic fatty acid desaturation and elongation activities were increased with increasing dietary RO. In another study by the same author (Bell *et al.*, 2002; Jobling, 2004) to evaluate the suitability of Palm oil (PO) as a replacement for FO in diets of Atlantic salmon, fishes

were fed four practical-type diets in which the added lipid was either 100% FO and 0%crude PO (0% PO); 75% FO and 25% PO (25% PO); 50% FO and 50% PO (50% PO); and 100% PO, for 30 weeks. The concentration of EPA was reduced significantly with increasing levels of dietary PO but the concentration of DHA was significantly reduced only in fish fed 100% PO, compared with the other three treatments. Hepatic fatty acid desaturation and elongation activities were 10-fold greater in fish fed 100% PO than in those fed 0% PO.

Lee *et al.* (2003) fed Starry flounder (*Platichthys stellatus*) with diets containing different n-3 HUFA levels ranging from 0.0% to 2.7% adjusted by either lauric acid as sole lipid source or different proportions of corn oil, linseed oil and squid liver oil at 10% of total lipid. Fatty acid compositions of neutral and polar lipid of fish were directly reflected by dietary fatty acid composition. The highest 14:0, LA, ALA and n-3 HUFA contents were observed in fish fed the diets containing lauric acid, corn oil, linseed oil and squid liver oil, respectively. The contents of n-3HUFA in fish linearly increased with increasing dietary squid liver oil.

Zheng *et al.* (2004) fed Atlantic Salmon with graded level of Linseed oil and found that after 20 weeks of feeding, desaturase and elongase gene expression in liver was increased in the graded manner by increasing dietary linseed oil.

2.9 Studies carried out on guppy

Guppy fish have served as a subject for numerous behavioural studies related to predator avoidance mechanisms and evolution related studies (Seghers 1974a, b: Reznick, 1982: Reznick *et al.*, 1990; Magurran and Seghers 1991: Godin and Briggs 1996: Reznick *et al.*, 2001): genetic models (Reznick 1982; Breden *et al.*, 1987): Shikano *et al.*, 2000); different factors affecting their reproductive behaviour and reproductive performance (Hester, 1964; Liley 1968; Endler 1980; Dzikowski *et al.*, 2001; Dahlgren, 1979; 1980; Shim and Chua, 1986; Ismihan *et al.*, 2006). Daikoku *et al.* (1982) studied the changes of fatty acid profile in guppy on seawater adaptation and

found that the proportion of SAFA to PUFA decreased in seawater adaptation. Lim *et al.* (2002a) carried out an experiment to study the feasibility of using decapsulated *Artemia* cysts for direct feeding to ornamental fish. Fatty acid profile of guppy fry was analyzed after feeding with brine cysts, dried cysts, *Artemia* nauplii and *Moina*. It was found that the dietary levels effects on the muscle fatty acid levels. Khozin-Goldberg *et al.* (2006) demonstrated that feeding with arachidonic acid-rich triacylglycerols from the microalga *Parietochloris incisa* improved recovery of guppies from infection with *Tetrahymena* sp. Ismihan *et al.* (2006) showed that the effect of temperature on sex ratio in guppy. In contrast to the vast number of studies on these subjects, research aims at better understanding the nutritional needs of these fish is scarce.

2.10 Principal Component Analysis (PCA)

Multivariate statistical analysis has been successfully applied in lipid research for particular fatty acids with respect to species, size and seasonal changes (De Silva, *et al*, 1997 and Ould El Kebir *et al.*, 2003). Principal Component Analysis (PCA) acts to reduce the dimensionality of multivariate data while preserving most of the variance within samples. The PCA has been used successfully to discern fatty acid patterns in steelhead trout (De Silva *et al.*, 1997).

Materials and Methods

3. MATERIALS AND METHODS

3.1 Site of the experiment

The first experiment (evaluation of feeds currently used in guppy farming) was conducted over a period of seven months from October 2005 to May 2006 and the second experiment (growth and reproductive performance of female guppy in response to dietary fatty acids) was conducted over a period of seven months from May 2006 to December 2006 at the Central Institute of Fisheries Education (CIFE), Mumbai, India. Culture and breeding trials were conducted at the wet lab of Inland Aquaculture Division of the same institute.

3.2 Experimental animals

Small fry of *Poecilia reticulata* were obtained from the ornamental fish breeding and culture section of the Central Institute of Fisheries Education, Mumbai. The mean initial weight of fry studied in the experiment was in the experiment one and two were 0.077-0.087g and 0.095-0.097 respectively.

3.3 Acclimatization of animals

The guppy fish fry were procured from ornamental fish breeding and culture section of CIFE and were acclimatized for 24 hours before stocking in experimental tanks. The sexes were separated in order to obtain virgin females.

3.4 Experimental setup

The first experiment was set up in three distinct experimental groups and second experiment was set up in four distinct experimental groups, each group having three replicates in nine uniform size rectangular plastic tubs (763 X 521 X 410 mm) of

160 L capacity were used in the first experiment and twelve same size tanks were used in the second experiment. The tubs were initially disinfected with 15 mg L^{-1} potassium permanganate for 24 hours and then thoroughly cleaned with water before the fishes were stocked.

3.5 Chemicals and Glassware

The glasses of Borosil and Vensil were used throughout the experiment. Chemicals of various companies viz Sigma, SRL, Hi-media, Qualigens, Merck, etc. were used.

3.6 Rearing

In the first and second experiments, each tub was stocked with sixty female fry of almost uniform size (0.077-0.087g) and (0.095-0.097) respectively. The sexes were separated at an early juvenile stage, in order to obtain virgin females. All tubs were filled with water and conditioned for 48 hours provided with aeration. The female guppies were randomly distributed to the plastic tubs. The tubs were covered with transparent acrylic sheets to allow the light and also prevent the fishes from jumping out. A uniform volume of 140 liters water was maintained in each tub throughout the experimental period. Round the clock aeration was provided to all the tubs with a 2 HP air blower. The aeration pipe of each tub was provided with air stone and $^{1}/_{8}$" diameter plastic regulator to adjust the air pressure uniformly for all tubs. Initial total body length, standard length and the total body weight were recorded before stocking.

3.7 Cleaning and Siphoning

Experimental tubs were cleaned manually by siphoning the water along with the faecal matter every alternate day and the same was replaced by 50% of fresh chlorine free bore-well water.

3.8 Experimental diets

3.8.1 Diets for first experiment

Three locally and commercially available diets (Diet-1, Diet-2 and Diet-3) were used in the first experiment. Diet-1 was made of mainly wheat flour, wheat bran, rice bran and maize bran while Diet-3 was made of rice bran, wheat flour, fishmeal and ground chicken feed. The composition of the Diet-2 is not known.

3.8.2 Preparation of diets for second experiment

Four isoenergetic, isoproteic and isolipidic diets were formulated with a constant lipid content of 10%. Coconut oil (CO), Sunflower oil (SO), Linseed oil (Lo), and Cod liver oil (FO) was used as the different lipid source. The feed ingredients such as Casein, Starch, and Cellulose were procured from S. D. Fine chemicals (Table 3). Cod liver oil, sunflower oil, Linseed oil, Coconut oil were procured from the local market. Vitamin and mineral mixture (Agrimin), vitamin C (S.D. fine Chemicals), vitamin B complex (Becosules) and carboxy methylcellulose (CMC) and soybean meal (Hi Media labs.) were taken for feed formulation (Table 4).

First, all the ingredients, Casein, Starch, Soybean Meal and Carboxy Methyl Cellulose were mixed thoroughly. Then the vitamins and 0.25% BHT was dissolved in the lipid source and that was mixed with the feed ingredients properly. Initially 100 g of feed was prepared in each diet and after that the each diet was prepared as per the requirement. Each diet was formulated to contain 100 g kg^{-1} lipid, 380 g kg^{-1} protein, 100 g kg^{-1} ash and 420 g kg^{-1} of nitrogen free extract. The prepared diets were labelled as Diet-CO, Diet-SO, Diet-LO and Diet-FO

Table 4. Composition of experimental diets

Ingredients	% Inclusion
Soybean Meal	30.00
Casein	25.00
Cellulose powder	15.9
Starch	14.00
Lipid source	10.00
CMC	2.00
Vitamin-Mineral mix[1]	2.90
Vitamin B Complex[2]	0.10
Vitamin C	0.10
Total	100.00

[1]Composition of vitamin-mineral mix (Agrimin) (quantity kg^{-1})

Vitamin A-6,25,000 IU; Vitamin D_3-62,500 IU; Vitamin E- 250 mg; Nicotinamide-1 g; Cu-312 mg; Co- 45 mg; Mg- 6 g; Fe- 1.5 g; Zn- 2.13 g; I-156 mg; Se- 10 mg; Mn- 1.2 g; Ca- 247.34 g; P- 114.68 g; S-12.2 g; Na- 5.8 mg; K- 48.05 mg.

[2]Composition of vitamin B complex (quantity g^{-1})

Thiamine mononitrate-20 mg; Riboflavin-20 mg; Pyridoxine hydrochloride-6 mg; Vitamin B_{12}-30 mcg; Niaciamide-200 mg; Ca pantothenate-100 mg; Folic acid-3 mg; Biotin-200 mcg.

3.9 Feeding of fish

A fixed amount of the experimental diet was weighted out for each tank of fish each week. Then sufficient amount of water was added to make the diet into paste. Feeding was carried out until satiation twice a day at 800 and 1700 hr throughout the experimental period. Each time a small amount of feed was dropped in to the tank and this process was repeated until satiation was observed.

3.10 Sampling

After the rearing period in the tubs, total body length, standard length and the total body weight were recorded. Before weighing, excess water was removed from the surface of the females by a paper towel. 20 fishes from each tank were separated for breeding purpose and the rest of fish in each tank were taken for the analysis of growth and biochemical parameters.

3.11 Physicochemical parameters of water

The water quality parameters (water temperature, dissolved oxygen, pH, ammonia, nitrite, nitrate and hardness) were monitored weekly throughout the experimental period (Table 5). The dissolved oxygen and temperature were measured using portable meter (Merck, Germany) and other parameters were measured using colourimetric methods (APHA-AWWA-WEF 1998).

Table 5. Water quality parameters monitored during the study period

Sl.No	Parameter	Method	Reference
01	Temperature	Membrane electrode method	Merck, Germany
02	Dissolved oxygen	Membrane electrode method	Merck, Germany
03	pH	Digital pH meter	WTW, Germany
04	Total hardness	Hardness test kit	Merck, Germany
05	Ammonia-N	Phenate method	APHA-AWWA-WEF, 1998
06	Nitrite-N	Colorimetric method	APHA-AWWA-WEF, 1998
07	Nitrate-N	Cadmium reduction method	APHA-AWWA-WEF, 1998

3.12 Growth Parameters

The growth performance in terms of specific growth rate of female guppy fry was assessed by taking their body length using Digimatic Vernier caliper and weight using electronic balance initially and after three months rearing period. Length and weight measurements were taken for all the animals and 20 fishes were separated for breeding and the rest were used for determination of ovary weight, ovary volume, ovum diameter, absolute fecundity, % GSI and biochemical analysis. The animals were kept without food overnight before body weight measurements. The specific growth rate and food conversion ratio were determined using the following formulae:

3.12.1 Specific growth rate (SGR)

$$\text{Specific growth rate} = \frac{\log_e (\text{Final weight}) - \log_e (\text{Initial weight})}{\text{Experimental periods in days}} \times 100$$

Where W_t = mean final weight, W_i = mean initial weight and T = Total experimental days

3.12.2 Food conversion ratio (FCR)

$$\text{Food conversion ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

3.12.3 Percentage survival of fish

The survival rate of the fish used in the experiments and the fry survival was calculated using following formula.

$$\text{Percentage survival} = \frac{\text{number of fish survived at the end of experiment}}{\text{number of fish at the beginning of experiment}} \times 100$$

3.13 Preparation of tanks for breeding

Sexual maturity is known to occur at 2-3 months (Svardson, 1943). Three replicates for each diet group were used and each tank was stocked with 20 female fishes and 10 males. Since the male mortality is known to be higher, a sex ratio of 2

females: 1 male was chosen (Breder and Coates, 1932; Shoemaker, 1947). Breeding tanks were provided with polythene strips arranged in bundles to offer shelter for new swimming fry from parental cannibalism. Each bunch of polythene strips was tied to a weight, which helped to keep it in a fixed position in the tank. All tanks were provided with mild aeration during the breeding period. After a gestation period of 21-25 days (Depeche and Schoffeniels, 1975; Stolk, 1951), the newly born fry in each tank were collected daily using a hand net during the feeding time and kept in separate tanks used for fry collection and the number was recorded. The fry collected from each replicate breeding tank during one month period was put into one tank to avoid cannibalism and hence three tanks were used to collect fry for each replicate during three months breeding period. Fry rearing tanks were also provided with mild aeration. Fry in each tank were fed with *Artemia* nauplii once and experimental feeds twice a day.

3.14 Reproductive performance

Reproductive performance was determined in terms of ovary weight, ovary volume, ovum diameter, %GSI, absolute fecundity and number of fry produced throughout the breeding period. 20 fishes of each treatment were used to determine the reproductive performance. Dissections were carried out under a binocular stereoscopic microscope. After taking all ovary measurements, one portion of ova were then preserved in 4% formalin for the measurement of egg diameter and the other portion was used to extract the lipids.

3.14.1 Absolute fecundity

Absolute fecundity: Number of mature oocytes in the ovary prior to spawning
(Bagenal, 1973)

3.14.2 Gonadosomatic index

Fish weight and the ovarian weight were measured using electronic balance and the accuracy of the balance was up to 4 decimal places.

$$\text{Gonadosomatic index (GSI) (\%)} = \frac{\text{Ovarian weight (g)}}{\text{Weight of fish (g)}} \times 100$$

3.14.3 Measure of ovary length, width and height

After excision of the gonad, the oviduct and mesovarium were separated and removed. Then the ovarian length, width, height and weight were measured using digital vernier caliper (Zoom digimatic caliper). Ovary volume (length × width × height) was calculated using these values.

3.14.4 Measurement of ovum diameter

The dissected out ovaries were kept in properly labeled glass vials, which contained 4% formalin. After three days the diameter of the preserved ova were measured using a microscope aided with computer software package (Mortic Image Conversion 3.1).

3.14.5 Total fry production

Total fry production: Total fry harvested throughout the experimental period

3.15 Collection and identification of eggs and embryos

Fishes were dissected out after maturation to obtain mature (yolky) eggs for fatty acid estimation. The female fishes with embryos were dissected out at the end of breeding experiment for the analysis of embryo fatty acid profiles in the first experiment. Embryos were identified based on the presence of developed eyes.

3.16 Biochemical analysis of tissues and diets

At the beginning of the experiment, fish fry were taken, weighed (2g) and subjected to proximate analysis (AOAC 1995). This was carried out to determine the moisture, crude protein, crude fat and ash content of the carcasses. At the end of the experiment the fishes which were remaining in the tanks (after obtaining fishes for breeding and determining ovary weight, ovary volume, absolute fecundity, ovum diameter and % GSI) were subjected to proximate analysis to determine the body composition.

Moisture

A known weight of the sample was taken and dried in an oven at 105 °C to constant weight and the moisture content was calculated by suing the following formula:

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of sample}}{\text{Wet weight of sample}}$$

Crude protein (CP)

Nitrogen content of the sample was estimated quantitatively by Kjeltec semi automated method (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden) using

titration as the means for determining nitrogen percentage and then crude protein was estimated by multiplying nitrogen percentage by a constant factor (6.25).

CP (%) = Nitrogen (%) X.6.25

Ether Extract (EE)

Ether extract was estimated by Soxtec method (1045 Soxtec Extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60°C) as a solvent.

$$\text{Ether Extract (\%)} = \frac{\text{Weight of initial sample} - \text{weight after extraction}}{\text{Weight of initial sample}}$$

Ash

Ash content was estimated by taking known weight of sample in silica crucible and placing it in a muffle furnance at 600 °C for 6 hours and calculated as follows

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Total carbohydrate

Total carbohydrate was calculated by difference method given by the formula.

Total carbohydrate= 100 – (CP+ EE + Ash)
CP=Crude protein; EE=Ether extract

Digestible energy value

The digestible energy value of experimental diets and carcasses was calculated on the basis of standard physiological value of 4 kcal g^{-1} proteins, 4 kcal g^{-1} carbohydrates and 9 kcal g^{-1} lipids (Halver, 1976). It was calculated as per the following formula:

Digestible energy (kcal/100 g) = Protein % × 4 + Lipid% × 9 + carbohydrate% × 4.

3.17 Lipid extraction

Total lipid was extracted by following the Folch (1957) method. The lipids were extracted from feeds, initial fry, muscle of fish after maturation, eggs and embryos of female guppy in the first experiment. In the second experiment, lipid was extracted from feeds, initial fry and muscle of fish after maturation, eggs, and fry of female guppy. Dissections were carried out under a binocular stereoscopic microscope. After excision of the gonad, the oviduct and mesovarium were separated and removed and directly used for estimation of lipid.

The tissue was homogenized in the 10 volume (of tissue w/v) methanol fortified with BHT (0.01%) (which was added to inhibit the oxidative degradation of lipids during analysis) followed by the 20 volume (of tissue w/v) chloroform in an Teflon coated tissue homogenizer (Superfit, India). After dispersion, the whole mixture is agitated during 15-20 minutes in an orbital shaker at room temperature.

The homogenate was filtered (Buchner funnel with a folded defatted filter paper) to recover the liquid phase and the filter residue re-homogenized with a second volume of chloroform-methanol. The filtered solvent was washed with 0.2 volumes (4 ml for 20 ml) of 0.9% NaCl solution and phases were vigorously mixed. The mixture was poured into a separating funnel and allowed to separate into two phases. The lower chloroform phase containing lipids was collected and evaporated under vacuum in a

rotary evaporator to bring down to a concentration of 2-3 ml. Further evaporation of chloroform was done under a nitrogen stream and residues are weighed to quantify the amount of lipid extracted. The lipid residue was redissolved in chloroform/methanol (2:1, v/v) and then stored in a 25ml conical flask with glass stopper under nitrogen at – 20°C until needed.

3.18 Preparation of Fatty Acid Methyl Esters (FAME)

The AOAC (1996) method was followed to esterify the lipid extract. FAME's were prepared from the isolated lipids by heating with the 0.5N methanolic NaOH attached to a condenser reflux. The solution was heated for 5 minutes from when steam evaporation and condensation was observed. Then 2.5 ml of BF_3 methanol was added from the top of the condenser and boiled for two minutes. 5 ml of n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and two phases were separated. The upper n-heptane phase was pipetted out and put into 10 ml glass vials. Then a small amount of preheated sodium sulphate was added and stored until further analysis.

3.19 Gas Chromatography-Mass Spectrometry

The extracted lipids from fish tissues and feed were esterified with BF_3 methanol and recovered in heptane (AOAC, 1995). The Fatty acid methyl ester (FAME) was analyzed by GC-MS (QP 2010, quadruple mass-spectrometer with ionization energy of 70eV) equipped with DB-WAX capillary column (30 m x 0.25 mm i.d., 0.5 µm film thickness, J & W Scientific, USA) with helium gas as carrier gas. The sample was injected at split mode injection port with 1:15 split ratio at 250°C and oven temperature was programmed from 50-230°C @ 10°C/min and held for 35 min. The mass spectrometer was tuned to get relative abundance of m/Z ranging from 40.00 to 550.00. The values of fatty acids are presented in area percentage of total identified fatty acids.

Suggested identifications of fatty acids from lipid extracted from feeds, muscle, eggs, embryo and fry are given in Table 6. Mass spectra of n-3 and n-6 highly unsaturated fatty acids are presented in figures 2, 3 and 4.

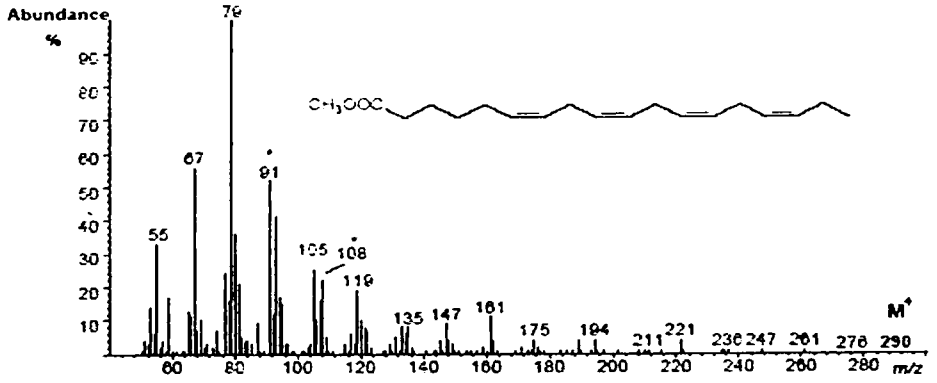
Table 6. Suggested identifications of fatty acids from lipid extracted from feeds, muscle, eggs, embryo and fry.

Serial No.	Retention Time	Fatty acid	Identification[1]
1	7.349	Caproic acid (C6:0)	GC-MS
2	10.546	Caprylic acid (C8:0)	GC-MS
3	13.443	Capric acid, (C10:0)	GC-MS
4	16.049	Lauric acid, (C12:0)	GC-MS
5	17.259	C13:0	GC-MS
6	17.861	Myristic acid, C14:0	GC-MS
7	18.984	Pentadecanoic acid, C15:0	GC-MS
8	20.057	Palmitic acid, C16:0	GC-MS
9	20.900	Palmitoleic acid, C16:1 n-9	GC-MS
10	20.974	Palmitoleic acid, C16:1 n-7	GC-MS
11	21.210	Margaric acid (C17:0)	GC-MS
12	23.363	Stearic acid, C18:0	GC-MS
13	23.805	Oleic acid, C18:1 n-9	GC-MS
14	23.889	Vaccenic acid, C18:1 n-7	GC-MS
15	24.696	Linoleic acid, C18:2 n-6	GC-MS,S
16	25.306	γ-Linolenic acid 18:3 n-6	GC-MS
17	26.077	α-Linolenic acid, C18:3 n-3	GC-MS,S
18	26.814	C 18:4 n-3	GC-MS
19	27.394	Arachidic Acid (C 20:0)	GC-MS
20	28.070	Gondoic acid (C 20: 1 n-9)	GC-MS
21	29.015	C 20:2 n-9	GC-MS

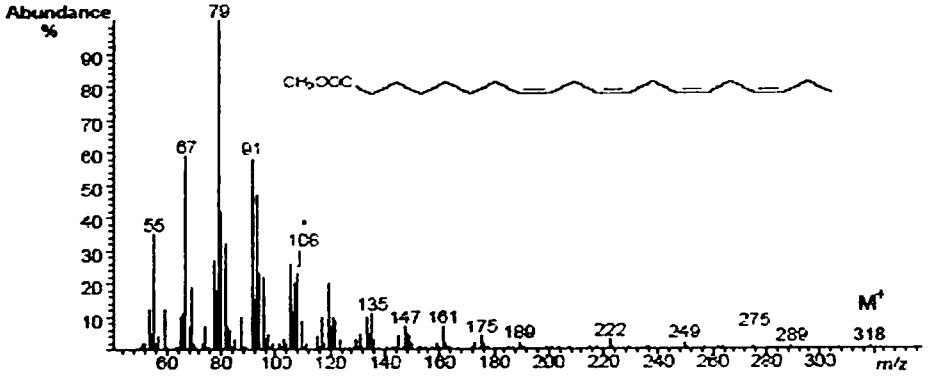
22	29.544	C 20: 2 n-6	GC-MS
23	29.787	C 20:3 n-9	GC-MS
24	30.525	C 20: 3 n-6	GC-MS
25	31.410	Arachidonic acid, C20:4 n-6	GC-MS,S
26	31.838	C 20:3 n-3	GC-MS
27	32.973	C 20:4 n-3	GC-MS
28	34.003	Eicosapentaenoic Acid C20: 5 n-3	GC-MS,S
29	34.693	Behenic acid (C 22:0)	GC-MS
30	35.124	Erucic acid (C 22:1 n-9)	GC-MS
31	39.111	C 22:3 n-6	GC-MS
32	39.596	C 23:0	GC-MS
33	40.729	C 22: 4 n-6	GC-MS
34	42.584	C 22:5 n-6	GC-MS
35	43.267	C 22:4 n-3	GC-MS
36	45.175	C 22:5 n-3	GC-MS
37	47.480	Docosahaexaenoic Acid, C22: 6 n-3	GC-MS,S

1: Means of Identification: Coinciding mass spectrum with library spectrum. Where, S stands for standard.

18:4 n-3



20:4 n-3



20:5 n-3

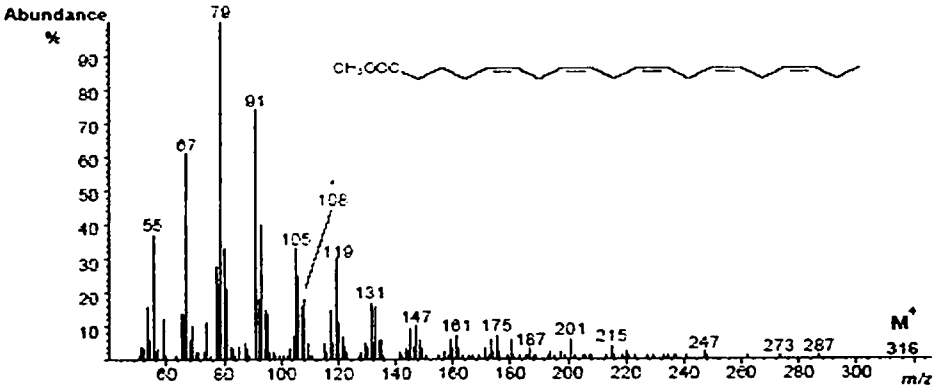
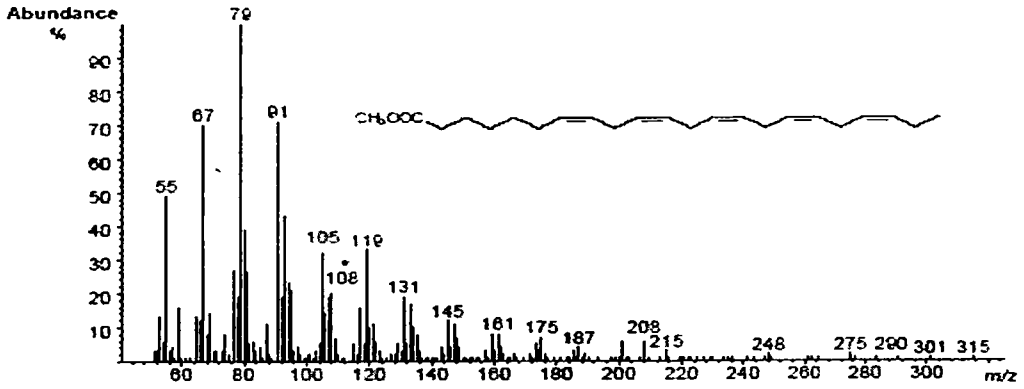


Figure 2. Mass spectra of n-3 highly unsaturated fatty acids
(octadeca tetraenoic acid, Eicosa tetraenoic acid and EPA)

* Characteristic ion (108) of n-3 fatty acids

22:5 n-3



22:6 n-3

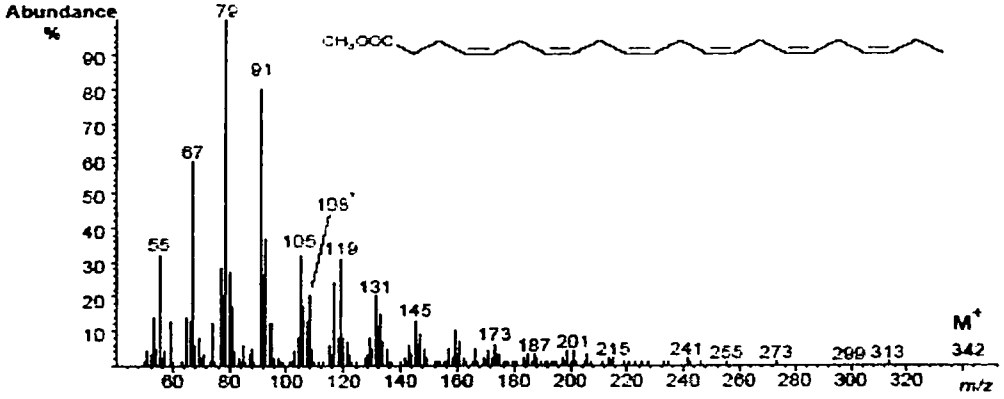
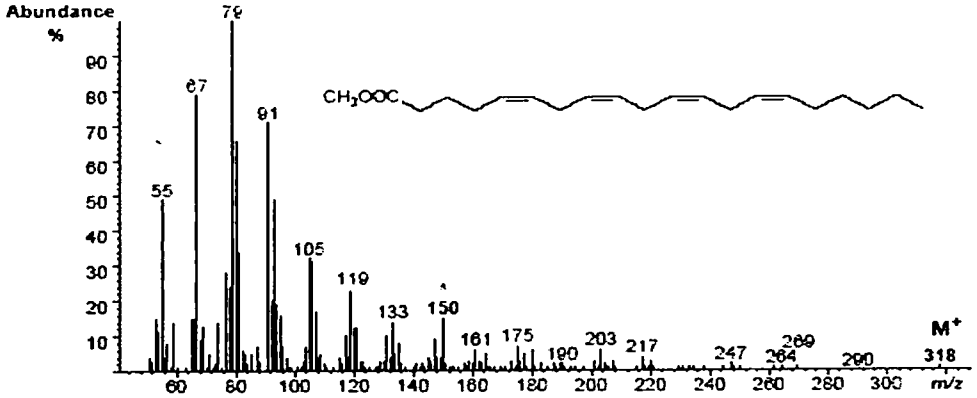


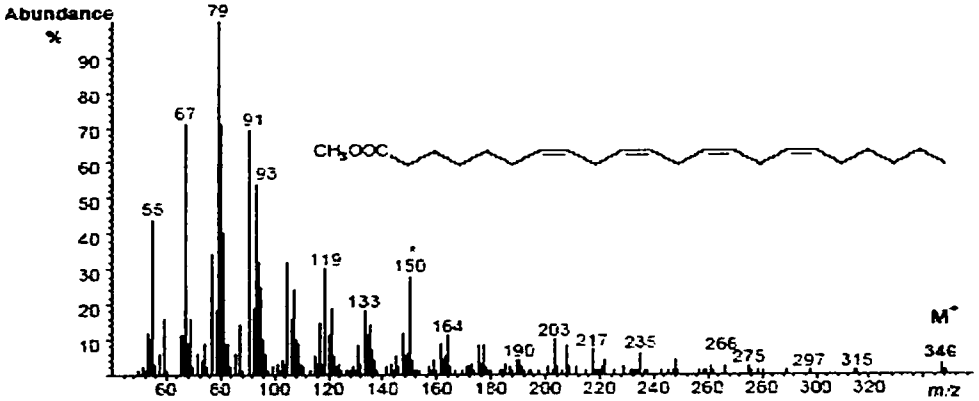
Figure 3. Mass spectra of DPA and DHA

* Characteristic ion (108) of n-3 fatty acids

(AA)
20:4 n-6



22:4 n-6



22:5 n-6

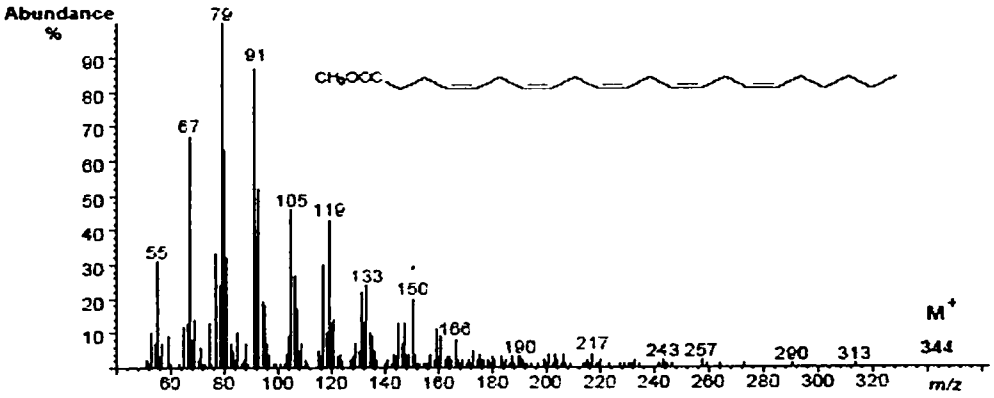


Figure 4. Mass spectra of arachidonic acid, docosatetraenoic acid and docosapentaenoic acid
* Characteristic ion (150) of n-6 fatty acids

Table 7. Details of characteristic ions used in the study of Extracted Ion Chromatograms (EIC)

Class of fatty acid	Characteristic ion (m/z)	Fragmentation
SAFA	74	Formation of McLafferty ion by McLafferty rearrangement
MUFA	M-74	Formation of stable and abundant ion at M-74 after the loss of McLafferty ion
HUFA	91	Formation of cyclic tropylium ion in fatty acids with four or more double bonds
n-6	150	Cleavage of tail from n-6 terminal moiety in methylene interrupted polyenoic fatty acids
n-3	108	Cleavage of tail from n-3 terminal moiety in methylene interrupted polyenoic fatty acids

3.20 Data and statistical analysis

All data are presented as means ±S.E. The effects of diets on muscle, eggs and embryos fatty acid composition in the first experiment and the effects of diets on muscle, eggs and fry fatty acid composition in the second experiment were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical package (SPSS, 2005) followed where appropriate by Duncan's test to determine significant differences ($p<0.05$) between individual treatments.

The PCA was carried out using the software The Unscrambler 9.5 (CAMO, Oslo, Norway). PCA is a data compression method based on the correlation among variables. Its aim is to group correlated variables, and replace them by new sets called principal components, PCs. PCs are completely uncorrelated and they are built as a simple linear combination of the original variables. Further, PCs contain most of the data set variability, but in a much lower-dimensional space. The first principal

component, PC1, is defined as the direction of maximum variance of the whole data set. PC2 is the direction that describes the maximum variance in the orthogonal subspace to PC1. The subsequent components are taken orthogonally to the ones previously chosen and describe the maximum of the remaining variance. When redundancy is removed, only the first few principal components are required to describe most of the information contained in the original data set.

The data matrix, X ($I \times J$), where I corresponds to the number of samples and J to the number of measurements taken on each sample, is decomposed into two matrices, T and L, so that $X = TL^T$. The matrix T, known as the "score" matrix, represents the positions of the compounds in the new coordinate system, i.e., in the PC coordinate system. L is the "loading" matrix whose columns describe how the new axes, i.e., the PCs, are built from the old axes.

Results

4. RESULTS

4.1 Evaluation of feeds currently used in guppy farming

4.1.1 Proximate composition of diets

The proximate compositions of the diets are presented in Table 8. The three diets contained three different levels of nutrients. Protein and crude lipid percentages in the Diet-3 were found higher than Diet-1 and 2. Diet-2 contained higher levels of protein and lipid than Diet-1.

Table 8. Proximate composition of diets

Nutrient	Percentage composition		
	Diet-1	Diet-2	Diet-3
Moisture	6.40	7.20	6.80
Crude protein	18.26	29.27	43.60
Crude lipid	4.17	4.55	9.47
Ash	8.43	12.69	8.45
Total carbohydrates	69.14	53.49	38.48
Energy*	387.13	371.99	413.55

*Calculated digestible energy, DE (Kcal/100g)= (CP%×4)+(EE%×9)+(TC%×4)
DE= Digestible Energy, CP=Crude Protein EE= Ether Extract
TC= Total Carbohydrates (calculated by difference)

4.1.2 Physico-chemical parameters of water

The essential physico-chemical parameters (temperature (^{0}C), pH, dissolved oxygen, total hardness, ammonia, Nitrite–N, Nitrate–N) were recorded during the study period and average values of all the treatments are presented in Table 9. The

water temperatures were monitored and maintained within the acceptable range (23.5-28.3°C). Dissolved oxygen (5.6-6.8 mg/l) and pH values (7.5-8.1) were within the acceptable range throughout the experimental period. Toxic ammonia and nitrite levels were maintained below the sub lethal levels.

Table 9. Physico-chemical parameters of rearing and breeding tanks during the study period

Parameter	Diet-1	Diet-2	Diet-3
Temperature (°C)	23.5-28.1	23.6-28.3	23.5-28.2
pH	7.6-8.0	7.7-8.1	7.5-8.1
Dissolved oxygen (mg/l)	5.6-6.4	5.8-6.6	5.8-6.8
Hardness (mg/l)	228-240	232-241	236-242
Total ammonia (mg/l)	0.18-0.32	0.18-0.33	0.20-0.34
Unionized ammonia (mg/l)	<0.025	<0.025	<0.025
Nitrite-N (mg/l)	<0.003	<0.003	<0.003
Nitrate-N (mg/l)	0.02-0.04	0.02-0.04	0.04-0.06

4.1.3 Body composition of guppy

The proximate compositions of the fish at the beginning of the experiment and after three months rearing period are presented in Table 10. The body moisture content of the fish before and after the experiments varied between 57.38 to 61.15%. The moisture content of fry was found significantly ($p<0.05$) higher than the adult fish. The percentage body protein in fish fry was 56.30 and after maturation, the values varied between 53.95 and 56.86%. The muscle of the guppy fed Diet-3 and Diet-2 had significantly ($p<0.05$) higher crude protein levels than the guppy fed Diet-1. Crude fat level in fish fry was 24.97% and after maturation the percentages varied between 26.56 and 29.83%. The crude fat levels in the guppy fed Diet-3 had significantly higher than the guppy fed other two groups. The guppy fed Diet-1 had the lowest crude fat levels in the body. In the present experiment the dietary lipid levels significantly influenced the crude fat levels of carcass.

Table 10. Carcass analysis (mean values ±SE) of female guppy

Diet type	Percentage on wet weight basis		Percentage on dry weight basis		
	Moisture	Dry matter	Crude protein	Crude fat	Ash
	At beginning of the experiment				
	61.15^{b}±0.19	38.21^{a}±0.16	$56.30^{a,b}$±0.90	24.97^{a}±0.13	18.73^{c}±0.11
At end of the experiment					
Diet-1	57.83^{a}±0.16	42.17^{b}±0.25	53.95^{a}±0.75	26.56^{b}±0.15	19.49^{c}±0.17
Diet-2	58.76^{a}±0.29	41.24^{b}±0.82	$56.21^{a,b}$±0.82	27.20^{c}±0.08	16.59^{b}±0.16
Diet-3	57.78^{a}±0.10	42.22^{b}±0.17	56.86^{b}±0.45	29.83^{d}±0.19	13.31^{a}±0.17

Values within a column with different superscript letters are significantly different ($p<0.05$)

4.1.4 Growth performance of female guppy reared on different diets

Growth parameters of female guppy reared on different diets are presented in Table 11. The highest total and standard lengths were recorded in the guppy fed Diet-3 and also significantly ($p<0.05$) higher than the fish fed Diet-1 and Diet-2. The guppy fed Diet-2 had significantly higher total and standard lengths than the guppy fed Diet-1. The final weight of guppy and the weight gain values were significantly ($p<0.05$) higher in guppy fed Diet-3 than other two diets. The specific growth rate and % survival values were also significantly higher in fish fed Diet-3. The fish fed Diet-3 had significantly lowest level of FCR than the fish fed other two diets. There was no significantly different % survival between the fish fed Diet-1 and 2.

Table 11. Growth parameters (mean values ±SE) of female guppy fed different diets

Parameter	Diet		
	Diet-1	Diet-2	Diet-3
Total length (mm)	21.8^{a}±0.87	29.14^{b}±0.79	36.03^{c}±1.76
Standard length (mm)	16.71^{a}±1.17	23.94^{b}±1.25	29.38^{c}±1.46
Initial weight (g)	0.077±0.01	0.081±0.01	0.087±0.02
Final weight (g)	0.32^{a}±0.04	0.48^{b}±0.06	0.89^{c}±0.07
Weight gain (g)	0.24^{a}±0.02	0.40^{b}±0.04	0.80^{c}±0.06
SGR (%)	0.69^{a}±0.03	0.86^{b}±0.02	1.12^{c}±0.02
FCR	3.50^{c}±0.10	2.21^{b}±0.11	1.70^{a}±0.01
Survival (%)	79.29^{a}±1.94	82.89^{a}±1.57	92.54^{b}±1.89

Initial total length of the fry range between 10-12 mm
SGR= Specific Growth Rate; FCR= Feed Conversion Ratio
Values within a row with different superscript letters are significantly different ($p<0.05$)

4.1.5 Reproductive performance of guppy

4.1.5.1 Ovary development

The ovarian weight, volume, ovum diameter, absolute fecundity and percentage GSI values are presented in Table 12. The fish fed Diet-3 had significantly ($p<0.05$) higher ovarian weight, ovarian volume, absolute fecundity and %GSI than the fish fed other two groups. Those values were found to be lowest in the guppy fed Diet-1. There was no significant ($p<0.05$) different between ovum diameters in the fish fed Diet-2 and Diet-3.

Table 12. Reproductive parameters (mean values ±SE) of guppy fed different diets

Feed type	Ovarian weight (g)	Ovarian volume (mm^3)	Ovum diameter (mm)	Absolute fecundity	GSI (%)
Diet-1	0.02^{a}±0.011	30.50^{a}±3.39	1.35^{a}±0.08	4.28^{a}±1.61	6.25^{a}±1.63
Diet-2	0.05^{b} ±0.013	73.52^{b}±5.40	$1.50^{a,b}$±0.01	7.98^{b}±1.89	10.41^{b}±0.91
Diet-3	0.12^{c} ±0.012	120.53^{c}±13.97	1.67^{b}±0.02	18.44^{c}±2.07	13.48^{c}±1.24

Values within a column with different superscript letters are significantly different ($p<0.05$)

Variations in size of ovaries of matured female guppy fed different diets are presented in Figure 5.The ovarian length, width and height values were highest in the guppy fed Diet-3 than the fish fed other two diets. The fish fed Diet-1 had the lowest ovarian length, width and height values.

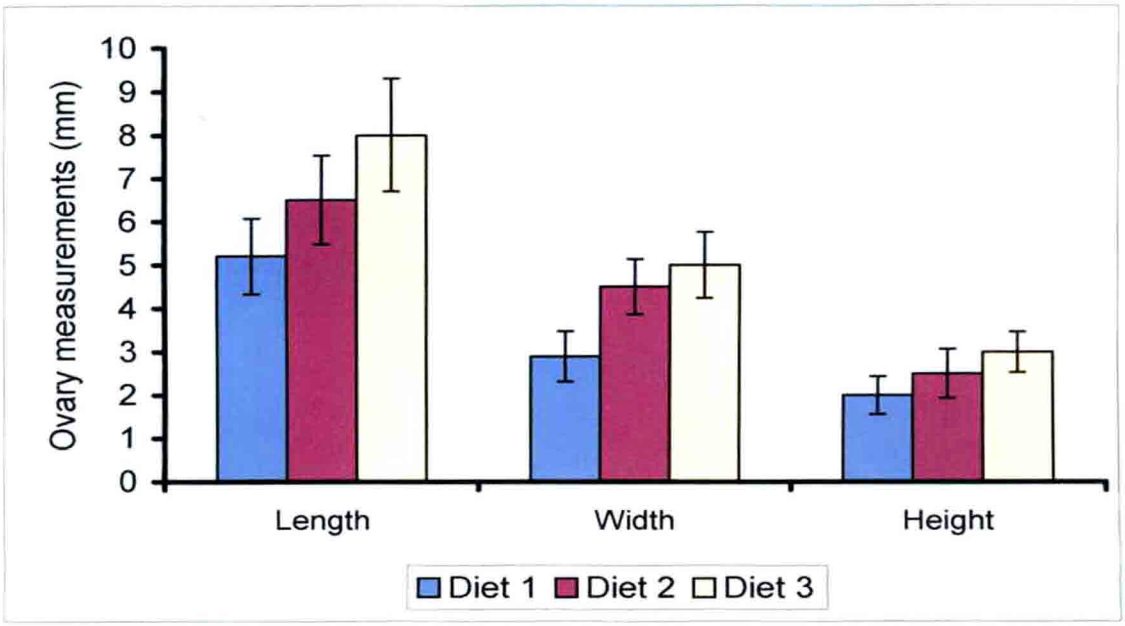


Figure 5. Variation in size of ovaries (±SE) of matured female guppy fed different diets

4.1.5.2 Fry production

The production of fry and the % survival of fry values are presented in Table 13. Mean total fry production was significantly ($p<0.05$) higher in guppy fed Diet-3 (43.6% protein and 9.47% lipids) than other treatments. The fry survival of the fish fed Diet-3 was significantly ($p<0.05$) followed by the fish fed Diet-2 and Diet-1.

Table 13. Mean total fry production, fry survival rate (mean values ±SE) after three months breeding period

Diet type	**Mean total fry production**	**Fry survival rate (%)**
Diet-1	43.00^{a}±6.0	82.8^{a}±1.6
Diet-2	85.5^{b}±8.5	86.8^{b}±0.19
Diet-3	133.5^{c}±9.5	90.37^{c}±1.7

Values within a column with different superscript letters are significantly different ($p<0.05$)

The mean weekly fry production values fed different diets are presented in Figure 6. The mean weekly fry production of the guppy fed Diet-3 was higher than the fish fed other two groups. There was no production of fry in the guppy fed Diet-1 during the first three weeks period. The lowest weekly fry productions were recorded in the guppy fed Diet-1.

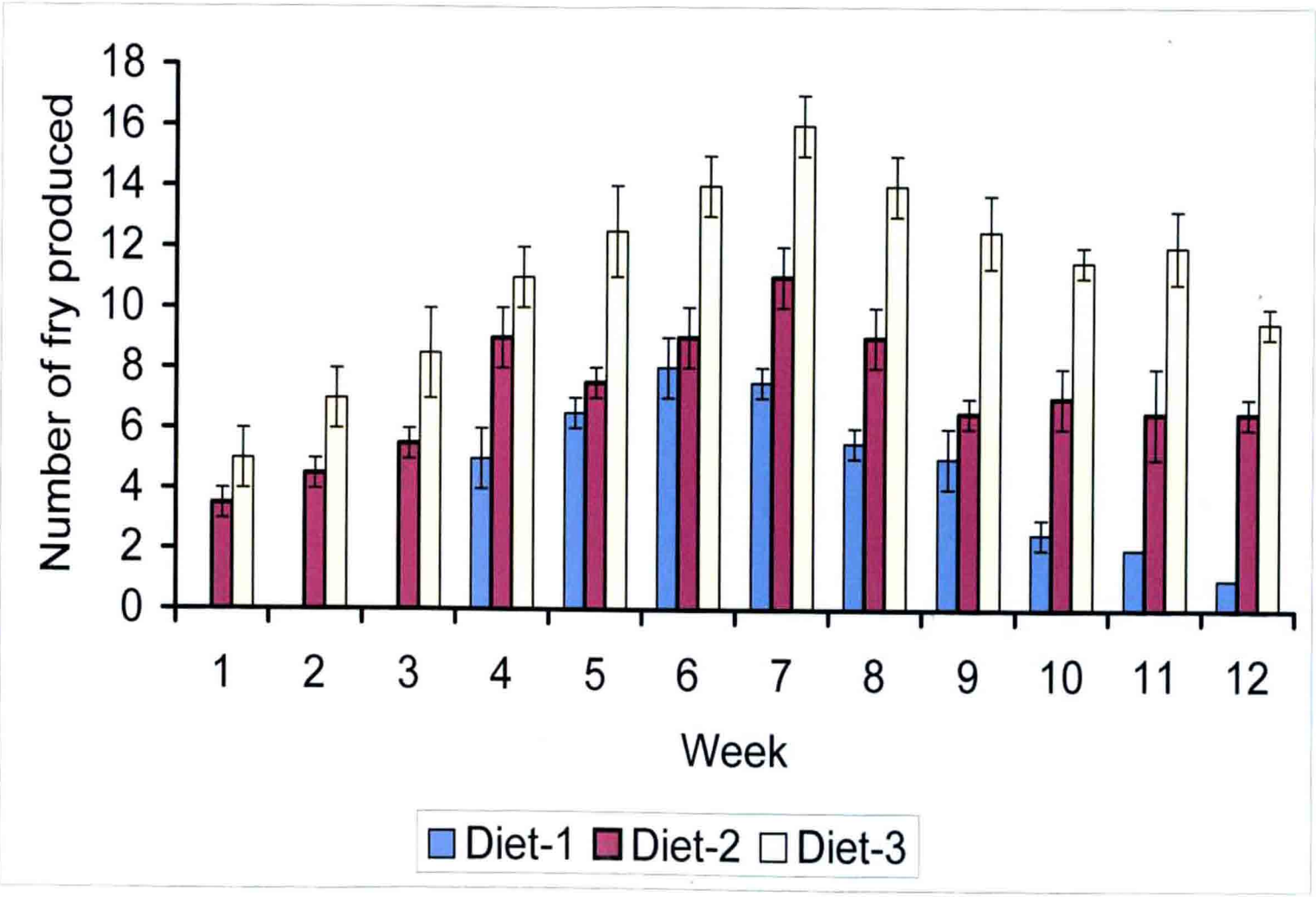


Figure 6. Mean weekly production of fry (± SE) of female guppy fed different diets

4.1.6 Fatty acid composition of diets, muscle, eggs and embryos of guppy

4.1.6.1 Fatty acid profile of diets

The fatty acid profiles of the experimental diets are presented in Table 14. The most available fatty acids in all the diets were 16:0, 18:0 18:1 n-9, 18:2 n-6, 18:3 n-3. In addition, Diet-2 and Diet-3 were also richer in EPA and DHA and hence their n-3 total PUFA, HUFA and n-3 HUFA were also significantly higher ($p<0.05$) than Diet-1. Diet-1 had significantly ($p<0.05$) higher PUFA and n-6 PUFA levels since it contained significantly ($p<0.05$) higher level of 18:2 n-6 compared to other diets. Diet-3 recorded the significantly higher ($p<0.05$) levels of EPA, DHA, and n-3 PUFA. Diet-1 contained the significantly highest ($p<0.05$) levels of 16:0 (palmitic acid) 18:1 n-9 (oleic acid) 18:2 n-6 (linoleic acid), 18:3n-3 (α-linolenic acid). Diet-2 contained significantly ($p<0.05$) higher level of 20:4 n-6 than Diets 1 and 3. The n-3/n-6 ratios varied in the three experimental feeds and the Diet-3 had significantly ($p<0.05$) higher ratio than other two diets.

Table 14. Fatty acid profile (% of individual fatty acids among total identified fatty acids) of diets

Fatty acids	Diet-1	Diet-2	Diet-3
8:0	$0.07^{b}\pm0.01$	$0.02^{a}\pm0.00$	$0.03^{a}\pm0.00$
10:00	$0.03^{a}\pm0.00$	$0.02^{a}\pm0.00$	$0.05^{a}\pm0.02$
12:00	$0.06^{a}\pm0.01$	$0.07^{a}\pm0.01$	$0.40^{b}\pm0.01$
13:00	$0.02^{a}\pm0.00$	$0.04^{a}\pm0.00$	$0.03^{a}\pm0.00$
14:00	$0.55^{a}\pm0.05$	$4.58^{c}\pm0.85$	$4.12^{b}\pm0.81$
15:00	$0.18^{a}\pm0.01$	$0.57^{c}\pm0.01$	$0.45^{b}\pm0.36$
16:00	$20.30^{c}\pm0.90$	$19.47^{b}\pm0.81$	$16.04^{a}\pm0.68$
17:00	$0.23^{a}\pm0.01$	$0.27^{b}\pm0.01$	$0.56^{c}\pm0.00$
18:00	$3.26^{a}\pm0.81$	$6.69^{b}\pm0.91$	$6.79^{b}\pm0.99$
19:00	ND	0.26±0.01	ND
20:00	$0.54^{a}\pm0.01$	$0.70^{c}\pm0.01$	$0.38^{b}\pm0.03$
22:00	1.24±0.20	ND	ND
23:00	$0.09^{a}\pm0.01$	$0.12^{b}\pm0.01$	ND
24:00	$1.61^{b}\pm0.24$	$0.97^{a}\pm0.01$	ND
SAFA	**$28.16^{a}\pm0.85$**	**$33.76^{b}\pm0.62$**	**$28.85^{a}\pm0.74$**
16:1 n-9	$0.18^{a}\pm0.01$	$0.35^{b}\pm0.01$	$5.45^{c}\pm0.05$
16:1 n-7	$0.57^{a}\pm0.01$	$5.37^{c}\pm0.15$	$1.29^{b}\pm0.27$
18:1 n-9	$26.37^{c}\pm0.80$	$20.11^{a}\pm0.55$	$21.50^{b}\pm0.26$
18:1 n-7	$1.85^{b}\pm0.05$	$2.59^{c}\pm0.10$	$0.23^{a}\pm0.02$
20:1 n-9	$0.68^{b}\pm0.05$	$0.31^{a}\pm0.05$	$3.90^{c}\pm0.05$
21:1 n-9	$0.13^{a}\pm0.01$	$0.04^{a}\pm0.00$	$4.91^{b}\pm0.05$
MUFA	**$29.77^{a}\pm0.62$**	**$28.13^{a}\pm0.65$**	**$37.28^{b}\pm0.36$**
18:2 n-9	ND	$0.08^{b}\pm0.00$	$0.02^{a}\pm0.00$
18:2 n-6	$38.21^{c}\pm0.46$	$25.00^{b}\pm0.31$	$14.51^{a}\pm0.35$
18:3 n-3	$3.32^{b}\pm0.06$	$1.98^{a}\pm0.01$	$1.82^{a}\pm0.10$
20:2 n-9	ND	$0.14^{a}\pm0.01$	$0.12^{a}\pm0.01$
20:2n-7	$0.07^{a}\pm0.00$	$0.15^{b}\pm0.00$	$0.22^{c}\pm0.00$
20:3 n-7	ND	0.08±0.00	ND

20:4 n-6	$0.05^{a}\pm0.00$	$1.18^{c}\pm0.02$	$0.76^{b}\pm0.00$
20:4 n-3	ND	$0.18^{b}\pm0.02$	$0.07^{a}\pm0.01$
20:5 n-3	ND	$4.42^{a}\pm0.06$	$6.39^{b}\pm0.07$
22:4 n-6	ND	$0.19^{b}\pm0.02$	$0.08^{a}\pm0.01$
22:5 n-3	ND	$0.38^{a}\pm0.01$	$0.30^{a}\pm0.00$
22:6 n-3	$0.43^{a}\pm0.02$	$3.70^{b}\pm0.15$	$9.60^{c}\pm0.05$
PUFA	**$42.07^{c}\pm0.41$**	**$38.11^{b}\pm0.53$**	**$33.87^{a}\pm0.42$**
HUFA	**$0.48^{a}\pm0.03$**	**$10.05^{b}\pm0.02$**	**$17.2^{c}\pm0.04$**
n-3 PUFA	$3.74^{a}\pm0.06$	$10.69^{b}\pm0.04$	$18.17^{c}\pm0.12$
n-3 HUFA	$0.43^{a}\pm0.01$	$8.71^{b}\pm0.03$	$16.36^{c}\pm0.04$
n-6 PUFA	$38.26^{c}\pm0.06$	$26.36^{b}\pm0.00$	$15.35^{a}\pm0.35$
n-6 HUFA	$0.05^{a}\pm0.00$	$1.37^{c}\pm0.01$	$0.84^{b}\pm0.01$
n-3/n-6 PUFA	$0.10^{a}\pm0.00$	$0.41^{b}\pm0.01$	$1.19^{c}\pm0.04$

ND=Not Detected

Values within a row with different superscript letters are significantly different

4.1.6.2 Fatty acid profile of initial fry and muscle of guppy after maturation

The guppy fry used in this experiment contained 35.65% SAFA, 34.35% MUFA, 30.00% PUFA and 15.08% HUFA (Table 15). The levels of PUFA were significantly ($p<0.05$) higher in fish fry than matured fish. After feeding experimental diets throughout the study period, the guppy fed with Diet-1 contained almost 47.58% SAFA, 38.59% MUFA, 13.83% PUFA and 2.8% HUFA while the guppy fed Diet-2 contained 41.09% SAFA, 41.10% MUFA, 17.81% PUFA and 4.79 HUFA. The fish fed Diet-3 had 33.68% SAFA, 37.04% MUFA, 29.28% PUFA and 14.7 HUFA. The most available fatty acids in the muscle were 16:0, 18:0, 16:1 n-7, 18:1 n-9, 18:2 n-6. The levels of 18:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3 in the guppy fed Diet-3 were significantly ($p<0.05$) higher than the fish fed other two diets. The total n-3 PUFA and n-3 HUFA were significantly higher in the guppy fed Diet-3 than other 2 diets (Figure 2). n-3/ n-6 ratios in muscle varied considerably (0.12-1.12) among the guppy fed different diets and the fish fed Diet-3 had significantly ($p<0.05$) higher ratio.

Table 15. Fatty acid profile (% of individual fatty acids among total identified fatty acids) of initial fry and muscle of guppy after maturation

Fatty acids	Initial	Diet-1	Diet-2	Diet-3
12:00	$0.13^{a,b}\pm0.03$	$0.10^{a,b}\pm0.01$	$0.08^{a}\pm0.01$	$0.17^{b}\pm0.02$
14:00	$2.38^{a}\pm0.21$	$2.93^{a}\pm0.29$	$2.79^{a}\pm0.25$	$3.70^{a}\pm0.54$
15:00	$1.48^{a}\pm0.18$	$3.74^{b}\pm0.39$	$2.68^{a,b}\pm0.58$	$1.55^{a}\pm0.35$
16:00	$15.90^{b}\pm1.45$	$26.92^{d}\pm1.24$	$21.18^{c}\pm1.59$	$13.18^{a}\pm0.86$
17:00	$0.91^{a}\pm0.12$	$1.61^{a,b}\pm0.24$	$1.15^{a}\pm0.18$	$2.09^{b}\pm0.26$
18:00	$14.04^{b}\pm1.52$	$11.91^{a}\pm0.84$	$12.42^{a,b}\pm1.50$	$12.13^{a}\pm0.45$
20:00	$0.58^{b}\pm0.05$	$0.24^{a}\pm0.04$	$0.45^{b}\pm0.05$	$0.60^{b}\pm0.05$
22:00	$0.25^{a,b}\pm0.03$	$0.16^{a}\pm0.04$	$0.35^{b,c}\pm0.03$	$0.48^{c}\pm0.06$
SAFA	**$35.65^{a}\pm1.67$**	**$47.58^{c}\pm1.56$**	**$41.09^{b}\pm2.51$**	**$33.68^{a}\pm1.05$**
16:1 n-9	$0.94^{c}\pm0.05$	$0.24^{a}\pm0.04$	$0.61^{b}\pm0.04$	$0.88^{c}\pm0.10$
16:1 n-7	$6.69^{a,b}\pm0.52$	$6.04^{a}\pm0.35$	$8.69^{b}\pm1.58$	$7.45^{a,b}\pm0.53$
18:1 n-9	$24.73^{b}\pm1.52$	$28.30^{b}\pm1.34$	$26.31^{b}\pm1.08$	$19.31^{a}\pm0.92$
18:1 n-7	$0.32^{a}\pm0.06$	$2.41^{b}\pm0.59$	$3.92^{c}\pm0.07$	$0.25^{a}\pm0.03$
20:1 n-9	$1.54^{a}\pm0.45$	$1.45^{a}\pm0.14$	$1.39^{a}\pm0.35$	$5.44^{b}\pm0.46$
22:1 n-9	$0.14^{a}\pm0.04$	$0.16^{a}\pm0.02$	$0.19^{a}\pm0.05$	$3.70^{b}\pm0.17$
MUFA	**$34.35^{a}\pm1.59$**	**$38.59^{a,b}\pm2.08$**	**$41.10^{b}\pm1.71$**	**$37.04^{a,b}\pm1.62$**
18:2 n-9	$0.30^{a}\pm0.02$	$1.06^{c}\pm0.07$	$0.61^{b}\pm0.04$	$0.55^{b}\pm0.04$
18:2 n-6	$11.24^{b}\pm0.75$	$7.82^{a}\pm0.26$	$9.83^{a,b}\pm0.60$	$9.67^{a,b}\pm0.55$
18:3 n-6	$0.62^{a}\pm0.05$	$0.55^{a}\pm0.11$	$0.67^{a}\pm0.06$	$0.66^{a}\pm0.02$
18:3 n-3	$0.92^{b}\pm0.07$	$0.26^{a}\pm0.03$	$0.42^{a}\pm0.04$	$0.90^{b}\pm0.04$
18:4 n-3	$0.38^{b}\pm0.04$	$0.15^{a}\pm0.04$	$0.26^{a}\pm0.03$	$0.57^{c}\pm0.01$
20:2 n-9	$0.03^{a}\pm0.02$	$0.37^{b}\pm0.02$	$0.34^{b}\pm0.02$	$0.35^{b}\pm0.05$
20:2 n-7	$0.59^{a,b}\pm0.05$	$0.29^{a}\pm0.04$	$0.46^{a,b}\pm0.04$	$1.04^{b}\pm0.31$
20:3 n-6	$0.81^{b}\pm0.06$	$0.52^{a}\pm0.05$	$0.41^{a}\pm0.03$	$0.47^{a}\pm0.03$
20:4 n-6	$4.43^{b}\pm0.51$	$1.64^{a}\pm0.35$	$1.39^{a}\pm0.33$	$1.86^{a}\pm0.80$
20:3 n-3	$0.05^{a}\pm0.01$	$0.04^{a}\pm0.01$	$0.04^{a}\pm0.02$	$0.38^{b}\pm0.03$
20:4 n-3	$0.026^{a}\pm0.01$	$0.03^{a}\pm0.01$	$0.02^{a}\pm0.01$	$0.26^{b}\pm0.05$
20:5 n-3	$1.02^{b}\pm0.04$	$0.04^{a}\pm0.01$	$0.25^{a}\pm0.04$	$1.83^{c}\pm0.13$

22:4 n-6	$1.37^{b}\pm0.21$	$0.36^{a}\pm0.04$	$1.56^{b}\pm0.40$	$0.39^{a}\pm0.03$
22:5 n-3	$1.19^{b}\pm0.08$	$0.06^{a}\pm0.01$	$0.23^{a}\pm0.02$	$2.46^{c}\pm0.06$
22:6 n-3	$7.06^{b}\pm0.42$	$0.69^{a}\pm0.03$	$1.34^{a}\pm0.22$	$7.91^{b}\pm0.39$
PUFA	**$30.00^{c}\pm1.26$**	**$13.83^{a}\pm0.52$**	**$17.81^{b}\pm0.23$**	**$29.28^{c}\pm0.57$**
HUFA	**$15.08^{b}\pm0.82$**	**$2.80^{a}\pm0.43$**	**$4.79^{a}\pm0.58$**	**$14.70^{b}\pm0.67$**
n-3 PUFA	$10.63^{c}\pm0.41$	$1.26^{a}\pm0.13$	$2.54^{b}\pm0.08$	$14.31^{d}\pm0.16$
n-3 HUFA	$9.28^{b}\pm0.52$	$0.81^{a}\pm0.05$	$1.84^{a}\pm0.16$	$12.46^{c}\pm0.16$
n-6 PUFA	$18.46^{c}\pm0.94$	$10.87^{a}\pm0.28$	$13.85^{b}\pm0.22$	$13.04^{b}\pm0.33$
n-6 HUFA	$5.80^{b}\pm0.3$	$1.99^{a}\pm0.38$	$2.95^{a}\pm0.73$	$2.25^{a}\pm0.83$
n-3/n-6 PUFA	$0.58^{c}\pm0.01$	$0.12^{a}\pm0.01$	$0.18^{b}\pm0.01$	$1.12^{d}\pm0.02$

ND=Not Detected

Values within a row with different superscript letters are significantly different ($p<0.05$)

4.1.6.3 Lipid content and fatty acid profile of eggs and embryos

The lipid content in the eggs and embryos are presented in Table 16. The percentage lipid content in the eggs and embryos of the guppy fed Diet-3 were significantly ($p<0.05$) higher than the guppy fed other two diets. The guppy fed diet-1 had the lowest lipid content. It was also observed that there was a little decrease of lipid levels in embryos.

Table 16. Percentage lipid content (wet weight basis) in eggs and embryos of guppy

Diet	Eggs	Embryos
Diet-1	$6.30^{a}\pm0.07$	$6.08^{a}\pm0.04$
Diet-2	$6.90^{b}\pm0.05$	$6.38^{b}\pm0.05$
Diet-3	$7.28^{c}\pm0.04$	$6.95^{c}\pm0.03$

Values within a row with different superscript letters are significantly different ($p<0.05$)

The fatty acid profiles of the eggs and embryos are presented in Table 17 and 18. The fatty acid profiles were dominated by a small number of fatty acids. The

most available fatty acids in the all treatments were 16:0, 18:0, 16:1 n-9, 18:1 n-9, 18:2 n-6, 18:3 n-3, 20:4 n-6 and 22:6 n-3. The guppy fed Diet-2 and Diet-3 had significantly ($p<0.05$) higher levels of PUFA and HUFA level in eggs and embryos. There was no significantly difference of 18:2 n-6 levels among the eggs of the guppy fed three different diets. However, the linoleic acid was higher in the embryos of the guppy fed Diet-1 than the other two diets. The18:3 n-3 level in the eggs of the fish fed Diet-3 was significantly higher than the guppy fed other 2 diets. In embryos, these levels were similar in the fish fed Diets 2 and 3 and significantly higher amount was observed in the fish fed Diet-3 than the fish fed Diet-1. There was no significant difference of 20:4 n-6 in the eggs of the fish fed three diets while levels in the embryos of the fish fed Diet-1 and 2 had significantly higher levels than the other. The EPA levels in the eggs of the guppy fed Diet-3 had significantly higher levels than other two diets. In embryos, there was no significantly difference between Diets 2 and 3 while Diet-1 contained the lowest level. The DHA levels in the eggs and embryos of the guppy fed Diet-3 were found to be significantly higher than the fish fed Diet-1 and Diet-2. The n-3/n-6 levels in the eggs and embryos of the guppy fed Diet-3 were significantly higher than the guppy fed other diets.

Table 17. Fatty acid profile (% of individual fatty acids among total identified fatty acids) of eggs

Fatty acid	Diet-1	Diet-2	Diet-3
14:0	$2.28^{a}\pm0.1$	$3.16^{b}\pm0.10$	$3.26^{b}\pm0.23$
15:0	$1.90^{a}\pm0.23$	$2.85^{b}\pm0.14$	$2.29^{a,b}\pm0.18$
16:0	$19.75^{b}\pm1.16$	$11.44^{a}\pm0.58$	$9.94^{a}\pm0.98$
17:0	$0.98^{a}\pm0.15$	$1.55^{b}\pm0.10$	$0.91^{a}\pm0.08$
18:0	$12.29^{a}\pm1.09$	$11.55^{a}\pm1.52$	$10.16^{a}\pm0.84$
20:0	$0.08^{a}\pm0.01$	$0.54^{b}\pm0.06$	$0.46^{b}\pm0.06$
22:0	$0.07^{a}\pm0.01$	$0.41^{b}\pm0.09$	$0.49^{b}\pm0.08$
SAFA	**$37.34^{b}\pm1.58$**	**$31.48^{a}\pm1.53$**	**$27.49^{a}\pm1.55$**
16:1 n-9	$6.96^{b}\pm0.25$	$1.59^{a}\pm0.40$	$0.79^{a}\pm0.07$
16:1 n-7	$0.62^{a}\pm0.03$	$8.57^{b}\pm0.75$	$8.23^{b}\pm0.42$
18:1 n-9	$28.22^{b}\pm1.11$	$20.54^{a}\pm1.83$	$22.92^{a}\pm1.36$
18:1 n-7	$0.41^{a}\pm0.06$	$0.24^{a}\pm0.12$	$0.31^{a}\pm0.08$
20:1 n-9	$1.01^{a}\pm0.11$	$2.10^{b}\pm0.22$	$2.71^{b}\pm0.27$
22:1 n-9	$0.30^{a}\pm0.09$	$0.09^{a}\pm0.01$	$1.03^{b}\pm0.19$
MUFA	**$37.50^{a}\pm1.63$**	**$33.12^{a}\pm1.66$**	**$35.98^{a}\pm1.67$**
18:2 n-9	$0.62^{a,b}\pm0.34$	$0.47^{a}\pm0.06$	$0.68^{b}\pm0.04$
18:2 n-6	$9.56^{a}\pm0.21$	$7.92^{a}\pm0.81$	$8.25^{a}\pm0.94$
18:3 n-6	$3.71^{b}\pm0.50$	$2.25^{a,b}\pm0.27$	$2.11^{a}\pm0.10$
18:3 n-3	$0.16^{a}\pm0.06$	$0.40^{a}\pm0.06$	$1.44^{b}\pm0.24$
18:4 n-3	$0.14^{a}\pm0.02$	$0.29^{a}\pm0.06$	$1.15^{b}\pm0.11$
20:2 n-9	$0.14^{a}\pm0.05$	$0.60^{b}\pm0.02$	$0.14^{a}\pm0.04$
20:2 n-7	$0.19^{a}\pm0.06$	$0.68^{c}\pm0.05$	$0.44^{b}\pm0.04$
20:3 n-6	$0.72^{b}\pm0.05$	$1.05^{c}\pm0.08$	$0.35^{a}\pm0.04$
20:4 n-6	$2.96^{a}\pm0.75$	$5.67^{a}\pm0.42$	$3.27^{a}\pm0.71$
20:3 n-3	$0.20^{a}\pm0.01$	$0.73^{c}\pm0.06$	$0.48^{b}\pm0.02$
20:4 n-3	$0.31^{a}\pm0.01$	$0.85^{b}\pm0.13$	$0.97^{b}\pm0.15$
20:5 n-3	$0.17^{a}\pm0.02$	$0.20^{a}\pm0.04$	$1.28^{b}\pm0.28$

22:4 n-6	2.84^{b}±0.50	2.26^{b}±0.14	0.80^{a}±0.10
22:5 n-3	0.38^{a}±0.04	2.37c±0.21	1.53b±0.13
22:6 n-3	3.10^{a}±0.45	9.69^{b}±0.58	13.69^{c}±0.53
PUFA	**25.16^{a}±1.06**	**35.40^{b}±1.19**	**36.53^{b}±1.08**
HUFA	**9.85^{a}±1.82**	**20.04^{b}±1.49**	**21.53^{b}±0.28**
n-3 PUFA	4.45^{a}±0.41	14.53^{b}±0.03	20.52^{c}±1.44
n-3 HUFA	3.95^{a}±0.47	13.11^{b}±0.20	17.46^{c}±1.08
n-6 PUFA	19.78^{b}±0.58	19.14^{b}±1.29	14.77^{a}±0.61
n-6 HUFA	5.80^{a}±1.25	7.93^{a}±0.29	4.07^{a}±0.81
n-3/n-6 PUFA	0.23^{a}±0.02	0.76^{b}±0.05	1.40^{c}±0.16

ND=Not Detected

Values within a row with different superscript letters are significantly different ($p<0.05$)

Table 18. Fatty acid profile (% of individual fatty acids among total identified fatty acids) of embryos

Fatty acid	Diet-1	Diet-2	Diet-3
14:0	$2.00^{a}\pm0.10$	$2.98^{b}\pm0.24$	$3.44^{b}\pm0.13$
15:0	$1.59^{a}\pm0.06$	$2.51^{b}\pm0.27$	$2.75^{b}\pm0.14$
16:0	$19.70^{c}\pm1.51$	$13.25^{a}\pm0.66$	$15.76^{b}\pm1.48$
17:0	$1.82^{a}\pm0.08$	$2.93^{a}\pm0.66$	$1.67^{a}\pm0.23$
18:0	$13.51^{b}\pm0.84$	$12.32^{b}\pm0.67$	$9.36^{a}\pm0.81$
20:0	$0.19^{a}\pm0.03$	$0.55^{c}\pm0.05$	$0.34^{b}\pm0.01$
22:0	$0.51^{a}\pm0.03$	$0.37^{a}\pm0.05$	$0.40^{a}\pm0.01$
SAFA	**$39.31^{b}\pm1.46$**	**$36.00^{a}\pm1.27$**	**$33.22^{a}\pm1.30$**
16:1 n-9	$6.30^{a}\pm0.65$	$6.83^{a}\pm0.50$	$7.36^{a}\pm0.38$
16:1 n-7	$0.54^{a}\pm0.09$	$0.78^{a,b}\pm0.06$	$0.90^{b}\pm0.08$
18:1 n-9	$28.54^{b}\pm0.10$	$21.24^{a}\pm1.12$	$23.07^{a}\pm0.83$
18:1 n-5	$0.33^{a}\pm0.05$	$0.23^{a}\pm0.02$	$0.23^{a}\pm0.04$
20:1 n-9	$0.25^{a}\pm0.04$	$1.76^{b}\pm0.33$	$2.79^{c}\pm0.03$
22:1 n-9	$1.22^{b}\pm0.01$	$0.08^{a}\pm0.01$	$1.19^{b}\pm0.05$
MUFA	**$37.17^{b}\pm1.75$**	**$30.91^{a}\pm1.35$**	**$35.53^{b}\pm1.14$**
18:2 n-9	$0.78^{c}\pm0.04$	$0.47^{b}\pm0.05$	$0.15^{a}\pm0.02$
18:2 n-6	$10.24^{b}\pm1.35$	$9.12^{a}\pm0.21$	$8.35^{a}\pm0.15$
18:3 n-6	$1.26^{a}\pm0.14$	$1.15^{a}\pm0.13$	$0.82^{a}\pm0.13$
18:3 n-3	$0.29^{a}\pm0.04$	$0.43^{a,b}\pm0.04$	$0.73^{b}\pm0.14$
18:4 n-3	$1.13^{b}\pm0.11$	$0.22^{a}\pm0.01$	$1.73^{c}\pm0.17$
20:2 n-9	$0.27^{a}\pm0.03$	$0.67^{b}\pm0.05$	$0.18^{a}\pm0.06$
20:2 n-7	$0.43^{a}\pm0.02$	$0.62^{a}\pm0.07$	$0.49^{a}\pm0.05$
20:3 n-6	$0.68^{b}\pm0.04$	$0.87^{b}\pm0.06$	$0.36^{a}\pm0.04$
20:4 n-6	$4.17^{b}\pm0.16$	$4.91^{b}\pm0.30$	$2.88^{a}\pm0.02$
20:3 n-3	$0.15^{a}\pm0.04$	$0.27^{a}\pm0.06$	$0.39^{a}\pm0.07$
20:4 n-3	$0.13^{a}\pm0.03$	$0.16^{a}\pm0.04$	$0.44^{a}\pm0.12$

20:5 n-3	$0.19^{a}\pm0.01$	$0.55^{b}\pm0.05$	$0.58^{b}\pm0.08$
22:4 n-6	$1.17^{b}\pm0.10$	$1.46^{b}\pm0.19$	$0.61^{a}\pm0.03$
22:5 n-3	$0.23^{b}\pm1.0$	$2.59^{a}\pm0.31$	$1.54^{a}\pm0.36$
22:6 n-3	$3.56^{a}\pm0.30$	$9.64^{b}\pm0.32$	$12.04^{c}\pm0.18$
PUFA	**$27.52^{a}\pm1.55$**	**$33.09^{b}\pm1.21$**	**$31.25^{b}\pm0.94$**
HUFA	**$9.43^{a}\pm0.09$**	**$19.29^{b}\pm0.55$**	**$17.92^{b}\pm0.88$**
n-3 PUFA	$5.65^{a}\pm0.01$	$13.84^{b}\pm0.17$	$17.43^{c}\pm1.10$
n-3 HUFA	$4.09^{a}\pm0.17$	$12.93^{b}\pm0.07$	$14.59^{b}\pm0.73$
n-6 PUFA	$17.51^{b}\pm0.08$	$17.50^{b}\pm0.88$	$13.01^{a}\pm0.24$
n-6 HUFA	$5.34^{b}\pm0.26$	$6.37^{b}\pm0.49$	$3.49^{a}\pm0.01$
n-3/n-6 PUFA	$0.55^{a}\pm0.00$	$0.79^{a}\pm0.03$	$1.34^{b}\pm0.11$

ND=Not Detected

Values within a row with different superscript letters are significantly different ($p<0.05$)

The comparison of SAFA, MUFA, PUFA and HUFA levels in the different stages are presented in Figure 7. SAFA levels were higher in Diet-2 while PUFA levels were higher in Diet-1. MUFA and HUFA levels were higher in Diet-3 than other two diets. Comparison of total n-3 and n-6 PUFA, HUFA and n-3/n-6 PUFA levels in different stages are presented in Figure 8. Comparison of total PUFA and HUFA levels in eggs and embryos eggs are presented in Figures 9 and 10.

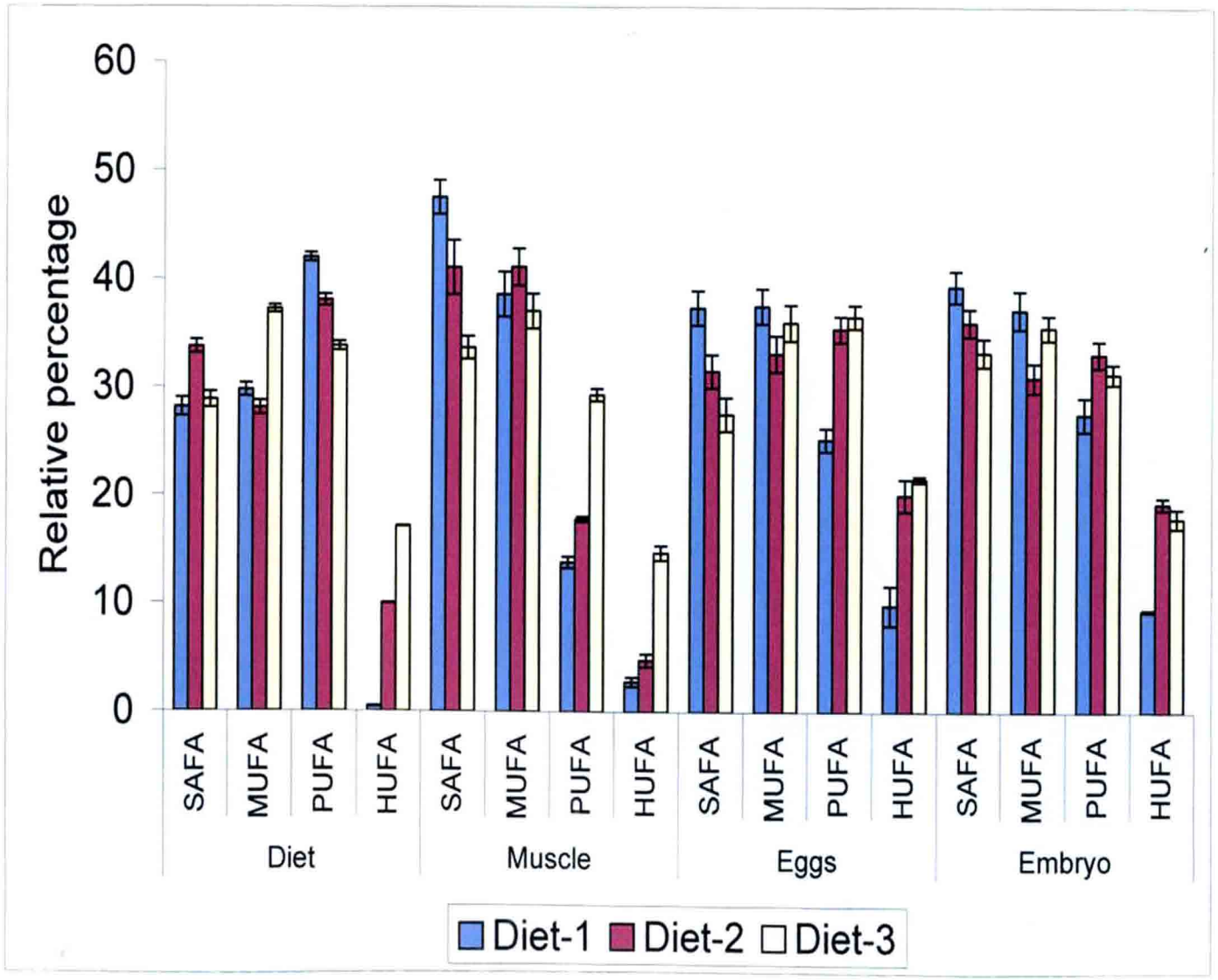


Figure 7. Comparison of SAFA, MUFA, PUFA and HUFA levels in different stages

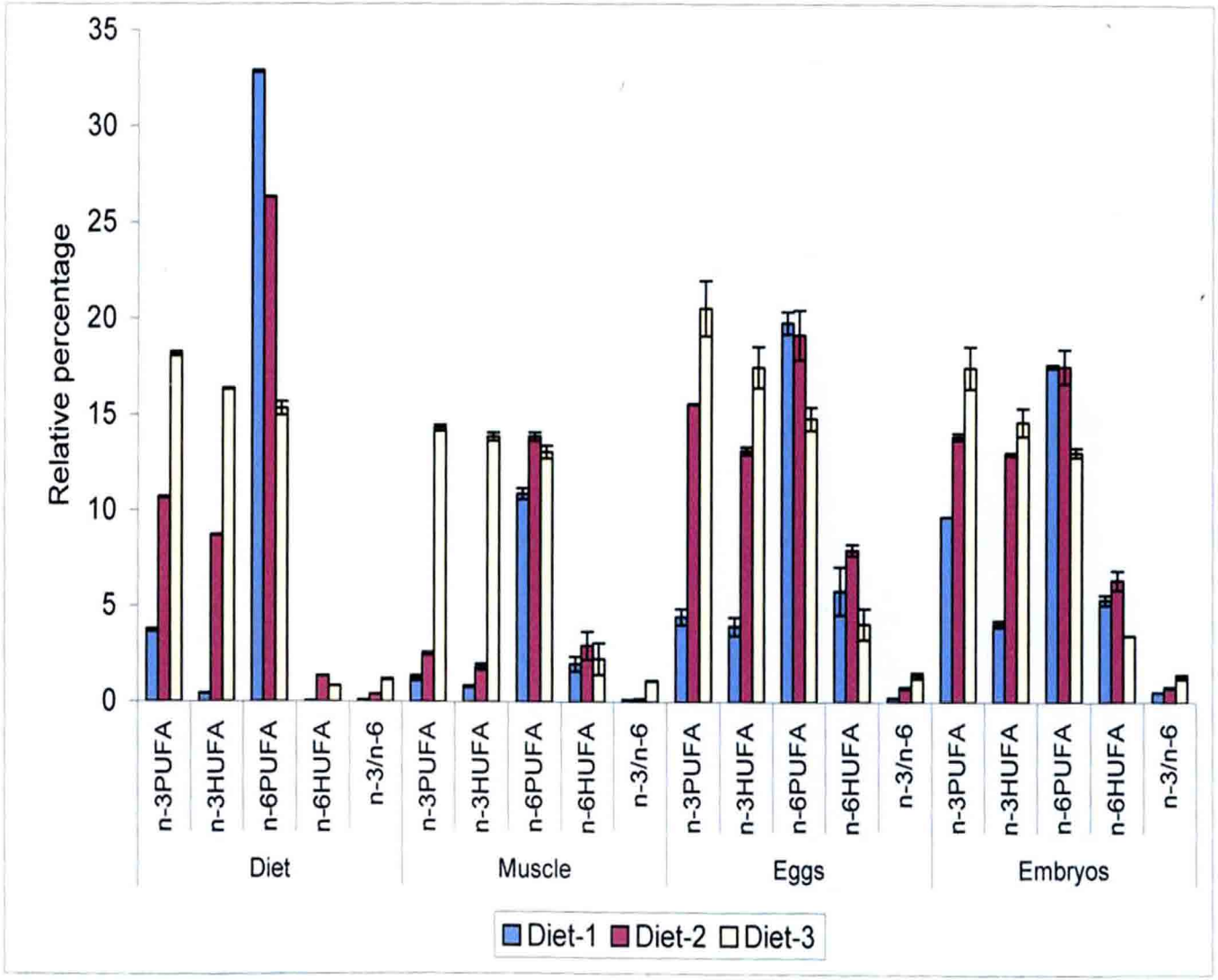


Figure 8. Comparison of polyunsaturated fatty acids (n-3 and n-6 PUFA) and n-3/n-6 levels in different stages

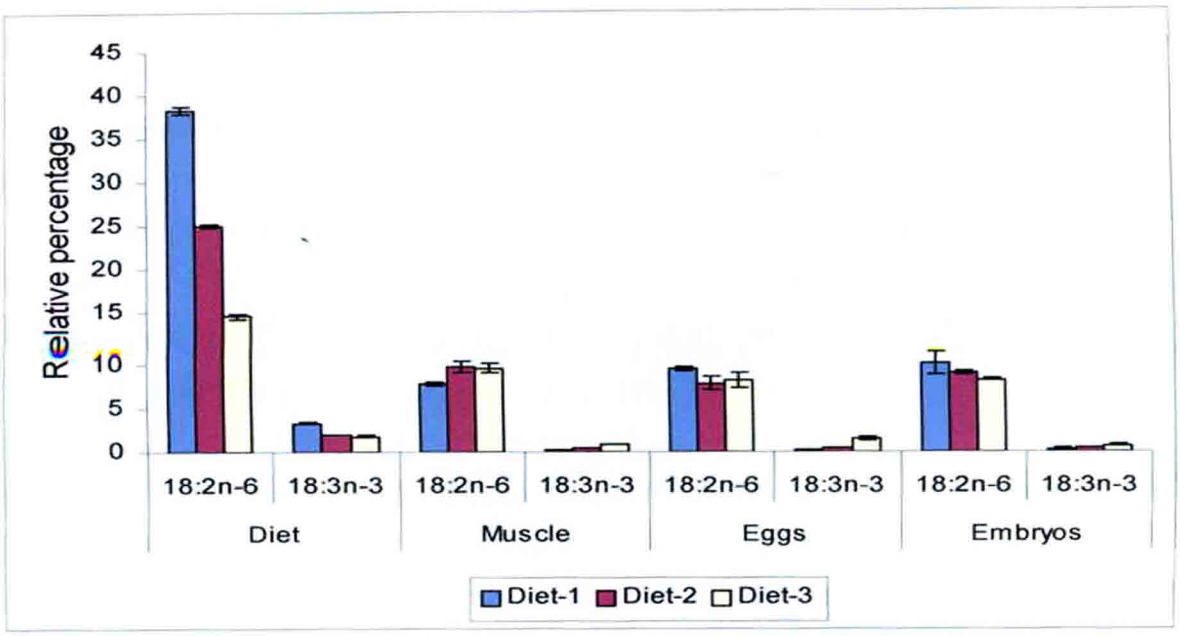


Figure 9. Comparison of important essential fatty acids levels in different stages

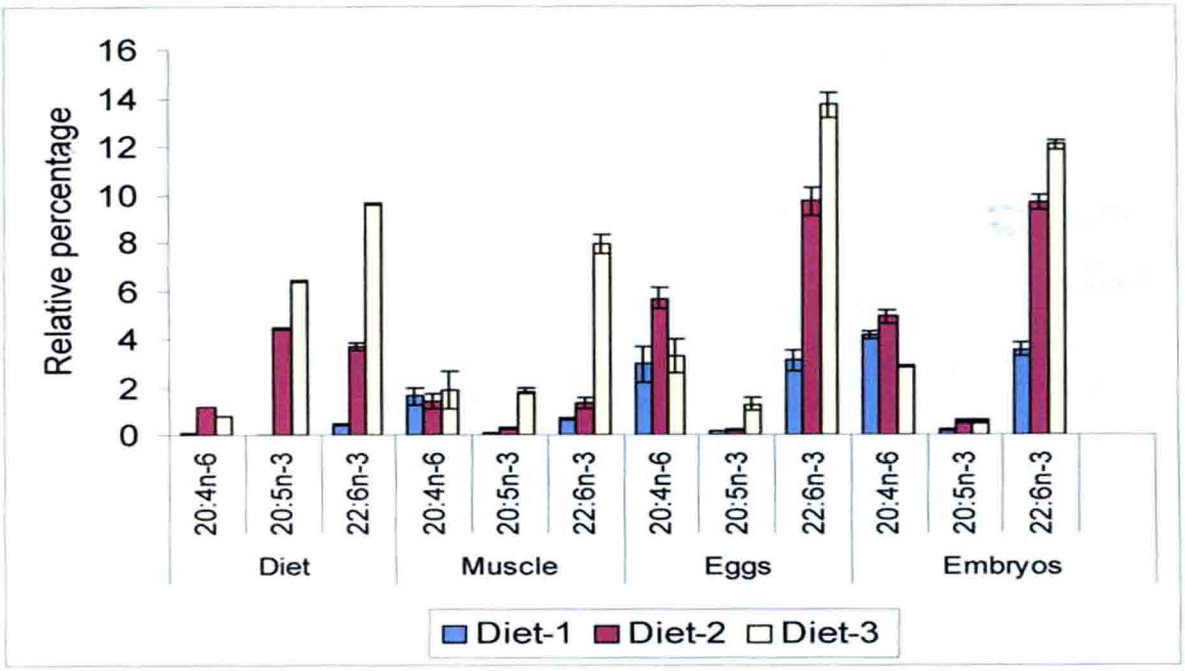


Figure 10. Comparison of important HUFA levels in different stages

4.1.6.4 Changes in fatty acid composition during embryogenesis

Important fatty acids in eggs and embryos are shown in Table 19. There was no significant difference in most of the fatty acids in eggs and embryos. However, the PUFA levels in the eggs of the guppy fed Diets -2 and 3 were found to be significantly higher than that of in embryos. The 18:2 n-6 fatty acid levels were found to be significantly ($p<0.05$) higher in embryos of the fish fed Diet-1 than eggs. All the fatty acids except 18:2 n-6 and SAFA levels were found to be higher in eggs than embryos, while there were slightly higher levels of all the fatty acids, except SAFA, 18:2 n-6, 18:3 n-3 and EPA in the eggs of the guppy fed Diet-2 than embryos. There was no significant difference in DHA levels in the eggs and embryos of the fish fed Diet-1 and Diet-2. n-3/n-6 ratios were significantly higher in embryos of the fish fed Diet-1 than eggs, while there was no significant difference of this ratio in the eggs and embryos of the guppy fed Diet-2 and Diet-3.

Table 19. Comparison of important fatty acid levels in eggs and embryos of guppy

Fatty acid	Diet-1		Diet-2		Diet-3	
	Eggs	Embryos	Eggs	Embryos	Eggs	Embryos
18:2 n-6	$9.56^{a}\pm0.21$	$10.24^{a}\pm0.35$	$7.92^{a}\pm0.81$	$9.12^{a}\pm0.21$	$8.25^{a}\pm0.34$	$8.35^{a}\pm0.15$
18:3 n-3	$0.16^{a}\pm0.06$	$0.29^{a}\pm0.04$	$0.40^{a}\pm0.06$	$0.43^{a}\pm0.04$	$1.44^{b}\pm0.24$	$0.73^{a}\pm0.14$
20:4 n-6	$2.96^{a}\pm0.75$	$4.17^{a}\pm0.16$	$5.67^{b}\pm0.42$	$4.91^{a}\pm0.30$	$3.27^{a}\pm0.71$	$2.88^{a}\pm0.02$
20:5 n-3	$0.17^{a}\pm0.02$	$0.19^{a}\pm0.01$	$0.20^{a}\pm0.04$	$0.55^{a}\pm0.05$	$1.28^{b}\pm0.28$	$0.58^{a}\pm0.08$
22:6 n-3	$3.10^{a}\pm0.45$	$3.56^{a}\pm0.30$	$9.69^{a}\pm0.58$	$9.64^{a}\pm0.32$	$13.69^{b}\pm0.53$	$12.04^{a}\pm0.18$
SAFA	$37.34^{a}\pm1.58$	$39.31^{b}\pm1.43$	$31.48^{a}\pm1.53$	$36.00^{b}\pm1.27$	$27.49^{a}\pm1.55$	$33.22^{b}\pm1.30$
MUFA	$37.50^{a}\pm1.63$	$37.17^{a}\pm1.75$	$33.12^{a}\pm1.66$	$30.91^{a}\pm1.35$	$35.98^{a}\pm1.67$	$35.53^{a}\pm1.14$
PUFA	$25.16^{a}\pm1.06$	$27.52^{a}\pm1.55$	$35.40^{b}\pm1.19$	$33.09^{a}\pm1.21$	$36.53^{b}\pm1.08$	$31.25^{a}\pm0.94$
HUFA	$9.84^{a}\pm1.82$	$8.71^{b}\pm0.09$	$20.04^{a}\pm1.49$	$19.29^{a}\pm0.55$	$21.53^{a}\pm0.28$	$17.92^{a}\pm0.88$
n-3 PUFA	$4.45^{a}\pm0.41$	$5.65^{a}\pm0.01$	$14.53^{a}\pm0.03$	$13.84^{a}\pm0.17$	$20.52^{b}\pm1.44$	$17.43^{a}\pm1.10$
n-3 HUFA	$3.95^{a}\pm0.47$	$4.09^{a}\pm0.17$	$13.11^{a}\pm0.20$	$12.93^{a}\pm0.07$	$17.46^{b}\pm1.08$	$14.59^{a}\pm0.73$
n-6 PUFA	$19.78^{a}\pm0.58$	$17.51^{a}\pm0.08$	$19.14^{a}\pm1.29$	$17.50^{a}\pm0.88$	$14.77^{a}\pm0.61$	$13.01^{a}\pm0.24$
n-6 HUFA	$5.80^{a}\pm1.25$	$5.34^{a}\pm0.26$	$7.93^{a}\pm0.29$	$6.37^{a}\pm0.49$	$4.07^{a}\pm0.81$	$3.49^{a}\pm0.01$
n-3/n-6 PUFA	$0.23^{a}\pm0.02$	$0.55^{b}\pm0.00$	$0.76^{a}\pm0.05$	$0.79^{a}\pm0.03$	$1.40^{a}\pm0.16$	$1.34^{a}\pm0.11$

Values of the same feed within a row with different superscript letters are significantly different ($p<0.05$)

4.2 Growth and reproductive performance of female guppy in response to dietary fatty acids

4.2.1 Proximate composition of diets

The proximate compositions of the diets are presented in Table 20. The prepared diets contained the equal amount of all nutrients. The crude protein levels were ranged between 37.64-38.01 and the crude lipid levels rang was 10.62-10.75. There were no significant different levels of nutrients in the prepared diets.

Table 20. Proximate composition of experimental diets

Nutrient	Percentage composition			
	Diet-CO	Diet-SO	Diet-LO	Diet-FO
Moisture	7.30	7.51	7.45	7.49
Crude protein	38.01	37.82	37.95	37.64
Crude lipid	10.66	10.68	10.62	10.75
Ash	9.66	9.72	9.71	9.51
Total carbohydrates	41.66	42.17	41.72	42.10
Energy*	414.62	414.52	413.64	415.71

*Calculated digestible energy, DE (Kcal/100g)= (CP%×4)+(EE%×9)+(TC%×4)
DE= Digestible Energy, CP=Crude Protein EE= Ether Extract
TC= Total Carbohydrates (calculated by difference)

4.2.2 Physico-chemical parameters of water

The essential physico-chemical parameters of water (temperature ^{0}C, pH, dissolved oxygen, total hardness, ammonia, Nitrite–N, Nitrate–N) were recorded during the study period and average values of all the treatments are presented in Table 21. The water temperatures were monitored and maintained within the acceptable range (25.5-29.3°C). Dissolved oxygen (5.2-6.8 mg/l) and pH values (7.5-8.2) were within the acceptable range throughout the experimental period. Toxic ammonia and nitrite levels were maintained below the sub lethal levels by changing water every alternate day.

Table 21. Physico-chemical parameters of rearing and breeding tanks during the study period

Parameter	Diet-CO	Diet-SO	Diet-LO	Diet-FO
Temperature (°C)	25.5-29.1	25.6-29.3	25.5-29.2	25.7-29.1
pH	7.6-8.0	7.7-8.2	7.5-8.2	7.6-8.2
Dissolved oxygen (mg/l)	5.2-6.8	5.3-6.7	5.2-6.8	5.2-6.6
Hardness (mg/l)	235-240	236-245	236-242	236-244
Total ammonia (mg/l)	0.19-0.21	0.18-0.33	0.20-0.28	0.20-0.28
Unionized ammonia (mg/l)	<0.025	<0.025	<0.025	<0.025
Nitrite-N (mg/l)	<0.003	<0.003	<0.003	<0.003
Nitrate-N (mg/l)	0.02-0.05	0.02-0.05	0.02-0.07	0.02-0.07

4.2.3 Body composition of guppy

The proximate compositions of the initial fish and mature fish at the end of rearing period are presented in Table 22. The body moisture content of the fish before and after the experiments varied between 63.26 to 66.29%. The percentage of body protein in fish fry was 58.97 and after maturation, the values varied between 56.01 and 56.76%. There were no significantly different protein levels among the treatments. Crude fat level in fish fry was 23.46% and after maturation the percentages varied between 26.38 and 27.54%. The crude fat levels in the fish fed Diet-FO and Diet-CO were found to be significantly higher than the other two groups.

Table 22. Carcass analysis (mean values ±SE) of female guppy

Diet type	Percentage on wet weight basis		Percentage on dry weight basis		
	Moisture	Dry matter	Crude protein	Crude fat	Ash
	At beginning of the experiment				
	66.29^{b}±0.03	33.71^{a}±0.03	58.97^{b}±1.87	23.46^{a}±0.12	17.57^{a}±0.06
	At end of the experiment				
Diet-CO	63.82^{a}±0.50	36.18^{b}±0.50	56.02^{a}±0.64	27.54^{c}±0.19	16.44^{a}±0.14
Diet-SO	63.56^{a}±0.11	36.44^{b}±0.11	56.75^{a}±0.61	26.10^{b}±0.10	17.15^{a}±0.17
Diet-LO	63.70^{a}±0.13	36.30^{b}±0.13	56.76^{a}±0.41	26.38^{b}±0.14	16.86^{a}±0.12
Diet-FO	63.26^{a}±0.14	36.74^{b}±0.24	56.01^{a}±0.84	27.37^{c}±0.15	16.62^{a}±0.24

Values within a column with different superscript letters are significantly different ($p<0.05$)

4.2.4 Growth performance of guppy in response to dietary lipids

Growth parameters of female guppy fed different diets are presented in Table 23. The total and standard lengths were significantly ($p<0.05$) higher in the fish fed Diet-FO than the fish fed Diet-CO, Diet-SO and Diet-LO. The fish fed Diet-CO had significantly higher total and standard lengths than the fish fed Diet-SO and Diet-LO. The final weight of fish and the weight gain values were significantly ($p<0.05$) higher in fish fed Diet-FO than other three diets. The specific growth rate value was also significantly higher ($p<0.05$) in fish fed Diet-FO than other three diets. The FCR value of the fish fed Diet-FO was found to be significantly lower than the fish fed other diets. The fish fed Diet-FO had significantly highest % survival rate than the fish fed Diet-SO and Diet-LO.

Table 23. Growth parameters (mean values ±SE) of female guppy fed different diets

Parameter	Diet			
	Diet-CO	Diet-SO	Diet-LO	Diet-FO
Total length (mm)	33.08^{b}±1.30	28.26^{a}±0.59	28.49^{a}±1.08	39.32^{c}±0.57
Standard length (mm)	28.61^{b}±1.03	23.38^{a}±0.45	23.06^{a}±0.99	34.32^{c}±0.55
Final weight (g)	0.78^{b}±0.03	0.40^{a}±0.05	0.37^{a}±0.04	0.96^{c}±0.02
Weight gain (g)	0.70^{b}±0.02	0.33^{a}±0.03	0.28^{a}±0.01	0.87^{c}±0.01
SGR (%)	1.03^{c}±0.01	0.70^{b}±0.05	0.66^{a}±0.05	1.13^{d}±0.01
FCR	2.34^{b}±0.02	2.69^{c}±0.07	2.91^{d}±0.05	2.07^{a}±0.05
Survival (%)	$85.00^{b,c}$±1.67	78.34^{b}±1.68	68.34^{a}±1.69	90.00^{c}±3.33

Values within a row with different superscript letters are significantly different ($p<0.05$)
Initial weight of fish fry range between 0.095-0.097
Initial total length of the fry range between 10-12 mm
SGR= *Specific Growth Rate*; *FCR= Feed Conversion Ratio*

4.2.5 Reproductive performance of guppy in response to dietary lipids

4.2.5.1 Ovary development

The ovarian weight, and volume, ovum diameter, absolute fecundity and percentage GSI values are presented in Table 24. The guppy fed Diet-FO had significantly ($p<0.05$) higher ovarian weight, ovarian volume, absolute fecundity and %GSI than the fish fed other three groups. Those values were found to be lowest in the fish fed Diet-SO and Diet-LO. There was no significant difference among treatments with respect to diameter of egg. All the parameters except ovum diameter and %GSI were significantly ($p<0.05$) higher in fish fed Diet-CO diet than the fish fed Diet-SO, Diet-LO. There was no significant ($p<0.05$) difference of % GSI among the fish groups reared on Diet-SO, Diet-LO and Diet-CO diets. The comparison of ovary length, width and height is presented in Figure 11. The ovary length, width and height values were higher in the fish fed Diet-FO than other three diets. Significantly higher correlations were observed between mean weight and number of ova (Fig 12).

Table 24. Reproductive parameters (mean values ±SE) of guppy fed experimental diets

Feed type	Ovarian weight (g)	Ovarian volume (mm^3)	Ovum diameter (mm)	Absolute fecundity	GSI (%)
Diet-CO	0.11^{b}±0.01	115.06^{b}±2.23	1.64^{a}±0.04	20.00^{b}±1.15	14.10a±0.77
Diet-SO	0.06^{a}±0.01	72.86^{a}±3.78	1.62^{a}±0.07	16.50^{a}±1.15	12.84^{a}±0.07
Diet-LO	0.05^{a}±0.02	81.00^{a}±7.67	1.61^{a}±0.02	15.00^{a}±0.58	12.90^{a}±0.40
Diet-FO	0.16^{c}±0.02	139.78^{c}±4.60	1.69^{a}±0.02	26.50^{c}±1.15	17.35^{b}±1.01

Values within a column with different superscript letters are significantly different ($p<0.05$)

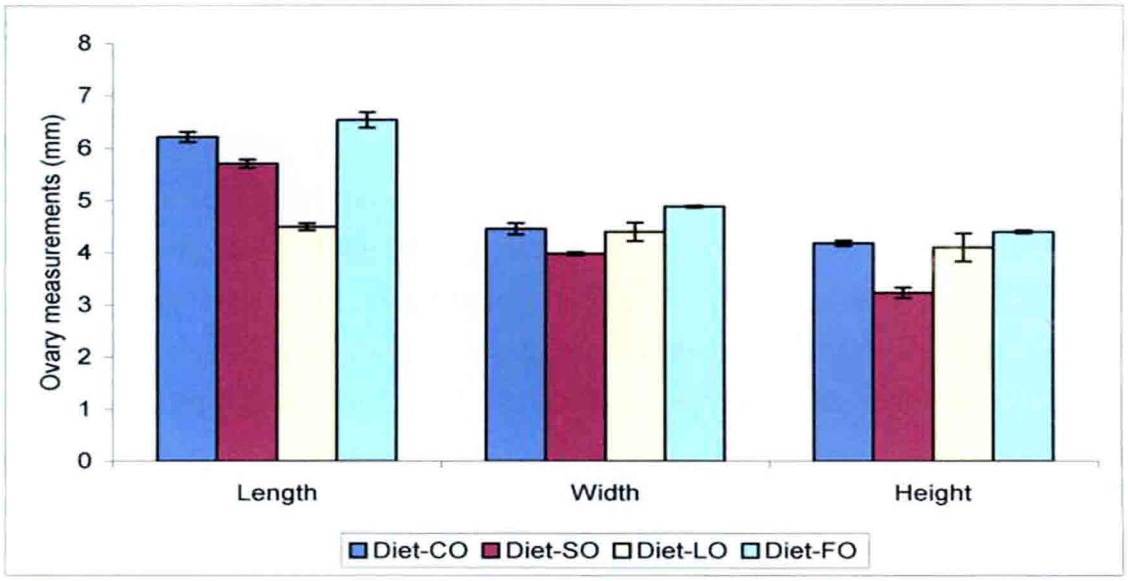


Figure11. Variation in size of ovaries (±SE) of matured female guppy fed different diets

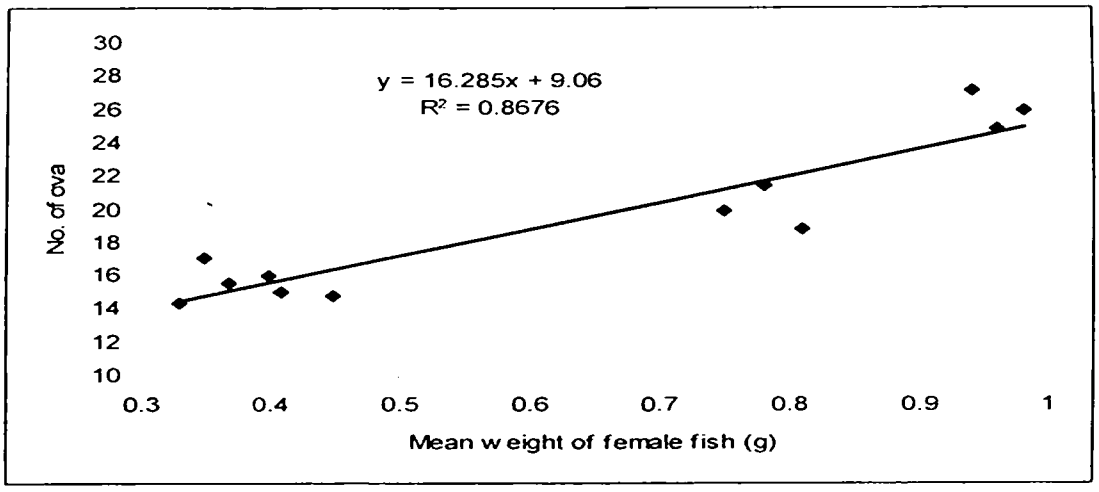


Figure 12. Relationship between number of ova and mean weight of female guppy

4.2.5.2 Production of fry and its survival

The production of fry and the % survival of fry values are presented in Table 25. The mean total production of fry was significantly ($p<0.05$) higher in the guppy fed Diet-FO than other three diets. The fish fed Diet-LO had the significantly lowest mean total fry production among three groups. The % survival of fry obtained from the fish fed Diet-FO was significantly higher than other three groups. There was no significant difference between the % survivals of fry obtained from the fish fed Diet-SO and Diet-LO. Mean weekly production of fry (± SE) of female guppy fed different diets are presented in Figure 13.

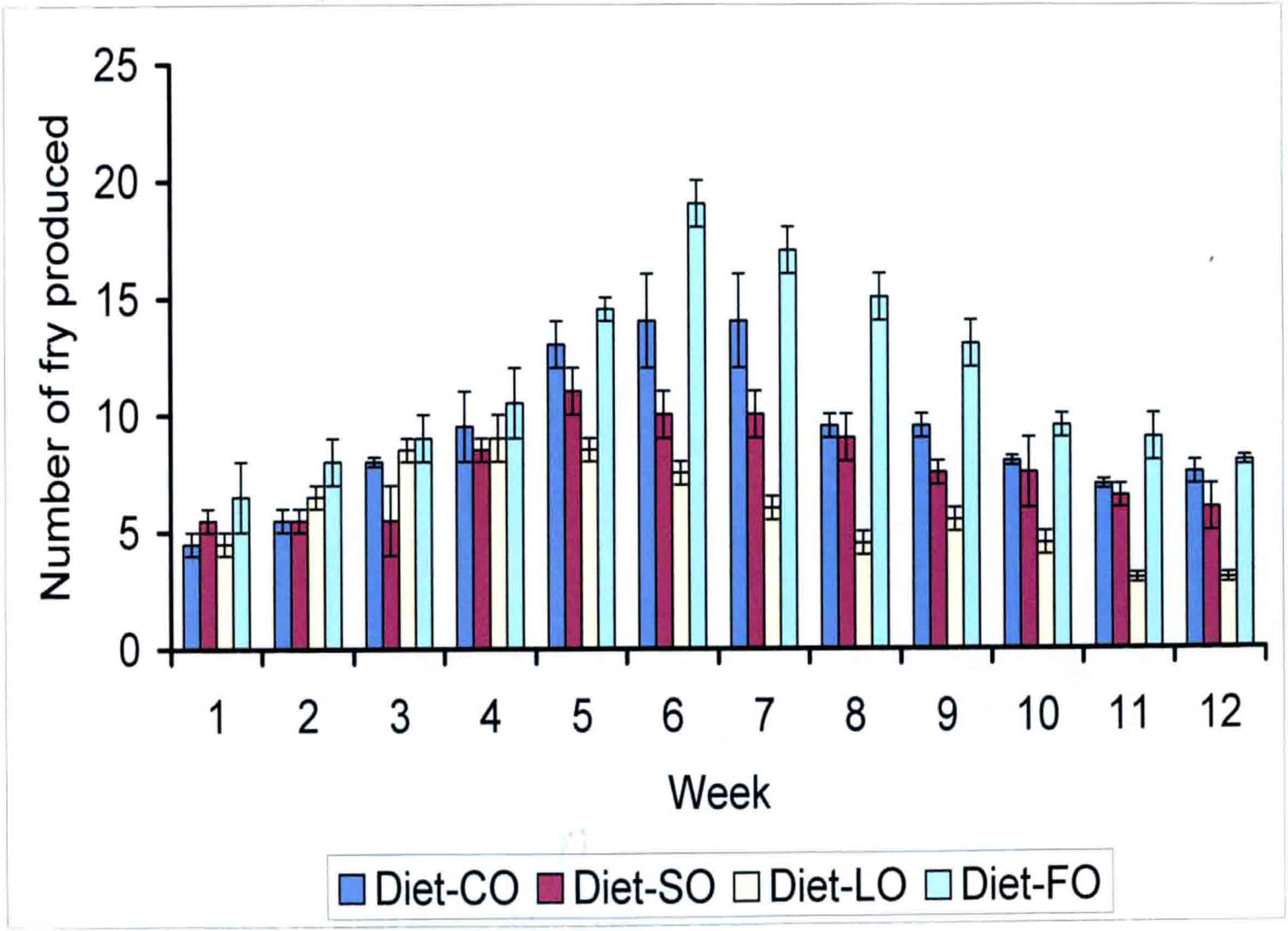


Figure 13. Mean weekly production of fry (± SE) of female guppy fed different diets

Table 25. Total fry production and fry survival rate (mean values ±SE) after three months breeding period

Diet type	Mean total fry production	Fry survival rate (%)
Diet-CO	110.0^{c}±5.20	86.38^{b}±0.42
Diet-SO	92.5^{b}±4.04	81.63^{a}±0.19
Diet-LO	71.0^{a}±4.62	80.27^{a}±0.56
Diet-FO	139.0^{d}±4.62	91.40^{c}±2.04

Values within a column with different superscript letters are significantly different ($p<0.05$)

Significantly higher correlations were observed between mean weight and number of fry produced by guppy (Fig 14).

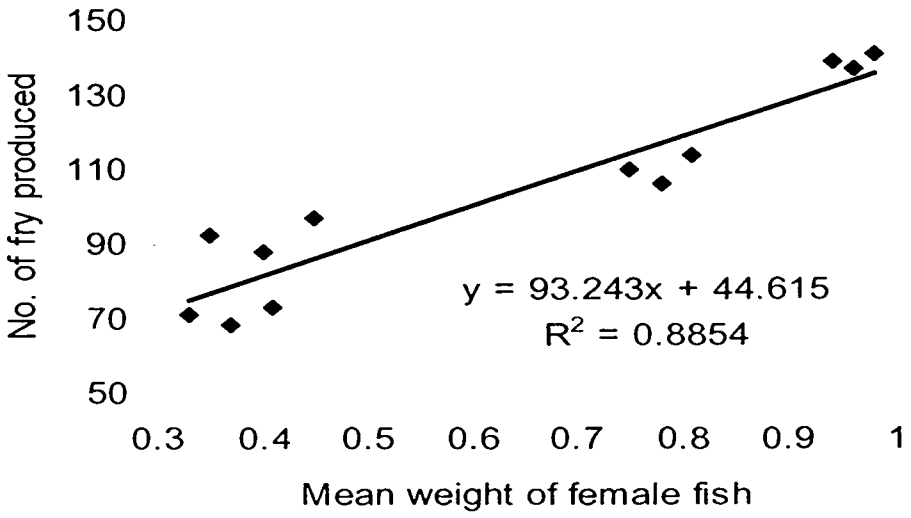


Figure 14. Relationship between mean fry production and final weight of female guppy ($p<0.05$)

4.2.6 Studies on fatty acid composition of diets and their effects on fatty acid profile of muscle, eggs and fry

4.2.6.1 Fatty acid profile of experimental diets

The fatty acid profiles of the experimental diets are presented in Table 26. 28 fatty acids were identified in the diets. All diets contain lower chain 6:0, 8:0, 10:0 and 12:0 levels. Diet-CO had significantly higher amount of SAFA than other three diets. The dominant fatty acids in the all diets were 16:0, 18:0 18:2 n-6, 18:2 n-6. Diet-CO had significantly higher amount of 12:0, 14:0 and 16:0. Diet-CO contained 20:2 n-9 and 20:3 n-9 fatty acids while those fatty acids were not reported in Diet-SO, Diet-LO and Diet-FO. Diet-SO and Diet-LO contained significantly higher amount of 18:2 n-6 and 18:3 n-3 respectively. Diet-FO had significantly higher amount of EPA and DHA than other three diets. Diet-CO, Diet-SO, Diet-LO did not contain any EPA and DHA levels. High levels of 18:1 n-9 were recorded in all diets.

Total PUFA was significantly ($p<0.05$) higher in Diet-LO than other three diets while total HUFA were significantly higher ($p<0.05$) in FO than other diets. Diet-FO contained significantly ($p<0.05$) higher amount of MUFA, HUFA, n-3 HUFA and n-6 HUFA than other diets. n-3 HUFA and n-6 HUFA were not recorded in Diet-CO, Diet-SO and Diet-LO. The n-3/n-6 was significantly higher in Diet-FO followed by Diet-LO, Diet-CO and Diet-SO. The chromatogram of Diet-FO is presented in Figure 15. The comparison of 18:2 n-6, 18:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3 levels in diets are presented in Figure 16.

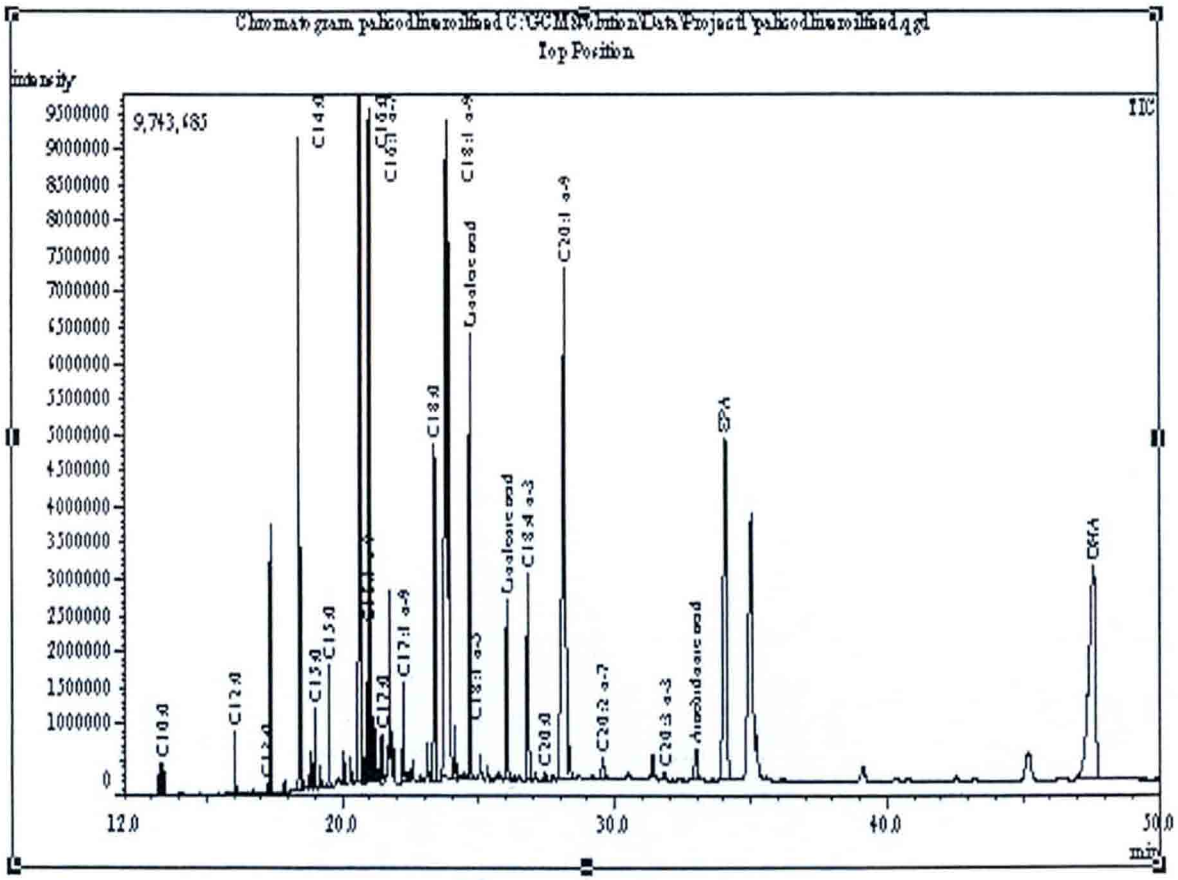


Figure 15. Chromatogram of Diet-FO

Table 26. Fatty acid profiles (% of individual fatty acids among total identified fatty acids) of the experimental diets

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
6:0	$1.46^{c}\pm0.01$	$0.14^{b}\pm0.01$	$0.06^{a}\pm0.00$	$0.12^{b}\pm0.01$
8:0	$8.66^{d}\pm0.01$	$0.44^{c}\pm0.01$	$0.08^{a}\pm0.00$	$0.1^{b}\pm0.00$
10:0	$7.54^{d}\pm0.01$	$0.43^{c}\pm0.03$	$0.1^{a}\pm0.00$	$0.17^{b}\pm0.01$
12:0	$24.21^{d}\pm0.06$	$2.63^{c}\pm0.02$	$0.19^{a}\pm0.01$	$0.31^{b}\pm0.01$
13:0	$0.13^{b}\pm0.01$	ND	ND	$0.04^{a}\pm0.00$
14:0	$15.03^{d}\pm0.20$	$1.91^{b}\pm0.05$	$0.76^{a}\pm0.04$	$5.44^{c}\pm0.01$
15:0	$0.22^{c}\pm0.01$	$0.18^{b}\pm0.00$	$0.13^{a}\pm0.01$	$1.15^{d}\pm0.01$
16:0	$11.27^{d}\pm0.31$	$10.45^{c}\pm0.03$	$9.04^{a}\pm0.05$	$9.73^{b}\pm0.04$
17:0	ND	$0.19^{a}\pm0.01$	$0.18^{a}\pm0.01$	$0.43^{b}\pm0.01$
18:0	$7.86^{b}\pm0.14$	$11.18^{c}\pm0.11$	$12.20^{d}\pm0.10$	$4.17^{a}\pm0.09$
20:0	$0.28^{b}\pm0.01$	$0.95^{d}\pm0.01$	$0.57^{c}\pm0.01$	$0.12^{a}\pm0.01$
22:0	ND	$2.13^{b}\pm0.01$	$0.62^{a}\pm0.01$	ND
SAFA	**$76.66^{d}\pm0.12$**	**$30.63^{c}\pm0.18$**	**$23.90^{b}\pm0.07$**	**$21.78^{a}\pm0.11$**
16:1 n-9	$0.11^{a}\pm0.01$	$0.53^{c}\pm0.01$	$0.44^{b}\pm0.00$	$1.12^{d}\pm0.02$
16:1 n-7	$0.47^{a}\pm0.05$	$0.63^{b}\pm0.01$	$0.61^{b}\pm0.01$	$7.82^{c}\pm0.01$
18:1 n-9	$13.48^{b}\pm0.05$	$24.93^{c}\pm0.29$	$20.71^{b}\pm0.01$	$19.83^{a}\pm0.12$
18:1 n-7	ND	ND	ND	0.44 ± 0.02
20:1 n-9	$0.28^{a}\pm0.01$	$0.70^{b}\pm0.01$	$0.62^{b}\pm0.01$	$11.85^{c}\pm0.06$
22:1 n-9	ND	ND	$1.61^{a}\pm0.02$	$8.07^{b}\pm0.05$
MUFA	**$14.43^{a}\pm0.01$**	**$26.79^{c}\pm0.29$**	**$23.99^{b}\pm0.01$**	**$49.13^{d}\pm0.10$**
18:2 n-6	7.62 ± 0.04	$41.87^{d}\pm0.15$	$16.14^{c}\pm0.04$	$5.07^{a}\pm0.05$
18:3 n-3	$0.54^{a}\pm0.01$	$0.53^{a}\pm0.01$	$35.84^{c}\pm0.16$	$2.09^{b}\pm0.06$
18:4 n-3	ND	ND	ND	2.37 ± 0.14
20:2 n-7	ND	ND	$0.06^{a}\pm0.01$	$0.31^{b}\pm0.00$
20:2 n-9	0.34 ± 0.01	ND	ND	ND
20:3 n-9	0.41 ± 0.02	ND	ND	ND
20:3 n-3	ND	$0.18^{b}\pm0.01$	$0.08^{a}\pm0.01$	$0.95^{c}\pm0.03$

20:4 n-6	ND	ND	ND	0.61±0.02
20:5 n-3	ND	ND	ND	7.92±0.04
22:6 n-3	ND	ND	ND	9.77±0.05
PUFA	**8.91^{a}±0.01**	**42.58^{c}±0.15**	**52.11^{d}±0.13**	**29.09^{b}±0.11**
HUFA	ND	ND	ND	18.30±0.04
n-3 PUFA	0.54^{a}±0.01	0.71^{a}±0.01	35.91^{c}±0.16	23.10^{b}±0.04
n-3 HUFA	ND	ND	ND	17.69±0.05
n-6 PUFA	7.62^{b}±0.01	41.87^{c}±0.15	16.14^{d}±0.04	5.68^{a}±0.07
n-6 HUFA	ND	ND	ND	0.61±0.02
n-3/n-6 PUFA	0.07^{b}±0.00	0.02^{a}±0.00	2.22^{c}±0.02	4.07^{d}±0.04

ND=Not Detected
Values within a row with different superscript letters are significantly different ($p<0.05$)

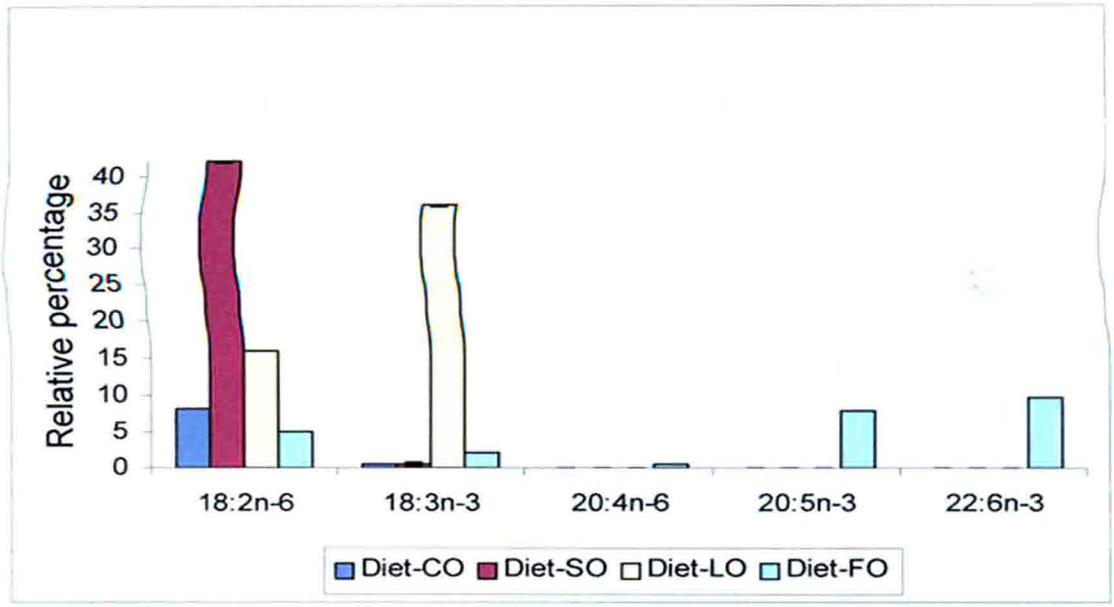


Figure 16. Comparison of some important PUFA levels in diets

4.2.6.2 Fatty acid profile of initial fry and muscle of fish after maturation

The fatty acid profiles of the initial fish fry and after maturation of the fish fed experimental diets are presented in Table 27. Thirty one fatty acids were identified in the muscle of the fish fed Die-SO, Diet-LO and Diet-FO while 32 fatty acids were identified in the muscle of guppy fed Diet-CO. The guppy fry used in this experiment contained 36.50% SAFA, 25.25% MUFA, 38.25% PUFA and 19.12% HUFA. The 18:2 n-6, 18:3 n-3, AA, EPA and DHA levels were 17.48%, 0.26%, 5.90%, 0.63% and 9.89% respectively. Initial fish also contained 12.65% n-3 PUFA and 24.37% n-6 PUFA levels.

After feeding experimental diets through the study period, the fish fed Diet-CO contained significantly higher amounts of 12:0, 14:0, 16:0 and SAFA levels. The fish fed Diet-SO contained significantly higher amount of 18:2 n-6, 18:3 n-6, 20:3 n-6, 20:4 n-6, 22:4 n-6, 22:5 n-6, n-6 PUFA and n-6 HUFA than the fish fed other three diets. The fish fed Diet-LO had significantly higher amount of 18:1n-7 and 18:3 n-3 levels than the fish fed other three diets. The muscle of guppy fed Diet-CO contained significantly higher level of 20:2 n-9 than the fish fed other three diets and 20:3 n-9 fatty acid was reported only in the fish fed Diet-CO while those fatty acid was not reported in the fish fed Diet-SO, Diet-LO and Diet-FO. The fish fed Diet-FO contained significantly higher amount of 16:1 n-7, 20:1 n-9, 22:1 n-9, MUFA, 20:3 n-3, EPA, DHA, HUFA, n-3 HUFA and n-3/n-6 than the fish fed other three diets. The chromatogram of muscle fatty acid composition of guppy fed Diet-FO is given in Figure 17.

Comparison of 18:2 n-6, 18:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3 levels in muscle after maturation are presented in Figure 18.

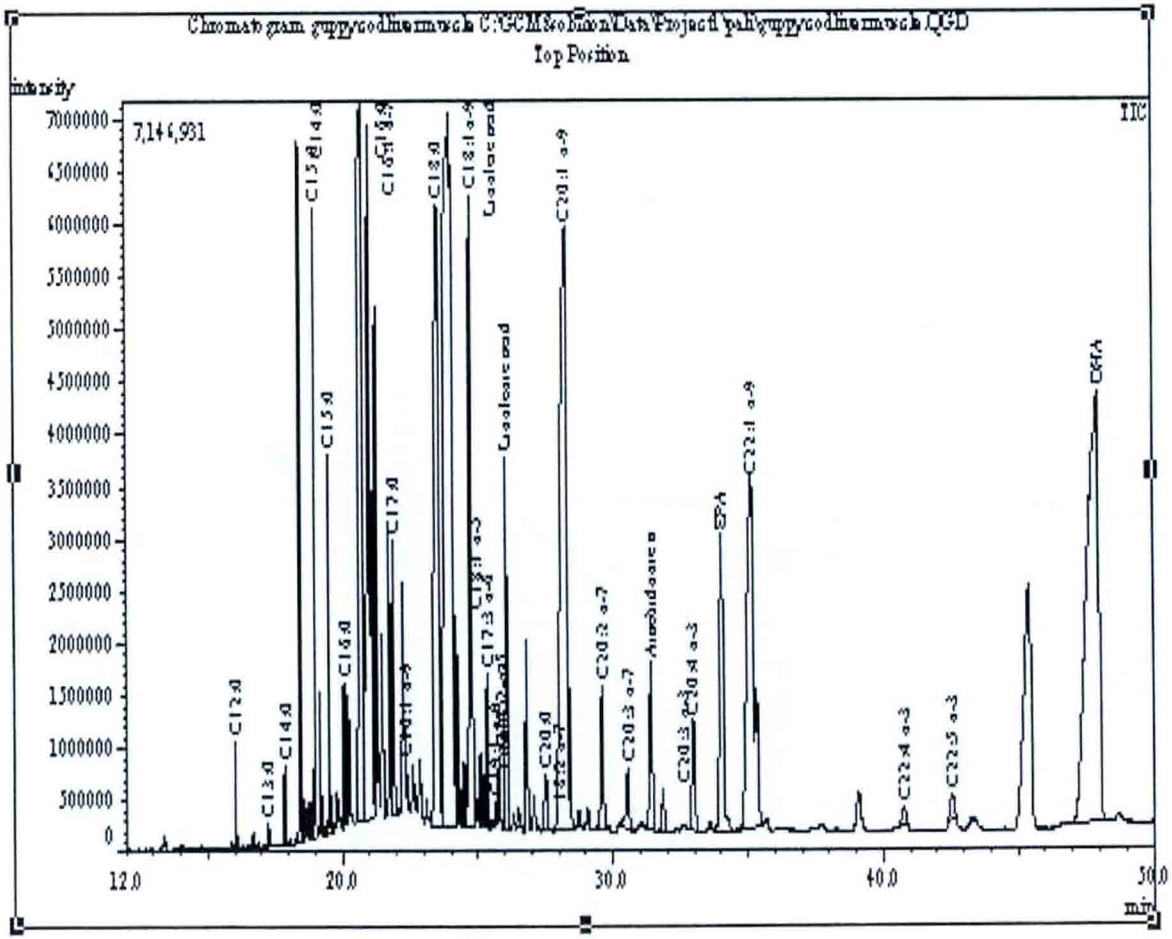


Figure 17. Chromatogram of muscle fatty acid composition of guppy fed Diet-FO

Table 27. Fatty acid profile (% of individual fatty acids among total identified fatty acid) of initial fry and after maturation

Fatty acid	Initial	Diet-CO	Diet-SO	Diet-LO	Diet-FO
10:0	$0.06^{a}\pm0.01$	$0.78^{b}\pm0.10$	$0.07^{a}\pm0.02$	$0.04^{a}\pm0.01$	$0.06^{a}\pm0.01$
12:0	$0.52^{a}\pm0.10$	$7.00^{b}\pm0.30$	$0.61^{a}\pm0.04$	$0.46^{a}\pm0.34$	$0.26^{a}\pm0.02$
14:0	$1.49^{a}\pm0.30$	$9.75^{d}\pm0.58$	$5.59^{c}\pm0.62$	$3.07^{b}\pm0.15$	$3.62^{b}\pm0.24$
15:0	$0.62^{a}\pm0.03$	$2.89^{c}\pm0.33$	$0.68^{a}\pm0.05$	$1.94^{b}\pm0.16$	$2.75^{c}\pm0.13$
16:0	$18.18^{d}\pm0.46$	$14.91^{c}\pm0.77$	$10.87^{a,b}\pm0.6$	$11.73^{b}\pm0.68$	$8.81^{a}\pm0.24$
17:0	$1.74^{a,b}\pm0.47$	$3.0^{c}\pm0.22$	$1.12^{a}\pm0.20$	$2.45^{b,c}\pm0.21$	$1.37^{a}\pm0.19$
18:0	$13.33^{c}\pm0.70$	$11.02^{b}\pm0.40$	$10.93^{b}\pm0.50$	$9.40^{b}\pm0.28$	$7.20^{a}\pm0.22$
20:0	$0.52^{a}\pm0.11$	$0.56^{a}\pm0.09$	$0.68^{a}\pm0.04$	$0.43^{a}\pm0.04$	$0.51^{a}\pm0.04$
22:0	$0.04^{a}\pm0.01$	$0.06^{a}\pm0.01$	$0.63^{c}\pm0.02$	$0.06^{a}\pm0.01$	$0.17^{b}\pm0.05$
SAFA	**$36.50^{c}\pm0.78$**	**$49.97^{d}\pm1.92$**	**$31.18^{b}\pm2.19$**	**$29.58^{a,b}\pm0.63$**	**$24.71^{a}\pm0.44$**
16:1 n-9	$0.17^{a}\pm0.06$	$2.97^{c}\pm0.11$	$1.10^{b}\pm0.10$	$1.15^{b}\pm0.05$	$0.25^{a}\pm0.05$
16:1 n-7	$2.19^{a}\pm0.21$	$4.79^{b}\pm0.58$	$1.82^{a}\pm0.03$	$4.07^{b}\pm0.38$	$7.42^{c}\pm0.26$
18:1 n-9	$21.98^{c}\pm0.34$	$18.40^{b,c}\pm0.75$	$18.71^{b}\pm0.97$	$17.53^{a,b}\pm0.17$	$16.03^{a}\pm0.85$
18:1 n-7	$0.32^{a}\pm0.02$	$0.68^{a}\pm0.04$	$0.28^{a}\pm0.04$	$3.59^{b}\pm0.28$	$0.69^{a}\pm0.02$
20:1 n-9	$0.45^{a}\pm0.05$	$2.62^{b}\pm0.28\pm$	$1.71^{b}\pm0.26$	$2.56^{b}\pm0.12$	$10.10^{c}\pm0.46$
22:1 n-9	$0.14^{a}\pm0.02$	$0.66^{a}\pm0.06$	$0.16^{a}\pm0.03$	$0.78^{a}\pm0.06$	$7.64^{b}\pm0.48$
MUFA	**$25.25^{a}\pm0.54$**	**$30.12^{b}\pm1.13$**	**$23.78^{a}\pm0.78$**	**$29.68^{b}\pm0.14$**	**$42.13^{c}\pm1.07$**
18:2 n-9	$0.26^{a}\pm0.03$	$1.25^{b}\pm0.21$	$0.48^{a}\pm0.07$	$0.26^{a}\pm0.05$	$0.25^{a}\pm0.04$
18:2 n-6	$17.48^{c}\pm0.65$	$4.12^{a}\pm0.45$	$20.28^{d}\pm0.85$	$11.11^{b}\pm0.75$	$5.78^{a}\pm0.20$
18:3 n-6	$0.34^{a}\pm0.04$	$0.79^{a,b}\pm0.07$	$4.94^{c}\pm0.58$	$1.60^{b}\pm0.22$	$0.60^{a,b}\pm0.03$
18:3 n-3	$0.26^{a}\pm0.06$	$0.21^{a}\pm0.03$	$0.43^{a}\pm0.02$	$10.85^{c}\pm0.21$	$1.58^{b}\pm0.40$
18:4 n-3	$0.22^{a}\pm0.03$	$0.55^{b}\pm0.07$	$0.41^{a,b}\pm0.03$	$0.50^{b}\pm0.08$	$0.24^{a}\pm0.03$
20:2 n-9	$0.52^{a}\pm0.05$	$1.46^{b}\pm0.21$	$0.27^{a}\pm0.02$	$0.18^{a}\pm0.02$	$0.26^{a}\pm0.02$
20:3 n-6	$0.43^{a,b}\pm0.05$	$0.36^{a}\pm0.06$	$2.47^{c}\pm0.39$	$0.67^{a,b}\pm0.05$	$1.12^{b}\pm0.12$
20:3 n-3	$0.24^{a}\pm0.01$	$0.48^{b}\pm0.03$	$0.30^{a}\pm0.07$	$0.28^{a}\pm0.04$	$0.92^{c}\pm0.03$
20:2 n-7	$0.45^{a}\pm0.03$	$0.97^{b}\pm0.05$	$2.22^{c}\pm0.11$	$0.58^{a}\pm0.01$	$0.56^{a}\pm0.04$
20:3 n-9	ND	1.11±0.02	ND	ND	ND

20:4 n-6	$5.90^{c}\pm0.40$	$3.13^{b}\pm0.34$	$4.94^{c}\pm0.38$	$2.45^{a,b}\pm0.34$	$1.48^{a}\pm0.16$
20:4 n-3	$0.17^{a}\pm0.03$	$0.16^{a}\pm0.03$	$0.11^{a}\pm0.02$	$0.81^{b}\pm0.17$	$0.36^{a}\pm0.03$
20:5 n-3	$0.63^{b}\pm0.04$	$0.16^{a}\pm0.04$	$0.03^{a}\pm0.01$	$0.80^{b}\pm0.12$	$4.67^{c}\pm0.21$
22:4 n-6	$0.05^{a}\pm0.01$	$0.25^{a}\pm0.04$	$1.76^{b}\pm0.20$	$0.37^{a}\pm0.05$	$0.17^{a}\pm0.02$
22:5 n-6	$0.17^{a}\pm0.05$	$0.06^{a}\pm0.01$	$5.00^{b}\pm0.38$	$0.25^{a}\pm0.03$	$0.04^{a}\pm0.01$
22:5 n-3	$1.24^{b}\pm0.19$	$1.47^{b}\pm0.26$	$0.15^{a}\pm0.03$	$0.97^{b}\pm0.08$	$0.35^{a}\pm0.04$
22:6 n-3	$9.89^{d}\pm0.68$	$2.99^{b}\pm0.63$	$1.25^{a}\pm0.22$	$7.55^{c}\pm0.42$	$14.81^{e}\pm0.17$
PUFA	**$38.25^{c}\pm0.24$**	**$19.91^{a}\pm0.49$**	**$45.04^{d}\pm1.42$**	**$40.74^{c}\pm0.49$**	**$33.16^{b}\pm0.63$**
HUFA	**$18.05^{c}\pm1.14$**	**$8.22^{a}\pm0.55$**	**$13.24^{b}\pm0.19$**	**$13.20^{b}\pm0.21$**	**$21.85^{d}\pm0.67$**
n-3 PUFA	$12.65^{c}\pm0.68$	$6.02^{b}\pm0.78$	$2.68^{a}\pm0.34$	$21.76^{d}\pm0.18$	$22.90^{d}\pm0.08$
n-3 HUFA	$11.93^{d}\pm0.79$	$4.78^{b}\pm0.91$	$1.54^{a}\pm0.38$	$10.13^{c}\pm0.47$	$20.16^{e}\pm0.48$
n-6 PUFA	$24.37^{c}\pm0.32$	$8.71^{a}\pm0.79$	$38.88^{d}\pm2.10$	$16.45^{b}\pm0.78$	$9.19^{a}\pm0.45$
n-6 HUFA	$6.12^{c}\pm0.35$	$3.44^{b}\pm0.36$	$11.70^{d}\pm0.56$	$3.07^{b}\pm0.26$	$1.69^{a}\pm0.19$
n-3/n-6 PUFA	$0.52^{b}\pm0.04$	$0.69^{b}\pm0.15$	$0.07^{a}\pm0.01$	$1.32^{c}\pm0.07$	$2.40^{d}\pm0.13$

ND=Not Detected
Values within a row with different superscript letters are significantly different ($p<0.05$)

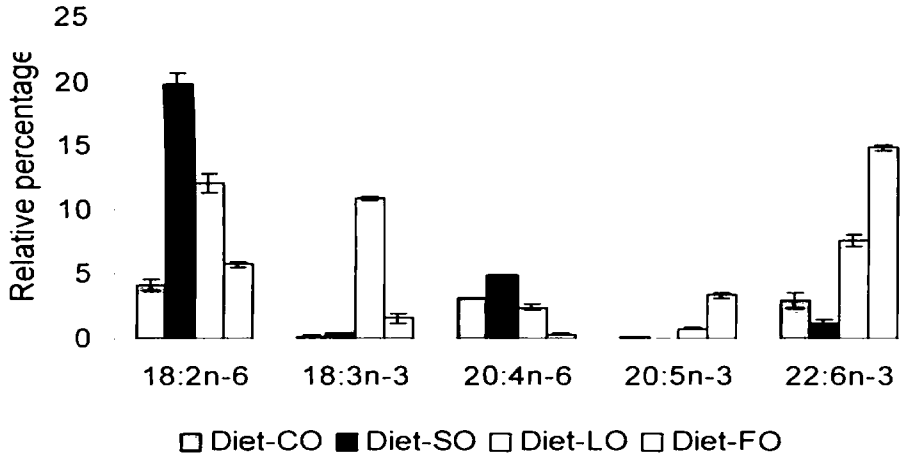


Figure 18. Comparison of some important PUFA in muscle after maturation

4.2.6.3. Fatty acid profile of eggs

The lipid content in the eggs of the fish fed Diet-CO, Diet-SO, Diet-LO and Diet-FO were 7.2%, 7.35%, 7.25% and 7.37% respectively in wet weight basis. There were no significantly different levels of lipids in the eggs of guppy fed different diets. The fatty acid profiles of the eggs of the fish fed experimental diets are presented in Table 28. 31 fatty acids were identified in the eggs of guppy fed Diet-SO, Diet-LO and Diet-FO while 33 fatty acids were identified in the guppy fed Diet-CO. Among them the eggs of the fish fed Diet-CO contained significantly highest amount of 12:0, 14:0 and SAFA while there was no significant difference among the SAFA levels in the eggs of the fish fed other three diets. In addition, eggs of the fish fed Diet-CO contained significantly higher amount of 18:1 n-9, and 20:2 n-9 than the fish fed other three diets. 20:3 n-9 fatty acid was reported only in the eggs of fish fed Diet-CO. The fish fed Diet-FO contained significantly the highest amount of MUFA in the eggs. Significantly lowest MUFA level was observed in the eggs of the fish fed Diet-SO and the fish fed Diet-CO and Diet-LO had the similar amounts of MUFA levels. The eggs of the fish fed Diet-FO had significantly higher amount of EPA, 22:5 n-3 and DHA levels than the fish fed other three diets. Significantly the highest level of PUFA was noticed in the eggs of the fish fed Diet-SO followed by Diet-LO, Diet-CO and Diet-FO fed fishes.

The eggs of the fish fed Diet-FO and Diet-CO contained significantly highest levels of HUFA. n-3 PUFA level was highest in the eggs of the fish fed Diet-FO and Diet-LO and n-6 PUFA levels were highest in the fish fed Diet-SO. Diet-CO and Diet-LO fed fish contained the equal amounts. The highest amount of n-3 HUFA contained in the eggs of the fish fed Diet-FO followed by Diet-CO, Diet-LO and Diet-SO fed fish. The highest amounts of n-6 HUFA levels were present in the fish fed Diet-SO followed by Diet-CO, Diet-FO and Diet-LO fed fish. The chromatogram of eggs of guppy reared on Diet-FO is presented in Figure 19. The n-3/n-6 ratios in the eggs of the fish fed Diet-FO diet contained significantly highest amount followed by the fish fed Diet-LO, Diet-CO and Diet-SO fed fish. Comparisons of some important PUFA in eggs are presented in Figure 20.

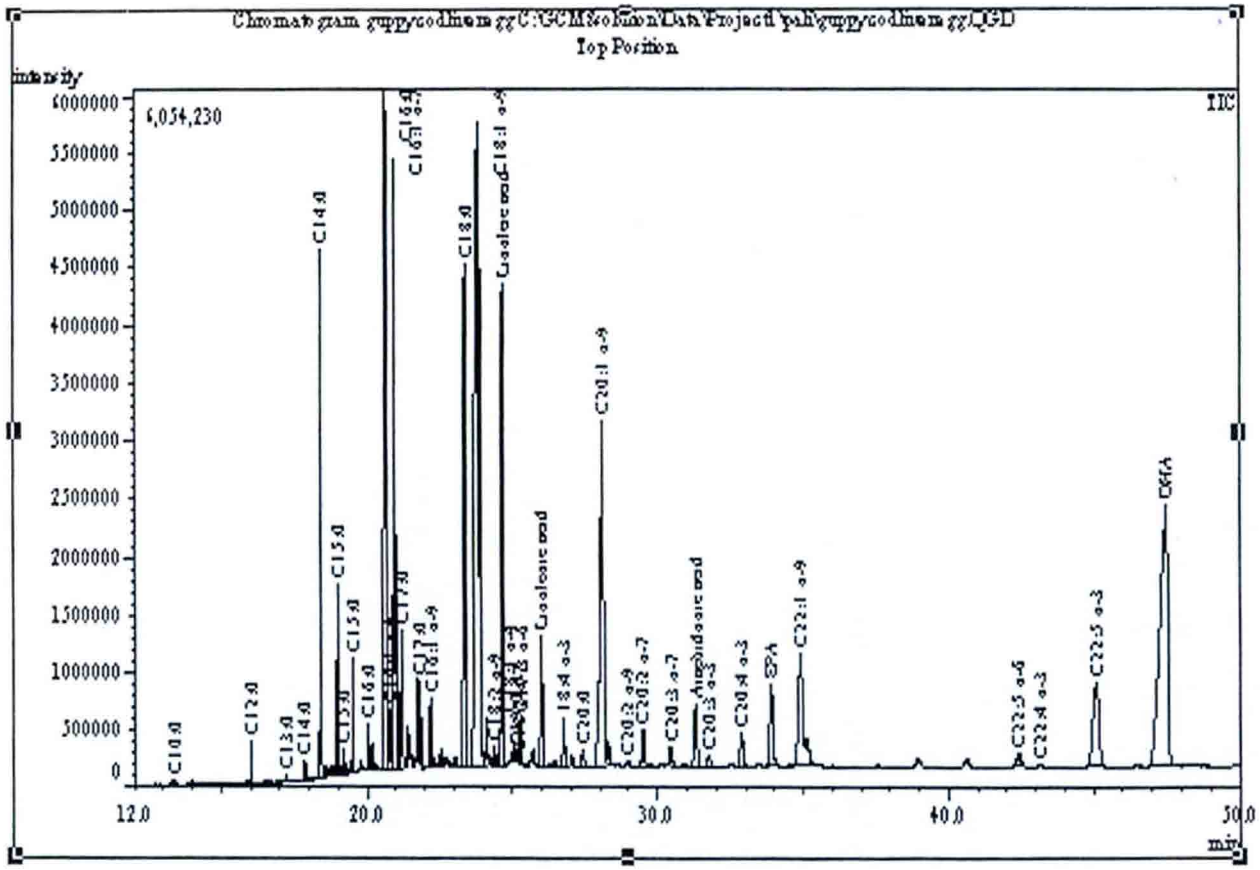


Figure 19. Fatty acid profile of eggs of guppy reared on Diet-FO

Table 28. Fatty acid profiles (% of individual fatty acids among total identified fatty acids) of eggs

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
10:0	$0.69^{b}\pm0.04$	$0.03^{a}\pm0.01$	$0.07^{a}\pm0.01$	$0.02^{a}\pm0.01$
12:0	$6.09^{c}\pm0.12$	$0.58^{a}\pm0.05$	$1.39^{b}\pm0.17$	$0.28^{a}\pm0.04$
14:0	$8.00^{c}\pm0.24$	$2.46^{a}\pm0.34$	$3.04^{a,b}\pm0.17$	$3.60^{b}\pm0.34$
15:0	$2.33^{b}\pm0.33$	$1.40^{a}\pm0.18$	$1.55^{a}\pm0.10$	$2.09^{a,b}\pm0.03$
16:0	$10.82^{a}\pm0.21$	$9.36^{a}\pm0.47$	$11.44^{a}\pm0.96$	$10.47^{a}\pm0.14$
17:0	$2.77^{c}\pm0.22$	$0.87^{a}\pm0.11$	$1.92^{b}\pm0.07$	$1.81^{b}\pm0.05$
18:0	$11.19^{b}\pm0.63$	$11.17^{b}\pm0.75$	$8.47^{a}\pm0.51$	$7.08^{a}\pm0.49$
20:0	$0.42^{b}\pm0.05$	$0.32^{a,b}\pm0.04$	$0.24^{a}\pm0.04$	$0.26^{a}\pm0.02$
22:0	$0.15^{a,b}\pm0.04$	$0.26^{b}\pm0.04$	$0.13^{a}\pm0.01$	$0.15^{a,b}\pm0.03$
SAFA	**$42.46^{b}\pm1.65$**	**$26.45^{a}\pm0.78$**	**$28.25^{a}\pm0.85$**	**$25.76^{a}\pm0.51$**
16:1 n-9	$1.68^{b}\pm0.21$	$0.27^{a}\pm0.01$	$0.21^{a}\pm0.04$	$0.54^{a}\pm0.03$
16:1 n-7	$5.71^{c}\pm0.27$	$2.38^{a}\pm0.33$	$3.67^{b}\pm0.19$	$7.23^{d}\pm0.34$
18:1 n-9	$16.02^{a}\pm0.72$	$18.99^{b}\pm0.23$	$20.08^{b,c}\pm0.37$	$21.04^{c}\pm0.54$
18:1 n-7	$2.53^{b,c}\pm0.30$	$2.14^{b}\pm0.12$	$3.06^{c}\pm0.16$	$0.53^{a}\pm0.05$
19:1 n-9	0.05±0.01	ND	ND	ND
20:1 n-9	$2.64^{b}\pm0.35$	$0.73^{a}\pm0.05$	$2.35^{b}\pm0.21$	$8.00^{c}\pm0.02$
22:1 n-9	$0.34^{a}\pm0.04$	$0.07^{a}\pm0.01$	$0.56^{a}\pm0.02$	$3.44^{b}\pm0.32$
MUFA	**$28.97^{b}\pm0.16$**	**$24.58^{a}\pm0.26$**	**$29.93^{b}\pm0.98$**	**$40.78^{c}\pm1.14$**
18:2 n-9	$1.55^{b}\pm0.26$	$0.42^{a}\pm0.06$	$0.58^{a}\pm0.06$	$0.23^{a}\pm0.01$
18:2 n-6	$3.63^{a}\pm0.13$	$23.25^{d}\pm0.67$	$13.22^{c}\pm0.96$	$5.99^{b}\pm0.05$
18:3 n-6	$0.55^{a}\pm0.43$	$4.79^{b}\pm0.24$	$1.28^{a}\pm0.16$	$0.58^{a}\pm0.06$
18:3 n-3	$0.13^{a}\pm0.02$	$0.52^{a}\pm0.02$	$14.13^{c}\pm0.44$	$1.71^{b}\pm0.05$
18:4 n-3	$0.57^{a}\pm0.06$	$0.28^{a}\pm0.01$	$1.25^{b}\pm0.21$	$1.00^{b}\pm0.02$
20:2 n-9	$1.29^{b}\pm0.08$	$0.25^{a}\pm0.04$	$0.24^{a}\pm0.03$	$0.08^{a}\pm0.02$.
20:3 n-9	1.34±0.02	ND	ND	ND
20:2 n-7	$0.43^{a}\pm0.02$	$1.77^{b}\pm0.21$	$0.45^{a}\pm0.04$	$0.66^{a}\pm0.03$
20:3 n-6	$1.18^{b}\pm0.16$	$2.35^{c}\pm0.23$	$0.53^{a}\pm0.06$	$0.36^{a}\pm0.04$
20:3 n-3	$0.13^{a}\pm0.01$	$0.05^{a}\pm0.02$	$0.89^{c}\pm0.03$	$0.27^{b}\pm0.06$

20:4 n-6	4.17[b]±0.21	5.39[c]±0.15	1.50[a]±0.08	1.35a±0.19
20:4 n-3	0.41a±0.03	0.25a±0.02	0.74b±0.11	0.71[b]±0.05
20:5 n-3	0.05[a]±0.01	0.03[a]±0.1	0.70[a]±0.05	2.30[b]±0.55
22:4 n-6	1.40[b]±0.08	1.42[b]±0.14	0.21[a]±0.02	0.35[a]±0.03
22:5 n-6	3.12[c]±0.12	6.75[d]±0.58	0.08[a]±0.01	0.35[b]±0.02
22:5 n-3	1.13[c]±0.06	0.15[a]±0.02	0.47[b]±0.05	3.59[d]±0.37
22:6 n-3	7.49[c]±0.65	1.27[a]±0.06	5.55[b]±0.17	13.93[d]±0.28
PUFA	**28.57[a]±0.47**	**48.97[d]±0.50**	**41.82[c]±0.11**	**33.46[b]±1.7**
HUFA	**17.77[c]±0.27**	**15.23[b]±0.87**	**9.25[a]±0.29**	**22.58[c]±1.48**
n-3 PUFA	9.91[b]±0.76	2.55[a]±0.62	23.73[c]±0.99	23.51[c]±1.37
n-3 HUFA	9.08[c]±0.68	1.70[a]±0.60	7.46[b]±0.38	20.53[d]±1.24
n-6 PUFA	14.05[b]±0.87	43.95[c]±0.41	16.82[b]±1.15	8.98[a]±0.29
n-6 HUFA	8.69[b]±0.41	13.56[c]±0.27	1.79[a]±0.09	2.05[a]±0.24
n-3/n-6 PUFA	0.71[b]±0.09	0.06[a]±0.01	1.42[c]±0.16	2.62[d]±0.07

ND=Not Detected

Values within a row with different superscript letters are significantly different ($p<0.05$)

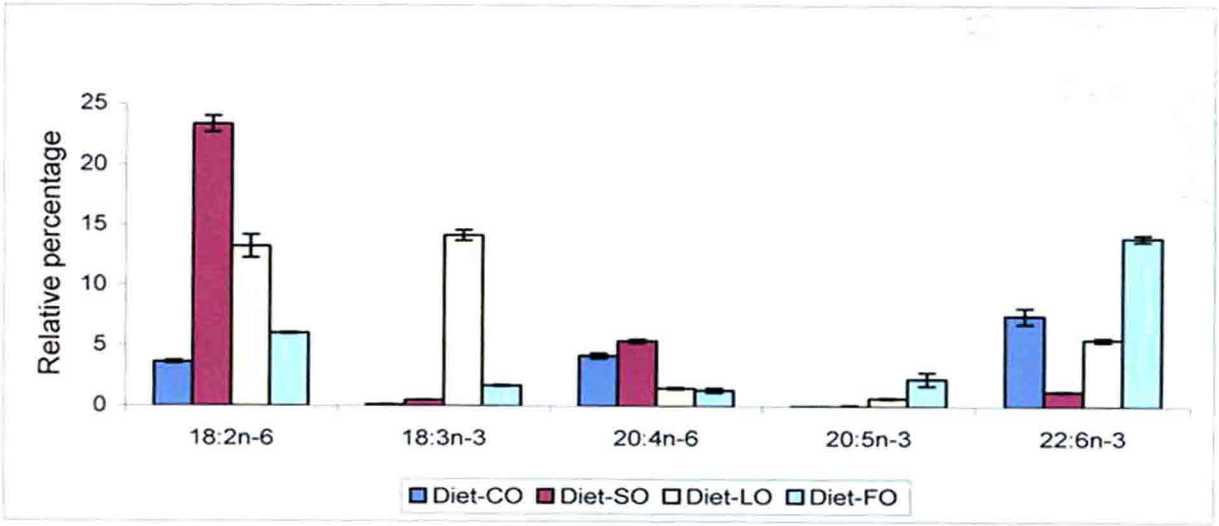


Figure 20. Comparison of some important PUFA in eggs

4.2.6.4 Changes in fatty acid composition during larval development

The fatty acid profile of fish fry is presented in Table 29. The fry of the fish fed Diet-CO contained significantly higher amount of 12:0, 14:0, SAFA, than the fish fed other three diets while the fish fed SO diet had significantly higher amount of fry fatty acid levels of 18:2 n-6, 20:3 n-6, 22:4 n-6, 22:5 n-6, n-6 PUFA and n-6 HUFA. The fry of the fish fed Diet-LO diet contained significantly higher levels of 18:3 n-3, 18:4 n-3 and n-3 PUFA than the fish fed other three diets while significantly higher levels of 16:1 n-7, 20:1 n-9, 22:1 n-9, MUFA, EPA, DHA, HUFA, n-3HUFA and n-3/n-6 PUFA were observed in the fry of the fish fed Diet-FO than other three diets. In addition, fry of the fish fed Diet-CO contained significantly higher amount of 20:2 n-9 than the fish fed other three diets. 20:3 n-9 fatty acid has been reported only in the fry of fish fed Diet-CO. The chromatogram of the fatty acid profile of fry collected from guppy brooders fed Diet-FO is presented in Figure 21. Comparison of some important PUFA levels in fry collected from breeding tanks are presented in Figure 22.

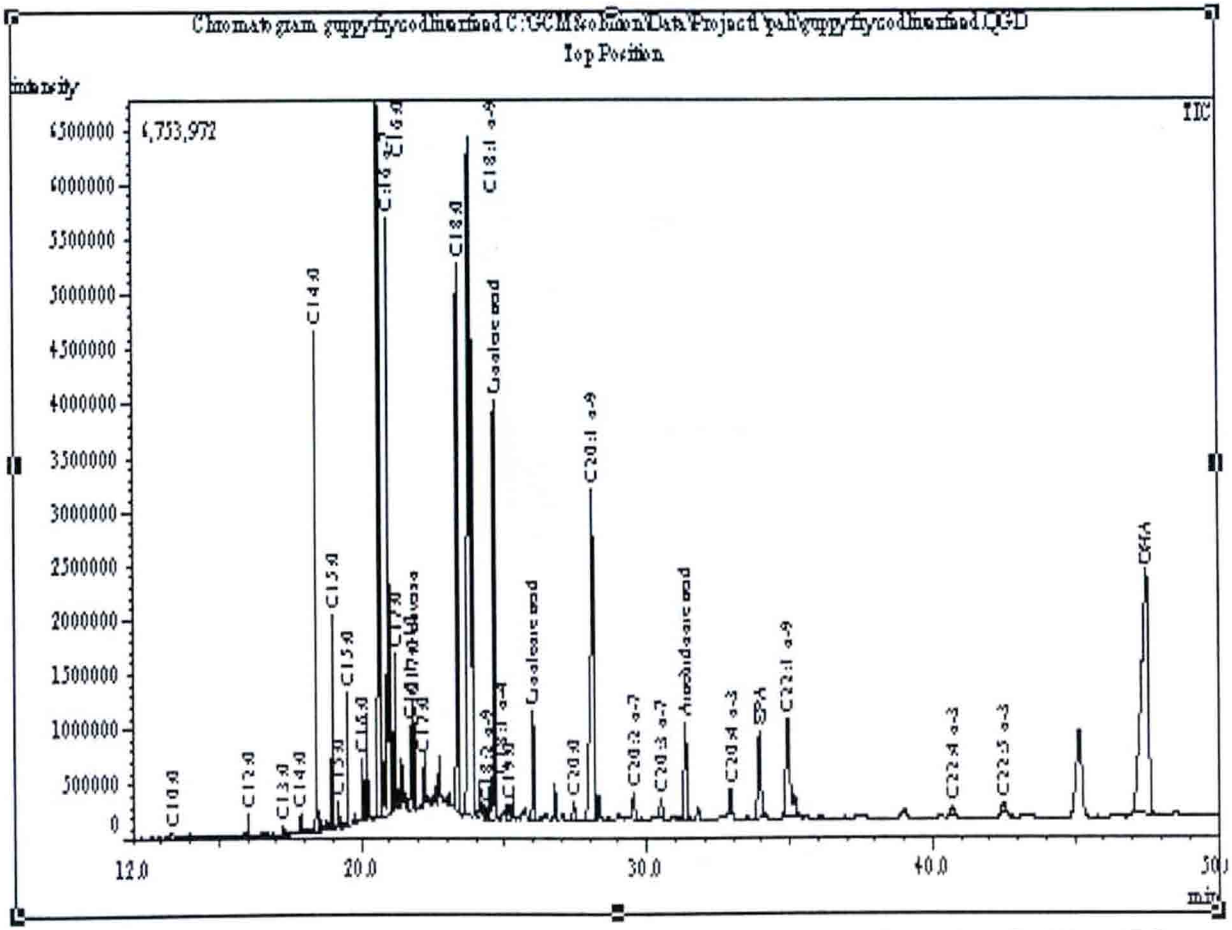


Figure 21. Fatty acid profile of fry collected from guppy brooders fed Diet-FO

Table 29. Fatty acid profiles (% of individual fatty acids among total identified fatty acids) of fish fry

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
10:0	$0.50^{c}\pm0.02$	$0.27^{b}\pm0.06$	$0.26^{b}\pm0.05$	$0.02^{a}\pm0.01$
12:0	$6.09^{b}\pm0.51$	$0.59^{a}\pm0.06$	$0.17^{a}\pm0.02$	$0.15^{a}\pm0.02$
14:0	$8.68^{c}\pm0.36$	$1.83^{a}\pm0.13$	$1.55^{a}\pm0.10$	$3.96^{b}\pm0.28$
15:0	$2.36^{a,b}\pm0.26$	$1.81^{a}\pm0.09$	$1.84^{a}\pm0.13$	$2.58^{b}\pm0.10$
16:0	$10.66^{b}\pm0.60$	$9.01^{a,b}\pm0.20$	$9.82^{b}\pm0.42$	$7.77^{a}\pm0.45$
17:0	$2.33^{b}\pm0.21$	$2.06^{b}\pm0.15$	$4.05^{c}\pm0.06$	$1.41^{a}\pm0.16$
18:0	$12.65^{c}\pm0.44$	$10.32^{b}\pm0.17$	$10.84^{b,c}\pm0.48$	$8.13^{a}\pm0.46$
20:0	$0.44^{b}\pm0.03$	$0.51^{b}\pm0.02$	$0.45^{b}\pm0.05$	$0.32^{a}\pm0.01$
22:0	$0.05^{a}\pm0.01$	$0.40^{c}\pm0.03$	$0.24^{b}\pm0.03$	$0.31^{b}\pm0.01$
SAFA	**$43.76^{c}\pm1.17$**	**$26.80^{a}\pm0.55$**	**$29.22^{b}\pm0.26$**	**$24.65^{a}\pm0.67$**
16:1 n-9	$0.45^{a}\pm0.04$	$1.70^{b}\pm0.49$	$0.22^{a}\pm0.01$	$0.83^{a,b}\pm0.05$
16:1 n-7	$5.58^{b}\pm0.38$	$2.43^{a}\pm0.31$	$2.62^{a}\pm0.03$	$7.39^{c}\pm0.41$
18:1 n-9	$15.50^{a}\pm0.49$	$21.18^{b}\pm0.60$	$17.61^{a}\pm1.29$	$21.96^{b}\pm0.65$
18:1 n-7	$3.27^{b}\pm0.15$	$0.14^{a}\pm0.02$	$2.71^{b}\pm0.25$	$0.42^{a}\pm0.01$
20:1 n-9	$1.61^{a}\pm0.35$	$0.75^{a}\pm0.05$	$1.54^{a}\pm0.11$	$7.95^{b}\pm0.36$
22:1 n-9	$0.47^{a}\pm0.02$	$0.07^{a}\pm0.02$	$0.31^{a}\pm0.02$	$3.23^{b}\pm0.25$
MUFA	**$26.88^{a}\pm0.35$**	**$26.27^{a}\pm1.39$**	**$25.01^{a}\pm0.88$**	**$41.78^{b}\pm1.71$**
18:2 n-9	$0.72^{b}\pm0.06$	$0.25^{a}\pm0.05$	$0.63^{b}\pm0.01$	$0.18^{a}\pm0.06$
18:2 n-6	$4.75^{a}\pm0.46$	$23.07^{c}\pm0.51$	$11.21^{b}\pm1.21$	$5.01^{a}\pm0.03$
18:3 n-6	$0.57^{a}\pm0.01$	$3.75^{b}\pm0.75$	$0.89^{a}\pm0.03$	$0.46^{a}\pm0.02$
18:3 n-3	$0.29^{a}\pm0.02$	$0.42^{a}\pm0.04$	$15.92^{c}\pm0.40$	$1.54^{b}\pm0.05$
18:4 n-3	$0.30^{a}\pm0.02$	$0.49^{a}\pm0.03$	$2.48^{c}\pm0.26$	$1.07^{b}\pm0.08$
20:2 n-9	$0.47^{b}\pm0.05$	$0.14^{a}\pm0.03$	$0.13^{a}\pm0.02$	$0.18^{a}\pm0.06$
20:2 n-7	$0.59^{a}\pm0.05$	$1.55^{c}\pm0.02$	$0.89^{b}\pm0.06$	$0.54^{a}\pm0.05$
20:3 n-9	1.21±0.02	ND	ND	ND
20:3 n-6	$1.44^{b}\pm0.21$	$1.93^{c}\pm0.05$	$0.54^{a}\pm0.03$	$0.40^{a}\pm0.03$
20:3 n-3	$1.30^{a,b}\pm0.09$	$0.27^{a}\pm0.01$	$0.58^{a}\pm0.04$	$2.00^{b}\pm0.71$
20:4 n-6	$5.47^{b}\pm0.50$	$4.98^{b}\pm0.04$	$2.44^{a}\pm0.06$	$1.31^{a}\pm0.26$

20:4 n-3	$0.11^{a}\pm0.02$	$0.04^{a}\pm0.01$	$0.45^{c}\pm0.02$	$0.19^{b}\pm0.02$
20:5 n-3	$0.27^{a}\pm0.02$	$0.13^{a}\pm0.02$	$0.58^{a}\pm0.05$	$3.88^{b}\pm0.71$
22:4 n-6	$0.26^{b}\pm0.03$	$1.54^{c}\pm0.02$	$0.13^{a}\pm0.01$	$0.13^{a}\pm0.02$
22:5 n-6	$3.03^{c}\pm0.18$	$5.15^{d}\pm0.17$	$0.80^{b}\pm0.06$	$0.28^{a}\pm0.01$
22:5 n-3	$2.52^{b}\pm0.08$	$1.60^{a}\pm0.05$	$1.97^{a}\pm0.01$	$2.48^{b}\pm0.04$
22:6 n-3	$6.46^{b}\pm0.36$	$1.62^{a}\pm0.08$	$6.13^{b}\pm0.05$	$13.92^{c}\pm0.29$
PUFA	**$29.36^{a}\pm0.83$**	**$46.93^{b}\pm0.34$**	**$45.77^{b}\pm0.38$**	**$33.57^{a}\pm2.37$**
HUFA	**$18.12^{c}\pm0.12$**	**$14.93^{b}\pm0.51$**	**$12.50^{a}\pm0.05$**	**$22.19^{c}\pm1.35$**
n-3 PUFA	$11.25^{b}\pm0.77$	$4.57^{a}\pm0.10$	$28.11^{d}\pm0.79$	$25.08^{c}\pm1.9$
n-3 HUFA	$9.36^{c}\pm0.65$	$3.39^{a}\pm0.17$	$9.13^{b}\pm0.09$	$20.47^{d}\pm1.06$
n-6 PUFA	$15.52^{b}\pm0.11$	$40.42^{c}\pm0.35$	$16.01^{b}\pm1.25$	$7.59^{a}\pm0.31$
n-6 HUFA	$8.76^{b}\pm0.77$	$13.60^{c}\pm0.05$	$3.37^{a}\pm0.04$	$1.72^{a}\pm0.29$
n-3/n-6 PUFA	$0.72^{b}\pm0.06$	$0.11^{a}\pm0.01$	$1.76^{c}\pm0.17$	$3.30^{d}\pm0.12$

ND=Not Detected

Values within a row with different superscript letters are significantly different ($p<0.05$)

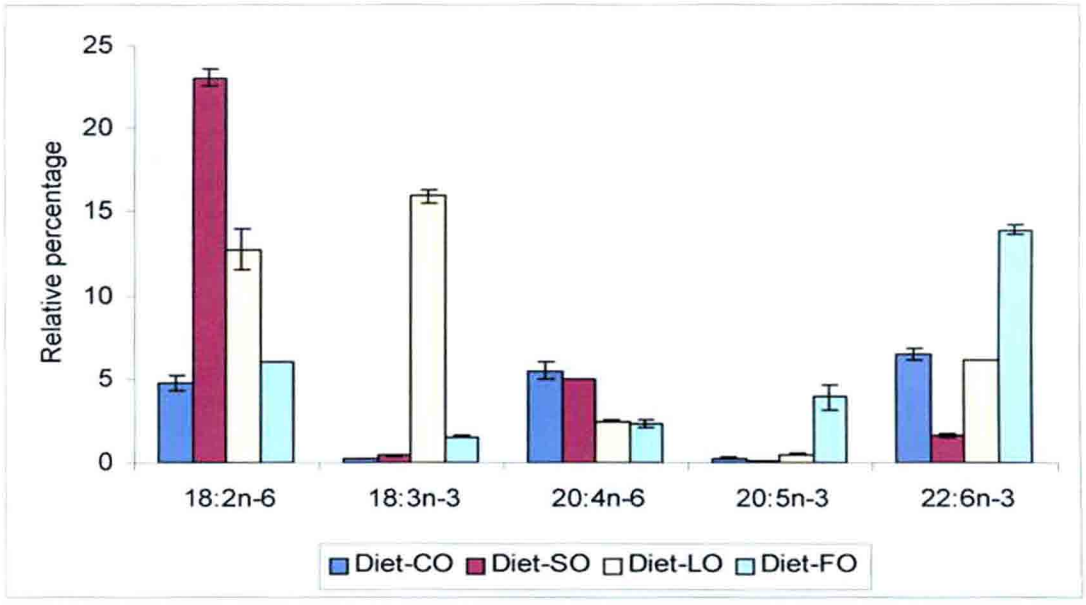


Figure 22. Comparison of some important PUFA levels in fry collected from breeding tanks

4.2.6.5 Comparison of fatty acids in different stages

Comparisons of fatty acid classes and important fatty acids in feeds and different stages of guppy are presented in Figures 23 to 27. SAFA levels were significantly higher ($p<0.05$) in all stages of guppy fed Diet-CO. MUFA levels were significantly higher ($p<0.05$) in all stages of the fish fed Diet-FO. Higher PUFA levels were observed in the all stages of the fish Diet-SO and Diet-LO (Figure 23). The HUFA levels were significantly higher ($p<0.05$) in all stages of the fish fed Diet-FO (Figure24). The n-3 HUFA levels were significantly higher ($p<0.05$) in all stages of the fish fed Diet-FO than the fish fed other three diets (Figure 25). n-3/n-6 ratios and the EPA and DHA levels were also significantly ($p<0.05$) higher in the fish fed Diet-FO than other three diets (Figure 26 and 27).

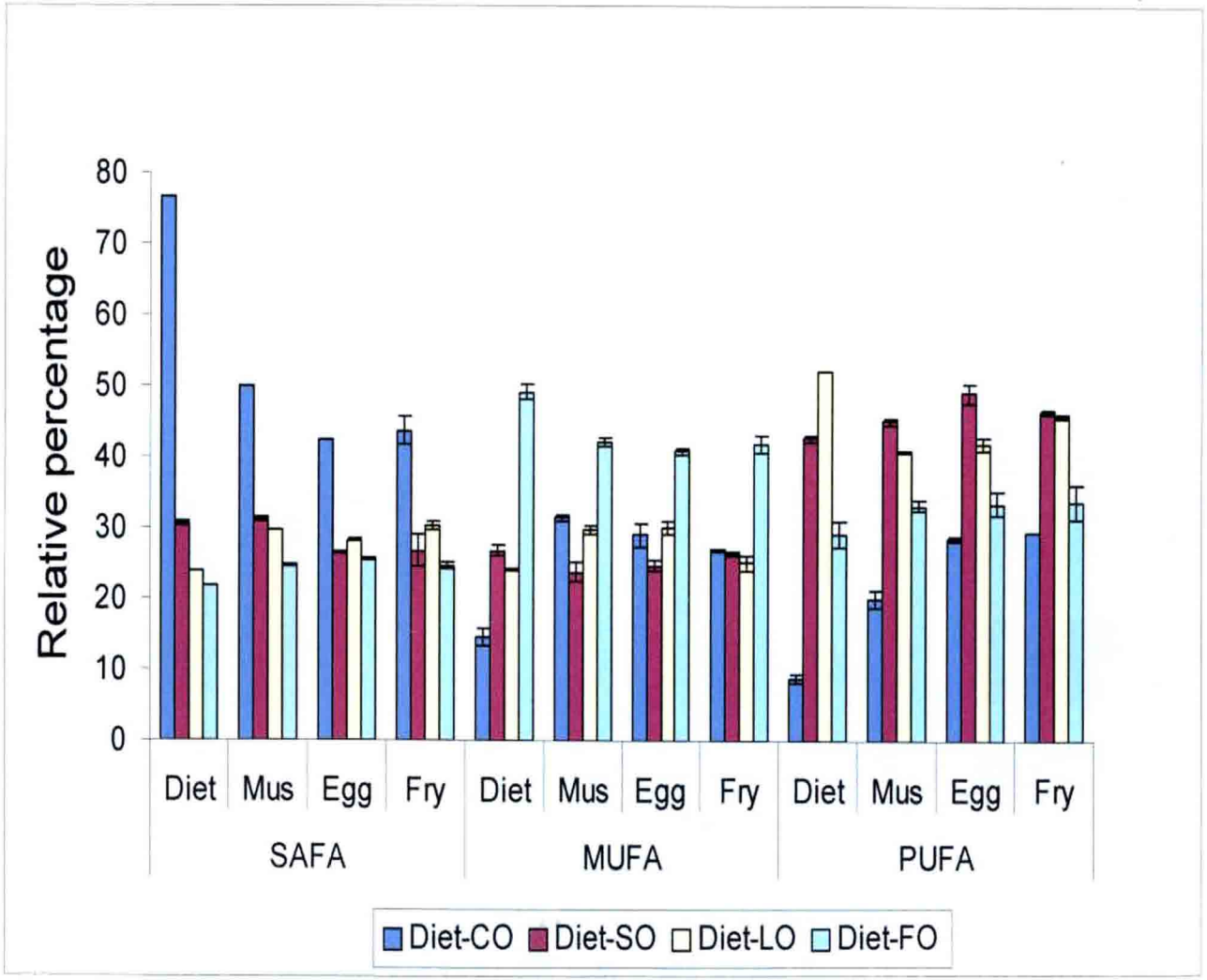


Figure 23. Comparison of SAFA, MUFA and PUFA levels in different stages

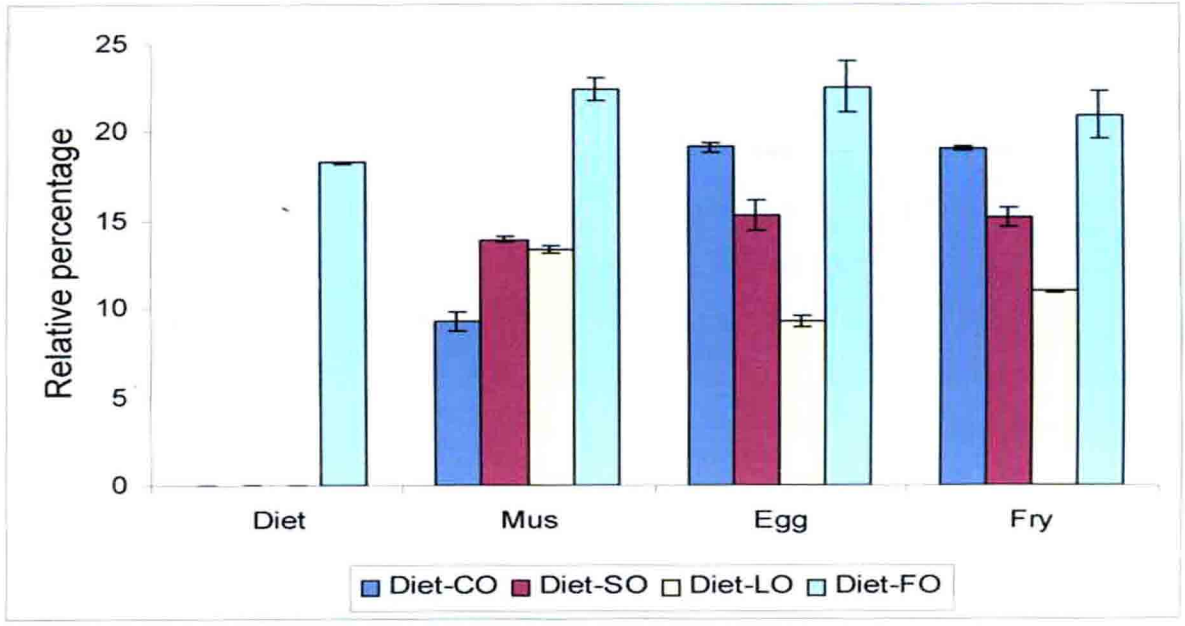


Figure 24. Comparison of HUFA levels in different stages

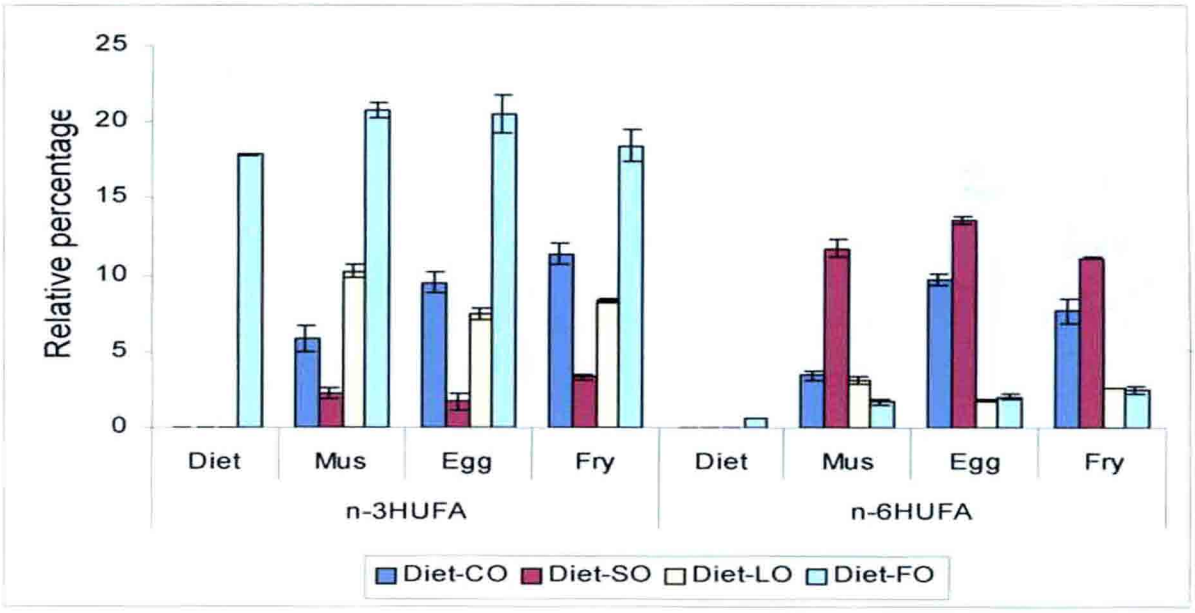


Figure 25. Comparison of n-3 HUFA and n-6 HUFA levels in different stages

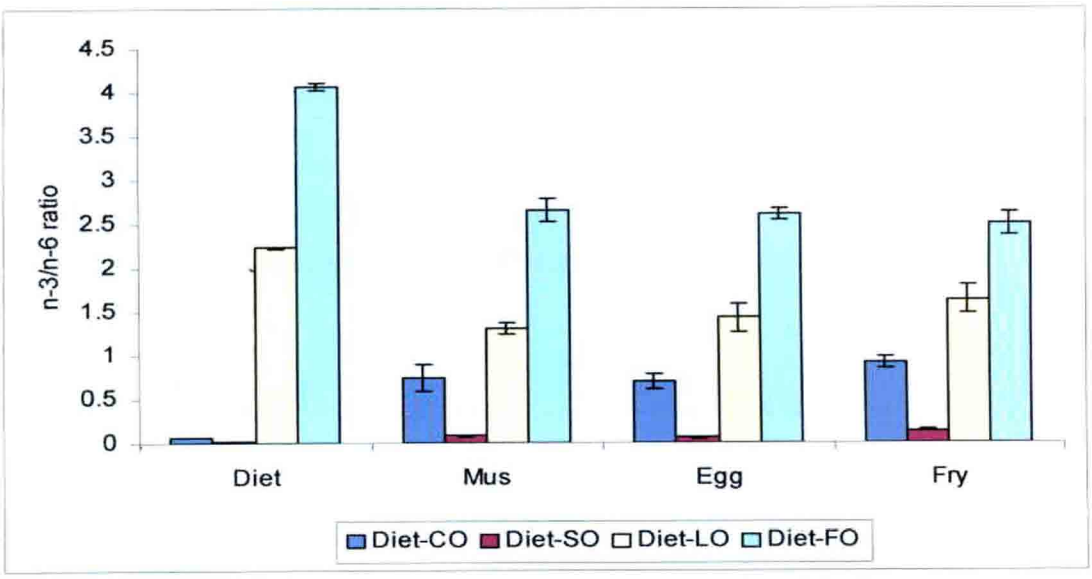


Figure 26. Comparison of n-3/ n-6 PUFA ratios in different stages

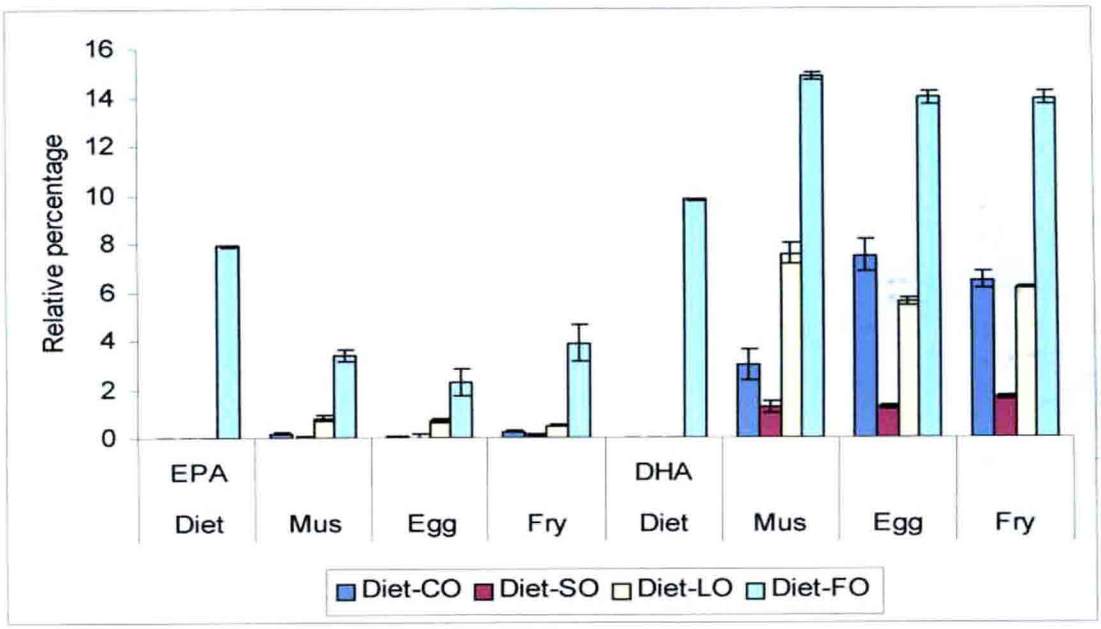


Figure 27. Comparison of EPA and DHA levels in different stages

In the present study it was observed that dietary fatty acid levels reflect he fatty acid levels in muscle, ovary and fry fatty acid levels. Correspondingly, significant correlations were obtained between dietary ALA/LA and muscle ALA/LAA

(Fig. 28). Similarly, significant correlations were also obtained between muscle ALA/LA and egg ALA/LA (Fig. 29) and egg ALA/LA and fry ALA/LA (Fig. 30). Significant correlations were also obtained between dietary n-3/n-6 and muscle n-3/n-6, muscle n-3/n-6 and eggs n-3/n-6 and eggs n-3/n-6 and fry n-3/n-6 (Figures 31-33).

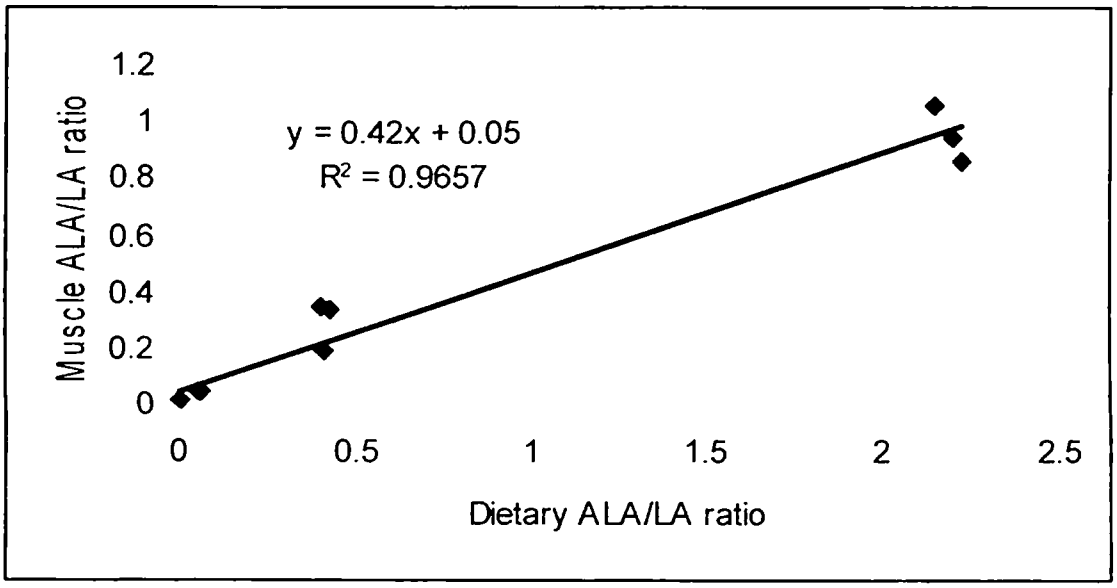


Figure 28. Relationship between dietary ALA/LA and muscle ALA/LA of female guppy ($p<0.05$)

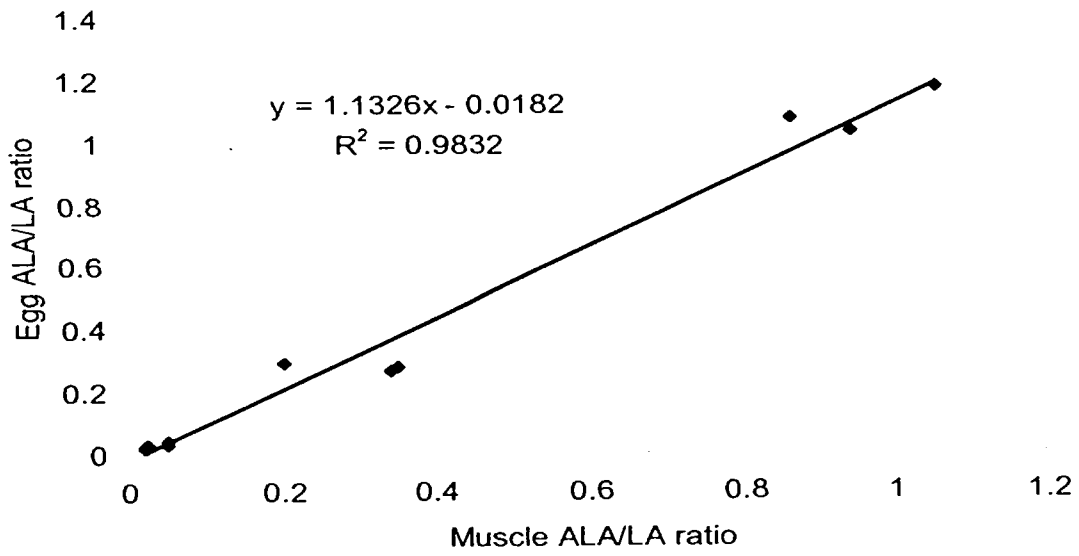


Figure 29. Relationship between muscle ALA/LA and egg ALA/LA of female guppy ($p<0.05$)

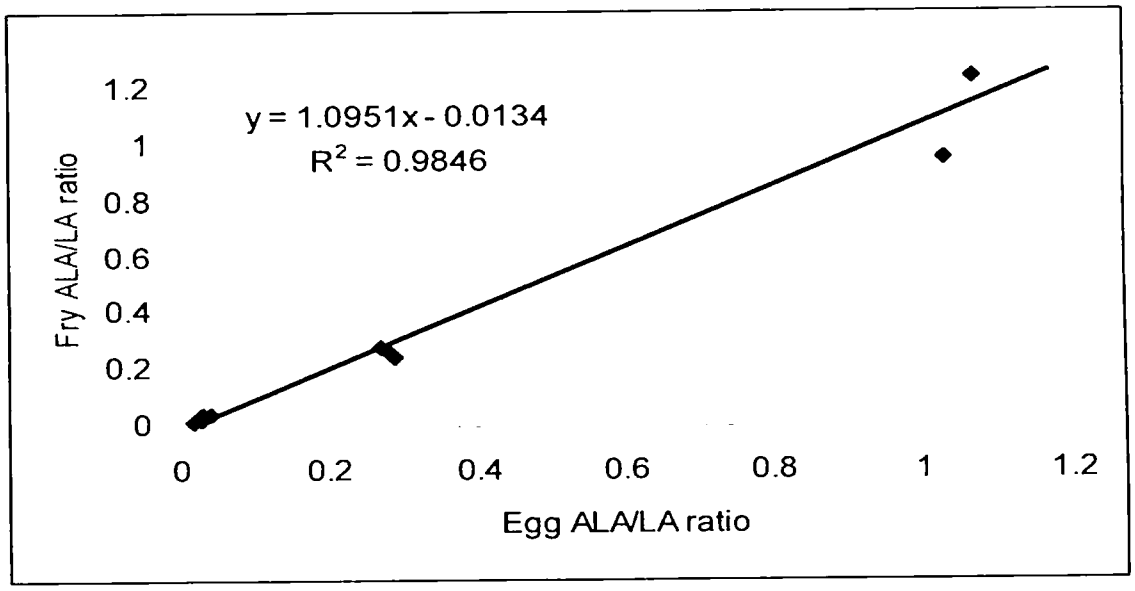


Figure 30. Relationship between egg ALA/LA and fry ALA/LA of female guppy ($p<0.05$)

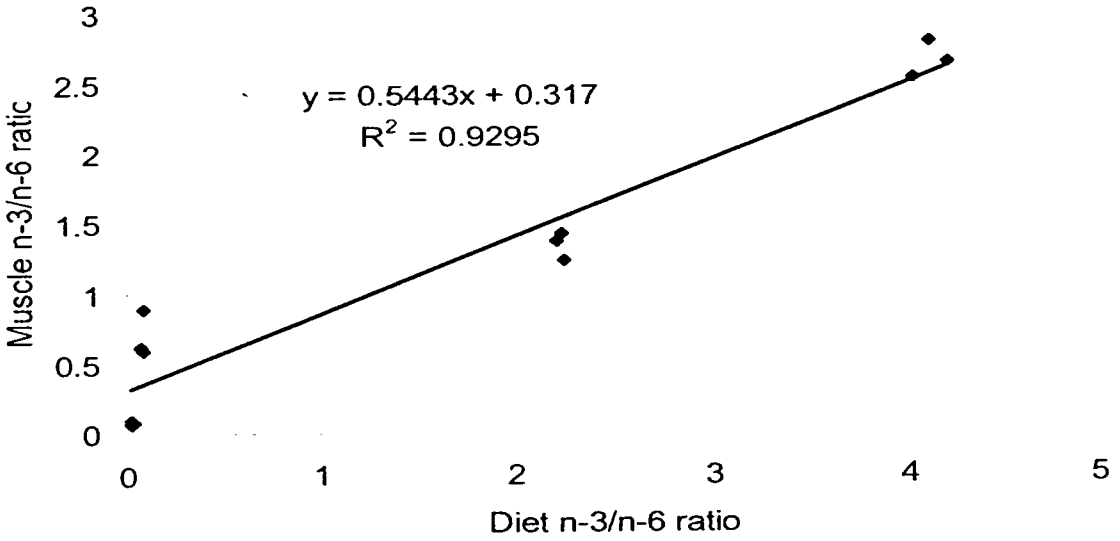


Figure 31. Relationship between dietary and muscle of female guppy with respect to ratio of n-3/n-6 fatty acids ($p<0.05$)

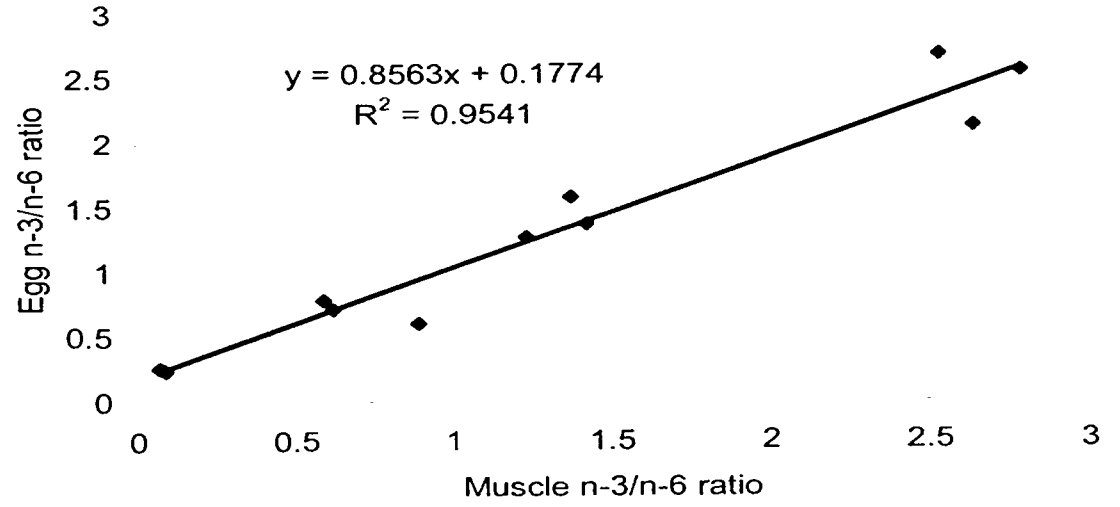


Figure 32. Relationship between muscle and egg of female guppy with respect to ratio of n-3/n-6 fatty acids ($p<0.05$)

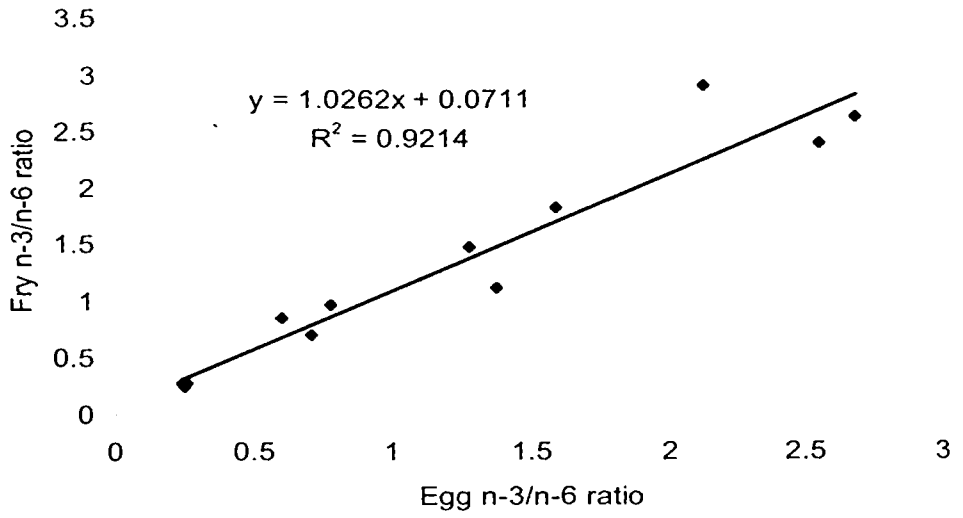


Figure 33. Relationship between egg and fry of female guppy with respect to ratio of n-3/n-6 fatty acids ($p<0.05$)

4.2.7 Fatty acid biosynthesis pathway

The fatty acid profiles of fish muscle were studied by Gas Chromatography and GC-Mass Spectrometry after three months rearing period. A total of 31 fatty acids were identified in the muscle of guppy fed Diet-SO, Diet-LO and Diet-FO. In addition to those fatty acids in the muscle of guppy fed Diet-CO had 20:3 n-9 fatty acid. Most of the freshwater fishes have a unique fatty acid composition. The proportions of different fatty acids of muscle of guppy fed different diets are shown in Table 27. The metabolites of n-3 pathway, 18:3, 18:4, 20:3, 20:4, 20:5, 22:5 and 22:6 appeared in the guppy fed all diets. With respect to n-6 pathway, the amounts of its metabolites (namely 18:2, 18:3, 20:3, 20:4, 22:4 and 22:5 were found in the fish fed all diets (Table 27). Relative percentage of ALA and its elongation and desaturation products in muscle in different treatments are presented in Figure 34 and relative percentage of LA and its elongation and desaturation products in muscle in different treatments are presented in Figure 35.

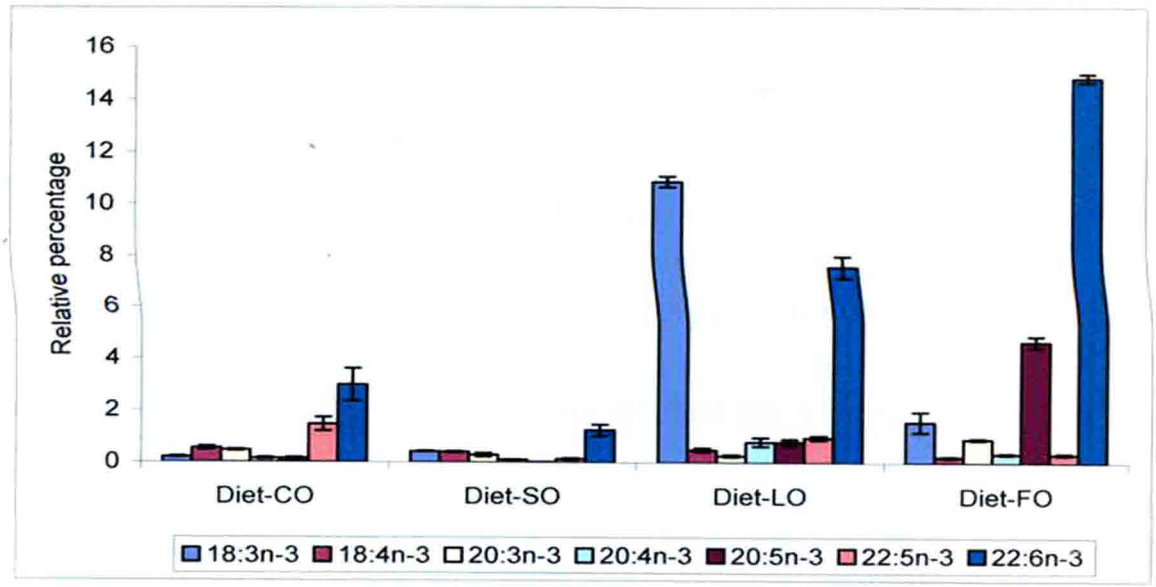


Figure 34 Relative percentage of ALA and its elongation and desaturation products in muscle in different treatments

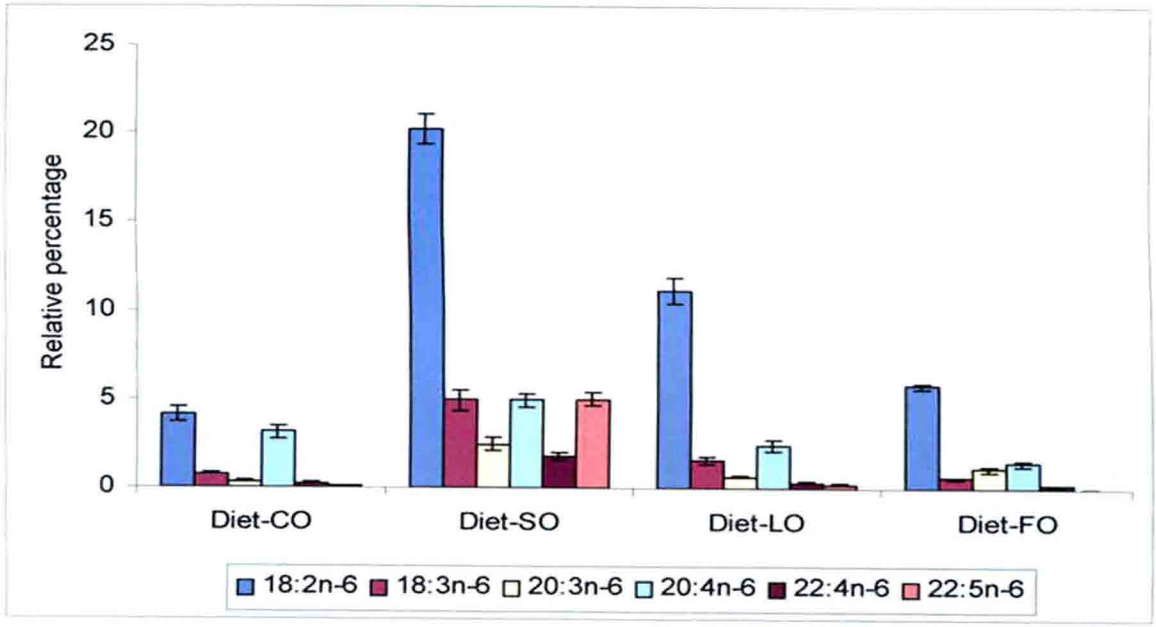


Figure 35. Relative percentage of LA and its elongation and desaturation products in muscle in different treatments

4.2.8 Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed on the data matrix of fatty acid composition of the diets used in the study and the fatty acid profiles of fry, muscle and eggs of guppy reared on different diets and dietary lipids. The PCA was done to express the main information in the variables by a lower number of variables, which are called principal components (PC1, PC2,). The main trends in the data were revealed by the 'score' plots, and the significant variables were identified by the 'loadings' plots. Both the 'score' and 'loading' plots were presented together in the form of Bi-plots (Figures 36-39).

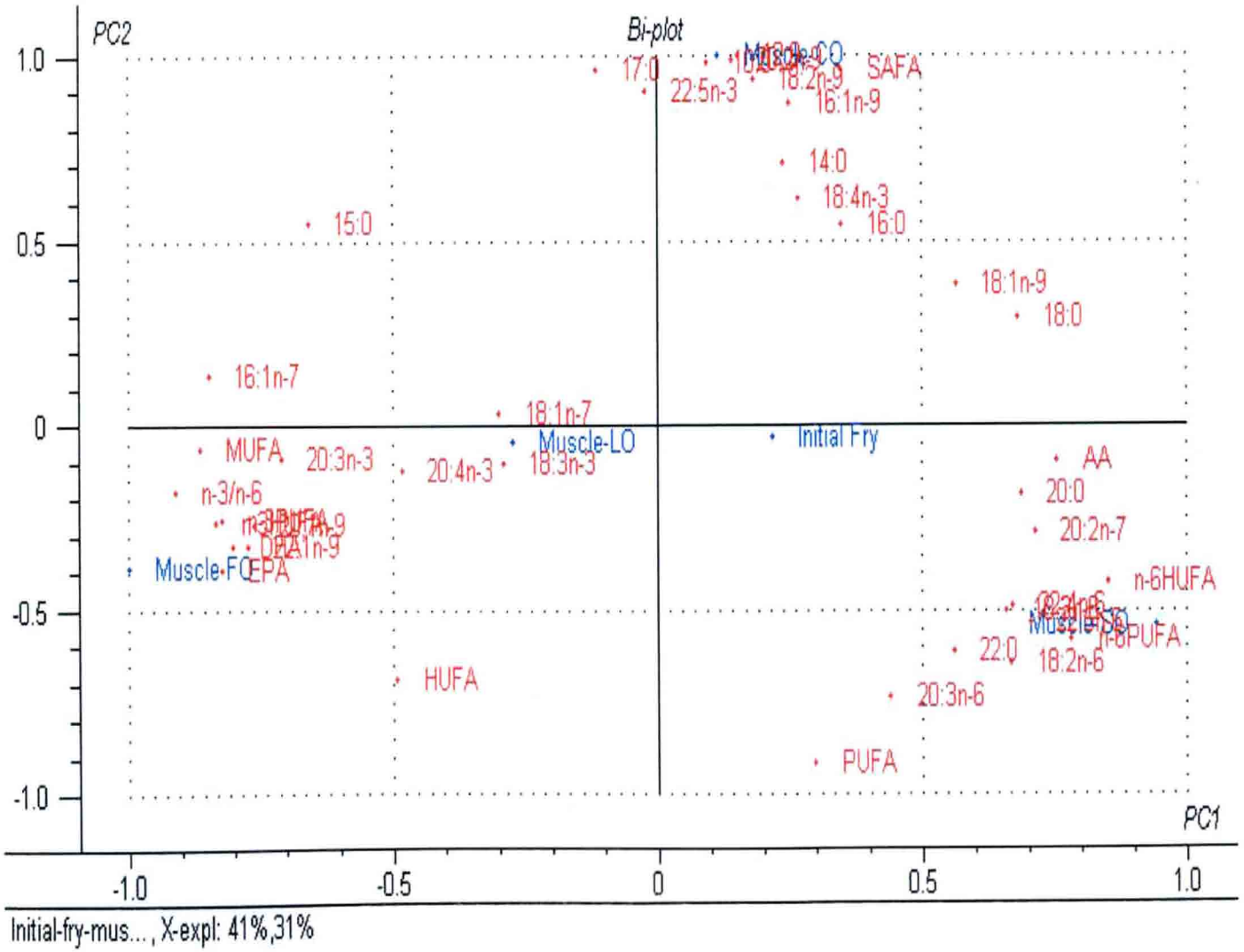
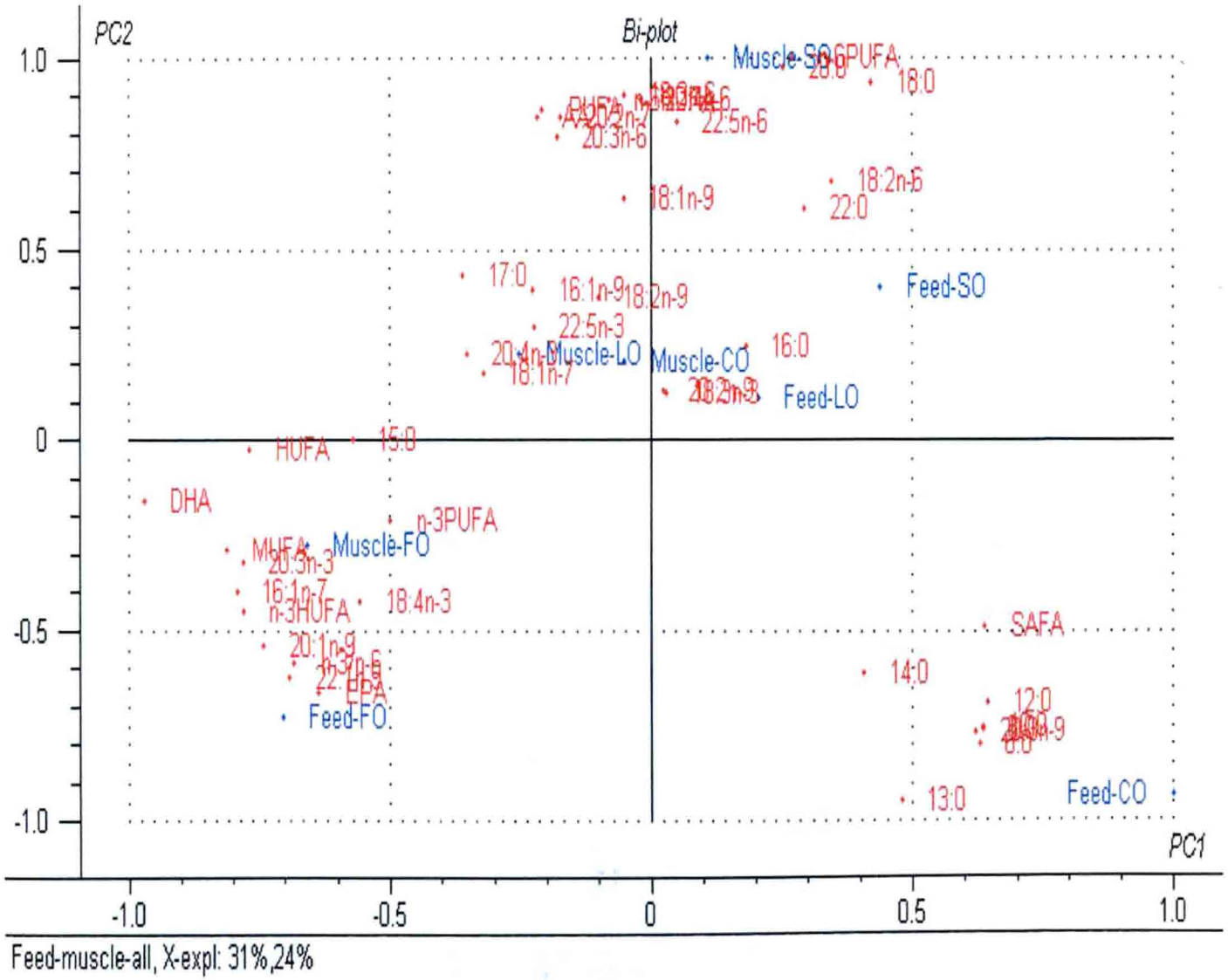
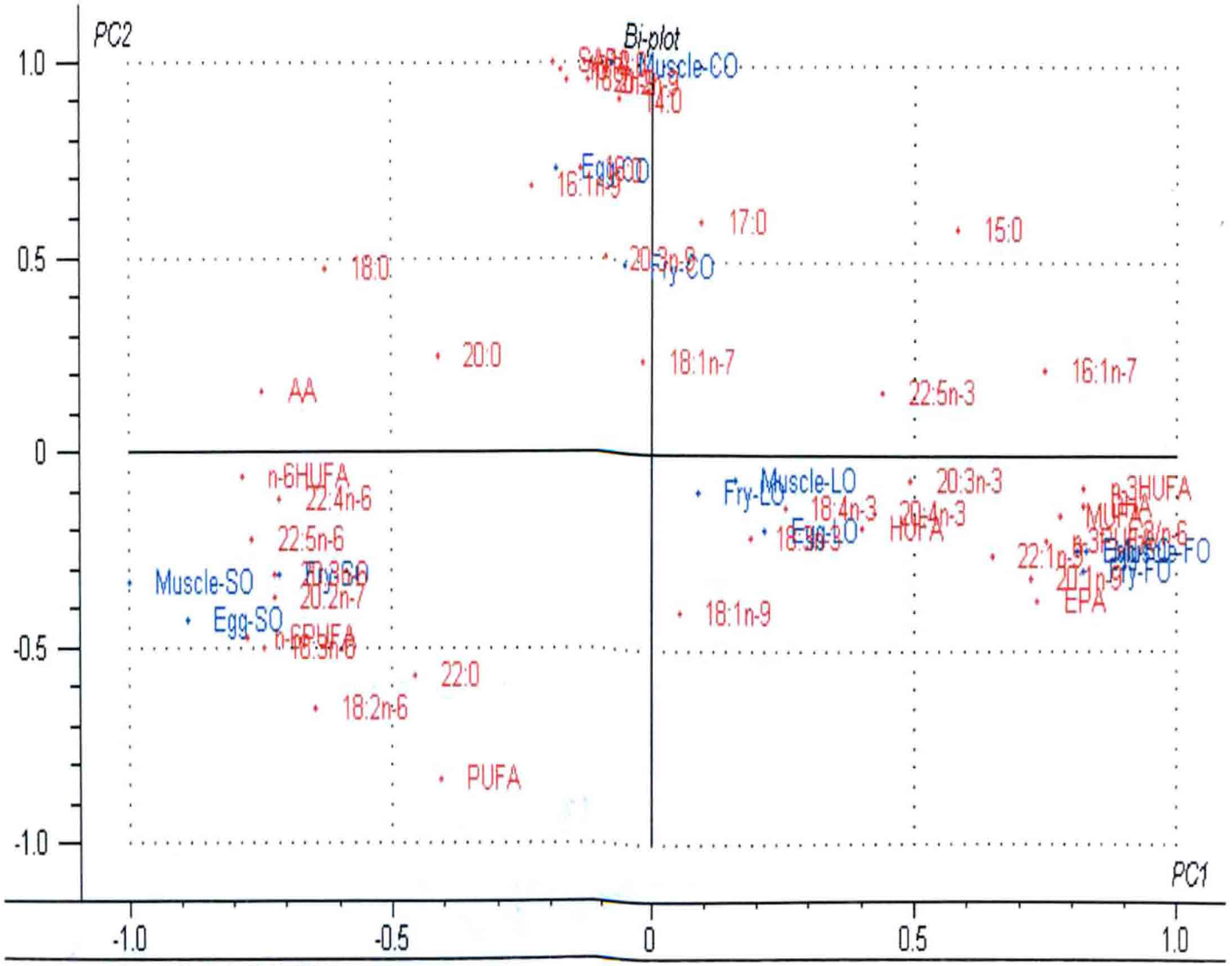


Figure 36. Changes in fatty acid composition of guppy with respect to initial fatty acid composition of fry



Feed-muscle-all, X-expl: 31%,24%

Figure 37. Effect of diets on muscle fatty acid composition of mature female guppy



Muscle-egg-fry-..., X-expl: 39%,25%

Figure 38. Correlations among muscle, egg and fry of guppy based on their fatty acid composition

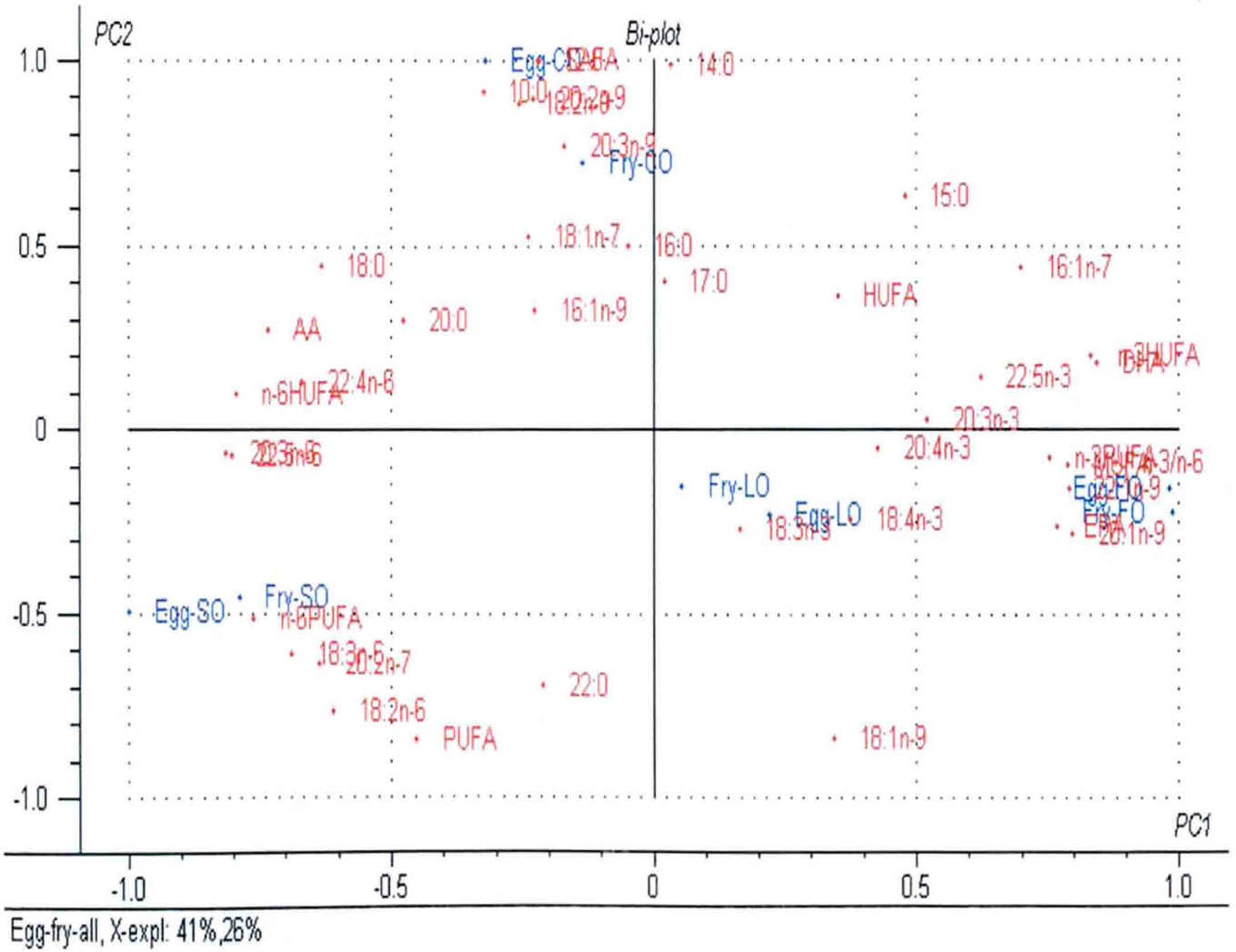


Figure 39. Correlations among egg and fry of guppy based on their fatty acid composition

Discussion

5. DISCUSSION

5.1 Evaluation of feeds currently used in guppy farming

Breeding and culture of guppy has been carried out in many countries due to the high demand for guppies in the world ornamental fish trade. In view of the easy breeding nature of the guppy, the farmers do not pay much attention to provide the fish with nutritionally well balanced diet. Owing to the use of unbalanced feed in guppy farming industry, problems of low brood size and low survival of fry have been reported by the farmers. It has also been observed that malnutrition and deterioration of genetic quality are leading to deformities of fry in guppy. Nutritionally unbalanced diets may also cause short broodstock replacement periods and lengthened brood interval. Ultimately this will lead to the production of poor quality fry with low survival rate. Therefore, in the first experiment three different types of feeds presently being used in the guppy farming were evaluated on growth and reproductive performance of female guppy.

The proximate composition of feeds namely Diet-1, 2 and 3 resulted 18.26%, 29.27% and 43.60% of protein and 4.17%, 4.55% and 9.47% of lipid respectively (Table 8). The three diets contained significantly different amounts of nutrient levels. The Diet-1 was found to be nutritionally deficient because of its feed ingredients which are mainly wheat flour and rice bran. These cereal based ingredients were highly rich in carbohydrates and poor in the quantities of protein and lipid. The Diet-2, a commercially available feed, was found to be moderately good with respect to protein having lipid only to limited extent. The list of ingredients used in the diet was not disclosed by the manufacturer. Diet-3 contained significantly high amount of protein and lipid resulting from its nutrient rich ingredients such as fishmeal.

In the present experiment the diets were used as powder type and hence the guppy could consume the provided feed easily since Sheenan *et al.* (2005) found that the growth of both male and female guppy fish was considerably enhanced when the diet was presented in the form of a finely ground powder compared with the flake form.

Water quality parameters such as temperature, pH and dissolve oxygen play a major role in the growth and reproduction of fish. Therefore, those parameters including ammonia and nitrite levels were monitored throughout the experimental period. All the tested water quality parameters were maintained within the acceptable range for the growth and reproduction of guppy (Table 9). The water quality was maintained by changing of water every second day.

Generally, nutrient levels in the diets influence the levels of nutrients in the carcass. The muscle nutrient levels are responsible for the fulfillment of the energy requirements and growth of fish. In the first experiment, the percentage body protein in fish fry was 56.30 and after maturation, the values varied between 53.95 and 56.86% (Table.10). Satia (1974) found that a general increase in the protein content in the carcass of rainbow trout (*Oncorhyncus mykiss*) in relation to the amount present in the diet. Ogino and Saito (1979) also reported that there was a linear relation between protein content of the diet and the body protein content of the young carp, *Cyprinus carpio*. Chong *et al.* (2004) found that the swordtails fed 30% dietary protein level had significantly higher muscle protein level than the fish fed 20% protein. Similarly, in the present study, significant difference ($p<0.05$) was observed in the muscle protein level of the guppy fed protein rich Diet-3 compared to the fish fed Diet-1 and Diet-2.

Crude fat level in guppy fry was 24.97% and after maturation the percentages varied between 26.56 and 29.83% (Table 10). In the present experiment, the dietary lipid levels significantly influenced the crude fat levels of carcass. The highest amount of lipid was found in carcass of guppy fed Diet-3. Earlier studies have also reported increased lipid concentration in fish carcass with provision of increasing

dietary lipids (Chou and Shiau, 1996; Chaiyapechara *et al.*, 2003; Lee and Sang, 2005). In a study carried out on female swordtail (*Xiphophorus helleri*), significant muscle lipid level has been reported in the female swordtails fed 12% dietary lipid level than the fish fed 8% lipid (Ling *et al.*, 2006a).

5.1.1 Growth performance of female guppy fed different diets

In aquaculture, achieving marketable size of fish within limited time period is very essential to minimize the production cost. On the other hand, the reproductive parameters are also mainly depending on the size of the fish. Therefore, supplementary feeding is very important especially in captive breeding and culture of fish since the entire essential dietary nutrient requirements only come through the feeds. The protein and lipid are major components of diets, which play a major role in growth of fish (Watanabe *et al.*, 1984; Watanabe and Kiron 1995; Furuita *et al.*, 2000). Growth of fish was determined in terms of body length and weight increment and specific growth rate.

The total and standard lengths of the guppy fed Diet-3 had significantly higher followed by the guppy fed Diet-2 and Diet-1. It clearly indicates that the Diet-3 was able to meet the dietary nutrient requirements especially protein and lipid for the growth of guppy (Table 11). Shim and Chua (1986) recommended 30-40% protein and 9-11% dietary lipid levels to achieve better growth in the female guppy. Therefore, the Diet-3 which contained the required amount of protein and lipid levels was found to be the right choice of the guppy females.

Gunasekera *et al.* (1997) reported that broodstock of *Oreochromis niloticus* fed low (10%) protein diet attained less weight than those fed high (20% and 35%) protein diets. Singh and Dhawan (1996) also observed that *Cyprinus carpio* showed higher weight gain when fed formulated diets containing 34% or 38% protein than those fed 31% protein diet. The Diet-2 which resulted moderate growth in guppy had only the required protein amount but not the required amount of lipid highlighting the importance of dietary lipids in guppy. Improved growth parameters have been reported previously in numerous fish species with provision of dietary lipid levels (Chou

and Shiau, 1996; Hemre and Sandnes, 1999; Vergara *et al.*, 1999; Chaiyapechara *et al.*, 2003).

It has been reported by many workers that decrease in body weight occurs due the fish fed with low protein diets (Dabrowski, 1977; Jauncey, 1982). Shim and Chua (1986) reported that the guppy fed with the diets having more than 20% protein levels had significantly greater gains in mean body weight than fish fed with below 20% of dietary protein levels. The guppy fed Diet-1, which had dietary protein and lipid levels far below the actual requirements showed the lowest level of length and weight increment. Though it has been reported that dietary protein levels play a major role in the growth of fish, many commercial feeds lack the required protein levels since it is an expensive ingredient compared to others.

Numerous studies have highlighted the interactive importance of dietary protein and lipid levels on fish growth performances (Miller *et al.*, 2005; Usman, 2005; Ozorio *et al.*, 2006). The higher weight gain resulted in higher % SGR in the fish fed Die-3 (Table 11). Chong *et al.* (2004) reported that the female swordtails fed diet containing 30% protein and 9% lipid levels showed significantly higher weight gain and % SGR than the fish fed 20% protein level. The SGR (%) value of the guppy fed Diet-3 was found to be significantly higher than the other two groups which indicate that the nutrients available in the Diet-3 fulfilled the dietary requirements of guppy to grow faster. The fish fed Diet-1 had the lowest SGR (%) value indicating that the diet did not contain the basic nutritional requirements for the growth especially protein which is absolutely essential for the buildup of new body tissues. The present study clearly indicated that the lipid levels present in the Diet-1 and Diet-2 were not adequate for the growth of guppy.

The feed conversion values of guppy fed on Diet-1 had a very high value (Table 11) indicating the non-availability of required nutrients for the growth of guppy. These fishes had only small length and weight increment throughout the experimental period. Feed conversion ratio in the group of guppy fed with Diet-3 was significantly

($p<0.05$) lower than the fish fed Diet-1 and Diet-2. It is obvious to observe significantly low FCR in the group Diet-3 as those guppies were fed with the diet loaded with the required amounts of protein and lipid. Furthermore, the guppy fed Diet-3 showed significantly higher survival of the fish after three months culture period compared to the other two groups. This may be due to the fact that the Diet-3 contained significantly high amount of protein and lipid levels, which are the source for essential amino acids and essential fatty acids. Based on these results, the present study proves that among the three different diets currently used in the guppy farming industry, only the Diet-3 fulfilled both dietary protein and lipid requirements for the growth of female guppy.

5.1.2 Reproductive performance of female guppy reared on different diets

Oocyte maturation involves transportation and accumulation of protein and lipid into oocytes (Tyler and Sumpter, 1996). Numerous studies elsewhere have shown that the dietary protein and lipid levels play a major role in reproductive performance of fish. Proteins and lipids, the main components of egg yolk, are considered to play pivotal role in reproduction. The quantity and composition of dietary protein are known to affect fish reproduction. Further, proteins act as a source of amino acids and reservoir of materials used during biosynthetic activities essential for early stages of embryogenesis (Watanabe *et al.*, 1984).

Results of the present study revealed that feeding higher levels of protein and lipid resulted in better gonadal development in female guppy. The ovary was expanded and large when containing numerous ova and/or embryos but was thin and elongated when only previtellogenic oocytes occurred. Thus the guppy fed Diet-3 had the highest mean ovary weight and ovary volume followed by Diet-2 and Diet-1 fed fish (Table 12). Shim and Chua (1986) also observed significantly higher ovary weight of the guppy fed 30-40% dietary protein levels and 9-11% lipid levels.

Dahlgren (1980) also observed that guppy females fed on a high protein diet developed comparatively heavier gonads indicating a higher ability to mobilize

nutrients for the protective reproductive tissue. The inferior ovarian mass in guppy fed Diet-1 therefore could indicate that 18% dietary protein and 4% dietary lipid level could be below the optimal requirement for proper oocyte development in female guppy. Similar results were observed in swordtail females fed 20% dietary protein levels yielding inferior ovarian mass (Ling *et al.*, 2006). Essential amino acids are provided with dietary protein and the essential n-3 PUFA and HUFA are provided with lipid for reproduction of fish. Therefore, adequate amount of protein and lipid should be provided for the better ovary development of female guppy.

The fecundity, total number of eggs produced by a fish in single spawn, has been used to determine reproductive performance of fish. Adequate amount of nutrients especially protein and lipid should be provided to fish to obtain higher number of eggs. On the other hand, number of ova in a fish is also related to the body weight. In the present experiment the highest absolute fecundity values were observed in the fish fed Diet-3 followed by Diet-2 and Diet-1 (Table 12). The guppy fed with the Diet-3, which contained essential amino acids as crude protein, and essential fatty acids as crude lipid may be the possible reason for higher absolute fecundity levels. It can also be explained that the fish fed Diet-3 showed highest mean weight and hence increased the number of ova. Shim *et al.* (1989) also demonstrated that female dwarf gourami (*Colisa lalia*) broodstock showed higher total weight and higher number of oocytes undergoing vitellogenesis when fed higher diet protein levels. Manissery *et al.* (2001) reported maximum fecundity in *C. carpio* at 35% protein diet.

In the present study, higher body weight showed the highest absolute fecundity. There was a significant positive correlation between final mean weight of the female and number of ova/female (r^2=0.89, $p<0.05$). Milton and Atherton (1983) reported that, fecundity was linearly related to body size in wild populations of swordtail. The guppy fed Diet-2 had comparatively lower fecundity values may be due to the poor growth of fish compared to the fish fed Diet-3. Larger females have also been reported to display higher success in spawning rates (Gunasekara *et al.*, 1996a; El-Sayed *et al.*, 2003). Shim and Chua (1986) observed significantly higher number of yolky oozytes in

the fish fed 30-40% protein and 9-11% lipid. In the present experiment, significantly higher numbers of yolky oozytes were also observed in the guppy fed Diet-3. Numerous studies have shown that once the dietary protein requirement of female broodstock is provided, further increase in dietary protein intake does not further enhance the fish fecundity (Dahlgren, 1980; Santiago *et al.*, 1983; Gunasekara *et al.*, 1996a; Al Hafedh *et al.*, 1999; Emata and Borlongan, 2003).

Egg diameter is considered an important criterion for the assessment of reproductive performance in fish. In the present study, the fish fed 43% dietary protein level showed the highest ovum diameter compared to the fish fed 18% dietary protein levels (Table 12). However, there was no difference between the ovum diameter of the fish fed 18% and 29% protein levels and 29% and 43% dietary protein levels. Several studies have shown that larger egg size will eventually result in larger fry at hatching (Seghal and Toor, 1991; Gisbert *et al.,* 2000). Though the absolute fecundity of females fed Diet 2 and 3 were significantly different, the diameters of ova were not significantly different. This may be due to the fact that the amounts of nutrients present in the mature fish contribute to the development of fish oocyte taking care not the number, but the quality of the ovum.

The fish fed Diet-3 showed significantly higher weight gain and the body weight that produce the large ova compared to the fish fed Diet-1, which had the lowest mean body weight. Positive correlation between egg size and parental body size has also been reported in several freshwater species (Seghal and Toor, 1991; Bromage *et al.*, 1992). The fish fed Diet-1, which contained 18.26% protein level probably indicates limited or insufficient protein for maintenance and oocytes development. The poor development of ovary in fish fed Diet-1 and 2 illustrates that the nutrient levels of these diets could be below the optimal requirement for ova development in female guppy.

It was observed that the guppy fed Diet-3 had significantly higher %GSI than the fish fed other two diets while the fish fed Diet-1 had significantly the lowest %GSI due to the lack of protein and lipid in the diet (Table 12). It has also been

reported previously that the female guppy fed 47% protein diet produced higher GSI than those receiving 15% and 35% protein diets (Dahlgren, 1980). Insufficient lipid in the Diet-2 may be one of the reasons for lower GSI (%) observed in the guppy fed Diet-2 compared with the fish fed Diet-3.

Ling *et al.* (2006a) found that the swordtail female fed 30% protein and 8% lipid levels showed significantly higher GSI (%) than the fish fed 20% protein and 8% lipid levels. This indicates that the importance of dietary protein on ovary development. They also reported that the swordtail females fed 20% protein and 12% lipid showed significantly higher GSI (%) than the fish fed 20% protein and 8% lipid levels (Ling *et al.*, 2006). This indicates that the dietary lipid levels also play a major role in gonadal development of Poeciliids. In another Poeciliid, the eastern mosquito fish (*Gambusia holbrooki*), lipid was found moving from bodily reserves to the ovary during vitellogenesis, further signifying the importance of providing adequate lipid supply to female broodfish (Meffe and Snelson, 1993). Another possible reason for significantly high gonadal development observed in the fish fed Diet-3 could be due to the provision of dietary highly unsaturated fatty acids for the ovary development.

The present study also demonstrated that feeding higher protein and lipid levels resulted in significantly higher levels of fry production. It was evident that the significantly low fry production from the fish fed Diet-1, which contained only 18.26% dietary protein, and 4.17% lipid levels (Table 13). In addition to the supply of essential fatty acids for reproductive physiology, dietary lipid can also provide energy to perform spawning activities (Halver and Hardy, 2002).

Ling *et al.* (2006) also observed that total fry production of swordtail female was influenced by dietary protein levels and fry production was higher in 30% dietary protein diets as compared to the 20% protein diets. It has also been demonstrated that the fish fed 30% protein and 12% lipid produced significantly greater number of fry than the fish fed 30% protein and 8% lipid. Significantly lowest number of fry production was observed in the swordtail fed 20% protein and 8% lipid diet. This

indicates that both the dietary protein and lipid levels affected total fry production by female swordtail broodstock. Since guppy is also a member of the family Poeciliidae, the minimum dietary requirement of protein and lipid may be the same for guppy. It can be supported by the fact that the guppy fed Diet-3 (43% protein and 9.5% lipid) showed significantly higher number of fry than the guppy fed Diet-1 (18% protein, 4% lipid) and Diet-2 (29% protein and 4% lipid). Though the Diet-2 contained adequate amount of protein, it did not fulfill the dietary lipid requirements for fry production (Table 13).

The mean values of weekly fry production are given in Figure 6. The mean weekly fry production was also found to be higher in the guppy fed Diet-3 than other two diets throughout the study period. Further, the guppy fed higher levels of nutrients (Diet-2 and 3) started producing fry three weeks earlier than the fish fed Diet-1. It has been reported that the dietary protein and lipid levels play a major role in weight gain in fishes and hence the adequate amount will lead to the higher fry production (Milton and Arthington, 1983). The higher fry production at a shorter period provides a great opportunity to the farmers especially in production of new strains, which generally have higher demand and higher price value when first introduced to market.

In tilapia, it has been reported that although 20% dietary protein limits the size, oocyte maturation and fecundity of female tilapia, successful hatching of fertilized eggs from this protein level still produce fry of equal quality in terms of weight and tolerance towards cold shock (Gunasekara *et al.*, 1995, 1996b). Similar observations were noticed in the present study in case of guppy fed Diet-1, which produced the lowest number of fry in order not to compromise the quality of the young fry. By observing the production of fry, though in small numbers, the farmers believe that the guppy breeds easily and do not pay the necessary attention to provide the nutritionally balanced diet.

Female fish need adequate proteins, lipids, vitamins and minerals for egg development and spawning/breeding. Protein is required for formation of follicle in the embryo. The deficiency of any of these nutrients can reduce larval survival (James and Sampath, 2004). Present study also revealed that the fry obtained from the guppy fed Diet-3, which contained significant amount of dietary protein, and lipid showed the highest fry survival (Table 13). There was no significantly different fry survival observed in the fish fed Diet-2 and Diet-3. Feed composition, quality and quantity and ration size are among the most important factors for better fry survival (Sampath and Pandean, 1984, James *et al.*, 1993, Jobling, 1998).

5.1.3 Effect of dietary fatty acid levels on growth and reproductive performance

The guppy fry stocked in this experiment contained 35.65% SAFA, 34.35% MUFA, 30.00% PUFA and 15.08% HUFA (Table 15). The levels of PUFA were significantly ($p<0.05$) higher in fish fry than matured fish. This may be due to the fact that HUFA levels came through the embryonic development and also the initial stages of fish fry were fed with live feed, *Artemia* and other zooplanktons, which contain high PUFA levels.

Lipid and fatty acid composition of broodstock diets have been identified as major dietary factors that determine successful reproduction and survival of offspring. Increased n-3 HUFA (particularly DHA) levels in broodstock diets have been reported to significantly enhance the weight of fish larvae and their resistance to osmotic shock (Aby-Ayad *et al.*, 1997). In a similar way, increasing n-3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk sack re-absorption. Many studies have reported that the dietary n-3 HUFA play a vital role in growth and reproduction of fish. In this experiment, it was found that the EPA, DHA, n-3 PUFA, n-3 HUFA and n-3/n-6 ratios were significantly higher in Diet-3 (Table 14) and the muscle (Table 15) and ovary (Table 17) of the fish fed this diet. This also may be one of the reasons for significantly higher growth, reproduction performances observed in the fish fed Diet-3. These fatty acids play a

major role during embryonic development. Higher fry survival was observed in the guppy fed Diet-3 and Diet-2, which contained significantly higher n-3 HUFA levels. Therefore, adequate lipid with essential fatty acids should be included in the broodstock diets to achieve better growth and reproductive performance of female guppy.

Based on the results of the first experiment, it was evident that the dietary protein and lipid play a crucial role in growth and reproduction of female guppy. Though the protein level in the Diet-2 was adequate for growth and reproduction of guppy female, significantly higher growth and reproductive performances were observed in the female guppy fed Diet-3. This may be possibly due to the availability of high lipid level with highly unsaturated fatty acids. Therefore, second experiment was carried out to determine the effect of dietary fatty acids on growth and reproduction of female guppy.

5.2 Growth and reproductive performance of female guppy in response to dietary fatty acids

Fatty acids not only are the major source of metabolic energy in fish for growth from the egg to the adult (Tocher *et al.*, 1985), but also the major source of metabolic energy for reproduction (Henderson and Tocher, 1987; Sargent *et al.*, 1999). Poly unsaturated fatty acids (PUFA) with 20 numbers of carbon atoms have been reported to regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes. Eicosanoids are important in the control of ovulation and probably involved in the embryogenesis, hatching and early larval development (Mustafa and Srivastrava, 1989). It has also been reported that certain dietary fatty acids content of broodfish significantly affect fecundity, fertility, hatching and viability of fish eggs, egg quality and larval growth (Mourente and Odriozola, 1990, Fernandez-Palacios *et al.*, 1995; Izquierdo *et al.*, 2001). In view of the importance associated with dietary fatty acids, the second experiment was designed to determine the effect of dietary fatty acids on growth and reproduction of female guppy.

Four purified diets were used in the second experiment and the proximate composition is given in Table 20. The amounts of protein (38%), lipid (10%), vitamin and mineral levels in all the prepared diets were same. However, the experimental diets were designed in such a way that each diet has different classes of fatty acids such as SAFA, n-6 PUFA and n-3 PUFA. Accordingly coconut oil (CO), sunflower oil (SO), linseed oil (LO) and fish oil (FO) were selected as lipid sources. Significantly higher amount of saturated fatty acids were observed in Diet-CO due to the fact that coconut oil mainly contained caprilic acid (8:0), capric acid (10:0), lauric acid (12:0), myrisric acid (14:0), palmitic acid (16:0) and stearic acid (18:0). Diet-SO and Diet-LO diets had the highest amount of PUFA levels due to the presence of high amount of linoleic acid and α-linolenic acid respectively. Diet-FO diet had the highest amount of MUFA and n-3 HUFA levels due to the high availability of 16:1 n-7, 18:1 n-9, 20:1 n-9 and EPA, DHA respectively. In the present experiment also the diets were used as powder type and hence there was no effect of diet type on the palatability and feed intake of guppy as

suggested by Sheenan *et al.* (2005). Generally, guppy consumes feed within two hours. At the beginning of the present study, it was observed that the Diet-SO and Diet-LO remain in the tanks for a long time without being consumed. Accordingly feeding regime was adjusted to provide feed till satiation.

As mentioned in the first experiment, all the water quality parameters such as water temperature, pH, DO, ammonia and nitrite levels were monitored regularly throughout the experimental period. The parameters were maintained within the acceptable range for the growth and reproduction of guppy by changing of water along with fecal matter every second day (Table 21).

In general, fish contain approximately 80-85 percent water, extreme values ranging from 53 to 89.3 percent (Vinogradov, 1953) The body moisture content of the guppy ranged from 63.26 to 66.29 percent, well within the limits defined above for fish (Table 22). The moisture content of the guppy was higher at the beginning of the experiments compared to the end of the experiment. The high moisture content of the young animals is a quite well known fact (Shim and Chua, 1986). All the four experimental diets had about the same moisture content, ranging from 7.3 to 7.51 percent. Thus, the four treatments did not affect the moisture content of matured guppy to any marked extent (Table 22).

The four feeds contained similar levels of protein and hence there was no significantly different level of protein content in the carcass of fish. Though the lipid levels were similar in the four experimental diets, the lipid levels in the carcass of the fish fed Diet-CO and Diet-FO were significantly higher compared to the fish fed Diet-LO and Diet-SO. Increased lipid concentration in guppy carcass may be due to the assimilation of high SAFA levels in the guppy fed Diet-CO and high PUFA levels in Diet-FO. It was also found that the dietary supplement of squid liver oil which contained EPA and DHA improved the weight gain and feed efficiency of starry flounder (Lee *et al.*, 2003). Lee *et al.* (2003) found that growth and feed utilization were lower in starry flounder (*Platichthys stellatus*) fed the n-3 HUFA deficient diets. The low lipid levels in

the fish fed Diet-LO can be explained based on the fact that the linseed oil has been reported to be poorly digested (Tinco, 1982). Muscle lipid content is an important indicator for broodstock reproductive performance as muscle act as an important source of lipid for ovary nutrient accumulation during maturation phase (Almansa *et al.*, 2001). There was no significantly different body ash levels present in the carcass of the fish before and after the experiment. It appeared to be unaffected by the different lipid sources.

5.2.1 Effect of dietary fatty acids on growth of female guppy

Several studies have shown the benefits of dietary HUFA in freshwater species despite their known ability to synthesize these fatty acids endogenously. The present study clearly demonstrated that the guppy fed Diet-SO and Diet-LO showed the poor growth and feed utilization compared to the fish fed Diet-FO, which contained significantly higher amount of HUFA levels. Interestingly, the guppy fed Diet-CO showed significantly higher level of length and weight gain than the fish fed SO and LO diets (Table 23).

Dietary lipids are important sources of energy and fatty acids that are essential for normal growth and survival of fish (Earle, 1995). Increased n-3 HUFA (particularly DHA) levels in broodstock diets have been reported to significantly enhance the weight of fish larvae and their resistance to osmotic shock (Aby-Ayad *et al.*, 1997). In the present experiment, the guppy fed Diet-FO showed the highest total and standard lengths, weights and SGR (%) after three months rearing period, compared to the fish fed other diets and hence the guppy fed Diet-FO had significantly higher SGR (%) followed by the fish fed Diet-CO, Diet-SO and Diet-LO. It can be explained based on fact that Diet-FO contained significant levels of n-3 HUFA especially EPA and DHA (Table 23). Francis *et al.* (2006) observed that the Murray cod, *Maccullochella peelii peelii* fed cod liver oil inclusion diet showed significantly higher final mean weight, %SGR and daily feed consumption than the fish fed linseed oil diet.

The diet refusal behaviour was observed in the guppy fed Diet-SO and Diet-LO, which remained in the tanks for a long time without being consumed. This may be one of the possible reasons that the fish fed Diet-LO and Diet-SO had the poor growth performance. Low palatability of Diet-LO and Diet-SO may be a possible reason for the high FCR values observed in the guppy fed those diets. The guppy fed Diet-FO diet, which had the highest amount of n-3 HUFA showed the lowest FCR indicating the importance of n-3 HUFA on growth and feed utilization of fish. However, the fish fed Diet-CO had significantly lower FCR than the fish fed Diet-SO and Diet-LO may due to the fact that, the palatability of this diet was comparatively higher than SO and LO diets. Highly unsaturated n-3 fatty acids principally DHA and EPA, play an important physiological role in fishes as components of membrane phospholipid and as precursors of biologically active eicosanoids. Consequently, fishes have a high dietary requirement for these fatty acids (Sargent *et al.*, 2002, 1995). Lanari *et al.* (1999) found that within the n-3 PUFA, particularly EPA and DHA in the muscle of *Dicentrarchus labrax* seem to be strictly determined by the dietary level of these acids. Therefore, in the present study it was clearly demonstrated that the dietary fatty acids play an important role on the growth of guppy.

5.2.2 Effect of dietary fatty acids on reproductive performance of female guppy

It has been reported that HUFA content of broodfish feed significantly affects fecundity, fertility, hatching and viability of fish eggs, egg quality and larval growth. However, very little is known about the requirements for dietary HUFA in freshwater fish species during reproduction. In the present study it was observed that the guppy fed Diet-FO, which contained significantly higher levels of n-3 HUFA (%) had significantly higher ovary weight than the fish fed other three diets (Table 24). It was also observed that ovary length, width and height values were significantly higher in the guppy fed Diet-FO than other three groups (Figure 11). The guppy fed Diet-CO showed significantly higher ovarian development compared to the fish fed Diet-SO and Diet-LO. A possible explanation for the higher ovarian development due to the significantly higher level of n-3 HUFA present in the eggs of the guppy fed Diet-CO than the fish fed

Diet-SO and Diet-LO. There was a selective deposition of n-3 HUFA especially DHA levels in the fish fed Diet-CO.

The effect of dietary oil source on fish fecundity (total number of yolky oozytes) was significant ($P<0.05$) (Table 24). The fecundity in the fish fed Diet-FO was significantly higher than the guppy fed other three diets due to the inclusion of fish oil in the diet, which contained significantly high levels of n-3 HUFA (Table 24). The inclusion of fish oil source (source of n-3 HUFA) has increased the fecundity and spawning frequency of Nile tilapia (Izquierdo *et al.*, 2001). The guppy fed Diet-CO showed significantly higher levels of fecundity than the fish fed Diet-SO and Diet-LO.

Yu *et al.* (1979) observed that the inadequate n-3 HUFA affected on the number and the viability of eggs of rainbow trout (*Salmo gairdneri*). The freshwater fish eggs were generally rich in n-3 HUFA (Henderson and Tocher, 1987), although not to such high that levels as found in marine fish (Weigand, 1996). The present study also demonstrated that the guppy fed Diet-FO had significantly higher egg n-3 HUFA levels followed by Diet- CO, Diet-SO and Diet-LO. Significantly higher fecundity was observed in the guppy fed Diet- CO may be due to the high level of n-3 HUFA present in eggs of those fish. This is in agreement with the results showing that absolute fecundity and number of fry increased with the increase of n-3 HUFA. Similarly, a positive correlation of the n-3 HUFA content between diet and egg has also been observed in several other fish species (Fernandez-Palacios *et al.*, 1995; Furuita *et al.*, 2000; Vassallo-Agius *et al.*, 2001a,b). In this study, the increase of n-3 HUFA was mainly due to the increase of DHA content (Tables 26, 27, 28), suggesting the selective retention of DHA during ovary development denoting the importance of this fatty acid for the development embryo and larvae in guppy. Therefore, it was clearly demonstrated that the dietary fatty acids especially n-3 HUFA play a major role in fecundity of guppy since this fatty acid class is very important for ovary development.

It was observed in the present study that the guppy fed Diet-FO, which contained significantly higher DHA levels, produced significantly higher number of mean weekly fry production (Figure 13) and total mean fry production than the fish fed other three diets (Table 25). In the freshwater common carp it has been demonstrated that dietary levels of dietary DHA significantly affected egg hatchability (Shimma *et al.*, 1977), as has been demonstrated in many species of food fishes (Rainuzzo *et al.*, 1997). Though the muscle of the guppy fed Diet-CO diet contained significantly lower amount of DHA compared to the fish fed Diet-LO, in the eggs, Diet-CO fed fish contained significantly higher amount of DHA which contributed to the higher fry production compared to the Diet-LO fed guppies. This selective retention of DHA in eggs of the fish fed Diet-CO also indicated the importance of DHA in the embryonic development of guppy. Selective retention of DHA has also been found during embryogenesis (Izquierdo, 1996) and during starvation (Tandler *et al.*, 1989) denoting the importance of this fatty acid for the developing embryo and larvae.

Increasing n-3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk sack re-absorption (Furuita *et al.*, 2000). Significantly higher number of fry and % fry survival observed in the guppy fed Diet-FO may be due to the availability of significantly high amount of n-3 HUFA levels in the eggs which were obtained from Diet-FO (Table 25). The highest fry production was also obtained in female swordtail fed with dietary n-3 HUFA (Ling *et al.*, 2006).

Coconut oil has been shown to have antiviral and antimicrobial properties due to the lauric acid fraction in the oil. It's fatty acid profile consists primarily of caprylic and lauric acids, which support immune function. This may be one of the possible reasons for observing high survival rate in the fry of the broodfish fed Diet-CO compared to the fish fed Diet-SO and Diet-LO. Bell and Sargent (2003) proposed that Arachidonic acid play a vital role during the times like pathogenic invasion and vitellogenesis. CO fed fish contained significant levels of AA in addition to the n-3 HUFA

levels and this combined effect of both may be responsible for the higher fry production and fry survival observed in the guppy fed Diet-CO than Diet-SO and Diet-LO fed fish.

A positive correlation between female weight and fry production in swordtail was reported by Chong *et al.* (2004) and Ling *et al.* (2006). In the present study, regression analysis showed significant correlations between final mean weight of female and mean fry production (r^2=0.89, P<0.05), mean weight of female and number of ova (r^2=0.87, P<0.05).

This is the first ever study on egg fatty acid composition of female guppy fed on diets with different lipid sources. 31 fatty acids were identified in the eggs of guppy fed Diet-SO, Diet-LO and Diet-FO while 33 fatty acids were identified in the guppy fed Diet-CO. Daikoku (1982) reported only 14 fatty acids in the ovary of guppy in the experiment carried out on seawater adaptation. It was observed that 16:0 and 18:0 were the most abundant SAFA in eggs. During embryonic development and yolk sac absorption, consistent with findings for the majority of species, the most abundant fatty acids were 16:0 and 18:0 which are main components of phospholipids, principally phosphatidycholine and phosphatidylethanolamine (Tocher *et al.*, 1985; Mourente and Vazquez, 1996). The abundance of MUFA especially 16:1n-7 and 18:1n-9 were very high compared to other MUFA in eggs and fry of guppy could be related with their importance as energy source. These two fatty acids are preferred substrates for catabolism (Izquierdo, 1996).

Generally eggs are rich in n-3 PUFA than parental body lipids (Tocher and Sargent, 1984). PUFA in the eggs are mainly EPA and DHA (Kairanta and Ackman, 1981; Tocher and Sargent, 1984). Eggs of the guppy fed Diet-LO and Diet-FO contained significantly higher levels of n-3 PUFA than n-6 PUFA. The n-6 PUFA in the eggs of the guppy fed all types of diets were higher than the respective dietary n-6 PUFA levels. The eggs of the guppy fed Diet-CO and Diet-SO contained significantly higher levels of n-3 PUFA than dietary levels while Diet-LO and Diet-FO fed guppy contained comparatively lower levels than the dietary level. However, it is obvious that

the dietary PUFA levels are directly related to the PUFA levels in the eggs of guppy. Further, the guppy fed Diet-SO, showed high n-6 PUFA in the eggs since Diet-SO and the muscle of guppy fed this feed contained significantly higher amount of linoleic acid (Tables 26, 27 and 28). Similarly, the guppy fed Diet-LO, showed high level of n-3 PUFA in the eggs since the Diet-LO and the muscle of the guppy fed this diet contained significantly higher level of α-linolenic acid.

Significant correlations were obtained between dietary ALA/LA ratio and muscle ALA/LA (r^2=0.97, P<0.05), muscle ALA/LA ratio and egg ALA/LA (r^2=0.98, P<0.05), egg ALA/LA ratio and fry ALA/LA (r^2=0.98, P<0.05), diet n-3/n-6 and muscle n-3/n-6 (r^2=0.93, P<0.05), muscle n-3/n-6 and egg n-3/n-6 (r^2=0. 95, P<0.05) and egg n-3/n-6 and fry n-3/n-6 levels (r^2=0.92, P<0.05) (Figs 28-33). These observations clearly indicate the influence of dietary fatty acids on the fatty acid profile of muscle, egg and fry of guppy.

5.2.3 Fatty acid metabolism in guppy fed varying levels of dietary fatty acids

After feeding the experimental diets for three months, fatty acid composition of guppy was studied by GC-MS. A total of 31 fatty acids were identified in muscle of guppy fed Diet-SO, Diet-LO and Diet-FO. 20:3 n-9 fatty acid was identified only in the fish fed Diet-CO. Most of the freshwater fishes have a unique fatty acid composition. The fatty acid composition of fish tissue lipids usually reflects those of the dietary lipids (Henderson and Tocher, 1987; Sargent *et al.*, 2002; Bell, 1998; Higgs and Dong, 2000; Jobling, 2001) even though there is a potential for modification and metabolism of fatty acids sequestered from the diet (Henderson 1996; Sargent *et al*, 2002; Bell, 1989).

To understand the biosynthetic capabilities and mobilization of dietary and endogenous fatty acids in guppy, the fatty acid profiles of its muscle were studied. The proportions of different fatty acids of muscle of guppy fed different diets are shown in Table 27. The fatty acid composition of fish lipids is determined by the ability of fish to

desaturate and elongate the *de novo* synthesized and dietary fatty acids (Henderson, 1996). Fish can desaturate the endogenous saturated fatty acid, 18:0, to the monounsaturated fatty acid 18:1 n-9, by 18:0-CoA desaturase, but lack both Δ12 and Δ15 n-3 desaturases which are required for the synthesis of C18 and C20 polyunsaturated fatty acids (PUFAs) (Henderson, 1996).

Significant amount of metabolites of n-3 pathway, 18:3, 18:4, 20:3, 20:4, 20:5, 22:5 and 22:6 appeared in the guppy fed Diet-LO (Figure 34). This clearly indicates about the biosynthetic capability of guppy to convert ALA into EPA and DHA. The 24:6 was not observed in the guppy and this may lead to propose the hypothesis that the guppy may directly convert 22:5 to 22:6. It has traditionally been accepted that 18:3 n-3 is converted to 22:6 n-3 by a pathway combining the sequential action of Δ6, Δ5 and Δ4 desaturases with chain elongation reactions (Henderson and Tocher 1987). However, Voss *et al.* (1991) after studying on rat hepatocytes, have proposed that Δ4 desaturase may not be participating in the pathway and 22:6 n-3 may be biosynthesized by the sequential desaturation and chain elongation of 20:5 n- 3 to 24:6, which is finally chain shortened by peroxisomes to yield 22:6 n-3 (Voss *et al.*, 1991; Voss *et al.*, 1992).

With respect to n-6 pathway, significant amounts of its metabolites (namely 18:2, 18:3, 20:3, 20:4, 22:4 and 22:5) were found in the fish fed Diet-SO indicating the biosynthesis capacity of guppy (Figure 35). 22:5 n-6 is the end product of n-6 HUFA biosynthetic pathway (Sprecher and Chen, 1999). However, other two intermediates of the proposed pathway (24:4 n-6 and 24:5 n-6) could not be detected in the present study by GC-MS.

The results of this study suggest that the activities of fatty acid desturases and elongases, involved in the n-3 and n-6 biosynthetic pathways, were higher in guppy fed on diets containing essential fatty acids which are rich in linseed oil and sunflower oil. Increases in hepatic desaturation and elongation activities have been observed in studies in which salmon were fed vegetable oils or vegetable oil blends (Bell *et al.*,

2001, 2002). In another study, Bell *et al.* (2003) also noted that hepatic desaturation and elongation of 18:3 n-3 were clearly enhanced progressively by replacing dietary fish oil with rapeseed oil.

Arachidonic acid was highest in the Diet-SO group and lowest in Diet-FO group. It is obvious to expect this as LA is the precursor for the biosynthesis of AA (Sprecher *et al.*, 1999) and high amount of AA in the Diet-SO group must have resulted from its precursor, which was significantly higher in the diet. The concentration of 22:5 n-6 varied markedly among the dietary groups, being the lowest (0.04%) in Diet–FO group and the highest in Diet–SO (5.0%) group. From the HUFA biosynthetic pathway, it can be noticed that $\Delta 6$ desaturase acts on C_{18} and C_{22} fatty acids in both n-6 and n-3 pathways (Sprecher *et al.*, 1995). The activity of desaturases was reflected in the conversion of EFA to HUFA.

The above-mentioned results and discussion demonstrate that both the n-3 and n-6 pathways are active in fatty acid metabolism of guppy. Interestingly, oleic acid, which is the precursor for the n-9 pathway was present in a significant quantity in Diet-SO, Diet-LO and Diet-FO. However, the amount of 20:2 n-9 found in the muscle of the guppy fed these diets was significantly lower. It can be explained on the basis of findings of Sargent *et al.* (2002), who observed the affinity of desaturases in the order of n-3>n-6>n-9 fatty acids. Tocher *et al.* (2001) concluded from their study that inclusion of high level of ALA in the diet inhibited the production of AA from LA. Furthermore, though the amount of oleic acid in the Diet-CO was significantly low, 20:3 n-9 was observed in the guppy fed Diet-CO due to the activation of $\Delta 9$ desaturates. An absence of n-6 or n-3 fatty acids in the diet led to the synthesis of n-9 fatty acids in the guppy fed Diet-CO.

5.2.4 Principal Component Analysis (PCA)

The correlations in the fatty acid profiles of muscle could be examined further using the principal component analysis. The PCA offered a better understanding of the fatty acid composition of muscle of guppy submitted to varying levels of dietary fatty acids. The "score" plot revealed information about patterns in the samples and it was found useful to interpret differences and similarities among the samples. The plot of PC1 and PC2 was used in this study since these two components summarized more variation (72%) in the data than any other pair of components. The fish fed different dietary lipids for 90 days (Diet-CO, Diet-SO, Diet-LO, Diet-FO) were differentiated from the initial position based on fatty acid composition. The "loading" plot clearly showed that the variables such as SAFA, MUFA, PUFA, n-3 HUFA, 18:2 n-6 and 22:6 n-3 were responsible to bring out the differences among the samples. The Bi-plot (Fig. 36) presented "score" plot (samples are marked in blue colour) together with the corresponding "loading" (variables are marked in red colour) plot, for the same two components.

The definite clusters for each of the four treatments were observed and the PC1 scores discriminated fish fed Diet-SO (positive scores) from fish reared on Diet-FO and Diet-LO (negative scores). The Diet-SO group was characterized by higher amounts of n-6 PUFA, n-6 HUFA, 18:2, 18:3 20:3, 22:4 and 22:5 belonging to n-6 class, whereas n-3 fatty acids were observed for Diet-FO and Diet-LO groups. Interestingly the saturated fatty acids were discriminated from unsaturated fatty acids by PC2. A higher similarity was observed for the guppy fed fish oil and linseed oil based feeds (Figure 36).

The PCA analysis has revealed clearly the correlation among samples and variables based on fatty acid composition. PC1 contains 41% of the original information of the data set, and the loadings indicated that there were significant contributions of n-6 PUFA and HUFA variables with positive loadings and of n-3 HUFA and MUFA variables, with negative loadings. The second PC described 31% of the total

variance and presented high positive loadings for saturated fatty acid variables. Moreover, the PUFA and HUFA variables presented high negative loading in the second principal component. The figure 37 exhibited the effect of different diets on the muscle fatty acid composition of respective fish groups. A close correlation was observed in the fish fed on Diet-FO, Diet-SO and Diet-LO with respect to their feeds. But, a clear difference was noticed in the case of fish fed Diet-CO which was loaded with high amount of SAFA. This can be explained based on the disappearance of SAFA in the muscle of the fish due to their oxidation.

Similarly, the correlations among the muscle, egg and fry fatty acid profiles of the guppy could also be examined. The plot of PC1 and PC2 was used in this study, since these two components summarized more variation (64%) in the data than any other pair of components. The scores and loading plots for PC1 vs. PC2 are presented in Bi-plot (Figure 38 and 39). Definite clusters for each of the four treatments were observed with each group contained the samples of muscle, egg and fry of the same treatment. This clustering has clearly demonstrated the correlation of parental body fatty acids with egg fatty acids and with that of resulting fry. The correlation was more evident when the PCA was subjected to the fatty acid data of egg and fry (Figure 39) indicating the influence in all the treatments.

Conclusions

- Of the three feeds, which are being used in the guppy farming, only Diet-3 with the required amounts of protein and lipid levels resulted better growth and reproductive performance of female guppy. The protein level in the Diet-3 was found to be above the recommended level, signifying the possibility of reducing the production cost since the protein source used in this diet was fishmeal, which is an expensive feed ingredient.

- The present study also revealed that the use of feeds (such as Diet-1 and Diet-2) with inadequate nutrients could be one of the reasons for poor reproductive performance observed in the guppy farming.

- Based on the results of growth and reproductive performance of the female guppy reared on Diet-2 and Diet-3, it can be concluded that dietary lipid levels play a major role in growth and reproduction of guppy.

- The reflection of fatty acid composition of guppy muscle, egg and embryo with those of the dietary fatty acid levels highlights the significance of broodstock nutrition with respect to inclusion of desired fatty acids.

- Both the experiments carried out in the present study clearly proved that the availability of n-3 HUFA either in the form of dietary source or endogenously produced from the dietary precursors enhance the growth and reproductive performance of female guppy.

- In view of the importance of HUFA, these beneficial fatty acids in the muscle and ovary of the guppy can be conserved by supplementing coconut oil which provides short chain fatty acids and those can be spared due to their preferential beta oxidation to meet the energy requirement.

- The production of fry with elevated levels of EPA (3.88%) and DHA (13.92%) in the present study demonstrates the potential of using these HUFA enriched fry (feeder guppy) as live feed for high value fishes.

- The identification of metabolites of n-3 and n-6 biosynthetic pathways in the guppy fed with the essential fatty acids established the enzyme activity of $\Delta 5$ and $\Delta 6$ desaturases and elongases, Since guppy completes its life cycle within short period of time, it could be used as a model for study of the fatty acid metabolism.

- The multivariate analysis of forty fatty acid variables carried out in the present study demonstrated that principal component analysis is an important tool to investigate the relationship of fatty acid contents in muscle, eggs and fry of guppy reared on different diets. The PCA has helped in the better understanding of the data as it has taken into account all the variables simultaneously.

Feeding strategies of ornamental fish are based on extrapolations of nutrient requirements and practices derived from food fish under intensive cultured conditions aimed at maximum growth in a short time period. The palatability, digestibility values of nutrients in feed ingredients and diets are not yet established in ornamental species. Availability of these values would not only result in least cost diet formulation for ornamental species, but would also be a valuable tool in reducing pollution of the living environment. Further investigations are required to test the palatability using different feed ingredients to find out the best combination of feed ingredients to formulate a cost effective feed for guppy. It is also needed to determine the actual optimized ratios of various dietary lipid sources to find out the best and cost effective combination on growth and reproduction of guppy.

Summary

6. SUMMARY

A conservative estimate of the annual wholesale value of the world trade in ornamental fish puts it at more than US$ 1 billion. Though there are about 1500 different species of ornamental fish that are traded annually, the quantities are dominated by only few species. Four of the top 10 species are live-bearing tooth carps belonging to the family Poeciliidae. Farming of Poeciliidae, (guppies, swordtails. platies and mollies), is now an established industry in several Asian countries. Among them, the guppy is considered as the most popular aquarium fish.

The guppy farming industry has been facing several problems of low brood size, low survival of fry, short broodstock replacement period and long period of brood interval. Review of literature indicates that there is a lack of information on the nutritional requirements of ornamental fish. Most of the nutritional studies have been carried out on protein requirements and few studies have been carried out on the lipid requirements of ornamental fish. In the ornamental fish industry, farmers believe that the live-bearer ornamental fish have the ability to use any kind of feed for their growth and reproduction. The farmers use either unbalanced or over balanced feeds to feed guppy without knowing the nutritional status in the feed.

In this background, the present study was undertaken with the following objectives: to evaluate the diets currently being used in the guppy fish farming on growth and reproduction of female guppy; to study variation in the fatty acid profile of female guppy during maturation in response to different dietary lipids; to study composition, accumulation and utilization of egg fatty acids in guppy and to investigate the effects of varying dietary fatty acid levels on the growth, gonadal development, fecundity and fry survival.

To fulfill the stated objectives, two feeding trials were carried out in the sequential order. In the first experiment, three feeds used in the guppy farming in South Asia were evaluated for their effects on growth and reproductive performance of

females. The feeds namely Diet-1, 2 and 3 contained 18.26%, 29.27% and 43.60% of protein and 4.17%, 4.55% and 9.47% of lipid respectively. Compared to the recommended nutritional requirements (30-40% protein and 9-11% lipid), Diet-1 did not contain the required protein and lipid levels while Diet-2 did not contain the required lipid level, Whereas, Diet-3 contained required amount of protein and lipid.

The growth and reproductive performance were evaluated based on growth parameters, gonadal development and fry production. The guppy fed Diet-3 had shown significantly ($p<0.05$) higher length and weight gain and specific growth rate than that of the fish fed Diet-1 and 2. The feed conversion ratios for the fish fed Diet-1, Diet-2 and Diet-3 were 3.5, 2.21 and 1.7 respectively. The ovary weight and ovary volume of guppy fed on Diet-3 were also significantly ($p<0.05$) higher than those on diets 1 and 2. The ovum diameter of the fish fed diet-3 was significantly ($p<0.05$) higher than the fish fed Diet-1. The absolute fecundity values were 4.28, 7.98 and 18.44 for the fish fed Diet-1, Diet-2 and Diet-3 respectively. The number of fry produced and their survival were also significantly ($p<0.05$) higher in the guppy fed Diet-3. The highest mean weekly fry production values were also observed in the guppy fed Diet-3. The fish fed Diet-1 started producing fry three weeks later compared to the fish fed other two diets.

The Diet-3, which fulfilled the required levels of protein and lipid compare to other diets had shown better growth and reproductive performance of female guppy. Thus, while a feed is formulated for guppy, the basic dietary requirements especially protein and lipid levels should be taken into account. The present study demonstrated that the use of feeds with inadequate nutrients could be one of the reasons for poor reproductive performance and this will lead to allocate more breeding area to fulfill the production targets especially in the commercial level guppy farming. On the other hand, the amount of protein available in the Diet-3 was found above the recommended level and nutritionally over balanced feeds will increase the production cost.

It has been observed that dietary fatty acids play a major role in growth and reproduction of fish. Fatty acids are used as a source of energy, as structural

components of membranes. Poly unsaturated fatty acids (PUFA) have been reported to regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes. In the present study total lipid was extracted by following the Folch (1957) method. The lipids were extracted from feeds, initial fry, muscle of fish after maturation, eggs and embryos of female guppy in the first experiment. The extracted lipid lipids were converted to fatty acid methyl ester (FAME), which were analyzed by GC-MS equipped with DB-WAX capillary column with helium as carrier gas. A total of 32 fatty acids were identified in the study and it was observed that the EPA, DHA, n-3 PUFA, n-3 HUFA and n-3/n-6 ratios were significantly higher in Diet-3 and the muscle and ovary of the fish fed this diet.

Literature survey to the best of our knowledge reveals that no work has been carried out to study the effect of dietary fatty acids on growth and reproduction of guppy and changes in fatty acid profile of muscle, egg and resulted fry of female guppy. Therefore, the second experiment was designed to determine the fatty acid composition of different organs in different developmental stages and to examine the effect of dietary fatty acids on the growth and reproductive performance of guppy. Four iso-nitrogenous, iso-calorific, iso-lipidic purified diets were formulated with 38% protein and 10% lipid. Coconut oil (CO), sunflower oil (SO), linseed oil (LO), and cod liver oil (FO) were used as the different lipid sources respectively to prepare the feeds Diet-CO, Diet-SO, Diet-LO and Diet-FO. The oils were selected in such a way to yield the diets with different types of fatty acid profiles: Diet-CO with 76.66% saturated fatty acids (SAFA), Diet-SO with 41.87% linoleic acid (18:2 n-6), Diet-LO with 35.84% α-linolenic acid (18:3 n-3) and Diet-FO with 17.69% n-3 HUFA (EPA and DHA) levels. In this experiment a total number of 34 fatty acids were identified.

The results of the second experiment clearly demonstrated that the guppy fed Diet-FO showed the highest total and standard lengths, weight gain and significantly higher specific growth rate after three months rearing period compared to the fish fed other diets, since Diet-FO contained high level of n-3 and n-6 HUFA especially significantly higher levels of EPA and DHA while other three diets did not contain any

HUFA level. The guppy fed Diet-SO and Diet-LO showed the poor growth and feed utilization compared to the fish fed Diet-FO and Diet-CO. The guppy fed Diet-FO, which had the highest amount of n-3 HUFA showed the lowest FCR indicating the importance of n-3 HUFA on growth and feed utilization of fish.

Reproductive performance in terms of ovary length, width, height, ovary weight, and GSI (%) values were significantly higher in the guppy fed Diet-FO. However, there were no significant differences with respect to ovum diameter. The guppy fed Diet-CO showed significantly higher ovarian development compared to the fish fed Diet-SO and Diet-LO. This may be possibly due to the significantly higher level of n-3 HUFA present in the eggs of the guppy fed Diet-CO and selective retention of n-3 HUFA in the muscle and egg of guppy resulting from preferential oxidation of short chain fatty acids.

The metabolites of n-3 pathway, 18:3, 18:4, 20:3, 20:4, 20:5, 22:5 and 22:6 were found in significant amount in the guppy fed Diet-LO. Though the Diet-LO did not contain any DHA, significantly higher levels observed in the guppy indicating the activity of desaturates and elongases in conversion of 18:3 n-3 precursor to DHA. With respect to n-6 pathway, the amounts of its metabolites (namely 18:2, 18:3, 20:3, 20:4, 22:4 and 22:5) were recorded. Though the Diet-SO did not contain any AA level, significantly higher amount of AA was found in the fish fed Diet-SO due to the desaturation and elongation of 18:2 precursor to AA. This clearly demonstrates the biosynthetic capability of guppy to convert essential fatty acids to HUFA. Since guppy complete its life cycle within short period of time it could be used as a model for study of fatty acid biosynthesis pathways.

Regression analysis showed that there were significant correlations between, final mean weight of female and mean fry production (r^2=0.89, P<0.05), mean weight of female and number of ova (r^2=0.87, P<0.05), dietary ALA/LA ratio and muscle ALA/LA (r^2=0.97, P<0.05), muscle ALA/LA ratio and egg ALA/LA (r^2=0.98, P<0.05), egg ALA/LA ratio and fry ALA/LA (r^2=0.98, P<0.05), diet n-3/n-6 and muscle n-3/n-6

(r^2=0.93, P<0.05), muscle n-3/n-6 and egg n-3/n-6 (r^2=0. 95, P<0.05) and egg n-3/n-6 and fry n-3/n-6 levels (r^2=0.92, P<0.05). These correlations clearly indicate that the dietary fatty acids reflect the fatty acid profile of muscle, egg and resulting fry of guppy.

Principal component analysis (PCA), performed on the data matrix of fatty acid composition of the diets used in the study and the fatty acid profiles of fry, muscle and eggs of guppy reared on different dietary lipids, revealed the relationship among samples and the responsible variables in the form of "score" and "loading". The "score" plot clearly demonstrated the differences among the diets and "loading" plot revealed the fatty acids, which were responsible for making this difference. This study has proved that the PCA is an important tool to investigate the relationship of fatty acid contents in muscle, eggs and fry of guppy reared on different diets. The PCA has helped in the better understanding of the data as it has taken into account all the variables simultaneously.

It can be concluded from the present study that the use of feeds with inadequate nutrients could be one of the reasons for poor reproductive performance observed in the guppy farming. Further, the availability of n-3 HUFA either in the form of dietary source or biosynthesis from the dietary precursors enhanced the growth and reproductive performance of female guppy. In view of the importance of HUFA, these fatty acids in the muscle and ovary of the guppy can be conserved by supplementing coconut oil which provides short chain fatty acids those can be spared due to their preferential b oxidation to meet the energy requirement. The identification of metabolites of n-3 and n-6 biosynthetic pathways in the guppy fed with the essential fatty acids established the enzyme activity of Δ5 and Δ6 desaturases and elongases, Since guppy completes its life cycle within short period of time, it could be used as a model for study of the fatty acid metabolism.

References

6. REFERENCES

Abi-Ayad, S., Melard, M.E.A. and Kestemont, P., 1997. Effects of n-3 fatty acids in Eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistance. *Aquac. Int.*, 5: 161-168.

Aki, T., Shimada, Y., Inagaki, K., Higashimoto, H., Kawamoto, S., Shiget, S., Ono, K. and Suzuki, O., 1999. Molecular cloning and functional characterization of rat D6 fatty acid desaturase. *Biochem Biophys. Res. Commun.*, 255: 575–579.

Al Hafedh, Y.S., Siddiqui, A.Q. and Al-Saidy, M.Y., 1999. Effects of dietary protein levels on gonad maturation, size and age at first maturity, fecundity and growth of Nile tilapia. *Aquac. Int.*, 7: 319–332.

Almansa, E., Perez M.J., Cejas, J.R., Badia, P., Villamados R. and Lorenzo A., 1999. Influences of brood stock gilthead sea bream (*Sparus aurata* L) dietary fatty acids on egg quality and egg fatty acids composition through out the spawning season. *Aquaculture*, 170: 323-336.

Anderson, A.J., Arthington, A.H. and Anderson S., 1990. Lipid classes and fatty acid composition of the eggs of some Australian fish. *Comp. Biochem. and Physiol., B* 96: 267-270

AOAC, 1995. Official Methods of Analysis of Association of Official Analytical Chemists, international, 16th edition. AOAC, Arlington, Virginia, USA.

APHA-AWWA-WEF, 1998. Standard methods for the Examination of Water and Wastewater, 20th edn. (ed. Clesceri, L.S., Greenberg, A.E. and Eaton A. D.). American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC.

Ashton, H.J., Farkvam, D.O. and March, B.E., 1993. Fatty acid composition of lipids in the eggs and alevins from wild and cultured chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences,* 50: 648-655.

Bagenal, T.B., 1973. Fish fecundity and its relations with stock and recruitment. *Rapp. P.v.Reun. Cons. perm. int. Explor. Mer.*, 164: 186-198.

Bell, M.V., Henderson, R.J. and Sargent, J.R., 1986. The role of polyunsaturated fatty acids in fish, *Comp. Biochem. Physiol.*, 83B: 711–719.

Bell, J.G., Tocher, D.R. and Sargent, J.R., 1994. Effect of supplementation with 20:3 (n-6), 20:4 (n-6) and 20:5 (n-3) on the production of prostaglandins E and F of the 1-2 and 3-series in turbot (*Scophthalmmus maximus*) brain astroglial cell in primary culture. *Biochimica. Et. Biophysica Acta.,* 1211: 335-342.

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M. and Carillo M., 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from seabass (*Dicentrarchus labrax*). *Aquaculture*, 149: 107-119.

Bell, J.G., 1998. Current aspects of lipid nutrition in fish farming. In:Black, K.D.,Pickering, A.D. (Eds) Biology of Farmed Fish. Sheffield Academic Press, Sheffield, pp 114-145.

Bell, M.V., Dick, J.R. and Porter, A.E.A., 2001. Biosynthesis and tissue deposition of docosahexaenoic acid (22:6n-3) in rainbow trout (*Oncorhynchus mykiss*). *Lipids*, 36: 1153– 1159.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R. and Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and hepatic fatty acid metabolism. *J. Nutr.*, 132: 222– 230.

Bell J.G. and Sargent J.R., 2003. Arachidonic acid and aquaculture feeds: current status and future opportunities. *Aquaculture*, 218: 491-499.

Berge, G. M., Ruyter, B. and Asgard, T., 2004. Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquaculture,* 237: 365–380.

Berglund, I., 1995. Effects of spring temperature and feeding regime on sexual maturation in Atlantic salmon (*Salmo salar* L.) male parr. In: Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish (Goetz, F.W. & Thomas, P. eds), pp. 170–172. Fish Symposium 95, Austin, TX, USA.

Bezard, J., Blond, J.P., Bernard, A. and Clouet, P., 1994. The metabolism and availability of essential fatty acids in animal and human tissues. *Reprod. Nutr. Dev.*, 34: 539-568.

Blom, J.H. and Dabrowski, K. 1995. Reproductive success of female rainbow trout (*Oncorhynchus mykiss*) in response to graded dietary ascorbyl monophosphate levels. *Biol. Reprod.*, 52: 1073–1080.

Blom, J.H. and Dabrowski, K. 1996. Ascorbic acid metabolism in fish: Is there a maternal effect on the progeny? *Aquaculture*, 147: 215-224.

Blom, J.H., Dabrowski, K., and Ebeling, J., 2000. Vitamin C requirements of the angelfish *Pterophyllum scalare*. *J. World Aquac. Soc.*, 31: 115–118.

Boonyaratpalin, N. and Lovell, R.T., 1977. Diet preparation for aquarium fishes, *Aquaculture*, 12: 53-62.

Breden, F., Scott, M. and Michel, E., 1987. Genetic differentiation for anti-predator behaviour in theTrinidad guppy *Poecilia reticulata*. *Animal Behaviour*, 35: 618-620.

Breder, C.M. and Coates, C.W., 1932. A preliminary study of population stability and sex ratio of *Lebistes*. *Copea*, 3: 147-155.

Brodtkorb, T., Rosenlund, G., and Lie, O., 1997. Effects of dietary levels of 20:5n-3 and 22:6n-3 on tissue lipid composition in juvenile Atlantic salmon *Salmo salar,* with emphasis on brain and eye, *Aquacult. Nutr.*, 3: 175-187.

Bromage, N.R., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 100: 141–166.

Bromage, N.R., 1995. Broodstock management and seed quality: general considerations. Pages 1-24 in N.R.Bromage and J.R.Roberts editors. Broodstock management and egg and larval quality. Black-well Scientific Publications, London.

Brooks, S., Tyler, C.R. and Sumpter, J.P. 1997. Egg quality in fish: what makes a good egg? *Rev. Fish Biol. Fish.*, 7: 387–416.

Bruce M., Oyen, F., Bell, G., Asturiano, J.F., Farnandale, B., Carrillo, M., Zanuy, S., Ramos, J. and Bromage, N.R., 1999. Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n-3 and n-6 highly unsaturated fatty acid to reproductive performance, *Aquaculture*, 177: 85-97.

Bulkley R.V., 1997. Diethylstibestrol in catfish feed. *Trans. Am. Fish Scoi.*, 101: 537-539.

Buzzi, M., Henderson, R.J. and Sargent, J.R., 1996. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. *Biochim. Biophys. Acta.*, 1299: 235–244.

Carillo, M., Zanuy, S., Oyen, F., Cerda, J., Navas, J.M. and Ramos, J., 2000. Some criteria of the quality of the progeny as indicators of physiological broodstock fitness. In: Proceedings of the seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM and FAO, *Zaragoza* (Spain), 1999, pp 1-9.

Castell, J.D., Sinnhuber, R.O., Wales, J.H. and Lee, D.J., 1972. Essential fatty acid requirements of juvenile seabass *Lates calcarifer* . Paper presented at Third International symposium on Feeding and Nutrition in fish. Toba, Japan, August 20- September 1, 1989.

Cerda´, J., Carrillo, M., Zanuy, S., Ramos, J. and Higuera, M., 1994. Influence of nutritional composition of diet on sea bass, *Dicentrarchus labrax* L., reproductive performance and egg and larval quality. *Aquaculture*, 128: 345–361.

Chaiyapechara, S. Liu, K.K.M., Barrows, F.T., Hardy, R.W. and Dong F.M., 2003. Proximate composition lipid oxidation and sensory characteristics of fillets from rainbow trout *Oncorhynchus mykis* fed diets containing 10% to 30% lipid. *Journal of World Aquaculture Society*, 34: 266-277.

Charlon, N., Durante, H., Escaffre, A.M. and Bergot, P., 1986. Alimintation artificielle des larves de carpe (*Cyprinus carpio*). *Aquaculture*, 54: 83-88.

Cho, H.P., Nakamura, M.T. and Clarke, S.D., 1999. Cloning expression and nutritional regulation of the human Δ6 desaturase. *J. Biol. Chem.*, 274: 471–477.

Chong, A.S.C., Hashim, R. and Ali, A.B., 2000. Dietary protein requirements for discus (*Symphysodon* spp.). *Aquac. Nutr.*, 6: 275–278.

Chong, A.S.C. Ishak, S.D., Osman, Z. and Hashim, R., 2004. Effect of dietary protein levels on the reproductive performance of female swardtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture*, 234: 381-392.

Chou, B.S. and Shiau, S.Y., 1996. Optimal dietary lipid level for growth of juvenile hybrid tilapia *Oreochromis niloticus* & *Oreochromis aureus*. *Aquaculture,* 143: 185-195.

Choubert, G. and Blanc, J.M., 1993. Muscle pigmentation changes during and after spawning in male and female rainbow trout, *Oncorhynchus mykiss*, fed dietary carotenoids. *Aquat. Living Resour.*, 6: 163–168.

Choubert, G., Blanc, J.M. and Poisson, H., 1998. Effects of dietary keto-carotenoids (canthaxanthin and astaxanthin) on the reproductive performance of female rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquacult. Nutr.*, 4: 249–254.

Christiansen, R. and Torrissen, O.J., 1997. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 153: 51–62.

Cook, H.W., 1996. Fatty acid desaturation and chain elongation in eukaryote. In: Vance, D.E., Vance, *J.E. Biochemistry of Lipids, Lipoproteins and Membranes,* 12: 129-152.

Cowey, C.B., Bell, J.G., Knox, D., Fraser, A. and Youngson, A., 1985. Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). *Lipids*, 20: 567-572.

Cowey, C.B., 1993. In: Nutrition and practice Colloq. INRA No.61. Institut National de la Recherche Agronomique, Paris. p. 227.

Craik, J.C.A. and Harvey, S.M., 1984. Egg quality in rainbow trout. The relation between egg viability, selected aspects of egg composition, and time of stripping. *Aquaculture*, 40: 115-134.

Cumaratunga, P.R.T. and Mallika, K.L.G.P., 1991. Effects of different levels of dietary protein and a legume Vigna catiang on gonadal development in *Oreochromis niloticus* (L.). In: Fish Nutrition Research in Asia (De Silva, S.S. ed.), pp. 125–133. Proceedings of the Fourth Asian Fish Nutrition Workshop, Asian Fisheries Society Special Publication 5. Asian Fisheries Society, Manila, Philippines.

D'Andrea, S., Guillou, H., Jan, S., Catheline, D., Thibault, J..N., Bouriel, M., Rioux, V. and Legrand, P., 2002. The same rat D6-desaturase not only acts on 18- but also on 24-carbon fatty acids in very-long-chain polyunsaturated fatty acid biosynthesis. *J. Biochem.*, 364: 49– 55.

Dabrowski, K., 1977. Protein requirements of grass carp fry (*Ctenopharyngodon idella* Val.). *Aquaculture*, 12: 63-67.

Dabrowski, K. and Poszyczyfiski, P., 1988. Comparative experiments on starter diets for grass carp and common carp. *Aquaculture*, 69: 317-322.

Dahlegren, B.T., 1979. The effects of population density on fecundity and fertility in guppy, *Poecilia reticulata* (Peters). *J. Fish. Biol.*, 15: 71-91.

Dahlegren, B.T. 1980. The effects of three dietary protein levels on the fecundity in the guppy. *Poecilia reticulata* (Peters). *J. Fish Biol.*, 16: 83-97.

Daikoku, T., Yano, I. and Masui, M., 1982. Lipid and fatty acid compositions and their changes in the different organs and tissues in guppy, *Poecilia reticulata* on sea water adaptation. *Comp. Biochem. Physiol.*, 73A, 2: 167-174.

De Antueno, R.J., Knickle, L.C., Smith, H., Elliot, M.L., Allen, S.J., Nwaka, S. and Winther, M.D., 2001. Activity of human D5 and D6 desaturases on multiple n-3 and n-6 polyunsaturated fatty acids. *FEBS Lett.* 509:77– 80.

De Silva, S.S. and Anderson, T.A., 1995. Fish Nutrition in Aquaculture. Chapman & Hall Aquaculture Series 1, Chapman & Hall, London, UK.

De Silva, S.S. and Radampola, K., 1990. Effect of dietary protein level on the reproductive performance of *Oreochromis niloticus.* In: The Second Asian Fisheries Forum (Hirano, R. & Hanyu, I. eds), pp.559–563. Asian Fisheries Society, Manila, Philippines.

De Silva, S.S., Gunasekara, R.M. and Austin, C.M., 1997. Changes in the fatty acid profiles of hybrid red tilapia, *Orechromis mossambicus* × *O. niloticus*, subjected to short-term starvation, and a comparison with changes in seawater raised fish. *Aquaculture*, 153: 273-290.

Degani, G., 1991. The effect of diet, population density and temperature on growth of larvae and juvenile *Trichogaster trichopterus* (Bloch and Schneider 1901) *J. Aquacult. Trop.*, 6: 135-141.

Degani, G., 1993. Growth and body composition of jevenile *Pterophylum scalare* at different densities and diets. *Aquacult. Fish Manag.*, 24: 725-730.

Degani G. and Gur, N., 1992. Growth of juvenile *Trichogaster leerii* (Bleeker 1852) on diets with various protein levels. *Aquacult. Fish Manag.*, 23: 161-166.

Degani, G. and Yehuda, R., 1996. Effects of diets on reproduction of angel *fish Pterophylum scalare* (Chchlidae) *Indian J. Fish*, 43: 121-126.

Depeche, J. and Schoffeniels, E., 1975. Changes in electrolytes, urea and free amino acids of *Poecilia reticulata* embryos following high salinity adaptation of the viviparous female. *Biochem. System. et. Ecol.*, 3: 111-119.

Dildine, G.C., 1936b. The effect of light and temperature on the gonads of *Lebistes*. *Anat. Rec.*, 67: 61-66.

Duray, M., Khono, H. and Pascual, F., 1994. The effect of lipid enriched broodstock diets on spawning and on egg and larval quality of hatchery-bred rabbit fish (*Siganus guttatus*) *Philipp.Sci.*, 31: 42-57.

Dussault, G.V. and Kramer, D.L., 1981. Food and feeding behaviour of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Can. J. Zool.*, 59: 684-701.

Dzikowski, R., Hulata, G., Karplus, L. and Harpaz, S., 2001. Effect of temperature and dietary L-carnitine spplementation on reproductive performance of female guppy (*Poecilia reticulata*). *Aquaculture,* 199: 323-332.

Earle, K.E., 1995. The nutritional requirements of ornamental fish. Vet. Q. 17 (Suppl. 1), S53–S55.

Elangovan, A. and Shim, K.F., 1997. Growth response of juvenile *Barbodes altus* fed isocaloric diets with variable protein levels. *Aquaculture,* 158: 321–329.

El-Sayed, A.F.M., Mansour, C.R. and Ezzat, A.A., 2003. Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. *Aquaculture,* 220: 619–632.

Emata, A.C. and Borlongan, I.G., 2003. A practical broodstock diet for the mangrove red snapper, *Lutjanus argentimaculatus. Aquaculture,* 225: 83– 88.

Endler, J.A., 1980. Natural selection on color patterns in *Poecilia reticulata. Evolution*, 34: 76-91.

Eskelinen, P., 1989. Effects of different diets on egg production and egg quality of Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 79: 275–281.

Falk-Petersen, S., Sargent, J.R., Fox, C., Falk-Petersen, I. B., Haug, T. and Kjrsvik, E., 1989. Lipids in Atlantic halibut (*Hippoglossus hippoglossus*) eggs from planktonic samples in Northern Norway. *Marine Biology*,101: 553-556.

Fernandaz-Palacios, H., Izquierdo, M.S. Robaina, L., Valencia, A., M. and Vergara J., 1995. Effect of n-3 HUFA level in broodstock diets on the egg quality of gilthead seabream (*Sparus surata*), *Aquaculture*, 132: 325-337.

Fernandaz-Palacios, H., Izquierdo, M.S., Robaina, L., Valencia, A. M. and Montero, D., 1997. The effects of dietary protein and lipid from squid and fishmeals on egg quality of broodstock for Gilhead seabream (*Sparus aurata*). *Aquaculture*, 148: 233-246.

Fernando, A.A., Phang, V.P.E. and Chan, S.Y., 1991. Diets and feeding regimes of Poeciliid fishes in Singapore. *Asian Fish Soc.*, 4: 99-107.

Fernando, A.A. and Phang, V.P.E., 1994. Freshwater Ornamental Fish Aquaculture in Singapore Singapore Polytechnique, Singapore, 123pp.

Finn, R.N., Henderson, J.R. and Fyhn, H.J. 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). II. Lipid metabolism and enthalpy balance. *Marine* Biology, 124: 371-379.

Fiogbé, E.D. and Kestemont, P., 1995. An assessment of the protein and amino acid requirement in goldfish (*Carassius auratus*) larvae. *J. Appl. Ichthyol.*, 11: 282–289.

Folch, J., Lees, M. and Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.*, 226: 497-509.

Fracalossi, D.M., Allen, M.E., Nichols, D.K. and Oftedal, O.T., 1998. Oscars, *Astronotus ocellatus*, have a dietary requirement for Vitamin C. *J. Nutr.* 128: 1745–1751.

Francis, D.S., Turchini, G.M., Jones, P.L. and De Silva, S.S., 2006. Effects of dietary oil source on growth and fillet fatty acid composition of Murray cod, *Maccullochella peelii peelii*, *Aquaculture*, 253: 547-556.

Fraser, A.J., Gamble, J.C. and Sargent, J.R. 1988. Challenges in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). *Marine Biology*, 99: 307-313.

Fremont, L., Leger, C. and Petridou, B., 1984. Effects of a polyunsaturated fatty acid deficient diet on profiles of serum vitellogenin and lipoprotein in vitellogenic trout (*Salmo gairdneri*) *Lipids,* 19(7): 522-528.

Furuita, H., Tanaka, H., Yamamoto, T., Shiraishi, M. and Takeuchi, T., 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 187: 387–398.

Furuita, H., Tanake H., Yamamoto, T., Suzuki, N. and Takeuchi, T., 2002. Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus. Aquaculture*, 210: 323-333.

Ghioni, C.,Tocher, D.R., Bell, M.V., Dick, S. and Sargent, J.R., 1999. Low C18 to C20 fatty acid elongase activity and limited conversion of stearidonic acid, 18:4n-3, eicosapentaenoic acid, 20:5n-3, in a cell line from the turbot, *Scophthalmus maximus. Biochim. Biophys. Acta.*, 1437: 170–181.

Gisbert, E., Williot, P. and Orvay-Castello, F., 2000. Influence of egg size on growth and survival of early stages of Siberian sturgeon (*Acipenser baeri*) under small-scale hatchery conditions. *Aquaculture*, 183: 83–94.

Gibson, M.B., 1954. Upper lethal temperature relation of the guppy, *Lebistes reticulates*. *Can. J. Zool.*, 32: 393-407.

Godin, J.G.J. and Briggs, S.E. 1996. Female mate choice under predation risk in the guppy. *Animal Behaviour*, 51: 117-130.

Guillaume, J., 1997. Protein and amino acids. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, vol. 6.World Aquaculture Society, Louisiana, USA, pp. 26–50.

Gunasekera, R.M., Shim, K.F. and Lam, T.J., 1995. Effect of dietary protein level on puberty, oocyte growth and egg chemical composition in the tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 134: 169–183.

Gunasekera, R.M., Shim, K.F. and Lam, T.J., 1996a. Effect of dietary protein level on spawning performance and amino acid composition of eggs of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 146: 121–134.

Gunasekera, R.M., Shim, K.F. and Lam, T.J., 1996b. Influence of protein content of broodstock diets on larval quality and performance in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 146: 245–259.

Gunasekera, R.M. and Lam, T.J., 1997. Influence of dietary protein level on ovarian recrudescence in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture,* 149: 57–69.

Gunasekera, R.M., Shim, K.F. and Lam, T.J., 1997. Influence of dietary protein content on the distribution of amino acids in oocytes, serum and muscle of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 152, 205–221.

Halver, J.E., and Hardy, R.W., 2002. Fish Nutrition. third ed. Academic Press, San Diego, CA, USA.

Hardy, R.W., Masumoto, T., Fairgrieve, W.T. and Stickney, R.R., 1989. The effects of dietary lipid source on muscle and egg fatty acid composition and reproductive performance of coho salmon (*Oncorhynchus kisutch*). In: Proceedings of Third International Symposium on Feeding and Nutrition in Fish (Takeda, M. & Watanabe, T. eds), pp. 347–355. Toba, Japan.

Harel, M. Tandler, A. and Kissil, G.M., 1992. The kinetics of nutrient incorporation in to body tissues of gilthead seabream *Sparus aurata* females and subsequent effects on egg composition and egg quality. *Isr. J. Aquacult-Bimidgeh*, 44 (4): 127.

Hashim, R., Ahyaudin, A. and Saat, N.A.M., 1992. Improvement of growth and feed conversion of hybrid catfish (*Clarias gariepinus*×*C. macrocephalus*) fry fed with diets supplemented with live *Tubifex. J. Aqua. Trop.*, 7: 239-248.

Hastings, N., Agaba, M., Tocher, D.R., Leaver, M.J., Dick, J.R., Sargent, J.R. and Teale, A.J., 2001. A vertebrate fatty acid desaturase with Δ5 and Δ6 activities. *Proc. Natl. Acad. Sci.*, U. S. A. 98: 14304–14309.

Hemre, G.I. and Sandnes, L. 1999. Effect of dietary lipid on muscle composition in Atlantic salmon *Salmo salar. Aquacult. Nutri.*, 5: 9-16.

Henderson, R.J. and Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research*, 26: 257-276.

Henderson, R.J., 1996. Fatty acid metabolism of fresh water fish with particular reference to polyunsaturated fatty acids. *Arch. Anim. Nutr.*, 49: 5-22

Henderson, R.J., Park, M.T. and Sargent, J.R., 1995. *Fish Physiol. Biochem.*14, 223.

Hepher, B., 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, UK.

Hester, F.J., 1964. Effects of food supply on fecundity in the female guppy, *Lebistes reticulates* (Peters) *Jour. Fish Res. Bd. of Can.*, 21: 757-764.

Higgs, D.A. and Dong, F.M., 2000. Lipids and fatty acids In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture, Wiley, New York, pp476-496.

Hillestad, M., Johnsen, F., Austreng, E. and Asgard, T., 1998. Long-term effects of dietary fat level and feeding rate on growth, feed utilization and carcass quality of Atlantic salmon. *Aquacult. Nutri.*, 5: 9-16.

Huang,Y.S., Chaudhary, S.,Thurmond, J., Bobik, E.G.,Yuan, L., Chan, G.E., Kirchner, S.J., Mukerji, P. and Knutson, D.S., 1999. Cloning of D12- and D5-desaturases from *Mortierella alpina* and recombinant production of gama-linolenic acid in *Saccharomyces cerevisiae. Lipids*, 34: 649– 659.

Ibeas, C., Cejas, J., Gomez, T., Jerez, S. and Lorenzo, A., 1996. Influence of dietary n-3 highly unsaturated fatty acids levels on juvenile gilthead sea bream growth and tissue fatty acid composition. *Aquaculture*, 142: 221-235.

Ibeas, C., Izquierdo, M.S. and Lorenzo, A., 1994. Effect of different levels of n-3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead sea bream. *Aquaculture*, 127: 177-188.

Ismihan, K., Orhan, A.K., and Karayucel, S., 2006. Effect of temperature on sex ratio in guppy *Poecilia reticulata* (Peters 1860). *Aquaculture Research:* 37: 139-150.

Izquierdo, M.S., 1996. Essential fatty acids requirements of cultured marine fish larvae. *Aquac. Nutr.* 2: 183–191.

Izquierdo, M.S., Fernandez-Palacios, H. and Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture,* 197: 25-42.

Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., and Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: Growth performance, tissue composition and flesh quality. *Aquacult. Nur.* 9: 397-407.

James, R. and Sampath, K., 2002. Effect of different feeds on growth and fecundity in ornamental fish *Betta splendens* (Regan). *Israeli J. Aquacult-Bamidgeh*, 49: 279-285.

James, R. and Sampath, K., 2004. Effect of feeding frequency on growth and fecundity in ornamental fish *Betta splendens* (Regan). *Israeli J. Aquacult-Bamidgeh,* 56: 138-147.

James, R., Muthukrishnana, J. and Sampath, K., 1993. Effect of food quality on temporal and energitics cost of in *Cyprinus carpio* (Cyprinidae). *J. Aqua. Trop.*, 8: 47-53.

James, S., and. Janssens, Geert P.J., 2003. Nutrient requirements of ornamental fish *Aquat. Living Resour.*, 16: 533–540.

Jauncey, K., 1982. The effects of varying dietary protein levels on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Saratherodon mossambicus*). *Aquaculture,* 27: 43-54.

Jobling, M., 1998. Feeding and nutrition in intensive fish farming. pp 68-113 In: K.Black, A.D.Pickering (eds). *Biology of farmed fish*. Sheffield Acad. Press Sheffield.

Jobling, M., Johnsen, H.K., Pettersen G.W. and Henderson R.J., 1995. Effect of temperature on reproductive development in Arctic charr, *Salvelinus alpinus* (L.). *Journal of Thermal Biology* 20: 157-165.

Jobling, M., 2001. Nutrient partitioning and the influence of feed composition on body composition. In: Houlihan, D., Boujard, T., Jobling, N. (Eds.), Food intake in Fish. Blackwell, Oxford, pp. 354-375.

Jobling, M., 2004. Are modifications in tissue fatty acid profiles following a change in diet the result of dilution? Test of a simple dilution model. *Aquaculture*, 232: 551-562.

Johnson, P.H., and Cross, J.A., 1980. Effects of sexual maturation and sex steroid hormone treatment on the temperature preference of the guppy *Poecilia reticulata* (Peters) Can. J. Zool., 58: 586-588.

Kairanta, J.K. and Ackman, R.G., 1981. Total lipids and lipid classes of fish roe. *Comp. Biochem. Physiol.*, 69B: 725-729.

Kairanta, J.K. and Linko, R.R., 1984. Fatty acids in the roe lipids of common food fishes. *Comp. Biochem. and Physiol.* B 79: 331-334.

Kanazawa, A., 1985. Essential fatty acid lipid requirement of fish. In: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds), Nutrition and Feeding in Fish. Academic Press, London, pp 281-298.

Khan, M.A. and Jafri, A.K., 1994. Replacement of fishmeal with soybean meal in diet formulated for fingerlings carp *Labeo rohita* (Hamilton) pp.663-666. In: 3rd Asian Fish Forum, Manila, Philippines.

Khozin-Goldberg, I, Cohen, Z., Pimenta-Leibowitz, M., Nechev, J. and Zilberg, D., 2006. Feeding with arachidonic acid-rich triacylglycerols from the microalga *Parietochloris incisa* improved recovery of guppies from infection with *Tetrahymena* sp. *Aquaculture*, 255: 142-150.

Kithsiri, H.M.P., Saurabh, S. and Venkateshwarlu, G., 2005. Breeding and culture of live-bearing ornamental fish, *Aqua International*, 21-25.

Kjorsvik, E., Mangor-Jensen, A. and Holmefjord, I., 1990. Egg quality in fishes, *Adv. Mar. Biol.*, 26: 71-113.

Knox, D., Bromage, N.R., Cowey, C.B. and Springate, R.C. 1988. The effect of brood stock ration size on the composition of rainbow trout egg (*Salmo gairdneri*). *Aquaculture*, 69: 93-104.

Kobayashi, H. and Iwamatsu, T., 2002. Fine structure of the storage micropocket of spermatozoa in the ovary of the guppy *Poecilia reticulata*. *Zool Sci* , 19: 545–555.

Kruger, D.P., Britz, P.J. and Sales, J., 2001. Influence of varying dietary protein content at three lipid concentrations on growth characteristics of juvenile swordtails (*Xiphophorus helleri* Heckel 1848). *Aquar. Sci. Conserv.* 3: 275-280.

Lahnsteiner, F., Weismann, T. and Patzner, R.A., 1999. Physiological and biochemical parameters of egg quality determination in lake trout, *Salmo trutta lacustris. Fish Physiol. and Biochem.*, 20: 375-388.

Lambert, J.G.D. and Van Ooordt, P.G.W.J., 1965. Preovulatory corpora lutea or corpora atretica in the guppy, *Poecilia reticulata.* a histological and histochemical study, *Gen. Comp. Endocrinol.*, 5: 693-694.

Lanari, D., Poli, B.M., Ballestrazzi, R., Lupi, P., D'Agaro, E. and Mecatti, M., 1999. The effects of dietary fat and NFE levels on growing European sea bass *(Dicentrarchus labrax* L.). Growth rate, body and fillet composition, carcass traits and nutrient retention efficiency. *Aquaculture*, 179: 351–364.

Lavens, P., Lebegue, H., Jaunet, A., Brunel, P. and Sorgeloos, P., 1999. Effects of dietary essential fatty acids and vitamins on egg quality in turbot broodstocks. *Aquacult. Internl.*, 7: 225-240.

Lee, O.K. and Sang, M.L., 2005. Effects of dietary protein lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvigraco. Aquaculture,* 243: 323-329.

Lee, S.M., 2001. Review of the lipid and essential fatty acid requirements of rockfish (*Sebastes schlegeli*). *Aquacult. Res.*, 32: 8-17.

Lee, S. M., Lee, J. H. and Kim, K. D., 2003. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture,* 225: 269–281.

Liley, N.R., 1968. The endocrine control of reproductive behaviour in the female guppy *Poecilia reticulata* Peters, *Anim. Behav.*, 16: 318-331.

Liley, N.R. and Seghers, B.H., 1975. Factors affecting the morphology and behaviour of guppies in Trinidada. In: Baerends, G., Beer, P., Manning, C. (Eds.), Function and evolution in behaviour. Oxford Univ. Press, Oxford, England, 92-118.

Lim, L.C. and Wong, C.C., 1997. Use of the rotifer, *Brachionus calyciflorus* Pallus, in freshwater ornamental fish larviculture. *Hydrobiologia,* 358: 269–273.

Lim, L.C., Sho, A., Dhert, P. and Sorgeloos, P., 2001. Production and application of on-grown *Artemia* in freshwater ornamental fish farm. *Aquac. Econ. Manage.* 5: 211–228.

Lim, L.C., Cho, Y.L., Dhert, P., Wong, C.C., Nelis, H. and Sorgeloos, P., 2002a. Use of decapsulated Artemia cysts in ornamental fish culture. *Aquacult. Res.*, 33: 575-589.

Lim, L.C., Dhert, P., Chew, W.Y., Dermaux, V., Nelis, H., and Sorgeloos, P., 2002b. Enhancement of stress resistance of the guppy *Poecilia reticulata* through feeding with vitamin C supplement. *J. World Aquac. Soc.*, 33: 32–40.

Ling, S., Hashim, R., Kolkovski, S. and Chong, A.S.C., 2006a. Effect of varying dietary lipid and protein levels on growth and reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquacult. Res.,* 1-9.

Ling, S., Kuah, M.K., Muhammad, T.S.T., Kolkovski, S. and Chong, A.S.C., 2006b. Effect of dietary HUFA on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNA in female swordtail *Xiphophorus helleri* (Poeciliidae). *Aquaculture,* 261: 204-214.

Lochmann, R.J. and Phillips, H., 1994. Dietary protein requirements of juvenile golden shiners (*Notemigonus crysoleucas*) and gold fish (*Carassius auratus*) in aquaria. *Aquaculture*, 128: 277-285.

Lovell, R.T., 1989. Nutrition and Feeding of Fish. Von Nostrand-Reinhold, NY, USA.

Macartney, A., 1996. Ornamental fish nutrition and feeding. In: Kelly, N.C., Wills, J.M. (Eds.), Manual of Companion Animal Nutrition and Feeding. British Small Animal Veterinary Association, Gloucestershire, UK, pp. 244–251.

Magurran, A.E. and Seghers, B.H., 1991. Variation in schooling and aggression amongst guppy *Poecilia reticulata* population inTrinidad. *Behaviour,* 118: 214-234.

Manissery, J.K., Krishnamurthy, D., Gangadhara, B. and Nandeesha, M.C., 2001. Effect of varied levels of dietary protein on the breeding performance of common carp *Cyprinus carpio*. *Asian Fish Sci.*, 14: 317–322.

Mazzorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E. Jordan, N., Rees, J., Papanikos, N., Porter, M. and Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, 227: 21-33.

Meffe, G.K. and Snelson, J.E.E., 1993. Lipid dynamics during reproduction in two live-bearing species, *Gambusia holbrooki* and *Poecilia latipinna*. *Can. J. Fish and Aqua. Sci.*, 50: 2185-2191.

Metcoff, J., 1986. Intracellular amino acid levels as predictors of protein synthesis. *J. Am. Coll. Nutr.*, 5, 107–120.

MillerC, L., Davis, D.A. and Phelps, R.P., 2005. The effects of dietary protein and lipid on growth and body composition of juvenile and sub-adult red snapper, *Lutjanus campechanus*. *Aquacult. Res.*, 36: 52-60.

Milton, D.A. and Arthington A.H., 1983. Reproductive biology of *Gambusia affinis* holbrooki Baird and Girard, *Xiphophorus helleri* (Gunther) and *X. maculates* (Heckel) (Pisces: Poecillidae) in Queensland. *Australia. J. Fish Biol.*, 23: 23-41.

Moore, P.K., 1995. Prostanoids: Pharmocological, Physiological and clinical Relevance, Cambridge University press In: Effect of brood stock nutrition on reproductive performance of fish, Izquierdo, M.S., H.Fernandez-Palacios and A.G.J.Tacon, *Aquaculture,* (2001) 197: 25-42.

Mourente, G. and Odriozola, J..M., 1990. Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream *Sparus aurata* L., *Fish Physiol. Biochem.*, 8: 93–101.

Mourente, G. and Tocher, D.R., 1993. The effects of weaning on to a pelleted diet on brain lipid and fatty acid composition in post-larval gilthead sea bream (*Sparus aurata* L.). *Comp. Biochem. Physiol.*, 104A(3): 605-611.

Mourente, G. and Vazquez, R., 1996. Changes in the content of the total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. *Fish Physiol. and Bioche.,* 15: 221-235.

Moyle, P.B. and Cech, J.J., 1982. Fishes: An Introduction to Ichthyology. Prentice-Hall, Inc., New Jersey.

Mustafa, T. and Srivastrava, K.C., 1989. Prostaglandins (eicosanoids) and their role in ectothermic organisms. *Advance of Comparative Environmental Physiology*, 5: 157-207.

Napier, J.A., Hey, S.J., Lacey, D.J., and Shewry, P.R., 1998. Identification of a *Caenorhabditis elegans* Δ6 fatty acid-desaturase by heterologous expression in *Saccharomyces cereviciae. Biochem. J.,* 330: 221-236.

NAQDA, 2005. Aquaculture News, National Aquaculture Development Authority, Sri Lanka, 2: 2pp.

National Research Council, N.R.C., 1977. Nutrient Requirements of Warmwater Fishes. National Academy Press,Washington, DC, USA.

National Research Council, N.R.C., 1983. Nutrient Requirements of Warmwater Fishes and Shellfishes. National Academy Press, Washington, DC, USA revised ed.

National Research Council, N.R.C., 1993. Nutrient Requirements of Fish. National Academy Press,Washington, DC, USA.

Navas, J.M., Trush, M., Ramos, J., Bruce, M., Carillo, M., Zanuy, S.,and Bromage, N., 1996. The effect of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European seabass (*Dicentrarchus labrax*) *Proc. V Int. Symp. Rep.Physiol.Fish.* Austin, TX, 1995 pp. 108-110.

Ng, W.J., Kho, K., Sim, T.S., Ho, J.M., and Tay, S.H., 1993. Preliminary estimation of tropical ornamental fish metabolite production rates. *Aquaculture*, 110: 263–269.

Ogino, C., Chiou, J.Y., Takeuchi, T., 1976. Protein nutrition of fish VI. Effects of dietary energy sources on the utilization of proteins by rainbow trout and carp. *Bull.Jap.Sci.Fish,* 42: 213-218.

Ogino, C. and Saito, K., 1979. Protein nutrition of fish. The utilization of dietary protein by young carp. *Bull. Jap. Soc. Sci. Fish*, 36: 250-254.

Olvera-Novoa, M.A., Gasca-Leyva, E., and Martinez-Palacios, C.A., 1996. The dietary protein requirements of *Cichlasoma synspilum* Hubbs, 1935 (Pisces: Cichlidae) fry. *Aquacult. Res.*, 27: 167–173.

Ould, El., Kebir, M.V., Barnathan, G., Siau, Y., Miralles, J. and Gaydou, E.M., 2003. Fatty acid distribution in muscle, liver and gonads of rays (*Dasyatis marmorata*, *Rhinobatos cemiculus* and *Rhinoptera marginata*) from the East Tropical Atlantic Ocean. *J. of Agric. and Food Chem.*, 51: 1942-1947.

Owen, J.M., Adron, J.A., Middleton, C., and Cowey, C.B., 1975. Elongation and desaturation of dietary fatty acids in turbot (*Scophthalmus maximus*) and rainbow trout (*Salmo gairdneri). Lipids*, 10: 528–531.

Ozorio, R.O.A., Valante, L.M.P. Pousao-Ferreira, P. and Oliva-Teles, A., 2006. Growth performance and body composition of white seabream (*Diplodus sargus*) juveniles fed diets with different protein and lipid levels. *Aquacult. Res.*, 37: 255-263.

Pannevis, M.C., 1993. Nutrition of ornamental fish. In: Burger, I.H. (Ed.), The Waltham Book of Companion Animal Nutrition. Pergamon Press, Oxford, pp. 85–96.

Pannevis, M.C., and Earle, K.E., 1995. Nutrition of ornamental fish: water soluble vitamin leaching and growth of *Paracheirodon innesi*. *J. Nutr.*, 124: 2633S–2635S.

Pathmasothy, S., 1985. The effect of three diets with variable protein levels on ovary development and fecundity in *Leptobarbus hoevenii*. In: Fish Nutrition Research in Asia (Cho, C.Y. ed.), pp. 107–112. IDRC, Ottawa, Canada.

Patino, R. and Sullivan C.V., 2002. Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiol. Biochem.*, 26: 57–70.

Pereira, J.O.B., Reis-Henriques, M.A., Sanchez, J.L. and Costa, J.M., 1998. Effect of protein source on the reproductive performance of female rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquacult. Res.*, 29: 751–760.

Pozernick, M. and Wiegand, M.D., 1997. Use of canola oil in the feed of larval and juvenile goldfish, *Carassius auratus*, *Aquacult. Res.*, 28: 75-83.

Rainuzzo, J.R., Reitan, K.I., and Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture,* 155: 103–115.

Raj, V.S.D., and Jesily, S., 1996. The interrelationship of NH3–N excretion with changing pH, selected metallic ions and food in *Puntius tetrazona*. *J. Ecobiol.* 8: 9–15.

Reznick, D.N., 1982. Genetic determination of offspring size in the guppy (*Poecilia reticulata*). *American Naturalist,* 120: 181-188.

Reznick, D.N., Bryga, H. and Endler, J.A., 1990. Experimentally induced life history evolution in a natural population. Nature 346,357-359. guppy (*Poecilia reticulata*). *Aquaculture*, 184: 233-245.

Reznick, D.N., Butler, M.J. and Rodd, H., 2001. Life history evolution in Guppies. VII. The comparative ecology of highland low-predation environment. *American Naturalist*, 157: 126-140.

Rodriguez, C., Perez, J.A., Diaz, M., Izquierdo, M.S., Fernandez-Palacios, H., and Lorenzo, A., 1997. Influence of the EPA/DHA ratio in rotifers on gilthead sea bream (*Sparus aurata*) larval development. *Aquaculture,* 150: 77-89.

Rose, S.M., 1959. Population control in guppies. *Am. Midl. Nat.*, 62: 474-481.

Rose, S.M. and Rose, F.C., 1965. The control of growth and reproduction in fresh water organisms by specific products. *Mitt. Int. Verein. Theor. Angew. Limnol.* 13: 21-35.

Rosen, D.E. and Gordon, M., 1953. Functional anatomy and evolution of male genitalia in Poeciliid Fishes. *Zoologica*, 38: 1–47.

Royes, J.B. and Chapman, F., 2003. Preparing your own fish feeds. University of Florida, IFAS Extension Report 4: 1-12.

Salhi, M., Hernandez-Cruz, C. M., Gonzalez, M. and Fernandez-Palacios, H., 1999. Effect of different dietary polar lipid levels and different n-3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture*, 179: 253-263.

Sampath, K., 1984. Preliminary report on the effects of frequency in *Channa striatus*. *Aquaculture*, 40: 301-306.

Sampath, K. and Pandian, T.J., 1984. Interaction of feeding frequency and density on feed utilization in air breathing murrel, *Channa striatus*. *Proc. Indian Acad. Sci.*, 93: 445-453.

Santiago, C.B., Aldaba, M.B. and Laron, M.A., 1983. Effect of varying dietary crude protein levels on spawning frequency and growth of *Sarotherodon niloticus* breeders. *Fish. Res. J. Philippines*, 8: 9–18.

Santiago, C.B., Camacho, A.S. and Laron, M.A., 1991. Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding in floating cages. *Aquaculture*, 96: 109–117.

Santiago, C.B. and Reyes, O.S., 1993. Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) broodstock. *J. Appl. Ichthyol.*, 9: 33–40.

Sargent, J.R., Henderson R. J., and Tocher D.R., 1989. The lipids. In: Halver, J.E. (Ed.), Fish Nutrition. Academic Press, San Diego, CA, pp. 154–219.

Sargent, J. R., Bell, M.V. and Tocher, D.R., 1992. Docosahexaenoic acid and development of brain and retina in marine fish. In: Omega-3 Fatty Acids: Metabolism and Biological Effects. (ed. By C.A. Drevon, Baksaas, I.and Krokan, H.E.,) pp 139-149.

Sargent, J. R., 1995. In: "Broodstock management and Egg and Larval quality" (N. R. Bromage and R. R. Roberts, (Eds.). *Black well Science, Oxford.* Pp 253-272.

Sargent, J.R., Bell, J.G., Mc-Evoy, L.A., Tocher, D. and Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture,* 177: 191-199.

Sargent, J.R., McEvoy, L., Este´ vez, A., Bell, G., Bell, M., Henderson, J. and Tocher, D. 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, 179: 217–229.

Sargent, J. R., Henderson, R. J., and Tocher, D. R., 2002. The Lipids. In: Halver, J., Hardy, E. (Eds), Fish Nutrition. Academic Press, Elsevier, San Diego, California, USA,. 181-257.

Sasayama, Y. and Takahashi, H., 1972. Effect of starvation and unilateral astration in male goldfish, *Carassius auratus,* and a design of bioassay for fish gonadotropin using starved goldfish . *Bull. Fac. Fish.*, Hokkaido Univ. 22: 267-283.

Satia, B.P., 1974. Quantitative protein requirements of rainbow trout. *Prog. Fish Cult.,* 36: 80-85.

Seghal, H.S., and Toor, H.S., 1991. Offspring fitness and fecundity of an Indian major carp, *Labeo rohita* (Ham.) in relation to egg size. *Aquaculture,* 97: 269–279.

Seghers, B.H., 1974a. Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia*, 14: 93-98.

Seghers, B.H., 1974b. Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution*, 28: 486-489.

Sellner, P.A. and Hazel, J.R., 1982. Time course of changes in fatty acid composition of gills and liver from rainbow trout (*Salmo gairdneir*) during thermal acclimation. *J. Exp. Zool.*, 221: 159-168.

Sheenan, H., Tatiyana, S and Segev, R., 2005. Effect of feeding guppy fish fry (*Poecilia reticulata*) diets in the form of powder versus flakes, *Aquacult. Res.,* 36: 996-1000.

Sheppard, A., and Bromage, N.R. 1998. Intensive fish farming. BSP professional books, Oxford, pp 404.

Shikano, T., Chiyokubo, T., Nakadate, M. and Fujio, Y., 2000. The relationship between allozyme heterozygosity and salinity tolerance in wild and domestic populations of the guppy (*Poecilia reticulata*). *Aquaculture,* 184: 233-245.

Shim, K.F. and Chua, Y. L., 1986. Some studies on the protein requirements of guppy, *Poecilia reticulata* (Peters). *J. Aquaricul. and Aqua. Sci.,* 4: 79-84.

Shim, K.F., Landesman, I. and Lam, T.J., 1989. Effect of dietary protein on growth ovarian development and fecundity in the dwarf gourami *Colisa lalia* (Hamilton) *J. Aquacult. in the Topics,* 4: 111-123.

Shim, K.F. and Ng, S.H., 1988. Magnesium requirements of the guppy (*Poecilia reticulata* Peters). *Aquaculture*, 73: 131–141.

Shim, K.F. and Ho, C.S., 1989. Calcium and phosphorus requirements of guppy *Poecilia reticulata. Nippon Suisan Gakkaishi,* 55: 1947–1953.

Shim, K.F. and Lee, T.L.. 1993. Zinc requirements of the guppy (*Poecilia reticulata* Peters). *J. Aquac. Trop*,. 8: 81–90.

Shim, K.F. and Ong, S.I., 1992. Iron requirement of the guppy (*Poecilia reticulata* Peters). *J. Aquar. Aquat. Sci.*, 6: 33–40.

Shimma,Y., Suzuki, R.,Yamaguchi, M. and Akiyama, T., 1977. On the lipids of adult carp raised on fishmeal and SCP feeds, and hatchabilities of their eggs. *Bull. Freshwater Fish. Res. Lab*,. 27: 35–48.

Shoemaker, H.H., 1947. A laboratory study of fish populations. *Trans. Am. Fish. Soc.*, 74: 351-359.

Siddiqui, A.Q., Al-Hafedh, Y.S. and Ali, S.A., 1998. Effect of dietary protein level on the reproductive performance of Nile tilapia, *Oreochromis niloticus* (L.). *Aquacult. Res.*, 29: 349–358.

Silliman, R.P., 1968. Interaction of the food level and exploitation in experimental fish populations. *Fish.Bull. Fish Wildl. Serv.* U.S., 66: 425-439.

Silversand, C., Norberg, B. and Haux, C. 1996. Fatty-acid composition of ovulated eggs from wild and cultured turbot (*Scophthalmus maximus*) in relation to yolk and oil globule lipids. *Marine Biology*, 125: 269-278.

Singh, R. and Dhawan, A., 1996. Effect of formulated diets on the growth and ovarian maturation in common carp *Cyprinus carpio* communis Linn. *Indian J. Fish.*, 43: 349–353.

Singh, T., 2005. Emerging trends in world ornamental fish trade, *INFOFISH International,* 3: 15-18.

Sprecher, H. and Chen Q., 1999. Polyunsaturated fatty acid biosynthesis: a microsomal-peroxisomal process. *Prostaglandins Leukot Essent Fatty Acids.* 60: 317-321.

Sprecher, H., Luthria, D. L., Mohammed, B.S. and Baykousheva, S.P., 1995. Re-evaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *J. Lipid Res.*, 36: 2471-2477.

Sprecher, H., 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta.*, 1486: 219–231.

Steffens, W., 1989. Principles of Fish Nutrition. Ellis Horwood, Chichester, UK, pp. 384.

Stolk, A., 1951. Histo-endocrinological analysis of gestation phenomena in the Cyprinodont *Lebistes reticulatus* Peters. II. The corpus luteum-cycle during pregnancy. *Proc. K. ned. Akad. Wet.*, C54: 558-565.

Svardson, G., 1949. Natural selection and egg number in fish. *Rep. Inst. Freshwat. Res. Drottningholm*, 29: 115-122.

Tacon, A.G.J., Stafford, E.A. and C.A. Edwards, 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout (*Oncorhynchus mykiss*), *Aquaculture*, 35: 187-199.

Tacon, A.G.J., 2004. Use of fishmeal and fish oil in aquaculture: A global perspective. *Aquat. Resour. Cult. Dev.*, 1: 3-14.

Tandler, A., Watanabe, T., Satoh, S., and Fukusho, K., 1989. The effect of food deprivation on the fatty acid and lipid profile of red seabream larvae (*Pagrus major*). *Br. J. Nutr.*, 62: 349-361

Tiku, P. E., Gracey, A. Y., MacArtney, A. I., Benyon, R. J. and Cossine, A. R. 1996. *Science,* 271: 815.

Tinoco, J., 1982. Dietary requirements and functions of α-linolenic acid in animals. *Progr. Lipid Res.* 21: 1-45.

Tocher, D.R. and Sargent, J.R., 1984. Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. *Lipids,* 19: 492-499.

Tocher, D. R., Fraser, A. J., Sargent, J. R. and Gamble, J. C., 1985. Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring (*Clupea harengus*, L.). *Lipids,* 20: 69–74.

Tocher, D.R., Leaver, M.J. and Hodgson, P.A., 1998. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. *Prog. Lipid Res.*, 37: 73–117.

Tocher, D.R. and Dick, J.R., 1999. Polyunsaturated fatty acid metabolism in a cell culture model of essential fatty acid deficiency in a freshwater fish, carp (*Cyprinus carpio). Fish Physiol. Biochem.*, 21: 257–267.

Tocher, D. R., Agaba, M., Hastings, N., Bell, J. G., Dick, J. R. and Teale, A.J., 2001. Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis nilotica*). *Fish Physiol. Biochem.*, 24: 309-320.

Townshend, T.J. and Wooton, R.J., 1984. Effects of food supply on the reproduction of the convict cichlid, *Cichlasoma nigrofaciatum. J. Fish Biol..*, 24: 91-104.

Tuner, C.L., 1937. Reproductive cycles and superfoetation in Poeciliid fishes. *Biol. Bull. Mar. Biol. Lab.*, Woods Hole, 72: 145-164.

Tyler, A.V. and Dunn, R.S., 1976. Ration, growth and measures of somatic and organ condition in relation to meal frequency in winter flounder (*Pseudopleuronectes americanus*), with hypothesis regarding population homeostasis. *J. Fish. Res. Bd. Canada*, 33: 63-75.

Tyler, C.R. and Sumpter, J.P., 1996. Ooocyte growth and development in Teleosts. *Reviews in Fish Biology and Fisheries,* 6: 287-318.

Usman, R., Laining, A., Ahmad, T. and Williams, K.C., 2005. Optimum dietary protein and lipid specifications for grow-out of humpback grouper *Cromileptes altivelis* (Valenciennes). *Aquacult. Res.*, 36: 1285-1292.

Vassallo-Agius, R., Watanabe, T., Imaizumi, H., Yamazaki, T., Satoh, S., and Kiron, V., 2001a. Effects of dry pellets containing astaxanthin and squid meal on the spawning performance of striped jack *Pseudocaranx dentex. Fish Sci.*,, 67: 667–674.

Vassallo-Agius R., Watanabe T., Yoshizaki G., Satoh S. and Takeuchi T., 2001b. Quality of eggs and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and its effects on the lipid and fatty acid components of eggs, semen and livers. *Fish Sci.*, 67: 818-827.

Vergara, J.M., Lopez-Calero, G., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.M. and Aksnes, A., 1999. Growth, feed utilization and lipid content of gilhead seabream *Sparus aurata* fed increasing lipid levels and fishmeals of different quality. *Aquaculture*, 179: 35-44.

Vinogradov, A.P., 1953. The elementary chemical composition of marine organisms (Efron and Setlow, translators), Yale University press, New Haven, 463-566.

Voss, A, Reinhart M, Sankarappa S, and Sprecher H., 1991. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of Δ4-desaturase. *J. Biol. Chem.*, 266: 19995-20000.

Voss, A, Reinhart, M, and Sprecher, H. 1992 Differences in the inter conversion between 20- and 22-carbon (n - 3) and (n -6) polyunsaturated fatty acids in rat liver. *Biochem. Biophys. Acta,*, 1127(1): 33-40.

Wallis, J.G., Watts, J.L. and Browse, J., 2002. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem. Sci.*, 27: 467– 473.

Warren, E.W., 1973. The effects of relative density upon some some aspects of the behaviour of the guppy, *Poecilia reticulata* (Peters). *J. Fish Biol.*, 5: 753-765.

Washburn, B.S., Frye, D.J., Hung, S.S.O., Doroshov, S.I. and Conte, F.S., 1990. Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 90: 179–195.

Watanabe, T., Arakawa, T., Kitajima, C., and Fujita, S., 1984. Effect of nutritional quality of broodstock diets on reproduction of red seabream. *Nippon Suisan Gakkaishi,* 50: (3), 495-501.

Watanabe, T., Itoh, A., Satoh, S., Kitajima, C. and Fujita, S., 1985. Effects of dietary protein levels and feeding period before spawning on chemical components of eggs produced by red sea bream broodstock. *Bull. Jpn. Soc. Sci. Fish.*, 51: 1501–1509.

Watanabe, T., Vassallo-Agius, R., 2003. Broodstock nutrition research on marine finfish in Japan. *Aquaculture,* 227: 35–61.

Weerasooriya, A.C., Edirisinghe, U., Silva, P. and Dematawewa, C.M.B., 1999. Feeding behaviour and acceptability of different feeds by *Puntius nigrofasciatus*. *Trop. Agric. Res.*, 11: 221–228.

Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biol. and Fish.*, 6: 259-286.

Wilson, R.P., 2002. Amino acids and proteins In: Fish nutrition. Eds, Halver J.E. and Hardy, R.W.

Yanong, R. P. E., 1999. Nutrition of ornamental fish. *Veterinary Clinics of North America: Exotic Animal Practice*, 2(1): 19-42.

Yone, Y., 1978. Essential fatty acid requirements of marine fish. *Jpn. Soc. Sci. Fish.* Dietary Lipids in Aquaculture. Koseisha-Koseik-Abu, Tokyo, pp 43-59.

Yu, T.C., Sinnhuber R.O. and Hendricks, J.D. 1979. Reproduction and survival of rainbow trout (*Salmo gairdneri)* fed linolenic acids as the only source of essential fatty acids. *Lipids*, 14: 572-575.

Zheng, X., Tocher, D. R., Dickson, C. A., Bell, J. G. and Teale, A.J., 2004. Effects of diets containing vegetable oil on expression of genes involved in polyunsaturated fatty acid biosynthesis in liver of Atlantic salmon (*Salmo salar*). *Aquaculture*, 236: 467– 483.