FATTY ACID ENRICHMENT AND POTENTIAL FOOD SOURCE FOR *Moina macrocopa* CULTIVATION

By

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ABSTRACT

FATTY ACID ENRICHMENT AND POTENTIAL FOOD SOURCE FOR *Moina macrocopa* CULTIVATION

Loh Jiun Yan

This study examined the potential of using *Moina macrocopa* in nutrient bio-encapsulation and propagation with aquaculture wastes and oil emulsions. This study discovered that *M. macrocopa* cultured with fish faeces produced the highest number of neonates as compared to those fed with *Spirulina sp.* and *Chlorella sp*. In this nutritional experiment, the arachidonic acid (AA) level of these organisms was found to increase significantly to between 28% and 38% at 12 h enrichment with 1 g L^{-1} A1 DHA Selco® (ADS) and 2 g L^{-1} canola oil (CO). The eicosapentaenoic acid (EPA) level was significantly increased to 253.3% with 1 g L^{-1} ADS after 12 h enrichment. Similarly, docosahexaenoic acid (DHA) significantly increased to 9% and 8% respectively at 1 g L^{-1} and 2 g L^{-1} of the same treatment diet (i.e. ADS) at 12 and 24 h enrichment. The overall AA, EPA and DHA levels increased when the diet concentrations were raised. AA/EPA and AA/DHA ratios could be

significantly improved by 2 g L^{-1} CO, whilst the DHA/EPA ratio was enhanced by 2 g L^{-1} squid oil (SO) after 12 h enrichment.

The demographical performances of *M. macrocopa* grown in ADS, SO, and CO were generally lower as compared to ground fish pellets (FP) and fish faeces (FF). Low concentrations of FF served as an excellent food source for *M. macrocopa* when compared to other diets tested in the study. The total offspring produced by *M. macrocopa* was the highest at 0.0625 g L^{-1} in the treatment diet of FF. An initial cultivation density of 4 individuals per 40 ml (or 100 individuals per liter culture volume) in captive condition was recommended for this cladoceran for a better aquaculture practice. In short, this study covered the most important aspects, which would provide a comprehensive dataset for an alternative solution of *M. macrocopa* propagation and utilization in hatchery.

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This project went through some major difficulties along the way which delayed its completion. However, thankful to several experienced individuals, I was able to finally complete this project.

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PERMISSION SHEET

It is hereby certified that **LOH JIUN YAN** (ID No: **07UEM08776**) has completed this thesis entitled "FATTY ACID ENRICHMENT AND POTENTIAL FOOD SOURCE FOR *Moina macrocopa* CULTIVATION" under the supervision of Assoc. Prof. Dr. Gideon Khoo (Supervisor) from the Department of Biological Sciences, Faculty of Science, Assoc. Prof. Dr. Alan Ong Han Kiat (Co-Supervisor) from the Department of Pre-clinical Sciences, Faculty of Medical and Health Sciences of Universiti Tunku Abdul Rahman, and Assoc. Prof. Dr. Hii Yii Siang from the Department of Fishery Science and Aquaculture, Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu.

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This thesis entitled "**FATTY ACID ENRICHMENT AND POTENTIAL FOOD SOURCE FOR *Moina macrocopa* CULTIVATION"** was prepared by LOH JIUN YAN and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

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DECLARATION

I hereby declare that the dissertation/thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Date _____8th February 2010_____

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LIST OF ABBREVIATIONS

HUFA	Highly-unsaturated fatty acid
PUFA	Poly-unsaturated fatty acid
MUFA	Mono-unsaturated fatty acid
SAFA	Saturated fatty acid
EFA	Essential fatty acid
AA	Arachidonic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
LnA	Linolenic acid
GLA	Gamma-linolenic acid
CLA	Conjugated linolenic acid
ATP	Adenosine triphosphate
FAME	Fatty acid methyl ester
VLDL	Very low density lipoprotein
ANOVA	Analysis of variance
ADS	A1 DHA selco ®
BW	Body weight
UV	Ultra violet
TAN	Total ammonia-nitrogen
N	Normality (equivalents per liter)
GC	Gas chromatography
FID	Flame ionized detector
RAS	Recirculating aquaculture system

C/N ratio	Carbon-to-nitrogen ratio
AIAR	Average initial age of reproduction
AL	Average longevity
GRR	Gross reproduction rate
r	The intrinsic rate of natural increase
R_o	Net reproduction rate
T	Generation time
l_x	Proportion of individual surviving to age x
m_x	Age specific fecundity
n_x	Actual number of individual alive for each class
e_x	Life expectancy
x	days
ind.	Individual

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CHAPTER 1.0

INTRODUCTION

Over the last two decades, aquaculture has gone through some significant changes, growing from small-scale homestead-level activities to large-scale commercial farming, exceeding landings from capture fisheries in many areas (NRC, 1992; FAO, 2003; DOF, 2009). In 1999, the output from capture fisheries grew at an annual average rate of 1.2%, while, output from aquaculture (excluding aquatic plants) grew at a rate of 9.1%. The latter is faster than other animal food producing systems such as fisheries and terrestrial farmed meat (FAO, 2003). This growth is expected to continue as the population grows and the per capita consumption of seafood increases while other protein source consumption decreases (USDA/ERS, 1999; Ng, 2009).

The importance of aquaculture has gained momentum in many countries, especially those whose economies rely mainly on food export. The successful operation of a farm or hatchery is attributed to the key knowledge and technical applications such as: aquaculture engineering, biotechnology, water and soil chemistry, fish physiology, disease management etc. Among these, larviculture has taken priority in the industry. Live food is a general basic requirement for fish and shellfish to grow. Small and semi-developing mouth sizes in newborns or newly hatched fries are only suitable to be fed with a certain size food.

Livefeed has become the most important secondary producers in aquatic food webs (Cary et al., 2004), and has been extensively used as high quality natural food in hatcheries for the maintenance or propagation of aquarium fishes for the past few decades (Rodolfo and Edmundo, 1980). The livefeed species most widely used in aquaculture industry are brine shrimp (*Artemia* spp.), rotifers, copepods, and *Daphnia*. *Moina* has recently become a potential livefeed, with respect to its availability, in most of the natural water resources and has a high nutritional value. This freshwater zooplankton is classified as a cladoceran. It occupies an important position in the aquatic food web especially in tropical countries and inhabits several types of water bodies throughout the world.

According to Shim and Bajrai (1982), *Moina* is a good protein source for fish fry. The survival of fish larvae reared with this zooplankton is better than with other natural foods. This cladoceran has been documented as a potential livefeed to substitute the use of the more expensive *Artemia* and other seasonal zooplankton, such as copepods, in larviculture (Das et al., 2007). However, studies on the use of *Moina* sp. as bio-carriers of nutrients for fish-larviculture are still very limited. In order to determine the potential application of this zooplankton in the larviculture industry, experiments on the propagation and nutritional enhancement of *Moina macrocopa* were carried out in this study.

1.1 Fish Faeces as a Potential Food Source for Cultivating the Water Flea, *Moina macrocopa*

In many South-East Asian countries, including Malaysia, domestic wastewater and sewage are commonly employed to cultivate cladocerans such as *Moina* sp. and *Daphnia* sp. for the aquaculture industry (Jhingran, 1991). Untreated wastewater, which has a high organic load (Cauchie et al., 1995), has been a traditional medium for raising high densities of freshwater cladocerans, and also for rotifers and copepods (Balasubramaniam and Kasturi, 1994; Roche, 1995). A recent study by Golder et al. (2007) demonstrated that human urine, cow urine, cow dung, poultry droppings, and vermin-compost could be utilized for zooplankton cultivation. Human urine was found to be a highly effective liquid nutrient source for mass production of cladocerans, which are commonly used as livefeed for larval and post-larval rearing of fish and shellfish (Alam et al., 1993a, b). Cladocerans have been found to be rich in essential nutrients, and are easily ingested and digested by larvae. The use of this type of livefeed could improve water quality by minimizing the need for artificial feeding (Tamaru et al., 1991; Sorgeloos and Lavens 1996; He et al., 2001).

The practice of fertilizing ponds with manures and organic wastes may introduce harmful microorganisms, antibiotics and hormones which may directly or indirectly affect the cultured animals as well as consumers (Sáenz et al., 2001; Nigawaba et al., 2008). However, aquaculture foods generally have a good safety record since the environment of each production cycle can

be relatively controlled. It has been the task of the aquaculture industry to hygienically produce livefeed organisms to ensure the safety of fish and crustacean larvae that possess low immunity, produce pathogen- and chemical-free stocks, and prevent disease outbreaks (Tamaru et al., 1991; Sorgeloos and Lavens 1996; He et al., 2001). Recent efforts at bioconversion of wastes have also led to products that are comparatively safer for the environment. In this study, faeces from drug-free Nile tilapia, *Oreochromis niloticus*, were harvested from a recirculating aquaculture system (RAS) and tested as a food source for the freshwater cladoceran, *Moina macrocopa*.

The objective of the experiment was:

i. To compare and determine the reproductive performance and survivorship of *Moina macrocopa* after being fed with fish faeces and lyophilized algae (*Chlorella* and *Spirulina*).

1.2 Life Table Demography of *Moina macrocopa* in relation to the Fish Wastes and Enrichment Lipid Emulsions

If this potential alternative livefeed is to be economically viable and environmentally sustainable, a cost effective means of obtaining large amount of these seasonal livefeed is required for fish larval cultivation, so that their growth performance is maintained at an optimal level during enrichment. Since a commercial supply of *Moina* is not readily obtainable from natural habitats, mass zooplankton cultivation is required for larval feeding purposes. Large scale propagation of *M. macrocopa* with green algae and yeast can be a

sustainable alternative, conversely, with a relative increase in operational costs. Animal droppings and manures have been demonstrated as effective food sources for *Moina* sp. cultivation (Golder et al., 2007; Patil et al., 2010). This approach produced large numbers of the livefeed. However, the use of animal faecal matter as a nutrient source to produce feed for fish and crustacean larvae ultimately destined for human consumption presents hygiene and food safety issues (Loh et al., 2009).

In an effort to promote sustainable aquaculture, recycling fish farm wastes from Recirculating Aquaculture Systems (RAS) which are under constant water quality and environmental protection, can provide a commercially viable and environmentally sustainable alternative without compromising on food or feed safety issues. Aquaculture effluents, especially uneaten fish food and faeces, contribute approximately 20% to 30% of the overall organic waste production from intensive aquaculture (Penczak et al., 1982; Beveridge, 1987). Most of the farm effluents are discharged directly into the waterways without further treatment. Aquaculture effluents like uneaten fish food and fish excretions contain high amounts of nutrients like phosphorus, nitrogen, and organic components (Hakanson et al., 1988; Kibra et al., 1997), as well as fish oil and other lipid components, e.g. free fatty acids, polar lipids and polyunsaturated fatty acids (Henderson et al., 1997). These unwanted waste materials could potentially be re-used as a safer and more productive alternative to terrestrial animal wastes for *M. macrocopa* propagation during larval feeding (Cheah et al., 1988; Nandini et al., 2004; Golder et al., 2007; Loh et al., 2009). Such implementation could reduce livefeed production costs

in an environmentally friendly manner, especially for large scale fish and crustacean hatcheries.

To date there has been no published research regarding the effects of aquaculture wastes for *M. macrocopa* propagation. Lipids, particularly highly unsaturated fatty acids (HUFA), have been reported to promote the growth of Cladoceran species; high zooplankton growth rates could be attainable when direct dietary sources of HUFA are available for the fast-growing zooplankton (Müller-Navarra, 1995). Therefore, the purpose of this study was to determine, through life history parameters, the growth performance of *M. macrocopa* in varying concentrations of aquaculture wastes like fish feaces, uneaten food, and common emulsified lipid media (DHA Selco®, squid oil and canola oil). The use of life table demographics provides an important tool for describing the life cycle strategies of zooplankton under continuously changing environmental conditions (Stearns, 1976). This study discusses the effects and potential of these aquaculture wastes as a cheap and safe food source for mass cultivation of *M. macrocopa*.

The objective of the experiment was:

i. To investigate the effects of aquaculture wastes and lipid emulsions on life table demography study of *Moina macrocopa*.

1.3 Effects of Concentration, Enrichment Time and Potential Enrichment Diets on the HUFA's Profile of *Moina macrocopa*

Artemia nauplii is the most widely used livefeed species in bio-encapsulation activities (Alam et al., 1991; Sargent et al., 1997; Sargent et al., 1999b; Tamaru et al., 2003). However, this livefeed involves some operational constraints, such as the high cost of importing the product in many countries. A major drawback in feeding these saltwater livefeed to freshwater cultured species is that the nauplii usually die in the freshwater after 30 to 60 min, and therefore intermittent feeding to the fish larvae is necessary by using *Artemia* nauplii (Merchie, 1996). To overcome these problems, freshwater zooplankton should be developed, e.g. *Moina* sp. specifically for freshwater fish and crustacean larval feeding (Alam et al., 1991, 1993a).

Moina macrocopa are rich in protein and nutrients. They are excellent live foods for fish and prawn larvae when compared to other livefeeds such as *Artemia* (Alam et al., 1991, 1993b). However, fatty acid contents of *Moina* are varied when they are cultivated with different culture media (Watanabe and Kiron, 1994). Like *Artemia, Moina,* for example, do not meet the HUFAs (highly unsaturated fatty acids) requirements for fish or crustaceans larvae in regards to fatty acid deficiencies (He et al., 2001). Thus, it is important to improve the nutritional quality by enriching them with HUFAs prior to feeding to fish larvae. Fatty acids have been reported to play a major role as an energy source and may influence cellular membrane functions which are in turn, vital for cell growth, differentiation, and metabolism (Sargent et al.,

1999a; Cahu et al., 2003; Tocher, 2003). Increasing the levels of dietary HUFAs in fish larvae helps to increase stress resistance (from starvation or osmotic shock) (Koven et al., 2003), assists in skeletal formation (Cahu et al., 2003), aids the development of neural and visual systems (Sargent et al., 1997, 1999b), improves survival rate (Watanabe, 1993), and enhances the pigmentation of fish larvae (Nicolaides and Woodall, 1962; Bransden et al., 2005).

In many hatcheries, common enrichment practices to enrich fatty acids composition in *Artemia* and other livefeeds such as rotifers consisted of microalgae, microalgae paste, and various marine fish oils (Naas et al., 1992; Papandroulakis et al., 2002). Improvement of fatty acid contents, particularly essential HUFAs in *Artemia* nauplii, has been achieved in many fin fish and crustacean hatcheries and laboratories (Alam et al., 1991, 1993a; Sargent et al., 1997, 1999b; Tamaru et al., 2003). Watanabe et al. (1983) and Das et al. (2007) found that the nutritional value of *Moina* could be similarly enhanced by enrichment with various culture media as demonstrated in *Artemia* nauplii and rotifer. However, there is limited information, and no notable study has been found on *M. macrocopa* enrichment, especially on the aspects of culture media selection such as potential aquaculture effluent, enrichment concentration, and appropriate treatment time.

With respect to these issues, the present study was conducted to investigate the HUFAs retention quality of *M. macrocopa* fed with two different lipid emulsions, i.e. squid and canola oil, and two different aquaculture wastes,

namely fish faeces and uneaten fish food. Fatty acids profiles of four treatment diets were compared with the commercial reference enrichment diet, A1 DHA Selco® in this study. Effects of dietary types, treatment concentration, and enrichment time on the fatty acid profile of *M. macrocopa* enriched with different lipid emulsions were analysed in the present study.

The objectives of the experiment were:

i. To investigate the enrichment capability of *Moina macrocopa* with locally available oil emulsions, and also the alternative natural sources of aquaculture effluent from the RAS system.

ii. To investigate the effects of treatment concentrations and enrichment times on the HUFA (highly unsaturated fatty acids) profile of *Moina macrocopa*.

1.4 Effects of Effluent Water from a Recirculating Aquaculture System (RAS) on the Population Growth of *Moina macrocopa*

Sustainable development in agriculture is a contemporary global issue. Recycling of water waste materials and the renewable energy from waste materials, and un-useable biological products are generated through the promotion of environmentally friendly strategies for sustenance and improvement in the quality of human life. Bio-conversion of liquid and solid wastes of animal origin has immense potential for industrial exploitation. Moreover, these bio-converted organic products are comparatively safer than chemical products to humans and the environment (Golder et al., 2007).

Some nutritional contents such as nitrogen and phosphorus are present in high amounts in liquid and solid organic wastes. For better management of organic wastes, these waste materials should be recycled in a useable form rather than eliminated in an unproductive way (Metcalfe, 1995).

Among zooplankton, *Moina* could be easily mass cultivated using organic fertilizers (Golder et al., 2007). In natural habitats, water quality, quantity and quality level of food resources, thermal conditions, competition, predation, and population density are the factors that interact to regulate the population growth of zooplankton (Gulati and DeMott, 1997). However, according to Gulati and DeMott (1997) and Nandini and Sarma (2000), parameters such as population density, food quantity and quality are the predominant factors in mass cultivation of *M. macrocopa*. Among the key factors, initial cultivation density plays a crucial role in determining production efficiency of *M. macrocopa* in a captive environment. This can be explained by the fact that intra-competition of space, food sources, and the accumulation of huge amounts of excretory products would lead to a decline in population. Therefore, in order to optimize *M. macrocopa* culture conditions using fish faeces as a potential culture medium, initial cultivation density should be determined for the livefeed production.

The objective of the experiment was:

i. To investigate the effects of initial cultivation density on the population growth of *Moina macrocopa*, which were cultivated using the potential culture medium-fish faeces.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Current Freshwater Aquaculture Status in Malaysia

Freshwater aquaculture contributed about 26% of the total aquaculture production in 2007 (DOF, 2009) with production coming from various culture systems such as ponds, former tin-mining pools, cages, and tanks per pens (18, 4, 3 and 1%, respectively) totaling 70,064 metric tons (Figure 1). The three major freshwater species farmed in Malaysia are tilapia, catfish, and carp constituting 46, 42 and 7% of the total freshwater aquaculture production, respectively (Figure 2). Other freshwater species include snakeheads (*Channa* spp.), marble goby (*Oxyeletris marmoratus*), and giant freshwater shrimp (*Macrobranchium rosenbergii*). About 32,023 metric tons of tilapia were produced in 2007. Unlike other countries where Nile tilapia is the major farmed species, the major tilapia species farmed in Malaysia is the red hybrid tilapia (*Oreochromis sp.*), accounting for about 82% of total tilapia production, and the remaining species being the Nile tilapia, *Mozambique* tilapia (*O. mossambicus*) and various other hybrids. The major farmed catfish species are the African catfish (*Clarias gariepinus* and their hybrids), *Pangasius* species, and the bagrid catfish, *Mystus nemurus*, locally known as 'Baung'. The total catfish production in 2007 was 28,875 metric tons. Various carp species such as grass carp (*Ctenopharyngodon idella*), Javanese (*Puntius gonionotus*), common species (*Cyprinus carpio*), and big head carp (*Aristichthys nobilis*),

are farmed, but the production was relatively small, amounting to about 5,005 metric tons in 2007. Aquaculture production of the giant freshwater prawn (*Macrobrachium rosenbergii*) amounted to only about 246 metric tons in 2007 (DOF, 2009; Ng, 2009).

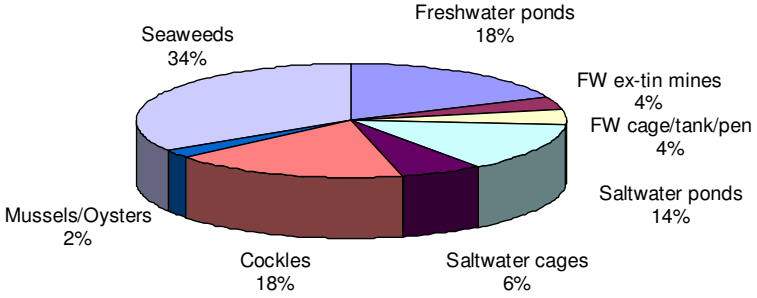

Figure 2.1: Aquaculture production in Malaysia by culture systems in 2007 (DOF 2009).

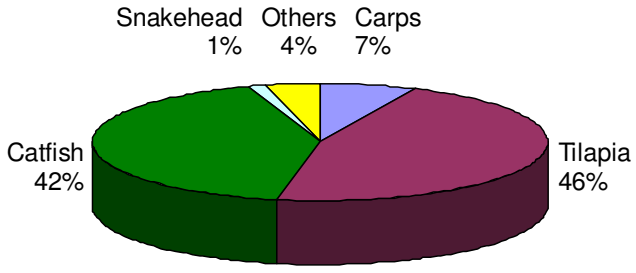

Figure 2.2: Freshwater aquaculture species cultured in Malaysia in 2007 (DOF, 2009).

2.2 Livefeed and Larviculture

Initial feeding of early larval stages is crucial in larviculture. The larvae of most fish and crustaceans feed naturally on motile prey organisms but they normally encounter problems when provided with inert or synthetic diets (Pedersen et al., 1987). Even if they are able to adapt to the diets, the low enzymatic activity and their poorly developed gastrointestinal system will restrict the digestion and absorption of formulated diets (Pedersen et al., 1987; Pedersen and Hjelmeland, 1988; Agh and Sorgeloos, 2005). Formulated diets, or inert diets, such as egg yolk suspension, milk powder, or powdered feed used for larval feeding have a tendency to pollute the water quality because the uneaten feed decays and pollutes the culture water (Lim et al., 2003). Live prey or feed is preferable as they do not have very much effect on the water quality when they are not completely consumed by the fish larvae. In terms of palatability, livefeed are indeed more preferable than artificial feeds for larval and early post larval stages of various fish and shellfish (Das et al., 2007). At the exogenous feeding stage, most fish larvae are small in size (approx. 2 to 3 mm). Their mouth gape is also small and is only suitable for ingesting small food items. This is one of the problems facing microdiet production technology (Conceição et al., 2007).

The success of the hatchery in producing fish fingerlings up to the grow-out stage is largely dependent on the availability of suitable livefeed for larviculture (Lim et al., 2003). The availability of livefeed for the different stages of larval production, has contributed to the successful fry production of

at least 60 marine finfish species and 18 species of crustacean (Dhert, 1996). Nowadays, rotifers, *Artemia*, *Moina*, *Daphnia*, copepods, and earth worms are the livefeed commonly used during the initial stage of freshwater and marine larval feeding in many countries (Murthy, 1998; Lim et al., 2003). In a normal feeding regime, newly hatched fish and crustacean larvae are fed with phytoplankton such as *Chlorella sp*., *Scenedesmus sp*., *Spirulina sp,* and dinoflagellates. As the larvae grow, the food size requirements also shift to larger items and from an algal diet to smaller zooplankton (Figure 2.3).

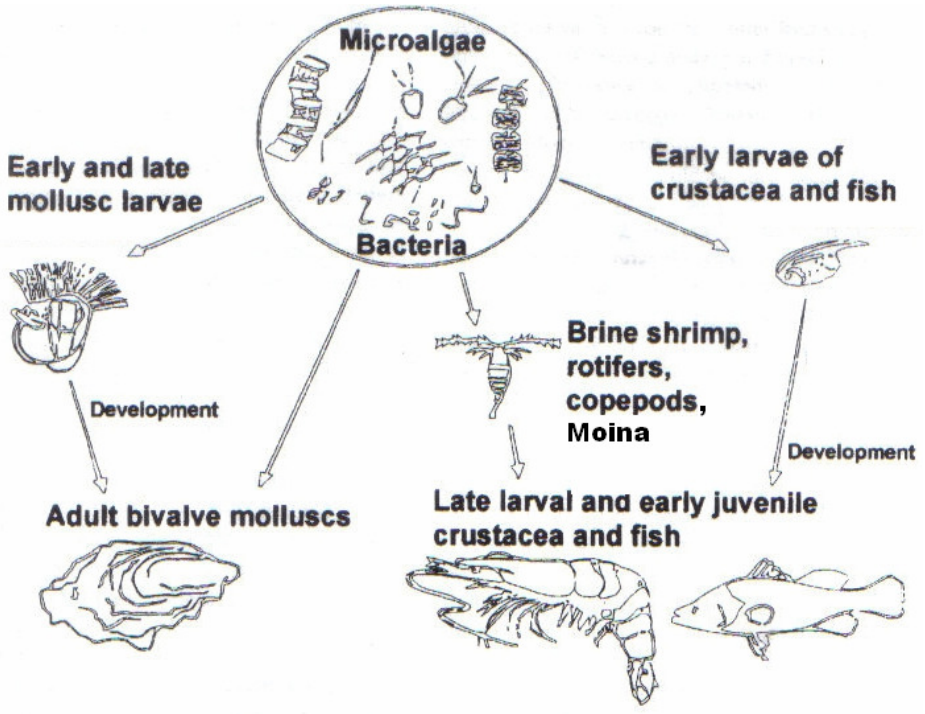

Figure 2.3: Live food chains used in marine aquaculture to feed larval and post-larval stages of fish, crustaceans and mollusks (modified from Coutteau and Sorgeloos, 1997).

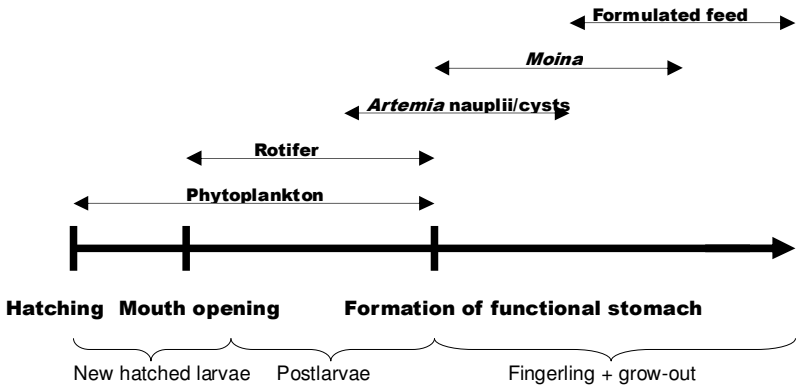

Figure 2.4: Feeding regimes for larviculture practice (modified from Sargent et al., 1997).

In most marine and freshwater fish hatcheries, the rotifer (*Brachionus plicatilis*) is widely used as starter food after phytoplankton for fish and crustacean larvae (Fulk and Main, 1991). Rotifers are then subsequently replaced by larger sized zooplankton, e.g. *Artemia*, from the fry to fingerling stages (Figure 2.4). Although *Artemia* has been used for many years, the application of *Artemia* in freshwater aquaculture still faces a lot of problems. Excessive feeding with *Artemia* should be avoided because these saltwater species easily die at low salinities and in freshwater, thus making them unsuitable for freshwater larval feeding. The resource crisis of *Artemia* in the Great Salt Lake (original source of *Artemia*) has also caused aquaculturists to seek alternative livefeed sources to substitute *Artemia* for larval feeding (He et al., 2001). Development of the culture technology for other livefeed such as copepods, *Tubifex,* and worm pupae for larval rearing has made some progress, however, the long generation time and complexities in the lifespan of these organisms make them difficult to produce in large numbers for intensive

culture systems (Stottrup and Norsker, 1997; Schipp et al., 1999; He et al., 2001; Lim et al., 2003).

Among cladoceran and copepods, most fish and crustacean larvae prefer the former because the jerky movement of cladocerans makes them more visible and attractive to fish larvae while copepods swim too fast for them to capture and consume (Mayer and Wahl, 1997). In freshwater aquaculture, cladecerans such as *Moina* and *Daphnia* have been successfully used as larval food for pond and tank cultures (Qin and Culver, 1996). Rapid population growth and short generation time of most cladocerans species makes them suitable for mass production because cladocerans reproduce asexually when the amount of food is adequate and the ambient conditions are favourable (Innes, 1997). The rapid reproduction of *M. macrocopa* and its adaptability to harsh environments such as water with high organic contents and mild salinity allows it to be used as an alternative livefeed in freshwater and even for brackish-water larviculture.

2.3 *Moina macrocopa*

Moina macrocopa is a species of freshwater cladocerans which is classified under the family Moinidae (Table 2.1). This zooplankton has important links in the aquatic food web especially in tropical countries. They are the secondary producers in the aquatic trophic hierarchy and can be found in a natural abundance in swallow swamps and rivers.

Shim and Bajrai's study (1982) on the nutritional comparisons of the artificial and natural foods for tropical fish found that *Moina sp*. are a good protein source for fish fry. Survival of the fish fry could be significantly improved when feeding on this species. According to Alam (1992), partial replacement of *Artemia* by *Moina* is able to improve overall fry growth performance of the freshwater prawn, *Macrobrachium rosenbergii*. In addition, *Moina macrcopa* has been used mainly as a live food source for carp larval rearing (Yamasaki and Uchiyama, 2001) and in the ornamental fish industry (e.g. guppies, sword tails, black mollies and platys) (Tamaru et al., 2003).

2.3.1 Morphology

Moina is ideally suited for freshwater fish fry feeding. Its gender can be easily recognized by its external morphology where the male's antennules are much longer and stouter than female (De Pauw et al., 1981). The body length of female *M. macrocopa* varies from 0.4 mm to around 1.65 mm. The observed length of the male's body is slightly smaller than the female's (0.4 to 1.13 mm), and has long graspers which are used for holding the female during copulation. Sexually mature females carry only two eggs enclosed in an ephippium which is part of the dorsal exoskeleton (Sorgeloos and Lavens, 1996). According to De Pauw et al. (1981), newly hatched young *Moina* are less than 400 µm. They are approximately the same size or only slightly larger than adult rotifers, but smaller than newly-hatched brine shrimp of the first larval stage, instar I (400 to 500 µm in length) (Van Stappen, 1996). Due

to its small size, *Moina sp*. is ideal for rearing newly hatched fish larvae, especially ornamental fishes which have small mouth sizes.

Moina belongs to the cladocera, and many of the biological and cultural characteristics that have been described for *Daphnia,* can also be applied to *Moina* (FAO, 1996). *Moina* is a tiny, transparent creature (Figure 2.5, 2.6 and 2.7). Its body comprises main structures such as the head, trunk (enclosed in a two-valve carapace), and the post abdomen. The head usually appears smooth and round in shape and ends with a slight depression. A large and spherical eye spot is located at the apex of the head and close to the anterior edge. It has numerous round and colourless lenses at the periphery of the eye spot. The eye spot is an important organ for it allows the organism to sense light and thereby locate food sources. The *Moina* has a head, strong and moveable antennules, followed by olfactory setae which are fairly long and of uniform length. The second antenna is the primary organ for locomotion such as swimming. Figure 2.5 shows the anterior part of the trunk. The post abdomen is turned ventrally and forward and bears special claws and spines to clean the carapace (FAO, 1996). Figure 2.6 shows the lateral view, and Figure 2.7 shows the dorsal view of *M. macrocopa*. The scientific classification and taxonomy of *M. macrocopa* are given in Table 2.1.

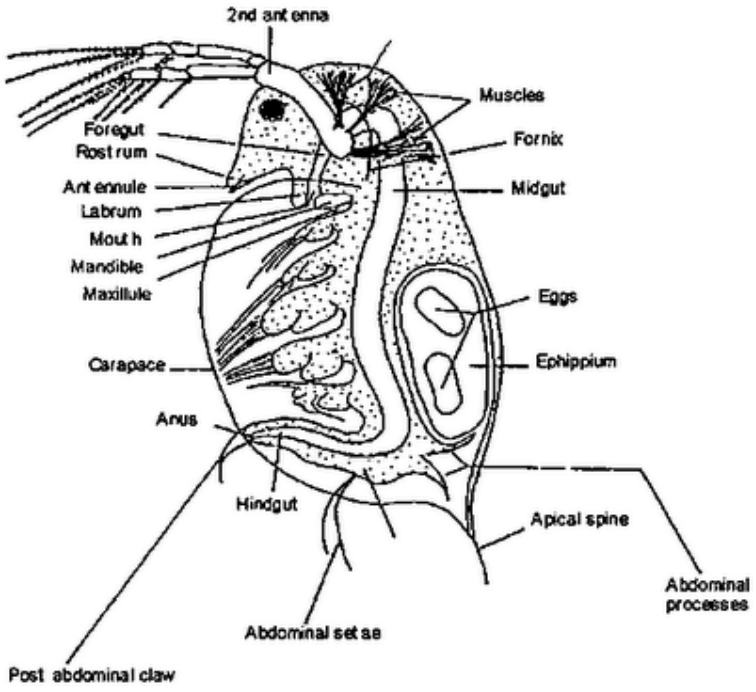

Figure 2.5: The illustration of cladocaran and her internal organs (adopted from www.fao.org).

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Table 2.1: Classification and taxonomy of *Moina macrocopa.*

Phylum	: Arthorpoda
Subphylum	: Mandibulata
Class	: Crustacea
Order	: Diplostraca
Suborder	: Cladocera
Family	: Moinidae
Genus	: *Moina*
Species	: *Moina macrocopa*

(Source: Goulden, 1968 as cited in Sick, 2003)

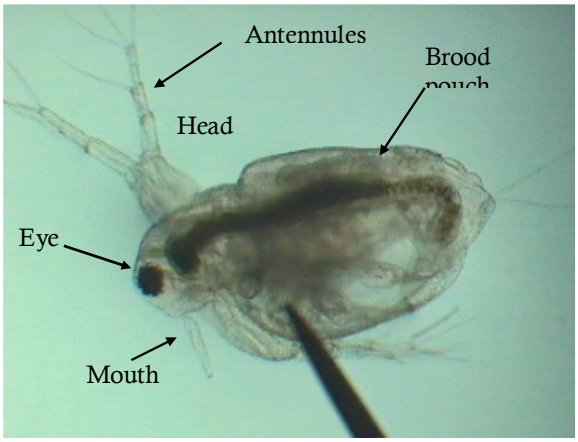


Figure 2.6: Lateral view of *Moina macrocopa* under a compound microscope at 100 x magnification (photo taken in April 2008).

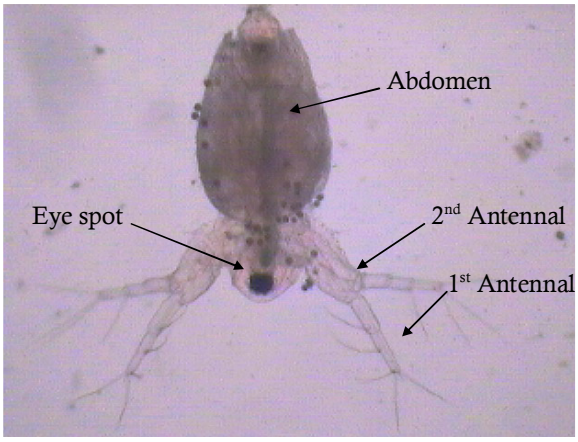


Figure 2.7: Dorsal view of *Moina macrocopa* under a compound microscope at 100 x magnification (photo taken in April 2008).

2.3.2 Ecology and Distribution

There are many species of *Moina* in the world e.g. *M. hutchinsoni, M. mongolica, M. salina, M. micrura,* and *M. macrocopa* (He et al., 2001). They are mostly found in different bodies of water, with some species (e.g. *M. mongolica* and *M. salina*) only inhabiting countries with temperate climates. *M. macrocopa* is one of the species that is commonly found in tropical

countries, for example – Malaysia. This species is found in large numbers in ponds, lakes, ditches, slow-moving streams, and swamps where highly decomposed organic materials exist. They live predominantly in temporary hyper-eutrophic ponds where they often constitute about 90% of the total zooplankton biomass (Burak, 1997). *Moina* are generally quite tolerant of the poor water quality in their habitats, such as low dissolved oxygen levels. They are able to regulate the amount of haemoglobin in their blood, causing their body colour to vary from pink to yellow to colourless, depending on the haemoglobin concentration in the blood (Kobayashi, 1981). This special regulatory mechanism makes them particularly resistant to changes in oxygen concentration, allowing them to reproduce in large quantities in water bodies heavily polluted with sewage. *Moina* has been reported to play an important role in the stabilization of sewage in oxidation ponds. However, this genus is very sensitive to some heavy metals and chemical pollutants (Chu et al., 1997).

2.3.3 Reproduction

Moina reproduce through either gamogenesis or parthenogenesis (Ahmad and Ahmad, 1992; Zadereev et al., 2003). Parthenogenesis implies that eggs laid by female *Moina* do not necessarily have to be fertilized by the male, but can still develop directly into a fully functioning organism (asexual reproduction) (Johnson, 1962), where the eggs develop immediately in the brood pouch. Figure 2.8 shows the resting egg which resulted from the changes of reproduction mode (parthenogenesis to gamogenesis) due to the unfavorable conditions or environmental stresses such as drastic changes in either: a)

photoperiod, which can affect the chromosome mechanism; b) temperature; c) food availability; d) population density, where the crowding of population resulted in accumulation of excretory products or e) predator's kairomones (Stross, 1987; Slusarczyk, 1995; He et al., 2001). Once the resting egg (Figure 2.8) is produced, the walls of the brood chamber are transformed into a protective, saddle-like ephippium. These eggs will then be cast off at the next mount (occurs during the process of metamorphosis), either separating from or remaining with the rest of the detached exoskeleton and settle down at the bottom or become attached to walls of cultured vessels. The eggs are hatched when conditions are optimal again (He et al., 2001). This phenomenon, called diapause, allows the population to survive in adverse conditions, so that the diapausing organisms are reactivated, and the population regenerates when favourable environmental conditions are established again (Zadereev et al., 2003).

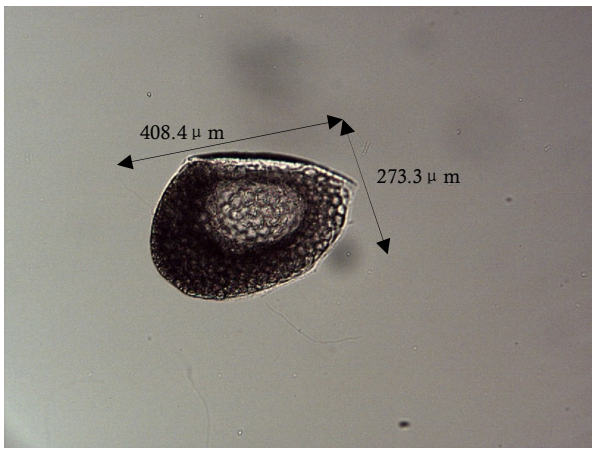


Figure 2.8: The resting egg (ephippium) under compound microscope at 400 x magnification (photo taken in March 2008).

Moina needs to undergo several moulting processes in order to grow continuously. At the first moult, the eggs are laid immediately, and the offspring are ready to be released before the next moulting occurs. Females reproduce asexually as long as the environmental conditions are favourable. Under adverse conditions, males are produced and sexual reproduction (gamogenesis) occurs. The eggs are fertilized in the capsular form (Vijverberg, 1989; Ahmad and Ahmad, 1992; Oda et al., 2004). Environmental factors such as temperature variation and availability of food sources directly affect the process of hatching, feeding, somatic growth, and reproduction of zooplankton (Lampert and Sommer, 1997). The general positive effect of temperature on the reproductive biology of zooplankton is correlated to the increase in growth rates (Vijverberg, 1980), a decrease in egg development times (Bottrell, 1975), and an increase in population growth rates (Armitage et al., 1973). Based on the study by Ahmad and Ahmad (1992), *Moina* reaches the adult stage within five days at 22 ^{o}C to 33^{o}C and a pH range of 6.6 to 6.7. Under optimal conditions, *Moina* begins to reproduce after four to seven days, with each female producing 4 to 22 broods. Broods are produced every 1.5 to 2.0 days with the females reproducing around 2 to 6 times during their entire life cycle. The male's lifespan is normally shorter than that of the females (Shim, 1988).

2.3.4 Life History and Demography

In an ecological study, the life table demographic approach is an important tool in understanding the life history strategies of zooplankton under

continuously changing environmental conditions. Parameters such as survivorship, average lifespan, life expectancy, fecundity, cumulative birth rate, net reproduction rate, intrinsic rate of natural increase, initial age of reproduction, and generation time provide valuable insights into the suitability of the ambient conditions for the zooplankton (Stearns, 1976). The definitions of some life table parameters such as survivorship, life expectancy, fecundity, net reproduction rate, intrinsic rate of natural increase, and generation time are as follows.

Survivorship is the proportion of survivors in a group, e.g., of rainbow trout, studied and followed over a time period, or the proportion of individuals in a specified group which survive from the beginning of a time interval until the end of the interval. It is often studied using life table methods (Krohne, 2001; Smith and Smith, 2003; Molles, 2005)

Life expectancy is the average number of years of life remaining at a given age. The term is most often used in the human context, but it can also be used in association with plant or animal ecology (Millar and Zammuto, 1983). The calculations are based on the analysis of life tables (also known as actuarial tables).

Fecundity is derived from the word fecund, which generally refers to the ability to reproduce. In biology and demography, fecundity is the potential reproductive capacity of an organism or population, measured by the number of gametes (eggs), seed sets, or asexual propagules. Fecundity is under both

genetic and environmental control, and is the major estimation of fitness. Fecundity is important and well studied in the field of population ecology. Fecundity can increase or decrease in a population according to current conditions and certain regulating factors. For instance, in times of hardship for a population such as a lack of food, juvenile and eventually adult fecundity has been shown to decrease (Lotka, 1913; Smith and Smith, 2003; Molles, 2005).

Net reproduction rate (NRR) is the average number of offspring that would be born to a female (or a group of females) if she passed through her lifetime conforming to the age-specific fertility and mortality rates of a given year. This rate is similar to the gross reproduction rate but takes into account the fact that some females will die before completing their childbearing years. The net reproductive rate of 'one' means that each generation of mothers has exactly enough daughters to replace themselves in the population (Krohne, 2001; Smith and Smith, 2003; Molles, 2005).

Intrinsic rate of natural increase is the rate at which a population increases in size, i.e. the change in population size over a particular period of time. The concept is commonly used in insect population biology to determine how environmental factors affect the rate at which pest populations increase (Jahn et al., 2005).

Generation time is the period of time required for the number of the population to double in size or value. It is applied to population growth which

tends to increase over time. When the relative growth rate (not the absolute growth rate) is constant, the quantity undergoes exponential growth and has a constant doubling time or period which can be calculated directly from the growth rate (Lotka, 1913; Krohne, 2001; Smith and Smith, 2003; Molles, 2005).

2.3.5 Food Requirement and Nutritional Value

In nature, *Moina* feeds on various groups of bacteria, phytoplankton, and detritus. They feed by ingesting these particulate organic substances that are filtered from water. The particles are gathered to the mouth by moving appendages located at the anterior part of body (He et al., 2001). The ingested food that remains in the digestive tract can be observed under a microscope or, if the organisms are appearing in large numbers, can be seen by the naked eye.

Moina has been reported to feed on various types of foods (He et al., 2001). Yeast has been demonstrated in mass culturing of *Rotifer* in fish farms and hatcheries (Fukusho, 1991), and now yeast is also widely used for *Moina* cultivation. Other than using yeast as culture medium, *Moina* are one of few zooplanktons which also feed on the blue green algae *Microcystis aeruginosa*, and various groups of phytoplankton such as *Chlorella sp*., *Anabaena sp*., *Scenedesmus sp.,* and *Staurastum sp*. (Rodolfo and Edmundo, 1980; Peña-Aquado et al., 2005). Combinations of *Chlorella vulgaris* and yeast as culture media are able to produce large densities of *M. macrocopa* (Peña-Aquado et al., 2005). According to Lee et al. (1985), rice bran, soya roughages, and

grain powder are also suitable to be used for culturing *Moina* in large numbers. A recent study in India has also shown that animal waste products such as human urine can be utilized to maximize the reproduction of *M. micrura* (Golder et al., 2007).

Moina has a higher protein content as compared to other livefeeds, e.g. *Artemia* (Sorgeloos and Lavens, 1996), and this makes it very suitable for rearing fish larvae. The protein content in the dry matter is 70% (Shim, 1988), which is much higher than *Daphnia magna*, which is 59.5% (Proulx and Noüe, 1985), *Brachionus plicatilis* (*Rotifer*), 38 to 42% (Tamaru et al., 1991), and *Artemia*, 59.1% (Alam et al., 1993a; Alam et al., 1993b). Tong et al. (1988) compared the essential amino acids in *M. mongolica* with other livefeed such as *Artemia* and *B. plicatilis* and found that the content of methionine in *M. mongolica* was 1.5% of the total amino acids, which is higher than in *Artemia* (0.9%) and *B. plicatilis* (0.8%). Kang et al. (2006) also found that the proportion of most essential amino acids in yeast-fed *M. macrocopa* was higher than *Artemia* and rotifers fed with the same diet. Moreover, the histidine and threonine contents of *Moina* were even higher than that of rotifers or *Artemia* fed on microalgae and commercial diets. Therefore, *Moina* may provide essential amino acids such as methionine, histidine, and threonine for fish larvae (He et al., 2001).

In a lipid study, He et al. (2001) found that the proportion of [20:5(*n*-3)] (eicosapentaenoic acid or EPA) in *M. mongolica* was 12.7% of the total fatty acids, while this fatty acid was only 2.3% and 1.9% of the total fatty acids in

Artemia nauplii and *Brachionus plicatilis*, respectively (Table 2.2). Some studies demonstrated that partial or full substitution of *Moina* with *Artemia* during larval feeding would increase the survival rate and weight gain of the larvae.

Table 2.2: Comparison of highly unsaturated fatty acids in *Moina mongolica* with *Artemia* and *Brachionus plicatilis* (adopted from He et al., 2001).

Fatty acids in percentage (%)	*M. mongolica*	*Artemia*	*B. plicatilis (Rotifer)*
16:4n-3	ND	ND	3.9
18:3n-3	0.6	27.6	10.2
18:4n-3	0.5	3.1	0.3
20:3n-3	ND	1.2	0.8
20:4n-3	2.4	0.3	1.1
20:5n-3	12.7	2.3	1.9
22:5n-3	ND	ND	0.3
18:2n-6	7.3	6.6	15.9

*ND = Non-detectable value.

2.4 *Moina* cultivation

There are four cultivation methods for *Moina* (He et al., 2001): the static batch method, the static continuous method, the aerated method, and the flow-through method.

2.4.1 Static Batch Method

Nannochloropsis oculata is commonly used as algal food for *Moina* and inoculated into 500 L fiberglass tanks with up to $^1/_5$ or ¼ of the tank volume at 2 to 5 x 10^6 cells mL^{-1} (He et al., 2001). *Moina* are introduced into each tank with the culture water at an initial stocking density of 250 ind. L^{-1}. During the

culture period, *N. oculata* is continuously maintained at > 2 x 10^6 cells mL^{-1}. The biomass will normally yield up to 9.8 to 47.4 g m^{-3} after 28 days. It is usually difficult to maintain a *Moina* culture by using algae as the sole food supply. Food type and supply can be a limiting factor for this type of culture due to the high food filtering rate of *Moina* (He et al., 1998b). Therefore, harvesting is recommended when the density of the culture reaches 500 to 1000 ind. L^{-1}. In Malaysia, the static batch culture is the typical culture method for the mass propagation of *Moina* spp. especially in sewage treatment plants and fin fish hatcheries (Figure 2.9 and 2.10).

Figure 2.9: Sewage treatment plant (adopted from www.ssd.sarawak.gov.my).

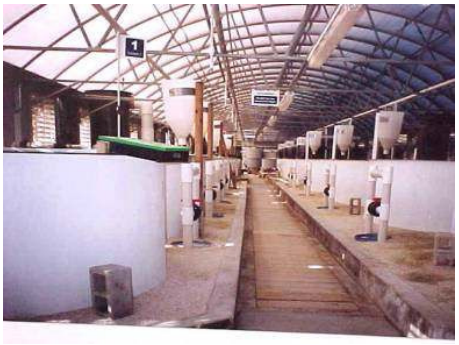


Figure 2.10: Fin fish larval hatchery (adopted from www.dof.gov.my).

2.4.2 Static Continuous Method

Moina are harvested periodically when the biomass reaches a targeted level (e.g. 500 to 800 ind. L^{-1}). Algae such as *Nannochloropsis sp*., *Anabaena sp., Scenedesmus sp., Chlorella sp*., *Schroederiu sp*., *Straurastum sp*., and *Coelastrum sp*. are used as live food for *Moina* (Ventura and Enderez, 1980; He et al., 2001; Nandini and Sarma, 2000; Chuah et al., 2007). They are inoculated into 500 L tanks in a way similar to static batch culture. Yeast is added as supplementary feed with the algal diet (He et al., 1998a). *Moina* is

harvested when the population density reaches 500 ind. L^{-1}. During harvesting, 10% to 50% of the tank volume is drained, and *Moina* are collected with a fine mesh filter. The tank is replenished with algae after each harvest. The remaining *Moina* in the tank are maintained as stock for further production. During the mass production of *Moina* with this method, dissolved oxygen can be a limiting factor for further increase of propagation when the density has reached 1225 to 5400 ind. L^{-1} (He et al., 2001).

The oxygen consumption of *Moina mongolica*, for example, is 0.008 mg per individual per day and the lowest oxygen tolerance of this zooplankton is 0.14 to 0.93 mg L^{-1} (He et al., 2001). In the static cultivation system, the minimum level of dissolved oxygen in the culture vessel should be maintained at > 0.5 mg L^{-1}. When the population density in the tank reaches 1000 ind. L^{-1}, the daily oxygen consumption may rise to 8 mg L^{-1}. Therefore, aeration should be provided when the density of *Moina* is more than 1000 ind. L^{-1}. The recommended level of dissolved oxygen for *Moina* culture is 6 to 8 mg L^{-1} (He et al., 2001).

2.4.3 Aerated Static Method

Air supply is normally provided by this method, especially when the dissolved oxygen level has dropped to a threshold level (He et al., 2001). Algal diet and *Moina* are inoculated into 500 L tanks similar to the way in which static continuous culture are inoculated. The culture is aerated 2 to 4 times daily when the dissolved oxygen level drops to < 6 mg L^{-1}.

2.4.4 Flow-through Method

An algal diet is continuously supplied to the *Moina* cultivation tank. At the initial stage, both algae and *Moina* are inoculated into 100 L tanks. The food is supplied through a 30 L algal tank with an algal density of 2×10^6 cells mL^{-1}. The algal tank is placed at an elevated position so that the algae can be supplied to the culture tank by gravity. In the *Moina* culture tank, the water exchange rate is 0.9 to 1.5 L min^{-1}. In the flow-through system, the density of *Moina* could increase to 6723 to 7890 ind. L^{-1} and the biomass production could reach to 33.5 to 88.6 g m^{-3}. This method generates a higher yield than that of static batch culture and static continuous culture, but it is lower than that of aerated static culture (He et al., 2001).

2.5 Aquaculture Nutrition

The dietary requirements of all aquaculture species can be categorized into five different classes of nutritional components: 1) proteins; 2) lipids; 3) carbohydrates; 4) vitamins; and 5) minerals. The quality of food is crucial for early larval development. Insufficient or sub-optimal feeding at the early stage will lead to poor performance and survival at later stages (Lim et al., 2003). In aquaculture nutrition, lipids are the most attractive part of nutritional research because it plays a very important role in the physiological synthesis and the mechanism of metabolism. Lipids and their benefits to fish and crustacean larvae will be discussed further in the following sub-sections:

2.5.1 Lipids

Lipids are the basic chemical substances found in both plant and animal cells and tissues. They are relatively insoluble in water, yet soluble in organic solvents such as ether, chloroform, and toluene. Lipids fall into two main categories (glycerol-based e.g. Triglycerides, and non glycerol-based e.g. sterols) according to the presence of glycerol (Sorgeloos and Lavens, 1996).

Adenosine triphosphate (ATP) is the energy source that allows plants and animals to maintain physiological fitness and the needs of daily activities. ATP is the main energy source generated using the available lipids in plants and animals bodies. The free fatty acids which are derived from fats and oils are the major source of metabolic fuel in fish muscle (Tacon, 1990). Each gram of fat (lipids) contains 2.5 times the energy found in a gram of carbohydrate or protein (Parker, 2002). Excessive consumption of fatty acids however, may result in an imbalance of the ratio of digestible energy to crude protein, and fat deposition in the body cavity and tissues.

Lipids are also essential components for cell membranes. They serve as carriers for fat soluble vitamins and are the source of essential fatty acids. Fatty acids are necessary for maintaining cell membrane integrity, lipid transport, and they form the building blocks of many hormones (Parker, 2002; Tamaru et al., 2003).

2.5.2 Lipid Composition and Chemical Structure

The most common compounds found in foodstuffs as well as the fat deposits of most animals are in the form of triglycerides and phospholipids (Stansby, 1981). In the chemical structure of triglycerides, one molecule of glycerol has the same fatty acid at three locations. The chemical (glycerol + fatty acids) linkages bond them together to form a lipid molecule with water as side product (Figure 2.11). The fatty acids of triacylglycerols can be oxidized for energy, and, once the dietary lipids are transferred to the target tissues, they are stored as energy in the form of re-synthesized triacylglycerols. The fatty acids are then further converted to eicosanoids or incorporated into the membranes within and around the cells (Berdanier and Zempleni, 2009).

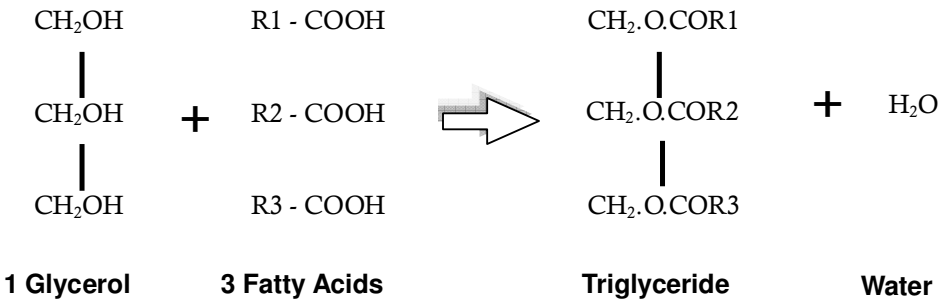


Figure 2.11: Chemical composition of triglyceride (modified from Tamaru et al., 2003).

All types of fish oil contain phospholipids. In the chemical structure of phospholipids, 'R1' represents a fatty acid, usually a saturated fatty acid. The 'R2' portion is also a fatty acid, often a HUFA such as arachadonate or docosahexaenoate. The 'z' portion after the P component (phosphate) is typically serine, choline, or ethanolamine (Figure 2.12). The fatty acids attach

to the structure, significantly affecting the physical and chemical properties of the fat or oil.

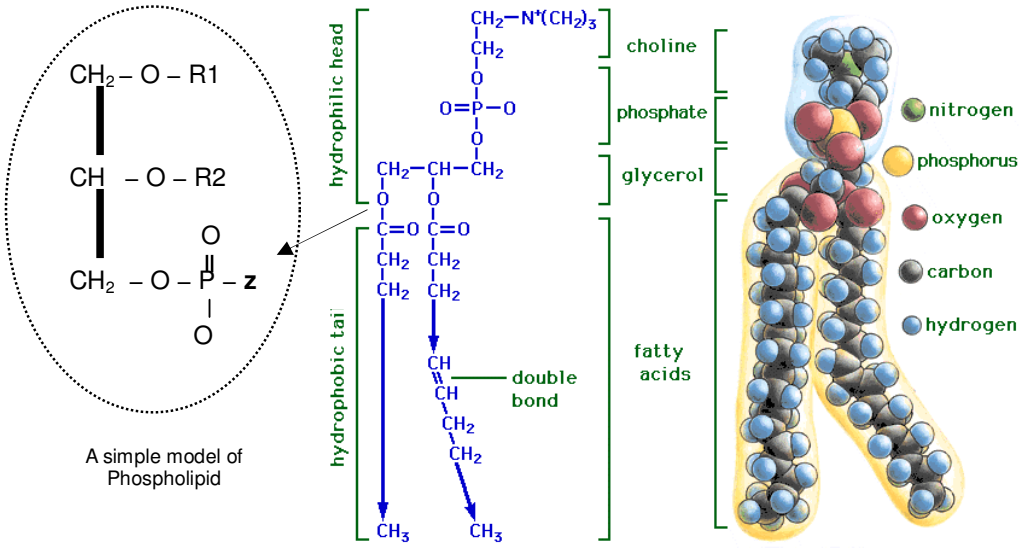


Figure 2.12: The chemical structure of phospholipids (modified from http://lipidlibrary.aocs.org/).

2.5.3 Fatty Acids

The common fatty acids of plant tissues are C_{16} and C_{18} straight-chain compounds with zero to three double bonds of a *cis* (or *Z*) configuration (Tamaru et al., 1991; 2003). Such fatty acids are also abundant in animal tissues, together with other even numbered components with longer chain-lengths and up to six *cis* double bonds separated by methylene groups (methylene-interrupted). Fatty acids with even numbers of carbon atoms predominate and are classified as short chain (C_4 to C_{10}), medium chain (C_{12}, C_{14}), long chain (C_{16} to C_{22}) and very long chain (> C_{22}) fatty acids, the C_{20} to C_{24} are sometimes referred to as long chain fatty acids (Dobson, 2002;

Wardlaw et al., 2004). The systematic and trivial names of these fatty acids are listed in Table 2.3.

At least 40 different fatty acids are known to occur naturally in plants and animals (Tamaru et al, 2003). They can all be represented by the following chemical formula:

$$CH_3 (CH_2)_n COOH$$

where;

n = 0 in acetic acid,

n = 1 in propionic acid,

n = 2 in butyric acid,

n = up to 24 where n is usually an even number

Unsaturated fatty acids are generally more chemically reactive and have lower melting points than the corresponding saturated fatty acids (i.e., carbon chain of the same length) (Tamaru et al., 1991; 1993; 2003). On the basis of the given classification, fatty acids are divided into three main families: saturated, monosaturated (or monoenoic), polyunsaturated or highly unsaturated fatty acids (Figure 2.13). In most cases, animals are incapable of synthesizing polyunsaturated fatty acids, for instance, fatty acids belonging to the *n*-6 (linoleic) and *n*-3 (linolenic) series. It is presumed that only plants are capable of synthesizing these families of fatty acids. Therefore, animals must have a diet containing these fatty acids in a ready-to-use form, referred to as essential fatty acids (EFAs) (Tamaru et al., 2003).

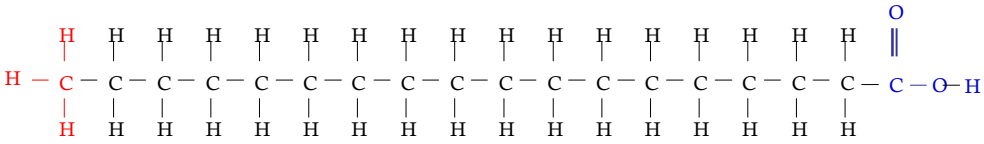
Table 2.3: The common fatty acids of animal and plant origins (source from http://lipidlibrary.aocs.org/).

Trivial name	Systematic name	Abbreviation
*Saturated FAs**		
Butyric	Butanoic acid	C4:0
Caproic	Hexanoic acid	C6:0
Caprylic	Octanoic acid	C8:0
Capric	Decanoic acid	C10:0
Lauric	Dodecanoic acid	C12:0
Myristic	Tetradecanoic acid	C14:0
Palmitic	Hexadecanoic acid	C16:0
Stearic	Octadecanoic acid	C18:0
Arachidic	Eicosanoic acid	C20:0
Behenic	Docosanoic acid	C22:0
Lignoceric	Tetracosanoic acid	C24:0
*Monoenoic FAs**		
Palmitoleic	*cis*-9-hexadecenoic	C16:1(*n*-7)
Petroselinic	*cis*-6-octadecenoic	C18:1(*n*-12)
Oleic	*cis*-9-octadecenoic	C18:1(*n*-9)
cis-vaccenic	*cis*-11-octadecenoic	C18:1(*n*-7)
Erucic	*cis*-13-docosenoic	C22:1(*n*-9)
Nervonic	*cis*-15-tetracosenoic	C24:1(*n*-9)
*Polyunsaturated FAs***		
Linoleic	9,12-octadecadienoic	C18:2(*n*-6)
γ-linolenic	6,9,12-octadecatrienoic	C18:3(*n*-6)
α-linolenic	9,12,15-octadecatrienoic	C18:3(*n*-3)
Arachidonic	5,8,11,14-eicosatetraenoic	C20:4(*n*-6)
EPA	5,8,11,14,17-eicosapentaenoic	C20:5(*n*-3)
DHA	4,7,10,13,16,19-docosahexaenoic	C22:6(*n*-3)

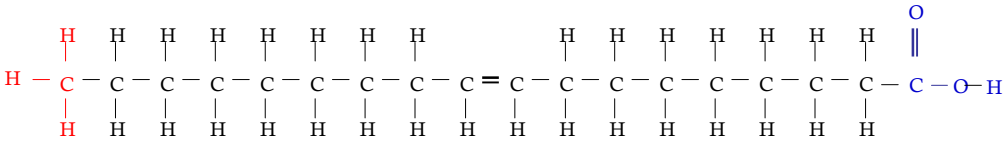
* *FAs* = Fatty acids

** All the double bonds are of the *cis* configuration

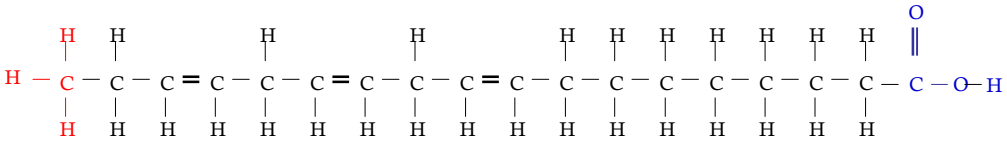
Saturated fatty acid
(Stearic acid; $CH_3(CH_2)_{16}COOH$; C18:0)



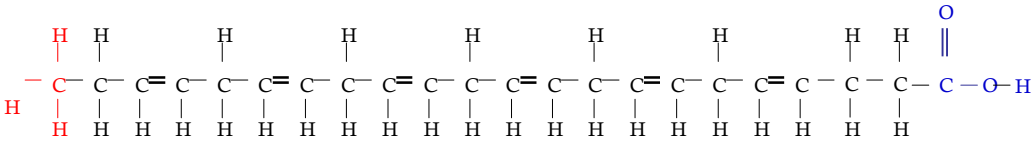
Mono-unsaturated fatty acid
(Oleic acid; $CH_3(CH_2)_7\ CH{=}CH(CH_2)_7COOH$; 18:1n-9)



Poly-unsaturated fatty acid
(Linolenic Acid; $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$; 18:3n-3)



Highly-unsaturated fatty acid
(Docosahexaenoic Acid; $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_2COOH$; 22:6n-3)



Highly-unsaturated fatty acid
(Eicosapentaenoic Acid; $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$; 20:5n-3)

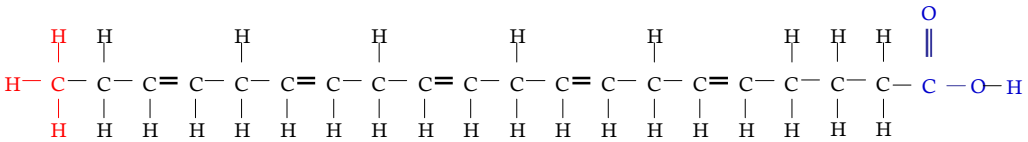


Figure 2.13 Examples of saturated, monounsaturated, and polyunsaturated and highlyunsaturated fatty acids (modified from http://lipidlibrary.aocs.org/).

EFAs are deemed essential because the body cannot synthesize them. They are important precursors to longer fatty acids, e.g. arachidonic acid, linoleic, linolenic acids etc, which are essential fatty acids that must be provided in the diet. EFA deficiency can result in the impairment of the glucose transporter activity in selected cells and an alteration in the insulin receptor number in an animal body (Berdanier and Zempleni, 2009). An EFA deficiency could cause skin lesions, shock syndrome, heart problems, reduced growth rate, reduced feed efficiency, reduced reproductive performance, and the increased mortality of some terrestrial animals (Parker, 2002).

2.5.4 Importance of Fatty Acids in Larval Nutrition

Fatty acids and their important values have been studied over many decades. Currently, *n*-3 or ω3 fatty acids (EPA, DHA and AA) are the major types of fatty acids incorporated into the functional foods because of their potential benefits to the health of many species of organisms (Dobson, 2002). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which are *n*-3 fatty acids, help in reducing the risk of cardiovascular disease (Ward and Singh, 2005). Some essential fatty acids, such as γ-linolenic acid (GLA) and conjugated linoleic acid (CLA), are able to reduce the effects of rheumatoid arthritis, atopic eczema, and may also be beneficial against other disorders, including some forms of cancer, depression, and other neural illnesses (Dobson, 2002; Nichols et al., 2004).

The benefits of dietary fatty acids are not just restricted to human health management. In aquaculture practices, there is documented evidence that highly unsaturated fatty acids (HUFAs) were able to enhance the strength and performance of fish larvae (Kanazawa, 1993a, b; Tamaru et al., 1993; Watanabe, 1993; Reitan et al, 1994; Hanaee et al., 2004). It has been well established that livefeed enrichment with HUFAs was able to enhance the strength and performance of fish larvae (Kanazawa, 1993; Tamaru et al., 1993; Watanabe, 1993; Reitan et al, 1994; Hanaee et al., 2004). Faulk and Holt (2005) found that enriching rotifers and *Artemia* with live algae can enhance the HUFAs content in the livefeed and consequently improve the growth and survival of cobia larvae (*Rachycentron canadum*). Increasing the levels of dietary HUFAs in fish larvae also increases the stress resistance of the larvae. This can be found in the study conducted on mahimahi *Coryphaena hippurus* (Kraul et al., 1993) and gilthead seabream larvae (Koven et al., 2003). Zooplankton such as *Artemia*, rotifers, copepods, and *Daphnia* are the common livefeed species used in most finfish and crustacean larviculture. Feeding livefeed with algae and phytoplankton containing HUFAs can improve the growth and survival of fish larvae (Sargent et al., 1997; 1999a, b; Faulk and Holt, 2005). Fatty acids play a major role as an energy source and influence cellular membrane functions which are, in turn, important for cell growth, differentiation, and metabolism (Sargent et al., 1999a; Cahu et al., 2003; Tocher, 2003; Villeneuve et al., 2005). Increasing the levels of dietary HUFAs in fish larvae also helps to increase stress resistance (from starvation or osmotic shock) (Kraul et al., 1993; Koven et al., 2003), assists in skeletal formation (Cahu et al., 2003), aids the development of neural and visual

systems (Sargent et al., 1997, 1999b), and the pigmentation of fish larvae (Nicolaides and Woodall, 1962; Izquierdo et al., 2000; Morais et al., 2004; Bransden et al., 2005).

Common essential fatty acids (EFAs) are arachidonic acid or AA [20:4(*n*-6)], eicosapentaenoic acid/EPA [20:5(*n*-3)] and docosahexaenoic acid or DHA [22:6(*n*-3)]. These EFAs have been recognized for playing an important role in HUFAs to promote the growth and survival of most fish and crustacean species, for instance, the gilthead seabream, *Sparus auratus* (Bessonart et al., 1999), the barramundi, *Lates calcarifer* (Rodgers and Barlow, 1987), the flounder, *Paralichthys dentatus* (Bisbal and Bengston, 1991), the freshwater prawn, *Macrobrachium rosenbergii* (Das et al., 2007) and the saltwater shrimp, *Penaeus japonicus* (Kanazawa et al., 1977; Guary et al., 1976) and *Penaeus indicus* (Read, 1981). Lipids are not just limited to health maintenance and the sustainability of cultured species with lipid supplementation; they also help to reduce feed cost in fish feeding. According to Blancheton (2000), the protein content in fish feed which is kept stable (around 45%) with the increase of feed lipid content from 10% to 30% in feed manufacturing, would reduce the voluntary feed intake of the fish. However, increasing the lipid content in feed allows the maintenance of a stable growth rate and lower nitrogen excretion.

Lipids are important in terms of an organism's well-being and the economic implementation. However, different species possess different nutritional requirements. The biochemical compositions of eggs and the yolk sac

contents of larvae have been suggested to reflect the basic nutritional requirements of first feeding larvae (Rainuzzo et al., 1997; Sargent et al., 1999c). Eggs and yolk sacs are examined initially for their nutritional profiles, and larval enrichment is subsequently manipulated based on the profile requirements. This concept has been adapted in many hatcheries to enhance the growth and performance of marine fish larvae. Despite this, it has been documented that some freshwater fish do not require long chain HUFAs in their dietary requirement as reported for marine species (Tamaru et al., 1993). According to Pickova et al. (2007) and Kamler et al. (2008), freshwater fish are more capable of desaturation and elongation of *n*-3 fatty acids than marine fish. Therefore, Tamaru et al. (1993) and Pickova et al. (2007) suggested that freshwater fish require *n*-6 rather than *n*-3 HUFAs. However, this data does not support the interpretation of non-necessity in supplementation of HUFAs in dietary requirement to other freshwater species. Long term HUFA deficiency has been associated with health problems in freshwater fish (Ng et al., 2003). Several studies conducted by private ornamental fish producers in Hawaii found that freshwater ornamental fish larval performances were improved significantly when their diet was supplemented with enriched livefeed, especially with long chain HUFAs. Growth and survival rates in discus and goldfish larvae were comparatively higher than that of non HUFA-supplemented fish larvae. The fecundity of angelfish and guppies also showed some promising results when fed with HUFA-enriched livefeed (Tamaru et al., 2003). These findings suggest that the supplementation of essential fatty acids is not only crucial to marine fish but equally important to some freshwater species.

A common practice for rearing fish larvae in captivity is to feed them with rotifers for several days or weeks, gradually switching them over to other livefeeds, and finally weaning them onto dry feed as shown in the feeding regime of Figure 2.4 (Sargent et al., 1997). However, fish larvae have an inadequate capability digesting or absorbing complex nutrients such as phospholipids (especially from an artificial microdiet) (Sargent et al., 1997; 1999a, b; Conceição et al., 2007). Since most of the livefeeds are naturally deficient in nutrition, HUFAs for example, it is necessary to enrich these livefeeds with essential fatty acids prior to feeding them to the larvae.

CHAPTER 3.0

MATERIALS AND METHODS

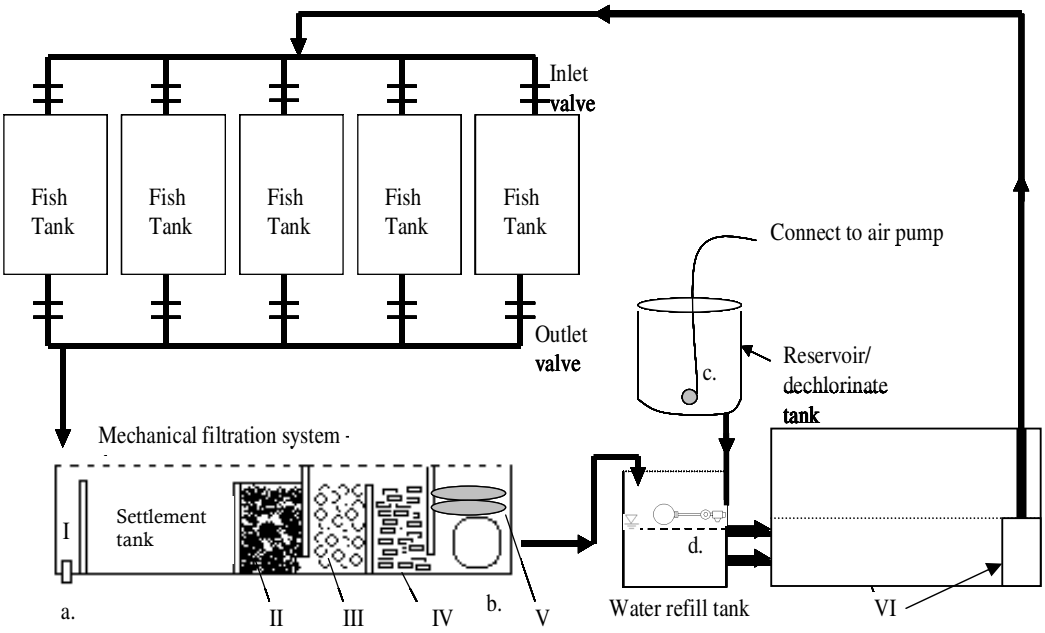
3.1 Fish Culture System

This project required the use of a research platform where the necessary equipment could be erected. The platform was built using 40 polyethylene pipes. Each pipe had a diameter of 3 inches and varied in length from between 5 m to 12 m. The PVC pipes were cut accordingly to the desired length and joined together with L and T-shaped pipe adaptors to form a strong supporting frame. To protect the project area from rain and direct sunlight, a waterproof plastic canvas, 2.68 m × 7.17 m, was securely tied to the frame with nylon cords.

In this experiment, a recirculating aquaculture system (RAS) (Figure 3.1 and 3.2) was built as a cultivation system for 40 juvenile tilapias (*Oreochromis niloticus*) with an average body length of 5.0 ± 0.2 cm. The RAS consisted of five fish rearing tanks (length x width x depth: 1 x 0.8 x 0.8 m per tank) connected to an effluent drainage system and clean water inlets (Figure 3.2). The effluent contained heavy particulates such as faeces and uneaten food discharged from the rearing tanks, which were collected in a sludge collection column (Figure 3.3). In order to remove suspended solids, wastewater was filtered through woven filter pads (approx. 20 μm). Prior to biological filtration, the wastewater was treated with hard coral skeletons to help

maintain the water pH at optimal levels (pH 6.5 to 8.3), while providing a carbon source for the nitrifiers (Kim et al., 2000; Van Rijn et al., 2006, Schneider et al., 2007). The biological filter medium was made from inert and insoluble substances, i.e. plastic bio balls, and silicon or ceramic rings which provided a surface area sufficiently wide enough for bacterial growth. Activated carbon (heat-treated charcoal) was added to the last compartment of the treatment tank to help eliminate bad odors and organic materials in the water. Treated water was channeled to fish tanks by a submersible mechanical water pump, 12W (Ocean Free^{TM}, Japan), with a water flow rate: 2200 L hour^{-1}, which was located in the compartment next to the water refill tank (Figure 3.2). Dechlorinated water from the reservoir (Figure 3.2) was added as needed to replace any water lost due to evaporation.

Figure 3.1: The laboratory scale recirculating aquaculture system and experimental area (photo taken in May 2008).



a. Sludge outlet; b., e. Water pump; c. Aeration tank; d. Auto water refiller; I. Sludge collection column; II. Woven medium; III. Coral sand and granules; IV. Bio-ball; V. Active carbon; VI. Electronic water pump.

Figure 3.2: Schematic diagram of recirculating aquaculture system (RAS) employed in the present study.

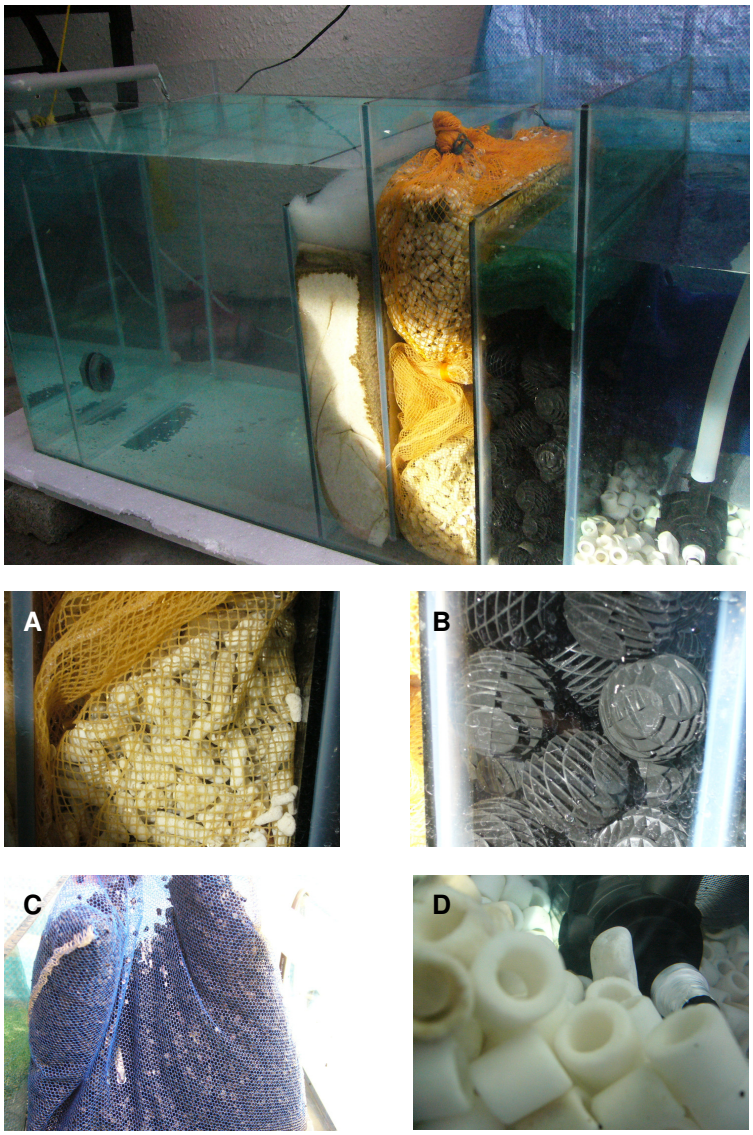


Figure 3.3: Filtration components in the RAS water treatment tank. Component: A: bio-balls; B: skeleton of hard corals; C: activated charcoal; D: bio-rings (photo taken in May 2008)

3.1.1 RAS Water Quality Monitoring

Monitoring of the water quality (dissolved oxygen, DO; total ammonia nitrogen, NH_3-N; nitrite-nitrogen, NO_2-N; nitrate-nitrogen, NO_3-N, water pH and temperature) was conducted monthly by using the Hach Methods (Hach,

2005) and laboratory methods (APHA, 1985) to ensure optimal condition for fish cultivation in the RAS system.

Dissolved oxygen levels were measured using a dissolved oxygen meter (YSI model DO 200, US) (Appendix A). The total ammonia nitrogen levels were determined using the salicylate method (Hach, 2005) (Appendix A). The nitrite-nitrogen levels were determined by the diazotization method (Hach, 2005) (Appendix A). The nitrate-nitrogen levels were determined with the cadmium reduction method (Hach, 2005) (Appendix A). The measurement of the water quality parameters was carried out using a Hach datalogging colorimeter (DR/890, Loveland, Colorado. U.S.), which was equipped with a silicon photodiode detector and a light-emitting diode (LED) lamp source with ± 0.005A photometric accuracy. The pH level of the water was measured with a pH mater (DELTA 320 pH meter China) (Appendix A). Water temperature was measured using a mercury thermometer (Allatrance, France) (Appendix A), and the total hardness and carbonate hardness were measured using water quality test strips (JBL, Germany).

Water quality monitoring was carried out at 2 different points: the influent inlet (I) connecting to the treatment tank, and water effluent outlet (E) from fish rearing tanks (Figure 3.4). The monthly data for water quality was recorded.

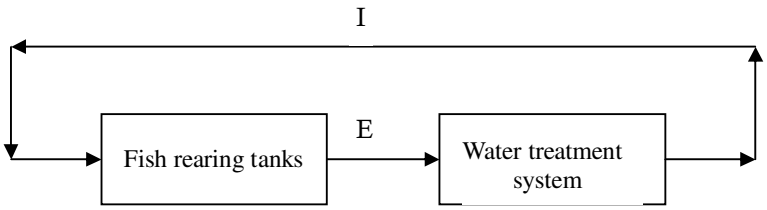


Figure 3.4 Water quality sampling points. *I*: Influent; *E*: Effluent.

3.2 Fish Faeces as a Potential Food Source for Cultivating the Water Flea, *Moina macrocopa*

3.2.1 Experimental Design

Moina macrocopa was obtained from the Cheras Aquarium in Kuala Lumpur, Malaysia and cultured at the Department of Bioscience and Chemistry, Universiti Tunku Abdul Rahman. Mass cultivation was carried out in transparent tanks with natural green water under natural light illumination. The green water was comprised of a mixture of *Chlorella* and *Scenedesmus* species of algae (Figure 3.5).

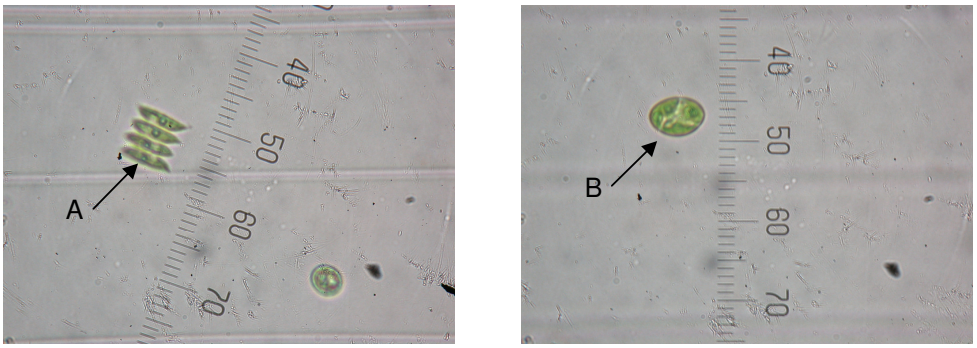


Figure 3.5: Algae species: A: *Scenedesmus sp.*, B: *Chlorella sp*. at 200 x magnification (photo taken in January 2009).

Only healthy adult *Moina macrocopa* females with brood pouches (Figure 3.6) were selected to produce offspring (neonates) in this study. These females were transferred into Petri dishes containing de-chlorinated tap water and fed with baker's yeast during the breeding period. Neonates of less than 24 hours were collected for the experiments.

Figure 3.6: A female with progeny in her brood pouches at 100 x magnification (photo taken in January 2009).

Treatment diets (i.e. *Chlorella*, *Spirulina* and fish faeces) were prepared at a concentration of 3.5 g L^{-1} based on the total ammonia-nitrogen (~ 3.5 mg L^{-1}), NO_3-N (~ 1.0×10^{-3} mg L^{-1}) and NO_2-N (~ 4.0×10^{-5} mg L^{-1}) levels which were detected in the rearing water of Nile tilapias (*Oreochromis niloticus*). The total ammonia-nitrogen (TAN), NO_3-N and NO_2-N levels were monitored with a DR/890 datalogging colorimeter (Hach Co., USA) following the standard methods (APHA, 1985).

The Nile tilapias were fed once a day with a Cargill™ formulated feed based on 2% to 3% body weight. Fish faeces (Figure 3.7), collected from the rearing

tanks, were blot-dried with filter paper. A dried weight of 3.5 g of fish faeces was blended for 5 minutes in 1 L de-chlorinated tap water. Two types of algal powder, *Chlorella* (Yakult Microbiological Central Institute, Japan), and *Spirulina* (YanLing Natural Hygiene Sdn. Bhd., Malaysia), were prepared in the same manner as the fish faeces. De-chlorinated tap water was used as control in this study.

Figure 3.7: Fish faeces was collected from the Nile tilapias *Oreochromis niloticus* (photo taken in January 2009).

M. macrocopa neonates were transferred into Petri dishes at a density of one neonate per 20 ml treatment diet. The diets with 3.5 g L^{-1} (in 20 mL) were fed to the *Moina* and the culture media was renewed every two days. Survival and reproduction (fecundity) of *M. macrocopa* were monitored daily. The length of time to the first reproduction and the total number of neonates produced by each parthogenetic female were recorded in order to evaluate its fecundity. Neonates were counted daily and discarded. To calculate cumulative birth, the total number of neonates produced by each female was summed up. All experiments were run in triplicate for up to 12 days or until the *M. macrocopa*

died. Life table parameters were tabulated to calculate net reproduction rate (R_o) and generation time (T) (Krebs, 1985), as shown below:

[1] Net reproduction rate (R_o) = $\sum I_x m_x$

[2] Generation time (T) = $\sum I_x m_x \, x \; / \; R_o$

where;

I_χ = the proportion of individuals surviving to age$_\chi$ (survivorship)

m_χ = the age specific fecundity (number of neonates produced per surviving female at age$_\chi$)

χ = days

The initial age of reproduction was referred to as the average age (in days) at which a female started to produce her first batch of offspring. Longevity was referred to as the average total number of days the female survived during the course of study.

3.2.2 Statistical Analysis

The data was tested using the Kolmogorov-Smirnov test for population distribution, and the population variances were tested by Levene's test (Carver and Nash, 2005). The reproduction rates of *M. macrocopa* were compared by one-way ANOVA (Minitab version 13, Minitab Inc., USA). Statistical significance among the different treatments was accepted at $p < 0.05$.

3.3 Life Table Demography of *Moina macrocopa* in relation to the Fish Wastes and Enrichment Lipid Emulsions

3.3.1 Test Organisms Preparation

Moina macrocopa were obtained from oxidation ponds located at residential areas in Kuala Lumpur, Malaysia. *M. macrocopa* females (body length approximately 1.1 to 1.6 mm) were isolated from the sampled population. The selected *M. macrocopa* were placed individually in several Petri dishes filled with de-chlorinated tap water (approx. 10 ml plate^{-1}) to allow them to breed (Figure 3.8). Mature *Moina* would start breeding overnight after being inoculated in the dish. Baker's yeast (Mauri-Pan®) at 1 g L^{-1} was added to the Petri dishes as a food source during the *Moina* breeding period. Newborn *M. macrocopa* were collected for the experiments (Figure 3.9).

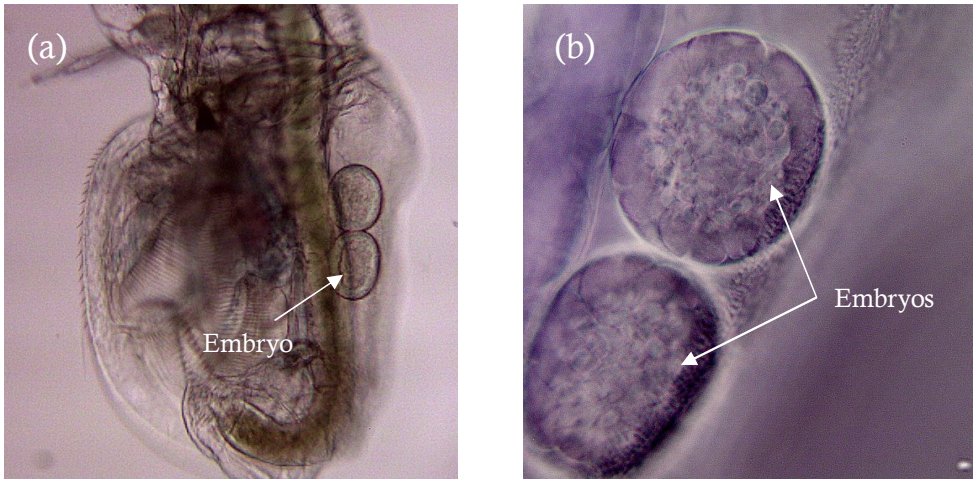


Figure 3.8: (a) Embryos appeared as spherical shapes in the female's brood pouch (asexual reproduction-parthenogenesis); (b) Embryos at higher magnification. Close up images at 100 x (a), and 400 x (b) magnifications (photo taken in February 2009).

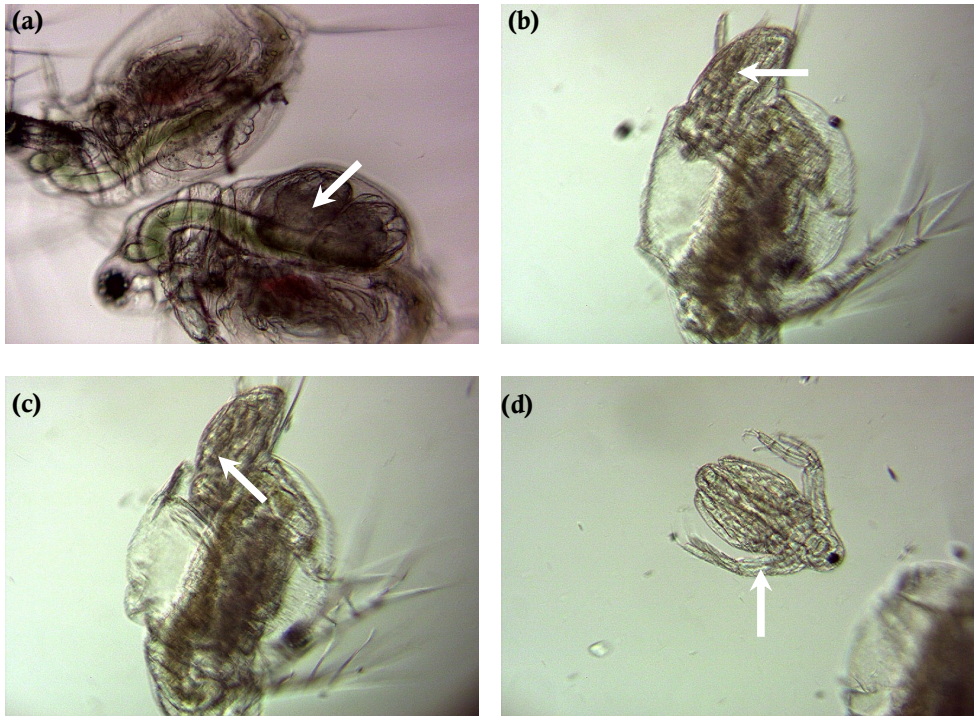


Figure 3.9: Arrows show: (a) Infants formed and developed in the pouch cavity; (b) Anus was opened to release the progeny; (c) Whole process completed in a few seconds; (d) Newly born neonate is approximately $^{1}/_{3}$ of the mother's body size. Images at 100 x magnification (photo taken in February 2009).

3.3.2 Diet Preparation

Canola oil (Sime Darby Edible Product Ltd., Singapore) (Figure 3.10), squid oil (Asia Star Laboratory Ltd., Thailand) (Figure 3.10), and commercial formulated emulsion, A1 DHA Selco® (INVE, Belgium) (Figure 3.10), fish faeces (from Nile Tilapias, *Oreochromis niloticus*), and ground fish pellets (Cargill Feed Sdn. Bhd., Malaysia) were used for the livefeed enrichment experiments. All diets were prepared in five concentrations: 0.0625, 0.125, 0.25, 0.5 and 1.0 g L^{-1}. The highest concentration of 1.0 g L^{-1} was determined based on the standard enrichment protocol of *Artemia* (Sorgeloos and Agh, 2005). The lower treatment concentrations were diluted two-fold from the highest concentration to the subsequent concentrations.

Emulsified CO (canola oil) and SO (squid oil) were prepared based on the modified *Artemia* enrichment methods of Estévez *et al* (1998) and Agh and Sorgeloos (2005). L-α-phosphatidylcholine (Sigma-Aldrich™, USA) (Figure 3.10) was mixed with the oil products at the ratio of 1:4 (w:w). These mixtures were blended vigorously with de-chlorinated warm water (40 to 60°C) using an electric blender (Waring Commercial®, USA) for 3 minutes. ADS (A1 DHA Selco®) emulsion was prepared in accordance with the manufacturer's instructions. All enrichment oil emulsions were prepared at 0.0625, 0.125, 0.25, 0.5 and 1.0 g L^{-1} and blended vigorously for 3 minutes with de-chlorinated water.

Commercial freshwater fish pellets (FP) (nutritional composition: protein: 18-20%; fiber: 6%; fat: 3%; moisture: 11%) were ground into fine particulate using mortar and pestle, and further pulverized into powder form (< 0.1mm) using a blender at maximum speed for 3 minutes. FF (fish faeces) was collected from the red Nile Tilapias recirculating aquaculture system (RAS) under laboratory conditions: dissolved oxygen, 6.89 ± 1.04 mg L^{-1}; pH, 7.36 ± 0.17; temperature, 26.60 ± 0.16 ^{o}C; total hardness: 7 to 14 dGH; ammonia-nitrogen: 0.088 ± 0.034 mg L^{-1}; nitrite: 0.042 ± 0.026 mg/L and nitrate: 23.85 ± 13.48 mg L^{-1}. Excessive water in FF was removed using filter paper (0.2 μm, DoubleRing, China) prior to weighing. The semi-solids were heat-dried in an oven at 55^{o}C overnight. Both FP and dried FF were prepared according to the five concentrations (0.0625, 0.125, 0.25, 0.5 and 1.0 g L^{-1}) and blended with de-chlorinated water for 3 minutes.

Figure 3.10: Materials for livefeed enrichment: A: canola oil (CO); B: L-α-phosphatidylcholine; C: A1 DHA Selco® (ADS); D: squid oil (SO).

3.3.3 Experimental Design

Moina macrocopa neonates of less than 24 h were harvested from breeding females in the Petri dishes for the tests. The experiments were carried out in four replicates. All neonates were divided equally (1 neonate dish^{-1}) in the Petri dishes, which contained 20 mL de-chlorinated water (control) and six

treatment diets. *M. macrocopa* in the Petri dishes were manually fed with the different diets using a pipette throughout their life span. The treatment diets were replaced with fresh medium at an interval of every two days. Petri dishes containing neonates were kept under well ventilated (28°C to 30°C), natural light conditions (12 h light: 12 h dark) (Figure 3.11). Newborn *M. macrocopa* were counted and discarded every day throughout the duration of the experiment. Mortality and fecundity were recorded for calculation of the life table. The experiments were terminated until all the *M. macrocopa* were found dead.

Figure 3.11: The experimental design of *Moina* population study, in random design (photo taken in February 2009).

The selected life history variables for the present study were the intrinsic rate of natural increase, survivorship curves, life expectancy, average longevity, gross reproduction rate, net reproduction rate, and generation time (Krohne, 2001; Smith and Smith, 2003; Molles, 2005 and Chuah et al., 2007). The intrinsic rate of natural increase (r) was calculated according to Lotka (1913) using the following formula:

[3] Intrinsic rate of natural increase (r) = $\sum l_\chi m_\chi e = 1$ (equal to one)

Where;

l_χ = the proportion of individuals surviving to age χ (survivorship);

m_χ = the age specific fecundity (number of neonates produced per surviving female at age χ);

χ = days.

The value r, calculated after 21 days, was indistinguishable from r estimated for the entire life span. Due to the importance of early reproduction (Van Leeuwen et al., 1985) all calculations would be based on 21 days of experimentation.

The variables related to survival and reproduction were derived based on the collected data using the standard procedures of Krebs (1985), Krohne (2001) and Molles (2005).

[4] Survivorship = $\sum l_\chi$;

[5] Average longevity = $\sum n_\chi / n$;

[6] Gross reproduction rate = $\sum m_\chi$;

[7] Life expectancy (e_χ) = T_χ / n_χ

Where;

n_χ = actual number of individuals alive for each age class;

n = total number of replicates.

T_χ = generation time at age χ

Net reproduction rate (R_o) and generation time (T) were calculated based on the formula in [1] and [2] respectively as described in Experiment 1 (Fish faeces as a potential food source for cultivating the water flea, *Moina macrocopa*). All calculations were listed in Appendix B and the life table was tabulated to compare the life history variables among the treatments.

3.3.4 Statistical Analysis

The initial age of reproduction was referred to the time when a female started to produce her first batch of offspring (as in average days). Longevity was referred to as the average total number of days the female survived during her life span. The results from the treatment diets and diet concentrations were tested by the Kolmogorov-Smirnov test (Normality) and the Levene's test (homogeneity-of-variance) (Carver and Nash, 2005), and the results were analyzed by using Two-way ANOVA followed by Tukey's comparison (Minitab version 13, Minitab Inc., USA), to determine the significant values and the interaction effects among the diet types and concentrations. Statistical significance among the different treatments was accepted at $p < 0.05$ or $p < 0.001$.

3.4 Effects of Concentration, Enrichment Time and Potential Enrichment Diets on the HUFA's Profile of *Moina macrocopa*

3.4.1 Diet Preparation

Canola vegetable oil, squid oil, fish faeces, A1 DHA Selco®, and commercial fish pellets were selected for the experiments. A1 DHA Selco® was used as the reference for the enrichment diet (the commercial enrichment product in larviculture) (Sorgeloos and Lavens, 1996). All treatment diets were prepared at the concentrations of 1.0 g L^{-1} and 2.0 g L^{-1}. The lower concentration of 1.0 g L^{-1} used in the study was based on the standard enrichment protocol for ADS commercial enrichment diet (Sorgeloos and Lavens, 1996), while the higher concentration of 2.0 g L^{-1} was selected to investigate whether doubling the dosage of enrichment would significantly increase the HUFA levels in *M. macrocopa* as compared to 1.0 g L^{-1}.

Canola oil or CO, squid oil or SO, A1 DHA Selco ® or ADS, fish faeces or FF and fish pellets or FP were prepared based on the methods described in section 3.3.2 of the experiment – "Life Table Demography of *Moina macrocopa* in relation to the Fish Wastes and Enrichment Lipid Emulsions". All treatment diets were prepared to 1 L for subsequent *Moina* enrichment studies.

3.4.2 *Moina macrocopa* Enrichment

An 80 L barrel was filled with tap water, which was de-chlorinated by strong aeration for several days prior to use. Wild-type *Moina macrocopa* were obtained from oxidation ponds located at the residential areas of Kuala Lumpur, Malaysia. *M. macrocopa* were acclimatized in the plastic barrel (stocking density: 100 ind. L^{-1}) overnight before the experiments commenced. A mild aeration was provided during the period to maintain the dissolved oxygen level at $\geq$ 4 ppm (APHA, 1985).

Only active (moving) *Moina macrocopa* were selected for the experiments. During the selection, aeration was turned off to reduce the water current in the tank. *Moina* was collected with a fine sieve net near the tank's edge. All of the adult *Moina* were concentrated into a glass beaker for quantification. Figure 3.12 shows the steps of sample counting, 40 mL of test organisms was transferred using a syringe onto a Petri dish, and counting was carried out under a dissecting microscope. Approximately 300 individuals per ml of *Moina* (estimation was made based on the number of *Moina* per volume, mL) were added into different treatment tanks (1.5 L per tank) for enrichment. A mild aeration was provided during the experiment (Figure 3.13). Enriched *Moina* were collected after 12, 24, and 36 hours of incubation. Collected samples were rinsed with distilled water thoroughly to remove excess oil emulsion prior to storage in 1.5 mL tubes at -20 ^{o}C for further analysis (Figure 3.14 to 3.15).

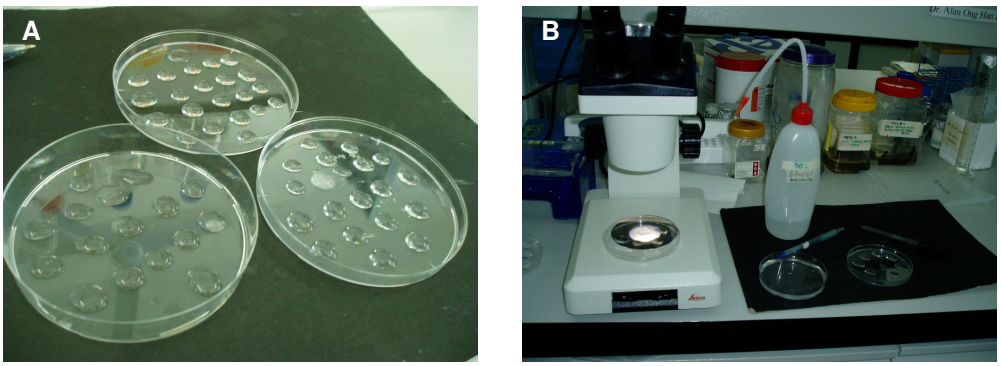


Figure 3.12: Experimental setup for *Moina* quantification. A: Droplets containing *Moina* were distributed on the surface of Petri dishes surface, B: Counting was performed with a dissecting microscope at 40 x magnification (photo taken in April 2009).

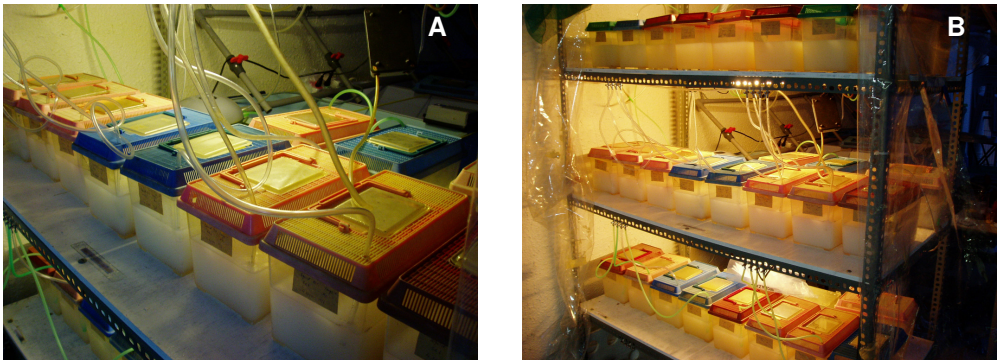


Figure 3.13: Experimental setup for *Moina* enrichment studies. A: *Moina* enrichment tanks connected to aeration, B: Tanks arranged on a 3-tier metal rack (photo taken in April 2009).

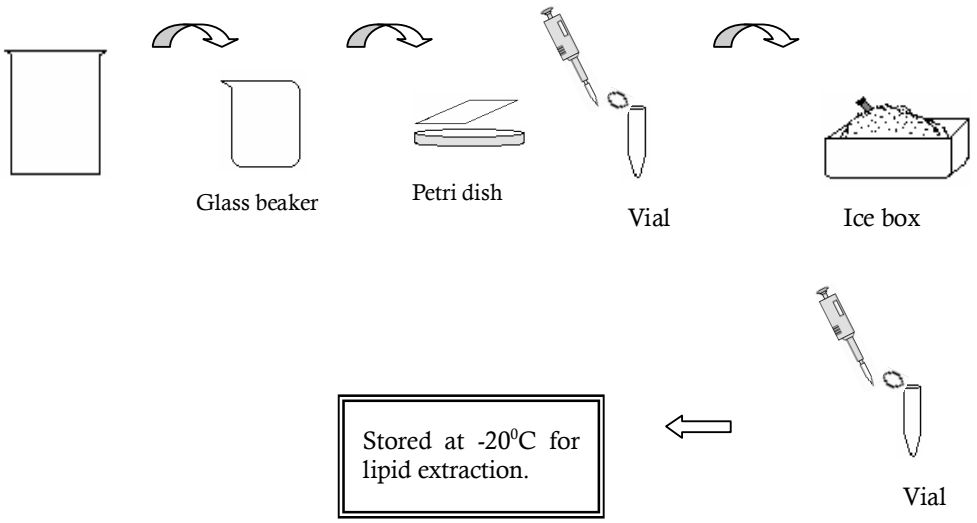


Figure 3.14: Diagram of the *Moina* collection. *Moina* were stored for FAME extraction.

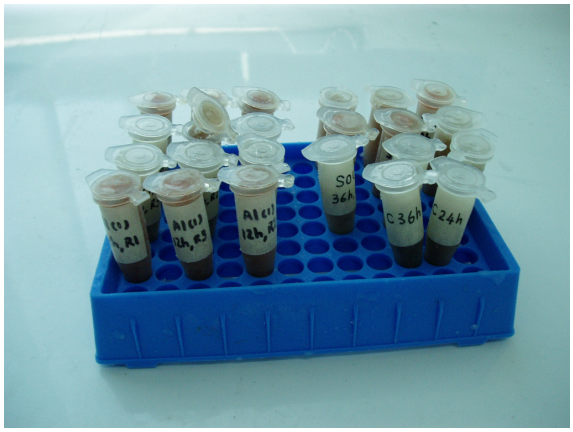


Figure 3.15: Samples of *Moina* were collected and stored at -20^{o}C prior to FAME analysis (photo taken in April 2009).

3.4.3 Lipid Extraction and Fatty Acid Determination

The lipid extraction procedure was as described in Appendix D. The preparation of fatty acid methyl ester (FAME) of *Moina macrocopa* was carried out according to the method modified from the AOAC (1995) as shown in Appendix E. Qualitative and quantitative analysis by gas chromatography (PerkinElmer Inc., USA) was carried out by a certified commercial food chemistry analytical laboratory (Chemical Laboratory Sdn. Bhd., Malaysia) (Appendix E).

3.4.4 Statistical Analysis

The fatty acids results of enriched *M. macrocopa* were analyzed by 4 sets of data, while the experimental diets were in 2 sets of data (Appendix F). The data were tested for normality (Kolmogorov-Smirnov test) and homogeneity-of-variance (Levene's test) (Carver and Nash, 2005), and analyzed by two-way ANOVA (Tukey's test) to find the effects of diet levels (1.0 and 2.0 g L^{-1}), enrichment periods (t_{12}, t_{24} and t_{36}) and the interactions of both factors (Appendix G). A 95% significance level ($p < 0.05$) was used throughout the data analysis (Sokal and Rohlf, 1981). All statistical analysis was performed using Minitab (version 13, Minitab Inc., USA).

3.5 Effects of Effluent Water from a Recirculating Aquaculture System (RAS) on the Population Growth of *Moina macrocopa*

3.5.1 Experimental Design

This experiment consisted of 4 initial culture densities (4, 20, 40 and 80 individuals per 40 mL) of *Moina macrocopa* and controls with the same initial culture densities. Forty eight polyether tubes containing 40 ml culture media were used in this study (Figure 3.16 and 3.17). Non-filter effluent water (NH_3-N: 0.44 ± 0.15 mg L^{-1}; NO_2-N: 0.10 ± 0.05 mg L^{-1}; NO_3-N: 23.32 ± 11.12 mg L^{-1}) from the outlet (E) (Figure 3.4) of recirculating aquaculture system (RAS) of Red Tilapia (*Oreochromis niloticus*) was used as the culture medium (no algae was observed in the culture medium), while de-chlorinated tap water (NH_3-N: 0 mg L^{-1}; NO_2-N: 0.01 ± <0.001 mg L^{-1}; NO_3-N: 1.37 ± 0.05 mg L^{-1}) was used in the control experiments. All experiments were repeated 6 times. The culture tubes were not capped throughout the experiment to allow gaseous exchange and to prevent suffocation of *M. macrocopa* during the experiment. The average ambient temperature was 26 to 30^{o}C and natural photoperiod (12 h light: 12 h dark). The culture medium was replenished with a new medium twice a week after population counting to reduce the effect of evaporation. Total ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen levels of the culture medium were measured during the refreshment. The level of medium in the culture tubes was maintained daily by replenishing with the respective medium.

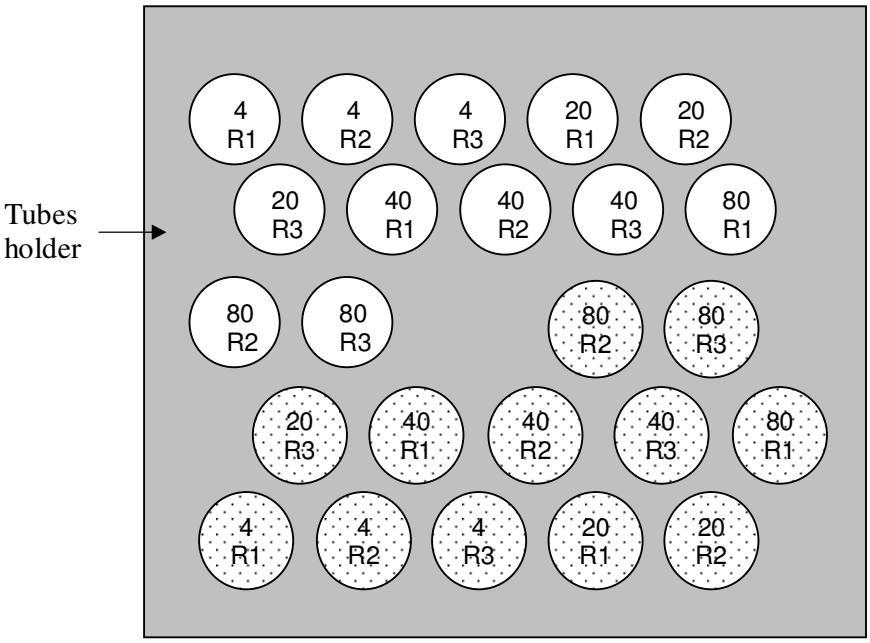


Figure 3.16: Arrangement of culture tubes in a holding platform: the clear circles indicate the controls and the dotted circles indicate the treatment culture tubes with different culture densities (4, 20, 40 and 80 individuals per 40 ml). "R" indicates the replication.

Figure 3.17: Experimental setup for *Moina* population culture studies. The experiments were conducted on a covered rack (photo taken in April 2009).

3.5.2 Data Collection

To collect *Moina* for quantification, fine mesh gauze was used to cover the mouth of the culture vessels (polyether tubes) (Figure 3.17), and the culture media with *Moina* were decanted slowly into a Petri dish. The gauze was then rinsed gently with a small volume (≈ 1 to 5 ml) of the culture medium using a glass pipette, in order to flush out *Moina* which were still on the gauze. The culture tubes were rinsed to ensure that all *Moina* were completely removed from the tubes for precise counting. The *Moina* was transferred gently into a Petri dish using a blunted glass pipette.

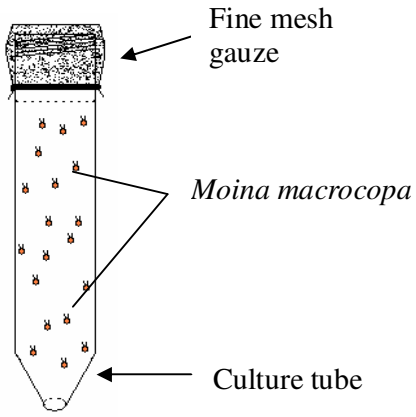


Figure 3.18 *Moina* were cultured in polyether tube covered by a piece of fine mesh gauze on top of the tube.

The first day of inoculation of the *M. macrocopa* was considered as Day 0. Population counting began on Day 1 and continued onwards. Counting was carried out by distributing droplets of culture medium containing *M. macrocopa* in the Petri dish, and observation was carried out under a

dissecting microscope at a range of 10x to 40x magnification (Figure 3.11). Live *M. macrocopa* were returned to the culture vessels, and dead organisms were discarded after the counting was completed. The number of individual *Moina* was counted and recorded using a Tally counter (Appendix H). The experiment was carried out for 16 days until all the *Moina* were dead.

3.5.3 Statistical Analysis

The data was statistically analyzed using Minitab version 13 (Minitab Inc., USA). The data was tested by the Kolmogorov-Smirnov test (Normality) and Levene's test (homogeneity-of-variance) (Carver and Nash, 2005), and the data was analyzed by one-way ANOVA and *Post Hoc* Tests (Tukey's tests) to compare population density between the different initial densities, different culture media, and experiment periods (Appendix I). Statistical significance among the different treatments was accepted at $p < 0.05$ or $p < 0.001$.

CHAPTER 4.0

RESULTS

4.1 Water Quality of the Fish Rearing System

In order to monitor the fish culture environment of the RAS system, testing of the water quality parameters was conducted on a monthly basis (October 2008 to March 2009). Table 4.1 shows the total water hardness and carbonate hardness of the effluent and the influent water in the rearing system. The level of the dissolved oxygen, temperature, pH, total ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen are shown in Figure 4.1.

Table 4.1: Total water and carbonate hardness of the effluent and influent water

Time	Effluent water		Influent water	
	Total hardness (GH, ^{o}d)	Carbonate hardness (KH, ^{o}d)	Total hardness (GH, ^{o}d)	Carbonate hardness (KH, ^{o}d)
Oct 08	7.0 -8.0	6.0	7.0 - 8.0	6.0
Nov 08	7.0 - 14.0	6.0	7.0 - 14.0	6.0
Dec 08	>14.0	6.0	14.0 - 21.0	6.0
Jan 09	>14.0	6.0 - 10.0	>14.0	3.0 - 6.0
Feb 09	7.0 - 8.0	6.0	>14.0	6.0
Mar 09	>14.0	10.0	>4.0	6.0

The dissolved oxygen (DO) levels of the recycled influent water ranged from 3.06 to 7.46 mg L^{-1}, which were slightly lower than the DO in the fish rearing tanks (Figure 4.1). Due to the use of an aerator, the DO level in the fish tanks was higher (5.78 to 7.84 mg L^{-1}) (Figure 4.1). The DO level met the standard DO requirement (5.0 mg L^{-1} to saturated level) for freshwater species (FAO,

1996). The pH levels of the effluent (waste water) and influent water (treated water) were found to be in the range of 7.20 to 7.69 (Figure 4.1). This indicated that the culture condition had a relatively neutral pH throughout the 6-month culture period. Water temperature did not fluctuate much in the culture water and in the treated water. The lowest temperature was 26.2 ^{o}C and the highest was 29.5^{o}C during the dry season of February 2009 (Figure 4.1).

The total water hardness and alkalinity (carbonate hardness) of the culture system were slightly below the standard water quality requirement (Table 4.1). According to the U.S. Environmental Protection Agency (1980), total water hardness for fish farming has to be between 10 to 400 mg L^{-1}, while alkalinity should fall within 10 to 100 mg L^{-1}. The total ammonia nitrogen (TAN) of the effluent ranged from 0.04 to 0.60 mg L^{-1} (Figure 4.1). After water treatment the TAN level was significantly reduced to less than 0.04 mg L^{-1}, which was less than the threshold level of < 0.1 mg L^{-1} (Figure 4.1). The nitrite levels in the fish rearing tanks ranged from 0.006 to 0.071 mg L^{-1} (Figure 4.1), which were considerably safe for the cultured fishes (< 0.1 mg L^{-1}, based on EPA (1980)). The nitrate (NO_3) levels were found to be highly concentrated in both effluent and influent water because of the intensive stocking density in the fish tank (averaging 300 g per 1 L culture water). However, the red Tilapia is a hardy fish species that is able to withstand high stocking density. For the effluent water, the nitrate levels ranged from 10.0 to 58.9 mg L^{-1}, and for the influent water, the nitrate levels were 9.4 to 60.9 mg L^{-1} (Figure 4.1), and were way above the range of the standard water quality (< 3.0 mg L^{-1}) recommended by the U.S. Environmental Protection Agency (1980).

Nevertheless, the high concentrations of nitrate did not cause any fish mortality throughout the 6-month culture period.

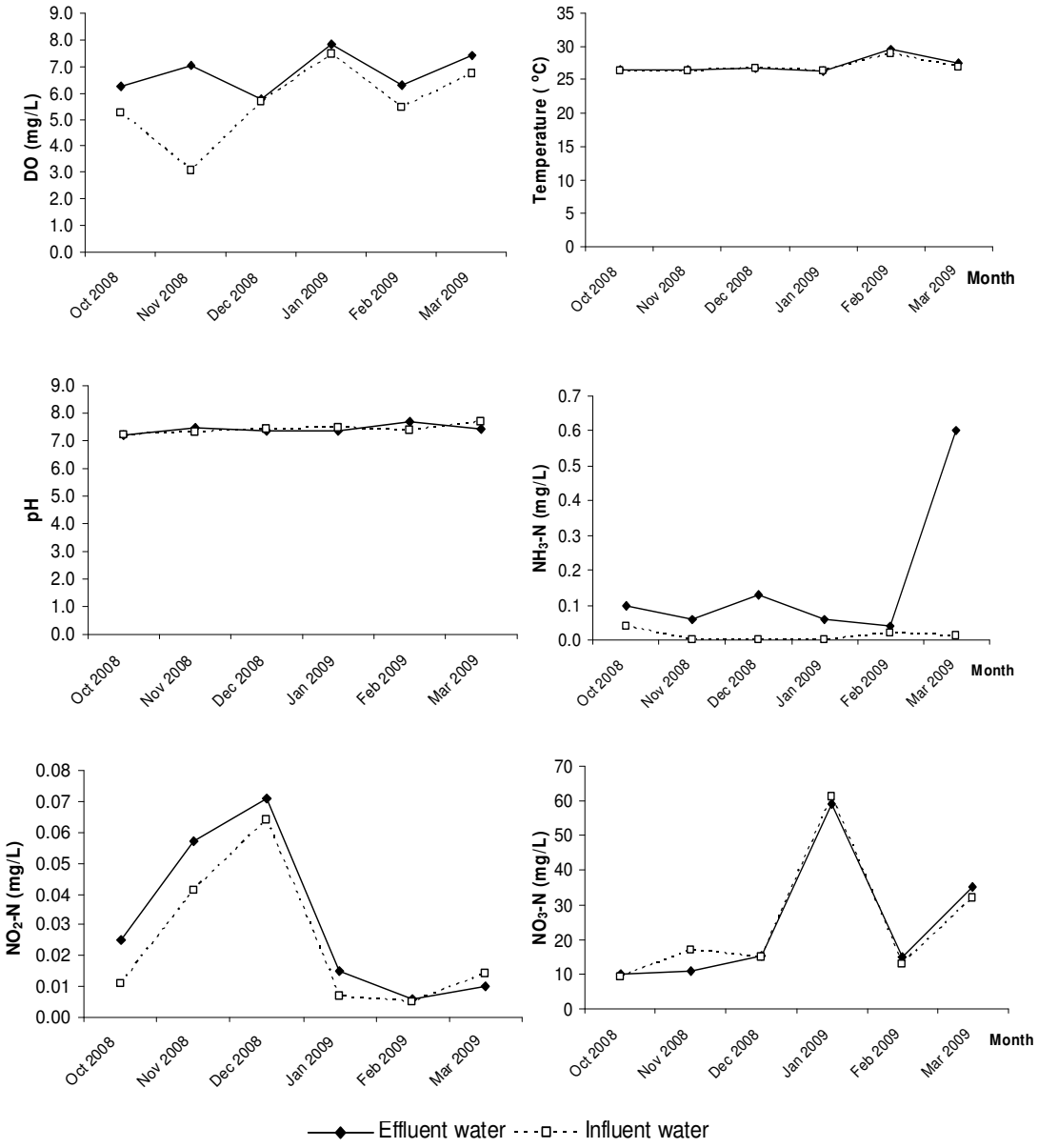


Figure 4.1: Water quality results of the effluent and influent water of the RAS system from October 2008 to March 2009.

4.2 Fish Faeces as a Potential Food Source for Cultivating the Water Flea, *Moina macrocopa*

This study showed that the average longevity of the cladoceran, *Moina macrocopa*, did not vary significantly (p = 0.226) among the three treatments, which were 7.0 days for *Spirulina*, 7.7 days for *Chlorella*, and 8.0 days for fish faeces (Table 4.2). In the de-chlorinated tap water (control), the neonates started to die from day 2 and complete mortality was observed by day 3 (Figure 4.2).

Table 4.2 Life table of *Moina macrocopa* fed with different diets at a concentration of 3.5 g L^{-1}

Diet types	Average longevity (Days)	Initial age of reproduction (Days)	Net reproduction rate (R_o)	Generation time (T)
Control	1.70 ± 0.58	–	–	–
Chlorella	7.70 ± 1.15	4	8.32	4.10
Spirulina	7.00 ± 0.00	5	4.01	4.50
Fish faeces	8.00 ± 2.65	3	17.27	4.03

Mortality did not occur in any of the treatments during the first 6 days. *M. macrocopa* fed with *Spirulina* began to die on day 7, but those fed with *Chlorella* showed a longer survivorship. Some *M. macrocopa* fed with fish faeces began dying on day 6 (Figure 4.2). Nonetheless, a stable population was achieved between day 8 and day 11, with complete mortality only on day 12.

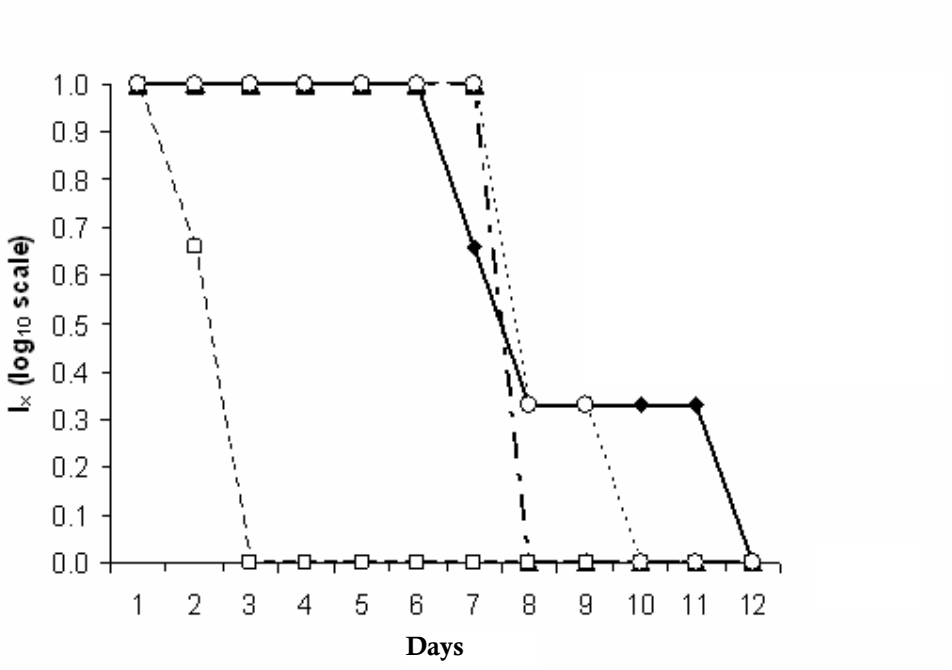


Figure 4.2: Survivorship of *Moina macrocopa* under different treatments (in triplicate). Symbols represent: control; *Spirulina sp.*; *Chlorella sp.*; fish faeces.

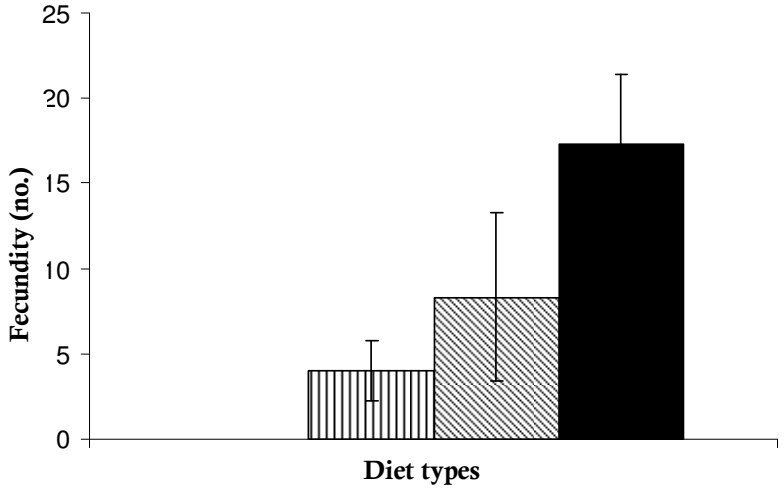


Figure 4.3: Average fecundity of *Moina macrocopa* under different treatments. No values are shown for the control experiment as the females did not produce any neonates. Symbols represent: control; *Spirulina sp.*; *Chlorella sp.*; fish faeces. Error bars = mean ± standard deviation.

The average number of neonates produced by the parthenogenetic females was significantly different (p = 0.022) among the three treatments and control experiments. Females fed with fish faeces showed the highest average fecundity followed by those treated with *Chlorella* and *Spirulina* (Figure 4.3). Females in the control experiments did not produce any neonates. *M. macrocopa* fed with fish faeces and *Chlorella* started to reproduce on day 3, while those under *Spirulina* treatment produced offspring one day later (Table 4.2, Figure 4.4). The peak population was reached on day 7, 9, and 11, for *Spirulina*, *Chlorella,* and fish faeces, respectively. *M. macrocopa*, fed with fish faeces, had the highest reproductive rates and produced the greatest number of neonates within a shorter generation time.

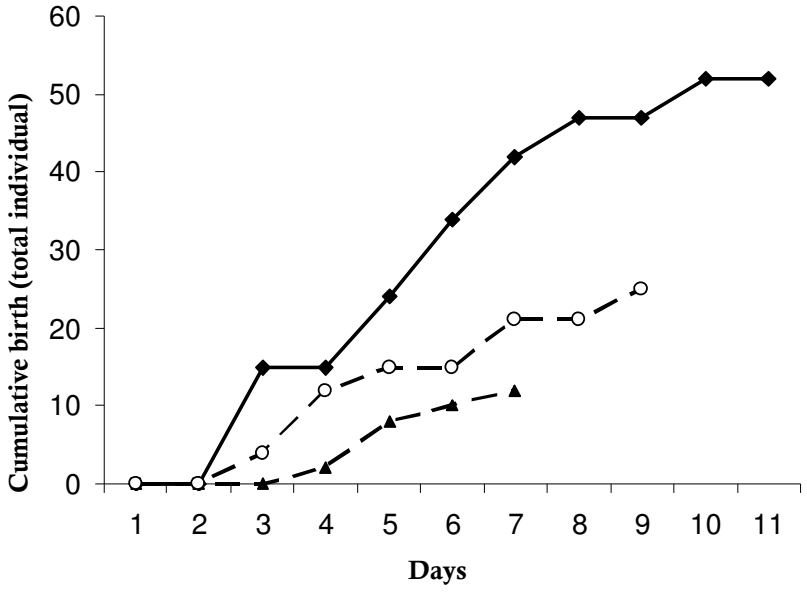


Figure 4.4: Cumulative birth of *Moina macrocopa* under different treatments (in triplicate). Symbols represent: control; *Spirulina sp.*; *Chlorella sp.*; fish faeces.

4.3 Life Table Demography of *Moina macrocopa* in relation to the Fish Wastes and Enrichment Lipid Emulsions

The fecundity of *Moina macrocopa* fed with 0.0625 g L^{-1} FF was the highest among all the treatment diets (43.75±7.37 ind.), followed by CO at 0.0625 g L^{-1} (21.75±3.59 ind.), and FP at 0.5 g L^{-1} (20.75±5.85 ind.) (Table 4.3). *Moina* fed on FP started to produce its offspring on day-2, which was one day earlier compared to the other treatment diets. More clusters of neonates were produced with the FF and FP diets at concentrations of less than 1.0 g L^{-1}. On the other hand, *Moina* produced only a small number (2.0 to 11.75 ind.) of offspring with the other two oil emulsion treatments (ADS and SO). *Moina* fed with the oil emulsions at lower concentrations i.e. 0.25, 0.125 and 0.0625 g L^{-1}, only started to produce their offspring on day-3. Notably, *Moina* fed on 0.25 g L^{-1} of CO showed a second cycle of reproduction on day-6 (Figure 4.5). Fecundity generally declined at the highest treatment concentration in every treatment diet of this study, except for the treatments of ADS, SO and CO, in which no offspring were produced at 1.0 g L^{-1} (Figure 4.5). The generation times of *M. macrocopa* fed with CO and FF were slightly lower when compared to other diets (Table 4.3). *M. macrocopa* started to reproduce from day-2 onwards in all treatments, except with the higher concentrations of emulsion oil diets (ADS, SO and CO) (Table 4.3).

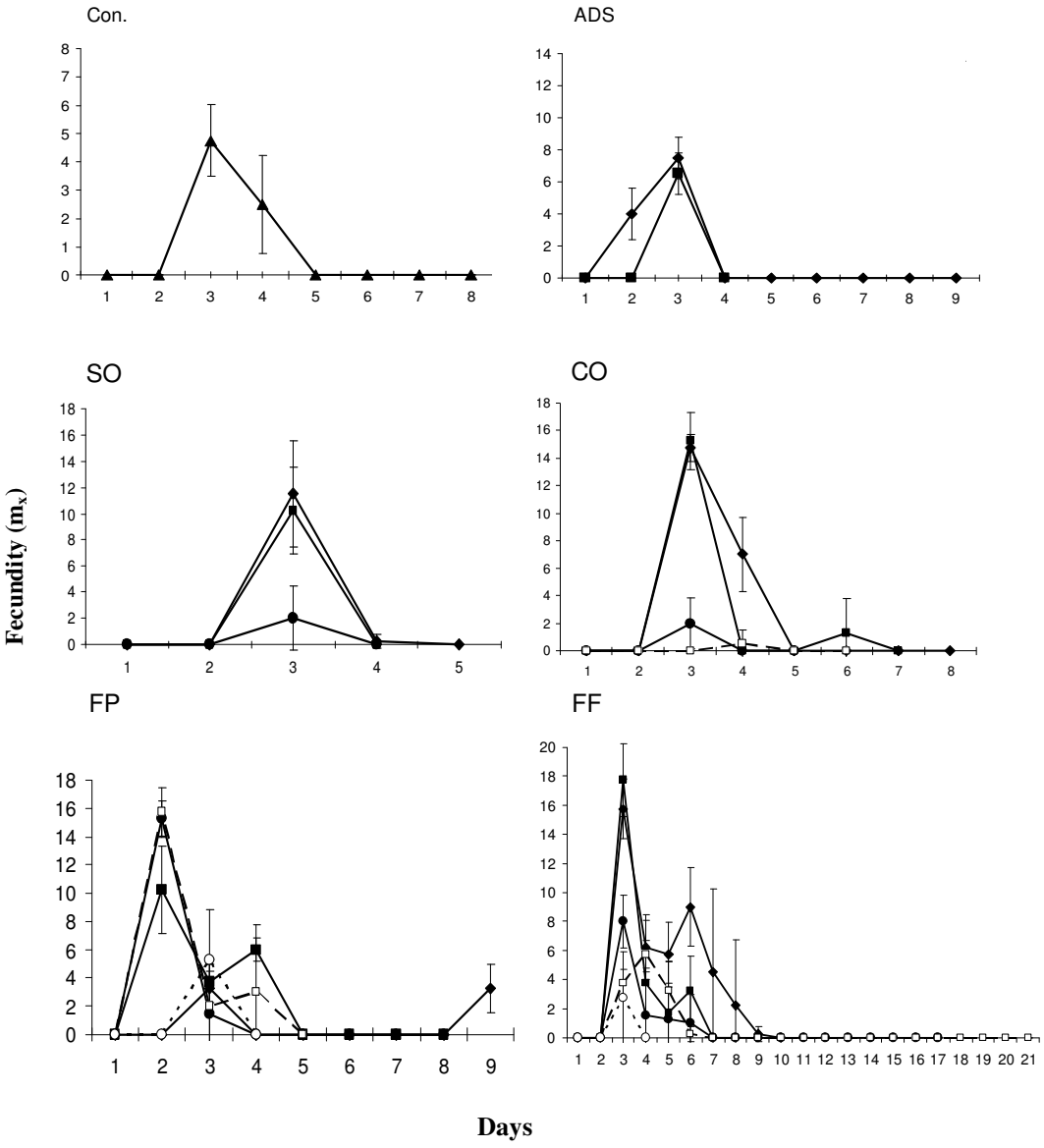


Figure 4.5: Fecundities of *Moina macrocopa* in control (Con.) and different concentrations of treatment diets: A1 DHA selco® (ADS); squid oil (SO); canola oil (CO); fish pellet (FP) and fish faeces (FF). Error bars indicate mean ± standard deviation. Symbols represent: —▲— Control; —◆— 0.0625 g L^{-1}; —■— 0.125 g L^{-1}; —●— 0.25 g L^{-1}; —□— 0.5 g L^{-1}; - -○- - 1.0 g L^{-1}.

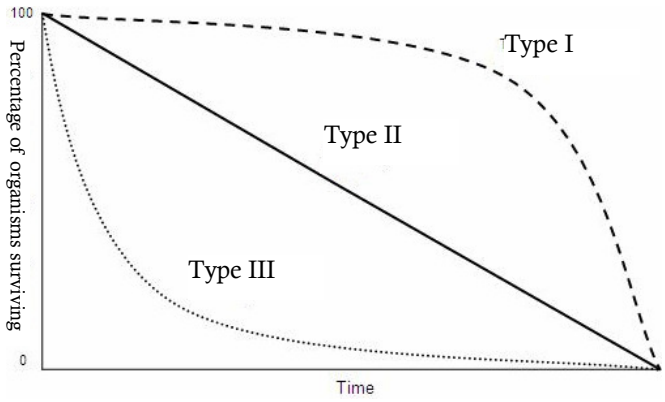


Figure 4.6: Survivorship curves: example of Type I, Type II and Type III Graph modified from Molles (2005).

The survivorship curves in Figure 4.7 were of Type-I deviating to Type-II as the pattern described in Figure 4.6, depending on the food type and treatment concentration in all of the treatments. *M. macrocopa* in Type-I showed a lower mortality rate in early and middle life spans, and the death rate shifted to a constant rate in the Type-II curve. Survivorship of *M. macrocopa* appeared to be significantly dependent ($p < 0.05$) on the food types and concentrations. *Moina* fed on 0.5 g L^{-1} FF showed the longest survival rate among all treatment diets (Figure 4.7). The average longevity of the *Moina* (21 days) at 0.5 g L^{-1} FF was almost three-fold higher compared to the control, which only survived until day-7 (*Moina* in de-chlorinated water). In contrast, the average longevity of *Moina* fed with SO was the shortest among the treatment diets. *Moina* fed with SO, CO and FP did not show any significant difference ($p > 0.05$) in the longevity compared to the control (Table 4.3).

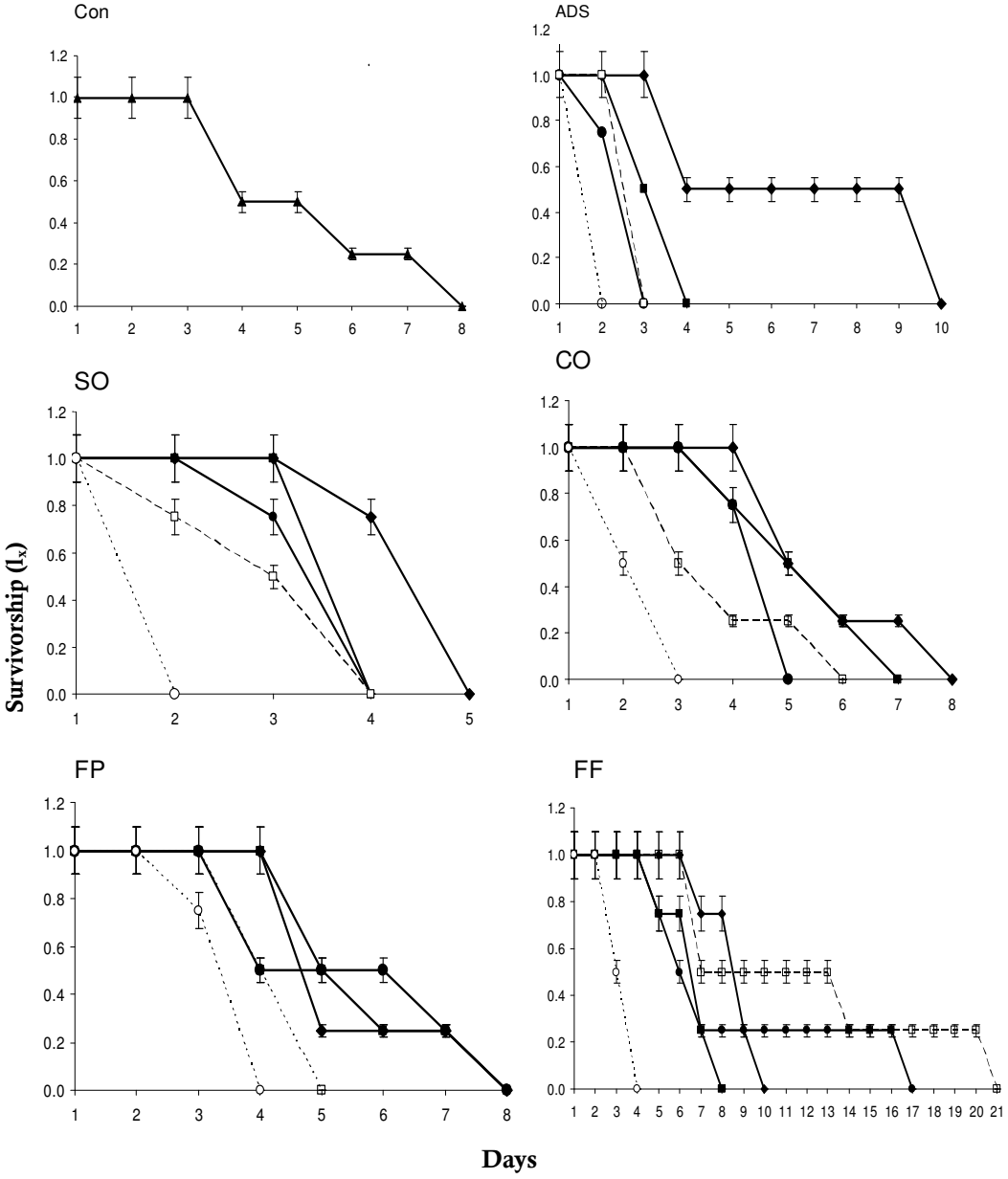


Figure 4.7: Survivorships of *Moina macrocopa* in control (Con.) and different concentrations of treatment diets: A1 DHA selco® (ADS); squid oil (SO); canola oil (CO); fish pellet (FP) and fish faeces (FF). Error bars indicate mean ± standard deviation. Symbols represent: Control; 0.0625 g L^{-1}; 0.125 g L^{-1}; 0.25 g L^{-1}; 0.5 g L^{-1}; 1.0 g L^{-1}.

Table 4.3: Life table of *Moina macrocopa* in relation to various treatment diets and concentrations.

Diet type	Life history variables						
	Conc. (g/L)	AIAR	AL	R_o	GRR	T	r
Control	–	3.00 ± 0.00^{b}	4.50 ± 1.66^{ab}	6.00	7.25 ± 1.50^{b}	3.21	0.56
ADS	1.0	$-^{a}$	1.00 ± 0.00^{a}	–	$-^{a}$	–	–
	0.5	$-^{a}$	2.00 ± 0.00^{ab}	–	$-^{a}$	–	–
	0.25	$-^{a}$	1.75 ± 0.43^{a}	–	$-^{a}$	–	–
	0.125	2.00 ± 0.00^{b}	2.50 ± 0.50^{ab}	3.25	6.50 ± 1.29^{b}	3.00	0.39
	0.0625	2.00 ± 0.00^{b}	6.00 ± 3.00^{bc}	11.50	11.50 ± 1.73^{b}	2.65	0.92
SO	1.0	$-^{a}$	1.00 ± 0.00^{a}	–	$-^{a}$	–	–
	0.5	$-^{a}$	2.25 ± 0.83^{ab}	–	$-^{a}$	–	–
	0.25	1.00 ± 1.00^{b}	2.75 ± 0.43^{ab}	1.50	2.00 ± 2.45^{b}	3.00	0.14
	0.125	2.00 ± 0.00^{b}	3.00 ± 0.00^{ab}	10.25	10.25 ± 3.30^{b}	3.00	0.78
	0.0625	2.00 ± 0.00^{b}	3.75 ± 0.43^{ab}	11.69	11.75 ± 4.03^{b}	3.02	0.82
CO	1.0	$-^{a}$	1.50 ± 0.50^{a}	–	$-^{a}$	–	–
	0.5	2.00 ± 2.00^{b}	3.00 ± 1.22^{ab}	0.13	0.50 ± 1.00^{b}	4.00	-0.52
	0.25	2.25 ± 1.30^{b}	3.75 ± 0.43^{ab}	2.00	2.00 ± 1.83^{b}	3.00	0.23
	0.125	3.00 ± 0.00^{b}	4.50 ± 1.12^{ab}	15.56	16.50 ± 2.06^{b}	3.06	0.90
	0.0625	2.00 ± 0.00^{b}	5.00 ± 1.22^{ab}	21.75	21.75 ± 3.59^{c}	3.32	0.93
FP	1.0	2.25 ± 1.30^{b}	2.75 ± 0.43^{ab}	3.94	5.25 ± 3.59^{b}	3.00	0.46
	0.5	2.00 ± 0.00^{b}	3.50 ± 0.50^{ab}	19.25	20.75 ± 5.85^{c}	2.26	1.31
	0.25	2.00 ± 0.00^{b}	4.75 ± 1.79^{ab}	16.75	16.75 ± 2.36^{b}	2.09	1.35
	0.125	2.00 ± 0.00^{b}	5.00 ± 1.22^{ab}	20.00	20.00 ± 3.56^{c}	2.79	1.07
	0.0625	3.00 ± 0.00^{b}	4.75 ± 1.30^{ab}	3.25	3.25 ± 1.71^{b}	3.00	0.39
FF	1.0	1.50 ± 1.50^{b}	2.50 ± 0.50^{ab}	1.38	2.75 ± 3.20^{b}	3.00	0.11
	0.5	3.00 ± 0.00^{b}	11.25 ± 5.80^{c}	13.00	13.00 ± 1.83^{b}	4.00	0.64
	0.25	3.00 ± 0.00^{b}	7.75 ± 4.82^{bc}	10.94	11.75 ± 3.10^{b}	3.45	0.69
	0.125	3.00 ± 0.00^{b}	5.75 ± 1.09^{bc}	25.25	26.50 ± 7.68^{c}	3.54	0.91
	0.0625	3.00 ± 0.00^{b}	7.75 ± 1.09^{bc}	41.88	43.75 ± 7.37^{d}	4.60	0.81

The abbreviations AIAR, AL, R_o, GRR, T and r refer to average initial age of reproduction (days), average longevity (days), net reproduction rate, gross reproduction rate, generation time and intrinsic rate of population increase, respectively. AIAR, AL and GRR are shown as mean ± standard deviation. Results are based on four replicate recordings. Dash (-) indicates no offspring was produced, $n = 4$. Superscripts a, b, c, d denote significant differences among dietary groups with $P < 0.05$ (Tukey's test).

Similar trends were also observed in the life expectancy curves for all treatments (Figure 4.8). The average longevity of *M. macrocopa* fed with FF at 0.5 g L^{-1} is the only treatment where the survivorship was better than the control organisms. At 1.0 g L^{-1}, there was no significant difference ($p > 0.05$) in the longevity of *Moina* among all diets (Table 4.3). Decreasing the concentration of ADS from 1.0 to 0.0625 g L^{-1}, however, improved the average longevity of *Moina*. Conversely, the average longevity of *M. macrocopa* was not significantly affected by different concentrations of SO, CO and FP (Table 4.3) (Appendix B).

Cumulative birth rates of *M. macrocopa* treated with FP and FF were higher than control organisms at 0.0625 g L^{-1} with the exception of the FP treatment (Figure 4.9). *Moina* showed good reproduction rates (expressed as the ratio of net reproduction rate and reproduction span) at the lowest concentration of all food types, except for FP, which showed the best reproduction rate at 0.25 g L^{-1} (Figure 4.10). The gross reproduction rate was the highest when *M. macrocopa* was fed with FF at 0.0625 g L^{-1}. There was no significant difference ($p > 0.05$) among the concentrations of ADS and SO with respect to gross reproduction rates. *M. macrocopa* fed with CO showed a similar trend in reproduction rate as did the FF treatment, where gross reproduction increased with decreasing emulsion concentration (Table 4.3) (Appendix B). However, this effect of diet concentration was not obvious in the FP diet treatment, in which the gross reproduction rate fluctuated as the FP concentration was decreased. A positive *r* value was observed for all treatment diets and

concentrations with the notable exception of CO at 0.5 g L^{-1}, which showed a negative value (Table 4.3) (Appendix B).

Analysis from the data shown in Table 4.4 (Appendix C) indicates that there are significant statistical differences between the nature of the treatment diets and their concentrations during the initial age of reproduction for *M. macrocopa*, average longevity and gross reproduction rate. All significant levels, including the interaction effects of both treatment diets and concentrations, ranged between $p = 0.001$ and 0.05.

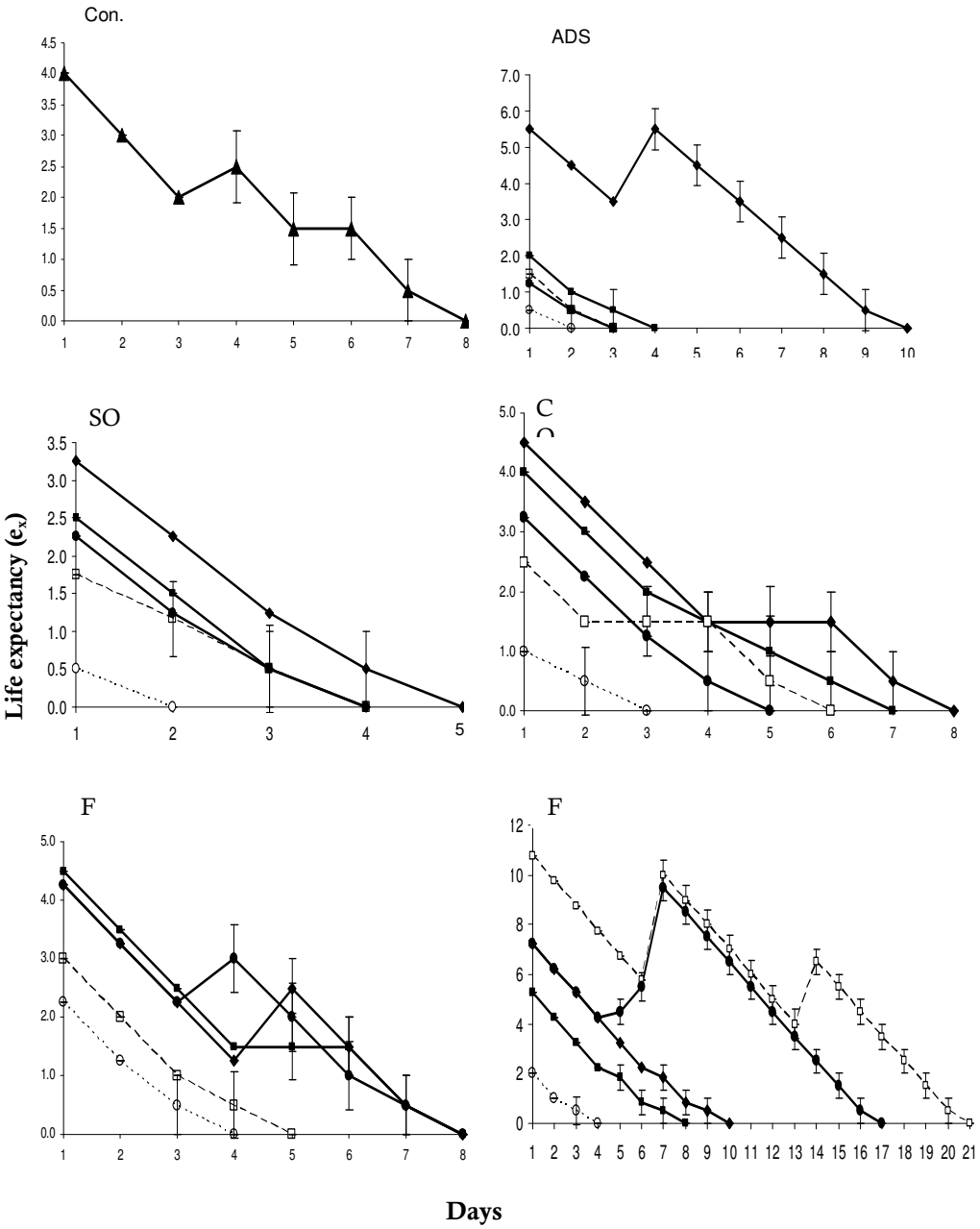


Figure 4.8: Life expectancies of *Moina macrocopa* in control (Con.) and different concentrations of treatment diets: A1 DHA selco® (ADS); squid oil (SO); canola oil (CO); fish pellet (FP) and fish faeces (FF). Error bars indicate mean ± standard deviation. Symbols represent: —▲— Control; —◆— 0.0625 g L^{-1}; —■— 0.125 g L^{-1}; —●— 0.25 g L^{-1}; —□— 0.5 g L^{-1}; - -○- - 1.0 g L^{-1}.

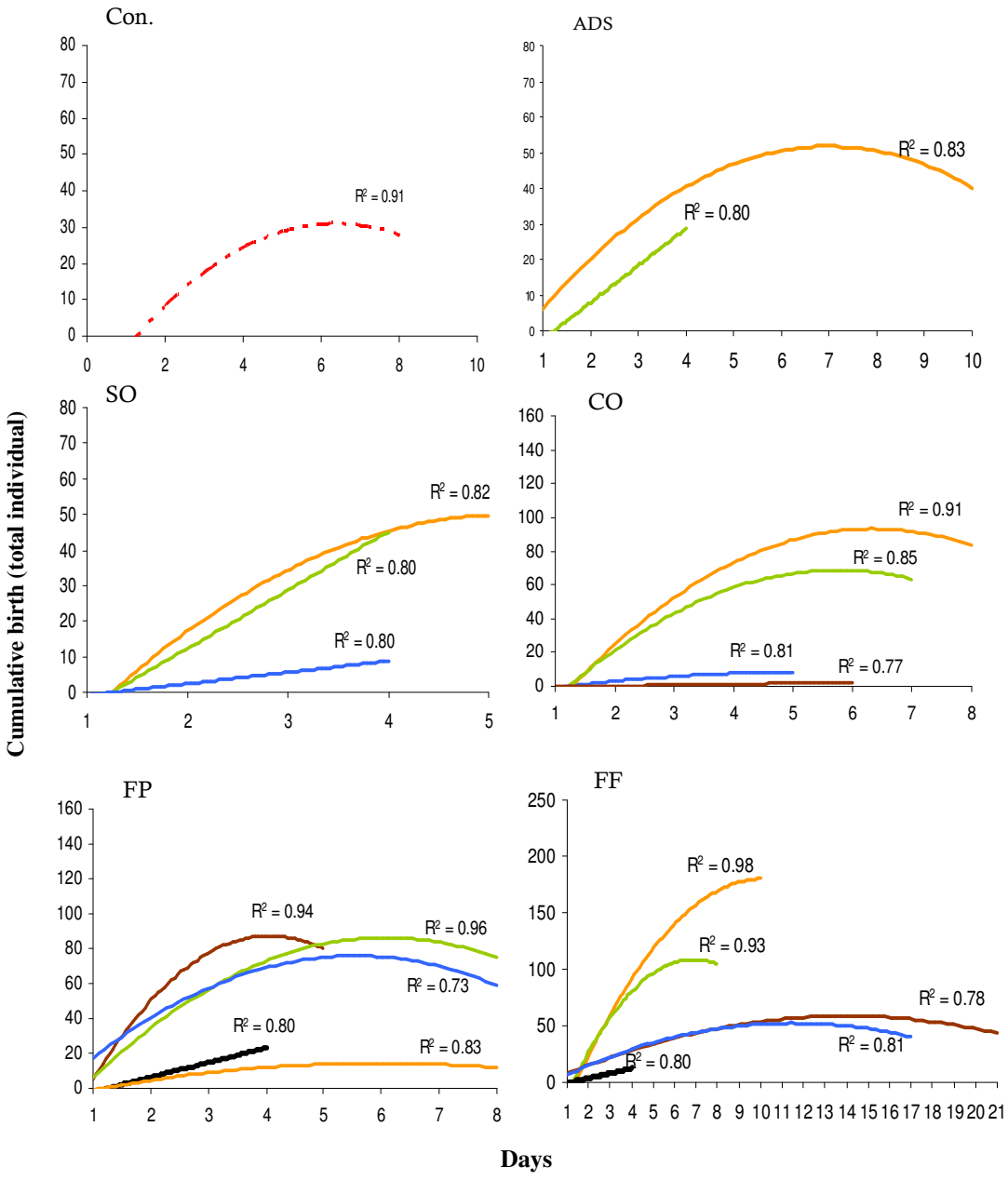


Figure 4.9: Cumulative births (polynomial curves) of *Moina macrocopa* in control (Con.) and different concentrations of treatment diets: A1 DHA selco® (ADS); squid oil (SO); canola oil (CO); fish pellet (FP) and fish faeces (FF). Symbols represent: Control; 0.0625 g L^{-1}; 0.125 g L^{-1}; 0.25 g L^{-1}; 0.5 g L^{-1}; 1.0 g L^{-1}.

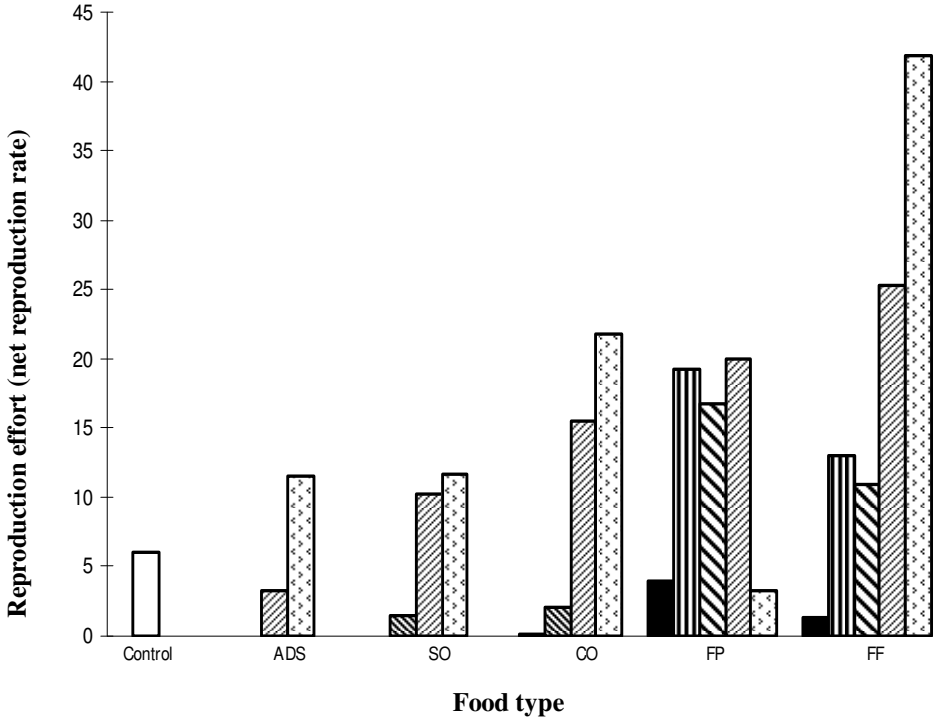


Figure 4.10: Net reproduction rates of *Moina macrocopa* in control and different concentrations of treatment diets: A1 DHA selco® (ADS); squid oil (SO); canola oil (CO); ground fish pellet (FP); fish faeces (FF). Symbols represent: Control; 0.0625 g L^{-1}; 0.125 g L^{-1}; 0.25 g L^{-1}; 0.5 g L^{-1}; 1.0 g L^{-1}.

Table 4.4: Results of two-way ANOVA (analysis of variance) performed for different life history variables

Variables and sources	**DF**	**SS**	**MS**	***F***	***P***
Initial age of reproduction					
Treatment diets	4	33.38	8.35	17.67	0.000***
Concentrations	5	39.37	7.87	16.67	0.000***
Diets x Conc.	20	64.22	3.21	6.80	0.000***
Error	90	42.50	0.47		
Average longevity					
Treatment diets	4	218.45	54.61	12.77	0.000***
Concentrations	5	152.08	30.42	7.11	0.000***
Diets x Conc.	20	185.05	9.25	2.16	0.007**
Error	90	384.75	4.28		
Gross reproduction rate					
Treatment diets	4	2894.53	723.63	78.85	0.000***
Concentrations	5	4021.27	804.25	87.63	0.000***
Diets x Conc.	20	4416.57	220.83	24.06	0.000***
Error	90	826.00	9.18		

DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F-ratio, P = P-value. Level of significance: *P<0.05; **P<0.01; *** P<0.001.

4.4 Effects of Concentration, Enrichment Time and Potential Enrichment Diets on the HUFA's Profile of *Moina macrocopa*

The fatty acid contents varied among the groups of un-enriched and enriched *Moina macrocopa*. Table 4.5 shows the fatty acid profile of un-enriched *M. macrocopa* (12 h) and the various lipid emulsions that were used as reference diets (Appendix F) in the study. Tables 4.6 & 4.7 show the fatty acid compositions of *M. macrocopa* after enrichment with various types of oil emulsion, with varying incubation times and concentrations. Figure 4.11 shows the *M. macrocopa* after being enriched with the oil emulsions.

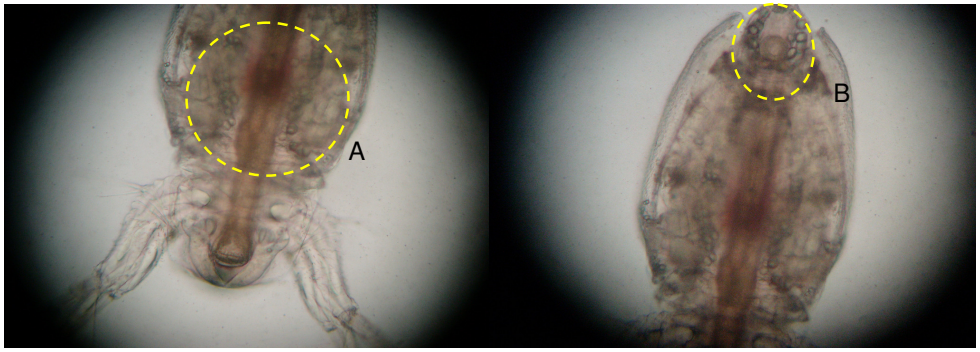


Figure 4.11: The encapsulated oil globules (yellow dash circles a; b) were found mainly on body trunk and the posterior area of the enriched *M. macrocopa* (200 x magnification)

The initial levels of linoleic acid [18:2(*n*-6)], and linolenic acid [18:3(*n*-3)] in *M. macrocopa* after 12 h starvation were 0.23 and 0.14 mg g^{-1} BW (body weight, wet weight), respectively. Linoleic acid increased to a maximum level of 0.50 mg g^{-1} BW after the *M. macrocopa* had been fed with 1 g L^{-1} SO diet for 12 h, and 0.40 mg g^{-1} BW with the 2 g L^{-1} CO diet at 36 h (Tables 4.6 & 4.7). The detected linolenic acid levels increased as the SO or CO

concentration was raised to 2 g L^{-1}, yielding a 0.70 mg g^{-1} BW for SO and 0.86 mg g^{-1} BW for CO after 12 h enrichment (Table 4.7).

Concentrations of essential fatty acids such as arachidonic acid (AA, 20:4*n*-6), eicosapentaenoic acid (EPA, 20:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3) were found to be low in un-enriched *M. macrocopa* (Table 4.5). With the addition of 1 g L^{-1} ADS (Table 4.6) after 12 h, the level of AA was elevated to 0.28 mg g^{-1} BW. AA levels were raised again to 0.38 mg g^{-1} BW by the addition of 2 g L^{-1} CO, after a 12 h enrichment period (Table 4.7). The level of EPA was significantly ($P < 0.05$) (Appendix G) increased to 0.38 mg g^{-1} BW through the enrichment with 1 g L^{-1} ADS for 12 h. EPA level of *M. macrocopa* fed with ground fish pellets (FP) showed better results at 12 and 24 h enrichment which were 0.20 and 0.19 mg g^{-1} BW at the concentration of 2 g L^{-1} (Table 4.7). The enrichment of the diet with ADS showed promising results with regard to levels of the fatty acid DHA. The level of DHA [22:6(*n*-3)] increased to 0.09 mg g^{-1} BW at 1 g L^{-1} and 0.08 mg g^{-1} BW at 2 g L^{-1} for ADS enrichment after 12 h and 24 h, respectively (Tables 4.6 & 4.7), while trace levels in the un-enriched culture were undetectable (Table 4.5).

Figures 4.12 and 4.13 show the fatty acid enrichment kinetics of *M. macrocopa* (in ratio per body weight in grams) following enrichment with the five types of treatment diets under varying conditions. The AA/EPA ratios of *M. macrocopa* were higher at FF (66.63) and FP (50.50) at 24 h and followed by CO (46.5) and SO (14.0), each at 1 g L^{-1}, at the 12 h of enrichment (Figure 4.12A). When the concentration of the oil component of the diet was increased

to 2 g L^{-1}, the AA/EPA ratios of *M. macrocopa* enriched with CO were noticeably elevated to the highest level (2476.3) at 12 h enrichment. When SO was added in the same concentration, the AA/EPA ratio of the *M. macrocopa* was the highest (167.2) at 24 h (Figure 4.13A). The AA/DHA ratio of CO, SO and ADS shared a similar trend with DHA/EPA at the concentration of 1 g L^{-1}, except for FF and FP. Both of the treatment diets of FP had the highest AA/DHA value which was 313.0 at 24 h enrichment and 42.43 at 12 h enrichment in the latter (Figures 4.12B and 4.12C). This trend changed at a concentration of 2 g L^{-1} where FF and FP were the lowest in these cases. With the treatment diet containing CO at 1 g L^{-1}, the AA/DHA and DHA/EPA ratios of the Cladocerans showed very similar trends, reaching the maximum after 24 h enrichment (Figures 4.12B & C). However, it did not occur at the treatment concentration of 2 g L^{-1}, where AA/DHA ratio of *M. macrocopa* fed with CO was the highest after 12 h enrichment (856.3) and decreased significantly ($P < 0.001$) (Appendix G) after 24 h (Figure 4.13B). The DHA/EPA ratio of *M. macrocopa* enriched with 1 g L^{-1} of CO was the highest (16.0) at 24 h (Figure 4.12C), however, it did not show a consistent trend where the DHA/EPA ratio of *M. macrocopa* fed with SO was the highest with 2 g L^{-1} enrichment at 12 h (210.0) (Figure 4.13C). On the other hand, the DHA/EPA ratio of *M. macrocopa* fed with 1 g L^{-1} FP was found to be high (43.0) at 12 h enrichment (Figure 4.12). However, this trend was not observed at 2 g L^{-1} of the same diet (Figure 4.13).

Table 4.8 shows the *F*-values and *P*-values of the two-way ANOVA that were used to analyze the effects of concentration, time, and the interaction of these

two factors on the *M. macrocopa* fatty acid composition (Appendix G). Diet concentration was shown to be the predominant factor in comparison with the enrichment time. AA, EPA and DHA levels increased significantly (*P*-value ranged from < 0.001 to 0.027) when treatment concentrations were raised. One notable exception was the AA level for ADS enrichment ($P = 0.292$) and DHA with FP which showed low measurable levels of this fatty acid in the *M. macrocopa* during enrichment (Table 4.8). The ratios of *n*-6 and *n*-3 HUFAs were significantly affected by the enrichment time in ADS. Positive trends were also observed for the AA/DHA ratios with all SO, CO, and FP enrichments, whilst there was no effect or change in the HUFA ratios for the diet concentration and enrichment time of FF. Continued enrichment using the treatment diets for more than 24 h with either treatment concentration neither altered the EPA and DHA levels, nor the AA/EPA and DHA/EPA ratios. *M. macrocopa* enrichment by ADS had the highest overall EPA and DHA contents, showing significant interactions ($P < 0.05$) between treatment concentration and enrichment time (Table 4.8).

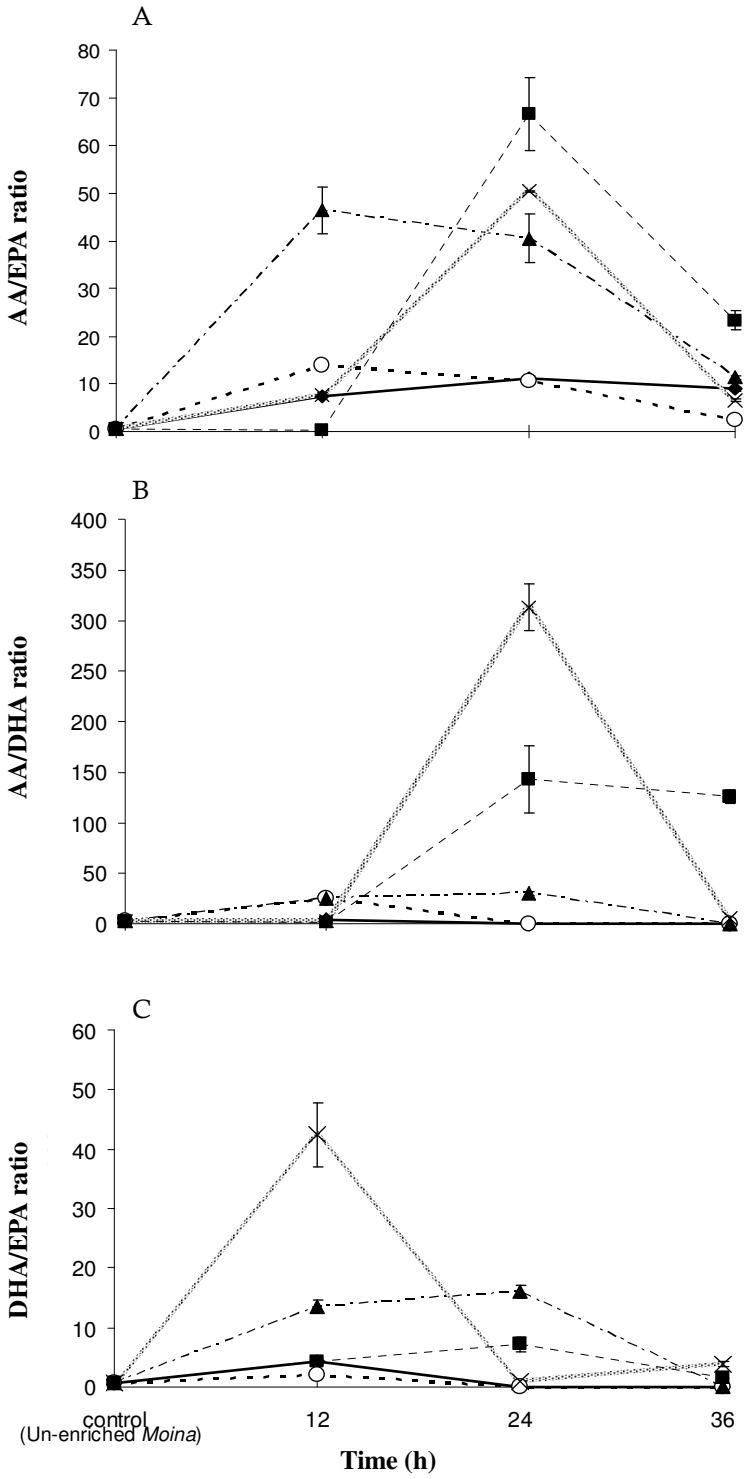


Figure 4.12: Changes of AA, EPA and DHA ratios (in ratio of fatty acids g^{-1} body of weight) of un-enriched *Moina* (control), ADS, CO, SO, FF and FP enriched *Moina* after 12, 24 and 36 h at the concentration of 1 g L^{-1}. Symbols represent: —◆— A1 DHA selco®; –○– squid oil; – –▲– – canola oil; ▒×▒ fish pellet; –■– fish faeces. Error bars = mean ± standard deviation.

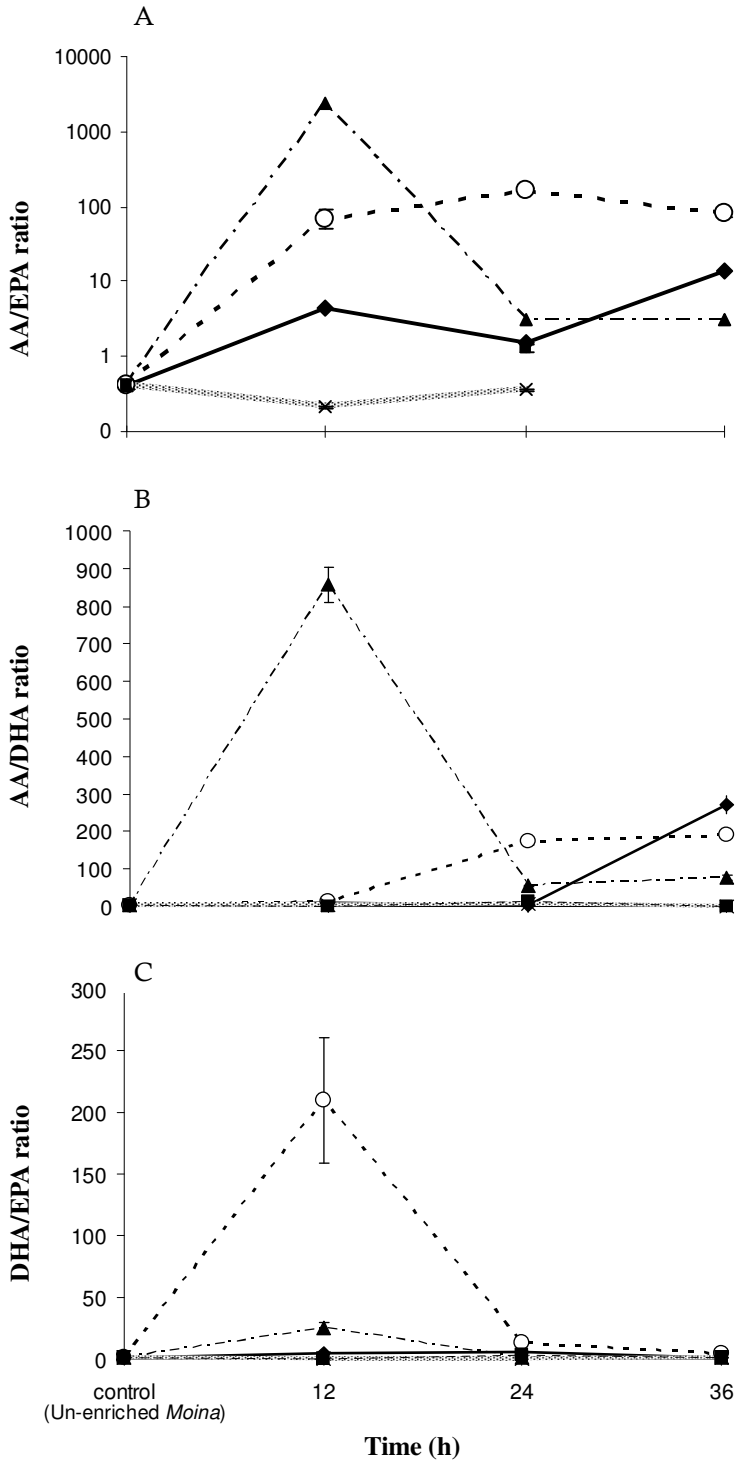


Figure 4.13: Changes of AA, EPA and DHA ratios (in ratio of fatty acids g^{-1} body of weight) of un-enriched *Moina* (control), ADS, CO, SO, FF and FP enriched *Moina* after 12, 24 and 36 h at the concentration of 2 g L^{-1}. Symbols represent: —◆— A1 DHA selco®; – ○ – squid oil; – ·▲· – canola oil; ▒▒ fish pellet; –■ - fish faeces. Error bars = mean ± standard deviation.

Table 4.5: The fatty acid composition of un-enriched *M. macrocopa* (12 h), lipid sources of emulsions and other diets in mg g^{-}

	un-enriched	lipid sources of emulsions				
Fatty acid	*Moina*	ADS*	SO*	CO*	FP*	FF*
12:0	—	—	—	0.01±<0.01	—	—
14:0	0.02±<0.01	—	0.01±<0.01	0.01±<0.01	—	0.02±<0.01
14:1	0.01±<0.01	—	0.01±<0.01	0.02±<0.01	—	—
15:0	0.10±<0.01	0.23±<0.01	0.05±<0.01	0.03±<0.01	0.01±<0.01	0.01±<0.01
16:0	0.04±<0.01	0.04±<0.01	0.02±<0.01	—	—	—
17:0	0.40±0.05	0.95±<0.01	—	0.11±<0.01	—	—
18:0	0.31±0.03	0.05±<0.01	0.07±<0.01	0.07±<0.01	0.01±<0.01	0.01±<0.01
18:1*n*-9	0.07±0.01	0.03±<0.01	0.03±<0.01	0.03±<0.01	—	—
18:2*n*-6	0.23±0.02	—	1.00±<0.01	0.79±<0.01	0.09±<0.01	0.06±<0.01
20:0	0.03±<0.01	—	0.03±<0.01	0.01±<0.01	—	0.02±<0.01
18:3*cis*-6	0.47±0.01	—	0.90±<0.01	0.84±<0.01	0.08±<0.01	0.12±<0.01
18:3*n*-3	0.14±0.01	—	0.01±<0.01	0.01±<0.01	0.01±<0.01	0.05±<0.01
22:0	0.01±<0.01	0.03±<0.01	0.01±<0.01	0.01±<0.01	—	—
20:3*n*-6	—	—	0.04±<0.01	0.06±<0.01	—	—
20:3*n*-3	0.04±<0.01	—	0.08±<0.01	0.09±<0.01	0.01±<0.01	0.01±<0.01
20:4*n*-6	—	—	0.01±<0.01	—	—	—
20:5*n*-3	0.15±0.01	—	0.05±<0.01	0.07±<0.01	0.01±<0.01	—
22:6*n*-3	—	0.04±<0.01	0.03±<0.01	0.01±<0.01	—	—
∑ SAFA	277.92±4.96	508.60±0.40	68.76±0.66	113.96±0.14	129.18±0.41	131.17±0.29
∑ MUFA	308.61±9.07	193.67±0.07	576.79±0.22	504.96±0.15	502.52±0.10	408.25±0.14
∑ PUFA	413.47±4.15	297.73±0.47	354.46±0.88	381.08±0.28	368.30±0.31	460.58±0.15
∑ *n*-3 PUFA	90.14±1.52	3.19±<0.01	27.55±0.05	35.41±0.25	37.43±0.05	114.27±0.19
∑ *n*-6 PUFA	1.51±0.05	1.13±0.01	8.10±0.01	12.50±0.11	0.04±0.01	—
AA/EPA	0.41±0.05	8.33±0.24	2.50±0.01	0.61±0.01	—	—
AA/DHA	3.13±0.18	12.50±0.35	4.81±0.03	3.21±0.02	ND	ND
DHA/EPA	0.66±0.08	6.67±<0.01	5.20±0.01	1.91±0.01	—	—

All values are given in mean ± S.E.M. with *M. macrocopa* (control) in 4 replicates and experimental diets in 2 replicates. Dash (—) indicates that the fatty acid was <0.01. ND indicates non-detected. Lipid emulsions and other diet sources are as below:

*ADS = A1 DHA selco® , INVE, Belgium *SO = Squid oil, Asia star laboratory Ltd., Thailand.

*CO = Canola oil (vegetable oil), Sime Darby edible product Ltd., Singapore.

*FP = Fish pellet (powder form), Cargill™, Malaysia *FF = Fish faeces, from red tilapia *Oreochromis niloticus*

Table 4.6: The fatty acid composition (mg g^{-1} body weight) of *M. macrocopa* enriched at 1 g L^{-1} with varied experimental diets over different enrichment

	ADS			SO			CO		
Fatty acid	12 h	24 h	36 h	12 h	24 h	36 h	12 h	24 h	36 h
12:0	—	0.03±<0.01	—	0.02±<0.01	0.01±<0.01	—	—[a]	0.01±<0.01[b]	—[a]
14:0	0.02±<0.01	0.01±<0.01	0.01±<0.01	0.01±<0.01	—	—	0.44±0.01[b]	0.02±<0.01[a]	—[a]
14:1	0.01±<0.01[b]	—[a]	—[ab]	—	—	—	0.04±<0.01[b]	—[a]	0.01±<0.01[a]
15:0	1.36±0.12[a]	0.11±<0.01[b]	0.44±0.05[ab]	0.01±<0.01	—	—	0.29±0.01[b]	—[a]	0.03±<0.01[a]
16:0	0.36±0.03[b]	0.02±<0.01[a]	0.05±<0.01[a]	0.01±<0.01	—	—	3.22±0.16[b]	—[a]	0.03±<0.01[a]
17:0	8.50±0.70[b]	1.36±0.14[a]	1.78±0.16[a]	0.05±0.01	0.01±<0.01	—	0.10±0.01	—	0.56±0.07
18:0	1.37±0.12[b]	0.05±<0.01[a]	0.23±0.03[a]	0.07±0.01	0.03±<0.01	—	—	0.04±<0.01	0.08±<0.01
18:1*n*-9	0.16±0.01	0.41±0.05	0.52±0.06	0.49±0.03[b]	0.45±0.01[b]	0.02±<0.01[a]	0.48±0.03[b]	0.13±0.01[a]	0.01±<0.01[a]
18:2*n*-6	0.21±0.01	0.28±0.03	0.08±0.01	0.50±0.06	0.01±<0.01	—	0.05±<0.01	0.01±<0.01	0.15±<0.01
20:0	0.20±0.02	0.06±0.01	0.01±<0.01	0.01±<0.01	0.14±0.01	—	0.04±<0.01	—	0.17±0.02
18:3*cis*-6	0.87±0.01[b]	0.10±<0.01[a]	0.06±<0.01[a]	0.05±<0.01	0.04±<0.01	—	—	—	1.07±0.12
18:3*n*-3	0.01±<0.01	0.01±<0.01	—	0.01±<0.01	0.01±<0.01	—	0.02±<0.01	—	0.06±<0.01
22:0	0.08±0.01[b]	—[a]	0.02±<0.01[a]	0.01±<0.01	0.02±<0.01	—	—[a]	0.10±0.01[b]	0.01±<0.01[a]
20:3*n*-6	0.37±0.04	0.10±0.01	0.07±0.01	0.07±0.01	0.03±<0.01	—	—	—	0.01±<0.01
20:3*n*-3	—	—	—	0.02±<0.01	0.02±<0.01	—	—	—	—
20:4*n*-6	0.28±0.01[b]	0.03±<0.01[a]	0.04±<0.01[a]	0.01±<0.01	0.01±<0.01	—	0.01±<0.01	0.02±<0.01	0.01±<0.01
20:5*n*-3	0.38±0.02[b]	0.03±<0.01[a]	0.05±<0.01[a]	0.02±<0.01	0.01±<0.01	—	—	0.01±<0.01	0.01±<0.01
22:6*n*-3	0.09±0.01[b]	—[a]	—[a]	—	—	—	—	0.01±<0.01	—
∑ SAFA*	447.73±2.99	410.63±30.59	411.29±22.91	250.49±10.09[a]	764.95±19.78[b]	968.20±3.68[b]	673.68±22.49[b]	343.60±4.57[a]	121.16±2.76[a]
∑ MUFA*	268.05±8.80[a]	423.65±38.69[ab]	510.32±26.06[b]	594.25±19.01[b]	222.18±19.77[a]	24.83±2.87[a]	290.76±21.56[a]	577.51±2.35[b]	214.18±15.96[a]
∑ PUFA*	284.22±11.67[b]	165.65±8.15[ab]	78.38±3.16[a]	155.26±9.13	12.86±0.22	6.96±0.81	35.56±0.95[a]	78.89±6.58[a]	664.65±13.97[b]
∑ *n*-3 PUFA	27.65±1.86[b]	6.25±0.20[a]	6.26±0.24[a]	7.94±0.59	3.49±0.09	1.35±0.16	4.97±0.36	2.43±0.10	11.14±0.72
∑ *n*-6 PUFA	21.22±0.39	24.78±2.48	15.50±0.95	18.59±1.42	3.87±0.07	2.77±0.33	2.49±0.16	2.86±0.18	3.34±0.14
AA/EPA	7.35±0.10	11.31±0.44	8.94±0.35	14.00±0.71	10.52±0.20	2.50±<0.01	46.50±4.94	40.45±5.03	11.35±0.41
AA/DHA	4.27±0.18	—	—	25.00±1.41	—	—	25.25±2.09	30.03±2.30	—
DHA/EPA	4.23±0.49	—	—	2.08±0.25	—	—	13.63±0.93	16.00±1.12	—

All the values are given in means (± S.E.M, n=4). Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet. Dash (—) indicates that the fatty acid was <0.01.

*∑ SAFA: total saturated fatty acids; ∑ MUFA: total monounsaturated fatty acids; ∑ PUFA: total polyunsaturated fatty acids.

Table 4.6 (continued): The fatty acid composition (mg g^{-1} body weight) of *M. macrocopa* enriched at 1 g L^{-1} with varied experimental diets over different enrichment periods

	FP			FF		
Fatty acid	12 h	24 h	36 h	12 h	24 h	36 h
12:0	—	0.01±<0.01	—	—	—	—
14:0	0.08±0.04^{b}	0.01±<0.01^{a}	0.01±<0.01^{a}	0.05±<0.01	0.20±0.02	—
14:1	0.06±<0.01^{b}	—^{a}	0.01±<0.01^{a}	0.01±<0.01	0.02±<0.01	—
15:0	0.43±0.03^{b}	—^{a}	0.06±0.01^{a}	0.09±<0.01	0.01±<0.01	—
16:0	1.54±0.17^{b}	—^{a}	0.05±<0.01^{a}	0.22±0.02^{b}	0.02±<0.01^{a}	0.02±<0.01^{a}
17:0	0.08±0.01	—	0.68±0.08	0.05±<0.01	—	—
18:0	—	0.05±<0.01	0.17±0.01	0.35±0.02	0.86±0.10	0.02±<0.01
18:1*n*-9	0.02±<0.01	0.10±0.01	0.01±<0.01	0.13±0.01	0.26±0.03	0.02±<0.01
18:2*n*-6	0.04±<0.01	—	0.33±0.01	0.22±0.03	0.02±<0.01	0.03±<0.01
20:0	0.07±0.01	—	0.43±0.05	0.02±<0.01	0.01±<0.01	0.01±<0.01
18:3*cis*-6	0.03±<0.01	0.01±<0.01	0.15±0.02	0.22±0.02	0.04±<0.01	0.10±<0.01
18:3*n*-3	0.12±0.01^{b}	—^{a}	0.02±<0.01^{ab}	0.09±0.01	0.01±<0.01	—
22:0	0.01±<0.01^{a}	0.03±<0.01^{b}	0.02±<0.01^{ab}	0.04±<0.01	0.02±<0.01	0.03±<0.01
20:3*n*-6	0.01±<0.01	—	0.02±<0.01	—^{a}	—^{a}	0.12±0.01^{b}
20:3*n*-3	0.04±<0.01	—	0.01±<0.01	0.04±<0.01	0.04±<0.01	—
20:4*n*-6	—^{a}	0.04±<0.01^{b}	0.01±<0.01^{a}	—^{a}	0.03±<0.01^{b}	0.03±<0.01^{ab}
20:5*n*-3	0.01±<0.01	—	0.01±<0.01	0.02±<0.01	—	0.03±<0.01
22:6*n*-3	0.02±<0.01^{b}	—^{b}	—^{a}	—	—	—
∑ SAFA*	759.64±10.99^{b}	185.71±5.28^{a}	389.78±7.14^{a}	311.45±9.47^{a}	735.18±7.04^{b}	454.01±37.32^{ab}
∑ MUFA*	148.48±8.42^{a}	476.87±31.25^{b}	380.81±17.17^{a}	437.21±2.96^{b}	165.34±7.08^{a}	50.34±3.16^{a}
∑ PUFA*	91.88±2.59	337.42±26.27	229.40±11.04	251.35±11.18	99.48±0.90	495.66±34.17
∑ *n*-3 PUFA	39.08±1.85^{b}	5.63±0.30^{a}	12.70±0.19^{a}	43.70±4.57	17.07±0.80	12.35±1.03
∑ *n*-6 PUFA	3.26±0.18^{a}	22.43±1.84^{b}	7.49±0.36^{a}	0.81±0.09^{a}	12.86±1.22^{a}	44.92±3.49^{b}
AA/EPA	7.61±0.63	50.50±0.14	6.55±0.24	0.14±0.02^{a}	66.63±7.53^{b}	23.29±2.09^{ab}
AA/DHA	3.09±0.17^{a}	313.00±23.09^{b}	5.00±<0.01^{a}	1.67±0.19	143.00±32.85	126.00±6.32
DHA/EPA	42.43±5.49	1.00±23.09	3.75±0.48	4.18±0.43	7.17±1.19	1.53±0.09

All the values are given in means (± S.E.M, n=4). Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet. Dash (—) indicates that the fatty acid was <0.01.

*∑ SAFA: total saturated fatty acids; *∑ MUFA: total monounsaturated fatty acids; *∑ PUFA: total polyunsaturated fatty acids.

Table 4.7: The fatty acid composition (mg g^{-1} body weight) of *M. macrocopa* enriched at 2 g L^{-1} with varied experimental diets over different enrichment

	ADS			SO			CO		
Fatty acid	12 h	24 h	36 h	12 h	24 h	36 h	12 h	24 h	36 h
12:0	—	0.01±<0.01	—	—	0.01±<0.01	0.01±<0.01	0.01±<0.01[a]	0.01±<0.01[b]	0.01±<0.01[b]
14:0	0.01±<0.01	0.01±<0.01	0.03±<0.01	—[a]	0.03±<0.01[b]	0.04±<0.01[b]	—	0.02±<0.01	0.04±<0.01
14:1	0.01±<0.01[a]	—[a]	0.01±<0.01[b]	—[a]	0.01±<0.01[b]	0.01±<0.01[b]	—	0.01±<0.01	0.01±<0.01
15:0	0.22±<0.01	0.23±<0.01	0.32±<0.01	—[a]	0.21±<0.01[b]	0.16±<0.01[b]	—[a]	0.13±0.01[b]	0.12±<0.01[a]
16:0	0.09±<0.01	0.09±<0.01	0.11±<0.01	0.03±<0.01[a]	0.11±<0.01[b]	0.09±<0.01[b]	0.06±<0.01	0.09±<0.01	0.07±<0.01
17:0	1.70±0.01	1.81±0.02	2.37±<0.01	—[a]	4.33±0.02[b]	3.83±0.04[b]	—[a]	2.21±0.13[b]	2.68±0.06[b]
18:0	0.16±<0.01	0.17±<0.01	0.14±<0.01	1.29±0.08[b]	0.14±<0.01[a]	0.11±<0.01[a]	2.31±0.12[b]	0.11±0.01[a]	0.09±<0.01[a]
18:1*n*-9	0.05±<0.01	0.01±<0.01	0.02±<0.01	0.05±<0.01	0.04±<0.01	0.04±<0.01	0.11±<0.01	0.01±<0.01	0.09±0.01
18:2*n*-6	0.08±<0.01	0.09±<0.01	0.08±<0.01	0.22±0.02	0.12±<0.01	0.19±<0.01	0.05±<0.01	0.18±<0.01	0.40±0.01
20:0	0.01±<0.01	0.02±<0.01	0.02±<0.01	1.55±0.13[b]	0.02±<0.01[a]	0.02±<0.01[a]	2.24±0.06[b]	0.02±<0.01[a]	0.03±<0.01[a]
18:3*cis*-6	0.61±<0.01[b]	0.65±<0.01[b]	0.10±0.01[a]	0.78±0.08[b]	0.05±<0.01[a]	0.08±<0.01[a]	1.19±0.17	1.04±0.12	0.14±0.01
18:3*n*-3	0.02±<0.01	0.02±<0.01	—	0.70±0.12	0.01±<0.01	0.01±<0.01	0.86±0.10[b]	0.04±<0.01[a]	0.01±<0.01[a]
22:0	0.01±<0.01	0.01±<0.01	0.02±<0.01	0.06±0.01	0.02±<0.01	0.02±<0.01	0.06±<0.01	0.02±<0.01	0.02±<0.01
20:3*n*-6	0.02±<0.01	0.01±<0.01	0.09±0.01	0.03±<0.01[a]	0.20±<0.01[b]	0.35±0.01[c]	0.03±<0.01[a]	0.01±<0.01[a]	0.79±0.01[b]
20:3*n*-3	0.01±<0.01	—	—	—	0.01±<0.01	—	—	0.01±<0.01	—
20:4*n*-6	0.04±<0.01	0.02±<0.01	0.07±<0.01	0.01±<0.01[a]	0.14±<0.01[b]	0.14±<0.01[b]	0.38±0.03[b]	0.03±<0.01[a]	0.03±<0.01[a]
20:5*n*-3	0.10±<0.01	0.14±<0.01	0.05±<0.01	—	0.01±<0.01	0.03±<0.01	—	0.10±<0.01	0.08±<0.01
22:6*n*-3	0.04±<0.01	0.08±<0.01	—	0.04±<0.01	0.01±<0.01	0.01±<0.01	—	0.01±<0.01	—
∑ SAFA*	384.22±1.01	376.27±1.17	485.66±0.92	300.12±3.99	532.77±0.27	504.66±1.08	511.76±14.78	250.63±13.89	222.18±3.34
∑ MUFA*	202.32±0.25	179.70±0.37	207.66±0.48	330.16±24.52	207.65±2.44	209.30±1.76	96.83±2.17	122.15±5.99	122.83±1.45
∑ PUFA*	413.46±0.87	444.03±1.37	306.68±0.51	369.72±27.54	259.58±2.58	286.03±2.51	391.40±12.66[a]	627.22±19.87[a]	654.99±4.67[b]
∑ *n*-3 PUFA	27.36±0.20[b]	36.32±0.29[b]	8.99±0.04[a]	17.63±0.98	4.71±0.10	6.03±0.29	69.34±7.92	13.97±0.08	6.67±0.10
∑ *n*-6 PUFA	9.88±0.53	4.60±0.07	25.32±1.21	3.73±0.31[a]	35.70±0.37[b]	54.88±1.10[c]	39.33±3.11[b]	3.13±0.23[a]	55.18±0.95[b]
AA/EPA	4.47±0.34[a]	1.57±0.05[a]	14.06±0.40[b]	70.00±19.80	167.18±8.54	80.01±7.64	2476.25±275.42	3.04±0.27	3.21±0.04
AA/DHA	10.84±0.86[a]	2.63±0.06[a]	271.07±23.06[b]	14.82±2.20[a]	172.50±9.84[b]	191.14±2.37[b]	856.25±48.14[b]	53.89±2.47[a]	75.75±6.33[a]
DHA/EPA	4.25±0.02[bc]	5.86±0.07[b]	0.93±0.07[ac]	210.00±50.91	12.73±1.16	4.46±0.44	25.00±4.24	0.87±0.09	0.58±0.03

All the values are given in means (± S.E.M, n=4). Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet. Dash (—) indicates that the fatty acid was <0.01.

*∑ SAFA: total saturated fatty acids; *∑ MUFA: total monounsaturated fatty acids; *∑ PUFA: total polyunsaturated fatty acids.

Table 4.7 (continued): The fatty acid composition (mg g^{-1} body weight) of *M. macrocopa* enriched at 2 g L^{-1} with varied experimental diets over different enrichment periods

	FP			FF		
Fatty acid	12 h	24 h	36 h	12 h	24 h	36 h
12:0	—[a]	0.01±<0.01[b]	—[a]	0.01±<0.01	0.01±<0.01	—
14:0	0.01±<0.01	0.02±<0.01	0.02±<0.01	0.01±<0.01	0.01±<0.01	0.01±<0.01
14:1	—	0.01±<0.01	0.01±<0.01	0.01±<0.01[ab]	—[b]	—[a]
15:0	0.08±<0.01	0.11±<0.01	0.14±<0.01	0.08±<0.01[a]	0.02±<0.01[ab]	0.04±<0.01[b]
16:0	0.05±<0.01	0.08±<0.01	0.09±<0.01	0.04±<0.01[b]	0.11±0.01[a]	0.03±<0.01[a]
17:0	1.03±<0.01	1.31±<0.01	1.67±0.03	0.82±0.02	0.32±0.03	0.51±0.01
18:0	0.10±<0.01	0.11±<0.01	0.10±<0.01	0.05±<0.01	0.19±0.01	0.05±<0.01
18:1*n*-9	0.03±<0.01	0.01±<0.01	0.05±<0.01	0.02±<0.01	0.03±<0.01	—
18:2*n*-6	0.07±<0.01	0.10±<0.01	0.10±<0.01	0.06±<0.01	0.12±<0.01	0.08±<0.01
20:0	0.01±<0.01	0.01±<0.01	0.01±<0.01	—	0.04±<0.01	0.01±<0.01
18:3*cis*-6	0.89±0.01[c]	0.67±0.01[b]	0.03±<0.01[a]	0.28±0.01	0.21±0.01	0.03±<0.01
18:3*n*-3	0.03±<0.01	0.02±<0.01	—	0.01±<0.01	0.03±<0.01	—
22:0	—	0.01±<0.01	0.01±<0.01	0.01±<0.01	0.02±<0.01	0.01±<0.01
20:3*n*-6	—	0.01±<0.01	0.01±<0.01	0.01±<0.01[a]	0.02±<0.01[ac]	0.01±<0.01[bc]
20:3*n*-3	0.01±<0.01	0.01±<0.01	0.03±<0.01	0.01±<0.01	0.01±<0.01	—
20:4*n*-6	—	0.01±<0.01	—	—[a]	0.01±<0.01[b]	—[ab]
20:5*n*-3	0.20±<0.01[b]	0.19±<0.01[b]	0.05±<0.01[a]	0.09±<0.01	0.09±<0.01	0.03±<0.01
22:6*n*-3	0.01±<0.01	0.01±<0.01	—	—	0.02±<0.01	—
∑ SAFA*	333.04±1.65[a]	419.78±0.33[ab]	587.47±3.01[b]	384.38±4.42[b]	323.99±7.56[a]	485.29±3.06[ab]
∑ MUFA*	200.32±0.54	205.57±2.22	280.72±1.52	222.00±4.77[b]	166.80±13.88[a]	329.18±4.57[a]
∑ PUFA*	466.64±2.18[b]	374.65±2.26[ab]	131.80±1.65[a]	393.62±1.74[ab]	509.22±21.37[bc]	185.53±1.77[a]
∑ *n*-3 PUFA	54.13±0.31[b]	53.03±0.50[b]	22.13±0.06[a]	36.61±1.01	39.20±2.30	22.09±0.13
∑ *n*-6 PUFA	1.66±0.03	2.79±0.06	2.24±0.05	2.21±0.10[a]	5.83±0.26[a]	4.26±0.08[b]
AA/EPA	0.21±0.01	0.36±0.01	—	—[a]	1.30±0.13[b]	—[a]
AA/DHA	4.93±0.21	6.85±0.25	—	—	13.20±1.10	—
DHA/EPA	0.44±<0.01	0.54±0.01	0.73±0.09	—	2.90±0.31	0.85±0.10

All the values are given in means (± S.E.M, n=4). Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet. Dash (—) indicates that the fatty acid was <0.01.

*∑ SAFA: total saturated fatty acids; *∑ MUFA: total monounsaturated fatty acids; *∑ PUFA: total polyunsaturated fatty acids.

Table 4.8: Results of two-way ANOVA analysis performed to study the effects of diet concentration, time and interaction of concentration vs. time on the enrichment experiment

Diet	Fatty acid	Concentration		Time		Concentration x Time	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
ADS	20:4*n*-6	14.76	0.000**	9.94	0.001*	10.71	0.000**
	20:5*n*-3	1.29	0.292	9.17	0.001*	5.62	0.002*
	22:6*n*-3	4.34	0.023*	4.71	0.018*	4.43	0.007*
	AA/EPA	19.33	0.000**	4.01	0.030*	8.59	0000**
	AA/DHA	5.69	0.009*	4.84	0.016*	5.44	0.002*
	DHA/EPA	8.42	0.001*	7.51	0.003*	3.99	0.011*
SO	20:4*n*-6	97.25	0.000**	18.88	0.000**	23.26	0.000**
	20:5*n*-3	11.21	0.000**	0.37	0.697	0.66	0.622
	22:6*n*-3	7.17	0.003*	2.95	0.069	2.66	0.054
	AA/EPA	11.12	0.000**	1.17	0.324	1.00	0.424
	AA/DHA	43.38	0.000**	7.12	0.003*	11.09	0.000**
	DHA/EPA	1.39	0.266	1.14	0.335	1.11	0.374
CO	20:4*n*-6	7.04	0.003*	5.13	0.013*	5.26	0.003*
	20:5*n*-3	9.11	0.001*	0.13	0.878	2.07	0.113
	22:6*n*-3	4.14	0.027*	4.91	0.015*	0.72	0.586
	AA/EPA	2.21	0.129	2.32	0.118	2.27	0.088
	AA/DHA	15.23	0.000**	10.74	0.000**	10.48	0.000**
	DHA/EPA	1.62	0.216	2.73	0.083	1.60	0.203
FP	20:4*n*-6	13.20	0.000**	14.76	0.000**	5.54	0.002*
	20:5*n*-3	16.06	0.000**	3.67	0.039*	2.68	0.053
	22:6*n*-3	1.92	0.166	3.36	0.050	2.22	0.093
	AA/EPA	4.47	0.021*	2.59	0.093	1.99	0.125
	AA/DHA	7.06	0.003*	7.84	0.002*	6.78	0.001*
	DHA/EPA	2.68	0.087	2.07	0.146	2.14	0.103
FF	20:4*n*-6	7.32	0.003*	4.31	0.024*	1.93	0.134
	20:5*n*-3	6.45	0.005*	0.98	0.390	0.85	0.504
	22:6*n*-3	18.44	0.000**	32.63	0.000**	21.96	0.000**
	AA/EPA	4.96	0.015*	2.59	0.093	2.06	0.114
	AA/DHA	3.08	0.062	1.01	0.376	0.77	0.555
	DHA/EPA	2.23	0.128	1.57	0.226	0.31	0.868

P-values with asterisk denote the significant level of study (*$p < 0.05$; **$p < 0.001$).

4.5 Effects of Effluent Water from a Recirculating Aquaculture System (RAS) on the Population Growth of *Moina macrocopa*

The population growth of *Moina macrocopa* in treatment media (RAS wastewater effluent) associated with the control media (in de-chlorinated water) is shown in Figure 4.14 to 4.18. Figure 4.14 shows the comparison of population densities of *M. macrocopa* in the control and treatment culture for 16 days, with both started at 4 ind. 40 mL^{-1} of initial density. The density of *M. macrocopa* cultured with wastewater effluent increased significantly ($p < 0.05$) on day-3 compared to the control treatment at 4 ind. 40 mL^{-1} (Figure 4.14 and Table 4.9). The population densities of *M. macrocopa* in the control culture were relatively constant at a low population density. The highest population density obtained was on day-11 with 9 individual *M. macrocopa* in 40 mL of cultivation medium. The population density then decreased until day-16 when no more test organisms were observed in the control culture.

There was a positive growth trend of the *M. macrocopa* population in the treatment culture (Figure 4.14), in which the population densities increased gradually from day-0 to day-4. A total of 40 individuals of *M. macrocopa* were observed to increase ($p < 0.05$) from day-4 to day-7 (Table 4.9), and the population densities reached their peak on day-7 (55 ind. 40 mL^{-1} of *M. macrocopa*) (Figure 4.14). However, population densities of *M. macrocopa* in the treatment culture decreased gradually from day-7 to day-11. Only 5 individuals of *M. macrocopa* remained in the culture tubes on day-16 (Figure 4.14).

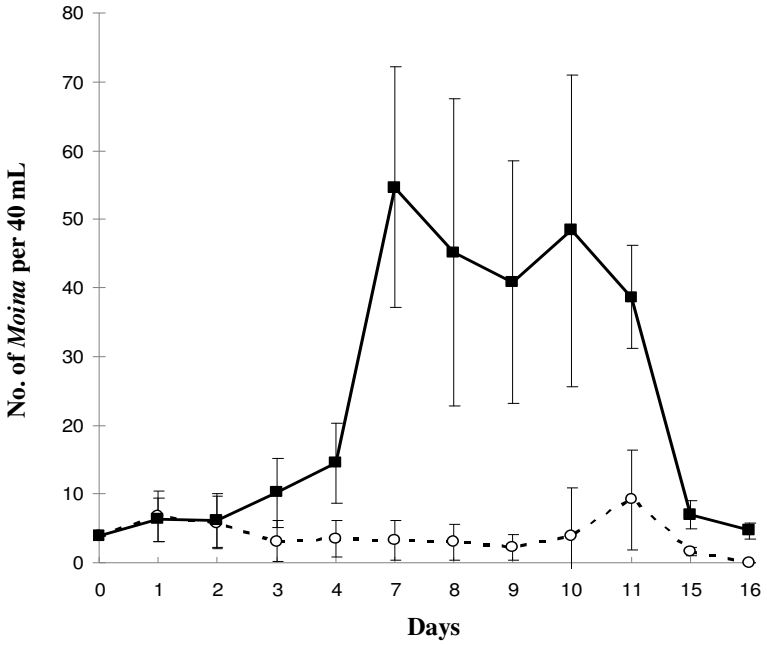


Figure 4.14: Population growth of *Moina macrocopa* at the initial stocking density of 4 ind. per 40 ml culture medium. —■— indicates wastewater effluent (RAS); – ○ - indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

Figure 4.15 shows the population growth of *M. macrocopa* over a duration of 16 days in the control and treatment culture at initial density of 20 ind. 40 mL^{-1}. *M. macrocopa* grew at a slow rate for the first 2 days in the treatment culture. However, the numbers dropped slightly on day-3 and day-4 (Figure 4.15). After day-4, the *Moina* population started to increase, and in an exponential scale on day-7 ($p < 0.05$) (Table 4.9). The population continued to increase and reached the peak density at 61 ind. 40 mL^{-1} on day-10. The population density then decreased drastically ($P < 0.05$) (Table 4.9) from 55 individuals

on day-11 to 4 individuals on day-16 (Figure 4.15). In the control culture, the numbers of *Moina* that survived in the culture tubes decreased from day-1 to day-8. The population density was increased from 3 individuals on day-8 to 9 individuals on day-11. However, *M. macrocopa* continued to die with absolute mortality on day-15 (Figure 4.15).

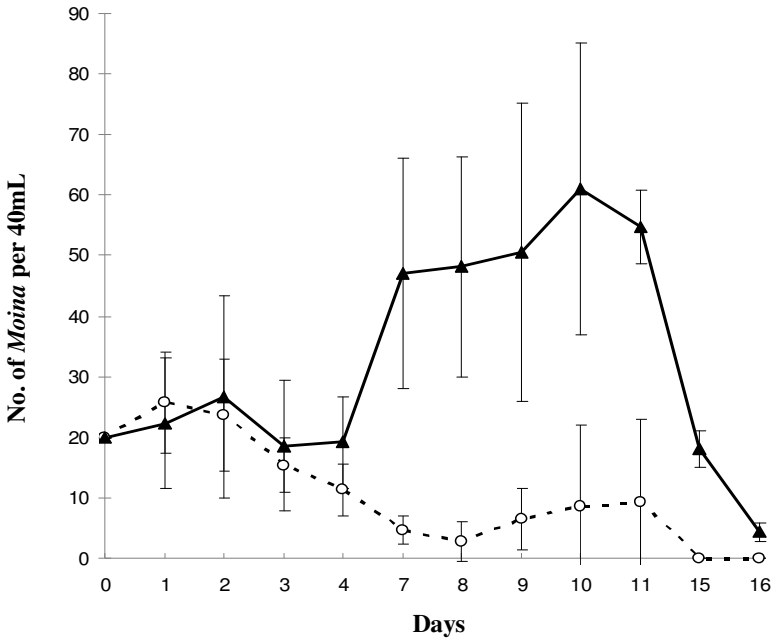


Figure 4.15: Population growth of *Moina macrocopa* at the initial stocking density of 20 ind. per 40 ml culture medium. —▲— indicates wastewater effluent (RAS); –○– indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

Figure 4.16 shows the population growth of *M. macrocopa* at the initial density of 40 ind. 40 mL^{-1}. The population density of *M. macrocopa* showed an increase of 4 individuals on day-1, and decreased during the following days to a total of 23 individuals on day-7 ($P < 0.01$) (Table 4.9) (Figure 4.16). The

number of *M. macrocopa* started to increase again on day-10 (42 ind. 40 mL^{-1}) before it declined to 4 individuals on day-16. The population density of control *M. macrocopa* declined linearly from day-1 to day-7, and no survivors were found from day-8 onwards (Figure 4.16).

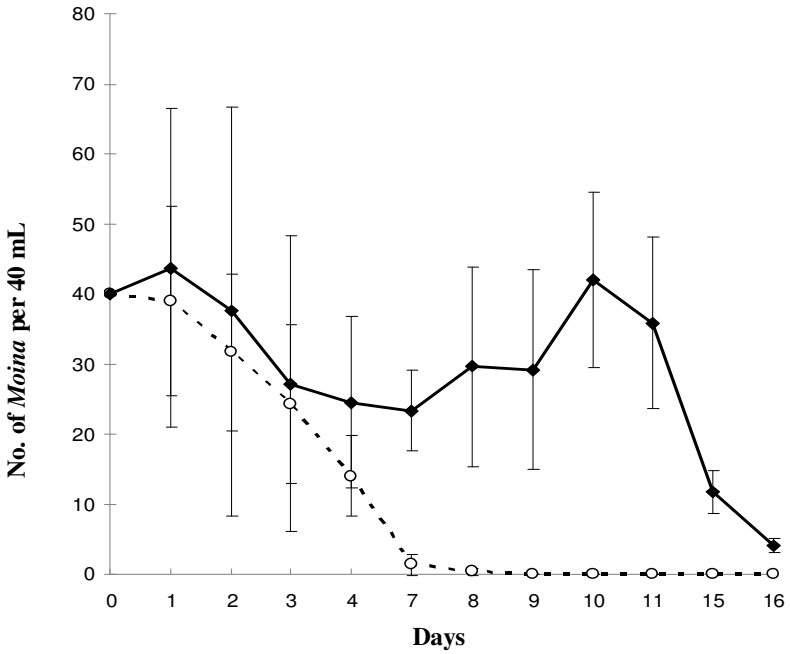


Figure 4.16: Population growth of *Moina macrocopa* at the initial stocking density of 40 ind. per 40 ml culture medium. —◆— indicates wastewater effluent (RAS); - -○- - indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

The population density of *M. macrocopa* cultured with 80 ind. 40 mL^{-1} is shown in Figure 4.17. The population growth in the treatment culture (wastewater effluent) reached its peak density on the first day with 108 individuals. Both population densities in wastewater and control treatments decreased drastically after day-1. By the end of the experiment (Figure 4.17)

in wastewater treatment, the *Moina* population had dropped from 108 to only 2 in 40 ml of culture medium. However, the population was only significantly different ($P < 0.01$) (Table 4.9) compared to *Moina* in the control treatment on day-7. In the control, the population showed negative growth from day-1 onwards. No *Moina* was found in the control tubes from day-10 (Figure 4.17).

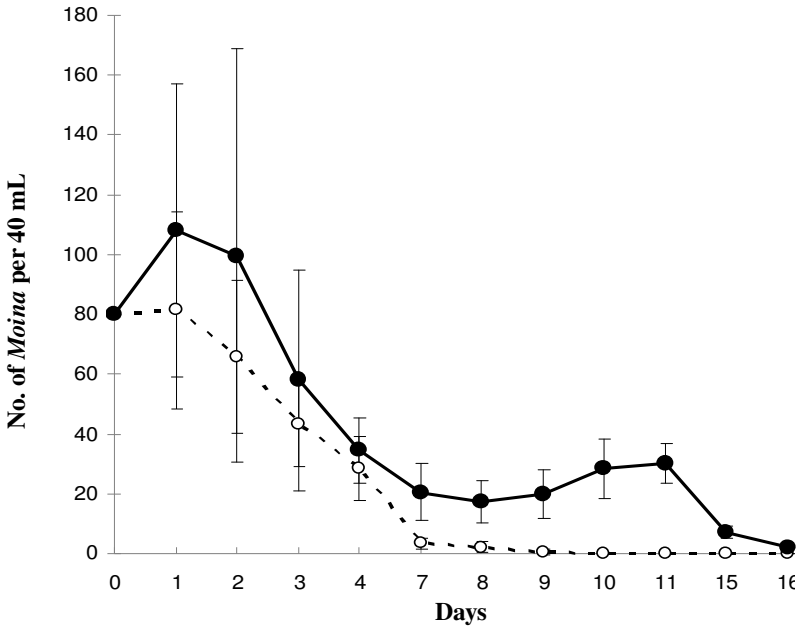


Figure 4.17: Population growth of *Moina macrocopa* at the initial stocking density of 80 ind. per 40 ml culture medium. —●— indicates wastewater effluent (RAS); –○– indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

All population densities were found to be significantly influenced ($P < 0.05$) by the duration of experiment (Table 4.10). During the culture period, there was no significant difference ($P > 0.05$) in the population density among the groups on day-10 and day-16 (Table 4.10).

Table 4.9 The *F* and *P*-value from one-way ANOVA analysis used in the comparison of population density between control and treatment cultures with 4, 20, 40 and 80 ind. 40 mL^{-1} initial population density for each day.

Day no.	Number of *Moina* in 40 mL culture treatments							
	4 ind.		20 ind.		40 ind.		80 ind.	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
1	0.063	0.806	0.356	0.564	0.186	0.068	1.245	0.291
2	0.022	0.886	0.148	0.708	0.209	0.657	1.268	0.286
3	8.368	0.016*	0.481	0.504	0.094	0.765	0.826	0.385
4	18.060	0.002**	4.946	0.050	3.637	0.086	0.910	0.363
7	49.574	0.000***	29.325	0.000***	80.369	0.000***	18.857	0.001**
8	21.076	0.001**	36.019	0.000***	25.490	0.001**	25.900	0.000***
9	28.212	0.000***	18.386	0.002**	24.690	0.001**	33.380	0.000***
10	10.485	0.031*	6.825	0.059	33.707	0.004**	24.103	0.008**
11	48.332	0.000***	55.944	0.000***	52.137	0.000***	128.273	0.000***
15	19.692	0.011*	108.000	0.000***	43.750	0.003**	36.750	0.004**
16	49.000	0.002**	24.143	0.008**	48.000	0.002**	36.577	0.004**

P-values with asterisk denote the significant level of study (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Table 4.10 The significance values (one-way ANOVA) of all population densities in each experimental period (in days) (left), and the interaction of density versus days in various population densities in the study (right).

Day no.	Initial densities (4, 20, 40, 80) ind. 40mL^{-1}	
	F-value	*P*-value
1	15.790	0.000***
2	6.561	0.003**
3	5.341	0.007**
4	4.926	0.010*
7	8.735	0.001**
8	4.505	0.014*
9	3.635	0.031*
10	1.640	0.256
11	9.500	0.000***
15	12.367	0.002**
16	3.690	0.062

Interaction	*F*-value	*P*-value
Population density:		
4 ind. 40 mL^{-1}		
Density × Days	14.464	0.000***
20 ind. 40 mL^{-1}		
Density × Days	7.388	0.000***
40 ind. 40 mL^{-1}		
Density × Days	2.208	0.028*
80 ind. 40 mL^{-1}		
Density × Days	7.743	0.000***

P-values with asterisk denote the significant level of study (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Table 4.11 shows the population growth rate (*r*) of *M. macrocopa* in each of the treatment cultures (wastewater effluent) with various initial population densities. Cultivation with 4 ind. 40 mL^{-1} initial density gave the highest *r*-value (0.374) followed by 80 ind. 40 mL^{-1} (0.300). The *r*-value of *M. macrocopa* with 20 ind. 40 mL^{-1} and 40 ind. 40 mL^{-1} initial density were 0.112 and 0.095, respectively.

Table 4.11 Population growth rate (*r*) of various population densities

Initial density, N_0 (ind. 40 mL^{-1})	Peak density, N_t (ind. 40 mL^{-1})	Time to reach peak density, t (Day)	Population growth rate (*r*)
4	55	7	0.374
20	61	10	0.112
40	44	1	0.095
80	108	1	0.300

The initial stocking density of *M. macrocopa* with 4 ind. 40 mL^{-1} (or equivalent to 100 ind. L^{-1}) showed a more superior performance in population growth than 20, 40, and 80 ind. in 40 mL. *M. macrocopa* in the density of 20 ind. 40 mL^{-1} was found to share a similar growth as in the density of 4 ind. 40 mL^{-1}. In terms of time and efficiency, *M. macrocopa* cultured at 4 ind. 40 mL^{-1} had the highest population growth rate and was able to reach the peak population on day-7, which was 3 days earlier than the density of 20 ind. 40 mL^{-1}.

CHAPTER 5.0

DISCUSSION

5.1 Fish Faeces as a Potential Food Source for Cultivating the Water Flea, *Moina macrocopa*

Under natural conditions, water fleas, rotifers, and other zooplankton species generally feed on fine organic particles, bacteria, phytoplankton, fungi and protozoans that are suspended in the water (Rodolfo and Edmundo, 1980). Mass cultivation of zooplankton for larviculture is widely carried out using baker's yeast, fish meal, and ground trash fish (Rottmann et al., 1992). The use of these foods is more convenient for large-scale production, but the production costs can be higher because zooplankton reared on yeasts and trash fish have an inconsistent nutritional value and may be deficient in essential fatty acids (EFAs). This would lead to additional costs due to the need to enrich the zooplankton with microalgae or artificial feeds high in EFAs prior to feeding to fish and crustacean larvae (Tamaru et al., 1991; Rottmann et al., 1992; Sorgeloos and Lavens, 1996; He et al., 2001).

In this study, the survival of *M. macrocopa* treated with 3.5 g L^{-1} *Chlorella* powder appeared similar to the findings with live *Chlorella vulgaris* (Nandini and Sarma, 2000). *M. macrocopa* that had been fed on fish faeces showed a superior result compared with algal types of food. Completely or partially digested, and totally undigested fish feed, which had passed through the

gastro-intestinal system of the Nile tilapia, was excreted as faecal material. The latter constituted a substantial proportion of total suspended solids in the rearing and treatment tanks of the freshwater RAS system. Burns (1968) reported that suspended particles, with an upper size limit of 50 μm, were filtered from the water by cladocerans using their setae and body movements, and utilized as a source of food. It is speculated that these faecal materials might also induce the growth of heterotrophic and autotrophic bacteria, which are in turn, ingested by *M. macrocopa* and other cladoceran species (Dodson and Frey, 2000; Schneider et al., 2007). Microbial degradation of organic and inorganic wastes depends on the amounts of carbon, nitrogen, and phosphorous available for their activity (Kim et al., 2008). According to Fivlstad et al. (1990) and Hakanson et al. (1988), approximately 70% of the total phosphorus and 15% of the total nitrogen fed to cultured fishes are lost through their faeces and subsequently utilized by bacteria. Some bacteria, for instance, the nitrogen-fixing species of the genus *Nitrosomonas* and *Nitrobacter*, are able to obtain nitrogen, phosphorus, and carbon from organic sources for optimal biomass production, and can convert ammonia to nitrites and nitrates through the nitrification process (Lechevallier et al., 1991; Schneider et al., 2007). These bacteria provide energy as well as essential vitamins and amino acids for the dietary requirements of *M. macrocopa* by decomposing dissolved organic compounds (Langis et al., 1988).

5.2 Life Table Demography of Freshwater Cladoceran, *Moina macrocopa* in Relation to Potential Diets of Fish Wastes and Enrichment Oil Emulsion

For more than two decades (mid 1980s to 2004s) (Nandini and Sarma, 2003), the cladoceran species have been extensively studied throughout the world on both fundamental and applied aspects. Among the Cladocera, *Moina* have been studied the most intensively in regards to the effects of food abundance, growth, and reproduction parameters (Burak, 1997; He et al., 2001). The quality and quantity of food are the most important factors determining biomass production by *Moina* species (He et al., 2001). In the current study, five different diets with a range of concentrations (0.0625 to 1.0 g L^{-1}) were investigated to optimise the food source for the cultivation of *Moina*. Fish faeces (FF), ground fish pellets (to mimic uneaten fish food waste from aquaculture) (FP), DHA Selco® (ADS), squid oil (SO), and canola oil (CO) were investigated as potential culture media for *M. macrocopa*.

The effects of diet types and concentrations on the life history variables of *M. macrocopa* indicated that 0.0625 g L^{-1} of CO, FP, and FF produced optimal growth and reproduction performance (Figures 4.6, 4.9 & 4.10 and Table 4.3). Growth performances, such as fecundity and longevity of *M. macrocopa* fed on fish faeces, were also found to be much higher than those propagated using algae (Nandini and Sarma, 2000), and human urine (Golder et al., 2007). The decline in the neonate production with increasing FP and FF concentrations was presumably caused by the increased effort associated with food gathering

due to the active filtering of the food particles (Nandini and Sarma, 2000). High concentrations of the treatment diets did not provide an optimal culturing condition. Such high levels of particles could actually lead to the starvation of Cladocerans as they are unable to clean their thoracic limbs once they are clogged by high particulate concentrations (Porter et al., 1982; Burak, 1997; Sarma and Nandini, 2000). These results corroborate those of Burak (1997), where a decline in the cultivated *Moina* population at high concentrations of algal diet (*Scenedesmus sp*.) was observed.

Diet type and concentration are the factors which strongly affect the density of a species in a community and its life history parameters (Lampert and Schoeber, 1980; He et al., 1998). In this study, both diet type and concentration exerted a significant influence on the maturation of *M. macrocopa*, which took a shorter time period to become sexually mature at high diet concentrations before production of the first brood (Table 4.3 and 4.4). This phenomenon indicated that the diet type and treatment concentration played a significant role in determining the initial age of reproduction (Table 4.4). The spawning intervals ranged from 2.09 days to 4.60 days for all of the treatment diets and concentrations. In this study, generation time did not seem to be influenced either by diet concentration or diet type (Table 4.3). However, fecundity was significantly affected by both diet type and concentration, where the total clusters of neonates produced by parthenogenesis showed an obvious difference among the concentrations and diet types. The gross reproduction rate (GRR) was also increased at lower treatment concentrations. Analysis showed that GRR was statistically higher when *M. macrocopa* was

fed with CO (canola oil), FP (fish pellets) or FP (fish faeces) (Table 4.3). These observations suggest that reproduction of this Cladoceran can be enhanced by providing organic or non-organic particulate material, or lipid compounds.

The *r* values did not seem to be changed to a trend when the diet type and concentration were different (Table 4.3). The *r* values could be explained by the change in population size over a particular period of time, and were elevated by the short lifespan and high fecundity of *M. macrocopa* (Stearns, 1976). This trend was clearly shown in this study, particularly with the FP treatment diet (Table 4.3). Nandini and Sarma (2003) discussed the population dynamics of some cladoceran genera with regard to the life history variables. They proposed that the *r* values should be in the range of 0.01 to 1.50. The ratio in the present study ranged from -0.52 to 1.35, which was slightly lower than the proposed range. This may be a consequence of different *Moina* species, food type, and temperature used in the previous studies (Deng and Xie, 2003; Nandini and Sarma, 2003). These factors could influence the life cycle parameters such as adult average size, mortality, first clutch size, and fecundity, and consequently lead to a change in the population dynamics of a species (Deng and Xie, 2003).

The results showed that *M. macrocopa* fed with fish faeces had the highest population growth and the longest lifespan (Table 4.3). This high rate of population can be attributed to the feeding preference of *Moina*. *Moina* tend to consume bacteria and filtered particles which are abundant in fish wastes

when other food sources, such as phytoplankton, are limited or unavailable (Nelson and Fernando, 2005). He et al. (1998) reported that *Moina* demonstrated the highest fecundity when a mixture of animal manure was used as the culture media. Kibria et al. (1997) also found that zooplankton grown on domestic wastes had superior nutritional quality compared to those raised solely with algal food sources. Some of these Cladocerans, such as *Moina*, were even able to propagate in the waste media as fast as those raised with algae (Nandini et al., 2004; Loh et al., 2009). Patil et al. (2010) found that *M. australiensis* could be batch cultured using piggery effluent, and the associated microbial biomass could also serve as a food source for the cladoceran.

The oil emulsions were not as good a cultivation food source as anticipated for *M. macrocopa* because their high concentrations resulted in a negative effect on the population dynamics of *Moina* (except for 0.0625 g L^{-1} of canola oil, $p < 0.05$). It is therefore essential to balance between the concentration of the oil emulsion needed for an optimum level of enrichment uptake and the maintenance of a stable population of *Moina* during enrichment. This consideration would ensure effective enrichment of *Moina* for larval feeding.

5.3 Effects of Concentration, Enrichment Time and Potential Enrichment Diets on HUFA's Profile of *Moina macrocopa*

Livefeed enrichment with oil emulsions is a common practice in fish and crustacean hatcheries. Various commercial enrichment products have been

formulated for this purpose. Although enrichment methods have been standardized and commercially adopted for *Artemia* nauplii and rotifer enrichments (Agh and Sorgeloos, 2005), an optimal enrichment protocol has not been clearly identified for use with *Moina macrocopa*. Lipids play crucial roles in the physiological and biological functions of living organisms (Sargent et al., 1997, 1999b). Marine and freshwater fish require *n*-3 and *n*-6 fatty acids in their daily diet (Mourente and Tocher, 1993; Ghioni et al., 1999), especially during the early stages of life because lipids are the main source of energy at the gastrula stage of fish embryonic development (Vetter et al., 1983). Inadequate lipid content in cultured fish diets can adversely affect the performance of larvae during the grow-out stage, since larvae often have low energy reserves and require substantial energy sources for their high somatic growth rates and development of their bodies (Fraser et al., 1988). Lipids contain 2.5 times the energy compared to carbohydrates or proteins per consumed weight (Parker, 2002). In addition, lipids which are the energy source for cellular housekeeping functions, are required for hormone synthesis, and serve as carriers for fat soluble vitamins (Parker, 2002; Tamaru et al., 2003). The data in the study clearly indicate that lipid levels in *M. macrocopa* can be improved through changes to the enrichment process, in a manner similar to that for *Artemia* nauplii (Das et al., 2007).

Enrichment diets and conditions (initial stage of neonate or nauplii, and duration and dose of the enrichment) are the most obvious factors known to influence the eventual outcome of enrichment (Léger et al., 1987). In this study, enrichment time, concentration, and different potential enrichment diets

i.e. canola oil, squid oil, fish feaces, and uneaten fish food together with a commercial reference (A1 DHA Selco®), were investigated to elucidate the lipid profile of *M. macrocopa*. The treatment concentration had a more predominant effect on the fatty acid content of *M. macrocopa* when compared with enrichment time as shown in the fatty acid profile of the study (Table 4.8). Essential fatty acids such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are desirable components of livefeed, are naturally low in un-enriched *Moina*. Piedecausa et al. (2007) demonstrated that fish species, for example *Diplodus puntazzo* which were fed soybean and linseed oil emulsions could lead to a low AA content in the muscles. The canola oil used in the present study increased the AA levels in the enriched *Moina* for 12 h to 0.38 mg g^{-1} at 2 g L^{-1} (Table 4.7). It was also noted that the *M. macrocopa* fed with aquaculture wastes (FF indicated fish faeces, and FP indicated fish pellets) also showed high values of EPA content (Table 4.6). AA is an essential dietary fatty acid which has been detected in relatively high amounts in neural and visual tissues of fish (Tocher and Harvie, 1988). This fatty acid is the main precursor to hormone-like compounds, e.g. eicosanoids, which are important in the development of neural tissues and the immune system, specifically in signal transduction (Sargent et al., 1999a) and inflammatory responses, respectively (Tocher, 2003).

The DHA levels in un-enriched *Moina macrocopa* (< 0.01 mg g^{-1}) indicated that this zooplankton is naturally deficient in this fatty acid. However, the EPA levels were higher (0.15 mg g^{-1}) than DHA in the un-enriched *M. macrocopa* (Table 4.5), as reported for *M. micrura* (Das et al., 2007). This could be

because *Moina* could not synthesize DHA *de novo* and metabolize PUFA as rapidly as *Artemia* (Estévez et al., 1998). Generally, the elevation of DHA will increase the level of EPA in the organisms, as shown by the marked retroconversion of DHA to EPA that takes place during enrichment (Navarro et al., 1999). This retroconversion occurred in *Moina* enriched with 1 and 2 g L^{-1} of ADS, whereas the zooplankton took a longer time to synthesize and metabolize the DHA from the reference diet (ADS) (Tables 4.6 & 4.7). According to Han et al. (2000), the DHA content in *Artemia* nauplii increased approximately two fold from 12 h to 24 h of the enrichment period. Although DHA levels did not increase greatly over the enrichment time, the DHA levels from the experimental diets were significantly higher than un-enriched *Moina* (range of 0.01 to 0.08 mg g^{-1}), except for FF and FP (Tables 4.6 & 4.7). Since DHA is important for vision and membrane fluidity functions (Koven, 2003), insufficient levels of this essential fatty acid in larval diets is likely to impair the neural and visual development in fish larvae (Sargent et al., 1999a). The EPA values of *Moina* dropped markedly after the enrichment, except with the 1 g L^{-1} ADS and 2 g L^{-1} FP treatments, which showed high values at 12 h enrichment (Tables 4.6 and 4.7). During the duration of the supplementation (Sargent et al., 1999a), it was anticipated that EPA would metabolize the eicosanoids, particularly the 3-series prostanoids and 5-series leukotrienes, The levels of EPA were not increased by SO and CO in this study.

Highly unsaturated fatty acids (HUFAs) are important for survival, growth and the pigmentation of almost all fish and crustacean larvae (Kanazawa, 1993a, b; Watanabe, 1993). However, the effects of HUFAs cannot be determined solely

on the absolute amount of a particular HUFA given to the fish. The absolute requirement for any given HUFA in fish is proportional to the absolute amounts of other HUFAs in the diet due to the competitive inhibition by fatty acids, which could affect the quality of the provided HUFAs (Rainuzzo et al., 1997; Sargent et al., 1999a; Corraze, 2001). According to Hamre et al. (2005), AA and EPA compete for binding to the cycloxygenase and lipoxygenase enzymes. These competitions can result in biological imbalances, ultimately affecting the cell membrane receptors (Sargent et al., 1999a). Within the fish tissues, EPA and DHA subsequently bind to these cycloxygenase and lipoxygenase, and subsequently determine the ratio of *n*-2 and *n*-3 eicosanoids in the muscles of fish larvae. These two series of eicosanoids cause different degrees of physiological changes (Logue et al., 2000), e.g. the malpigmentation of fish due to excessive of eicosanoids which induce biochemical stress in the fish (Sargent et al., 1999b). It has been suggested that increasing the ratio of *n*-3 over *n*-6 could reduce or inhibit the synthesis of AA-derived prostaglandins (Whelan, 1996), which consequently influenced the rate and type of eicosanoids produced (Lund et al., 2007).

The results presented in this study indicate that the lipid ratio is strongly influenced by the lipid ratio of livefeed given to the targeted species (Piedecausa et al., 2007). The commercial enrichment diet, ADS was found to be superior in enhancing DHA levels. However, it does not efficiently improve *n*-3 over *n*-6, or *n*-6 over *n*-3 ratios as shown by canola oil and squid oil in the present study (Figures 4.12 & 4.13). For a better enrichment process, the AA/EPA and AA/DHA ratios were comparatively more effective when the

Moina was enriched with FF, FP, CO, and SO. Reitan et al. (1994) found that the malpigmentation of some species, e.g. turbot, could be mitigated if the *n*-6 over *n*-3 ratio was increased in the lipid content of the diet during larval feeding. The DHA/EPA ratio of *M. macrocopa* could be increased when enrichment was performed with 2 g L^{-1} SO at 12 h in this study. This gave better results when compared with FP. Sui et al. (2007) reported that the megalopa stage of the Chinese mitten crab (*Eriocheir sinensis*) could tolerate more salinity fluctuation when the diet was supplemented with DHA/EPA. Increase in the ratios of HUFAs also reduces solidification of the total bio-membrane lipid pool, which acts as an anti-freeze mechanism for many aquatic poikiloterms, such as fish and freshwater Cladocerans to protect them from cold water temperatures (Brett and Müller-Navarra, 1997).

The bio-encapsulated lipid ratios in *M. macrocopa*'s tissue during enrichment indicated a positive trend with the levels measured in the experimental diets. Other fatty acids such as linoleic acid [18:2(*n*-6)] and linolenic acid [18:3(*n*-3)] are also relatively important for larval performance. The LA levels showed better improvement (0.50 mg g^{-1}) when *M. macrocopa* was enriched with 1 g L^{-1} SO (0.50 mg g^{-1}) at 12 h (Table 4.6) followed by 2 g L^{-1} CO (0.40 mg g^{-1}) at 36 h (Table 4.7). Linolenic acid (LnA) levels could also be better improved (0.86 mg g^{-1}) by using 2 g L^{-1} CO (Table 4.7) followed by 2 g L^{-1} SO (0.70 mg g^{-1}), both at 12 h (Table 4.7). Linoleic acid (LA) is a metabolic precursor of gamma-linolenic acid (GLA) to AA, and it functions to prevent abnormal colouration in freshwater fish (Nicolaides and Woodall, 1962), whilst LnA could be converted to DHA via EPA catabolism (Ahlgren *et al*., 1999; Tocher,

2003). This study has demonstrated that LnA enriched *M. macrocopa* could be an alternative source of the essential DHA and EPA for freshwater fish. LA and LnA are important to fish and crustacean species (Kanazawa et al., 1979; Brett and Müller-Navarra, 1997; Das et al., 2007) because almost all aquatic or terrestrial organisms that lack chlorophyll cannot synthesize either fatty acid (Brett and Müller-Navarra, 1997). Nevertheless the levels of LA and LnA of the livefeed could be increased as shown in Tables 4.6 and 4.7, although there is no direct relationship with treatment concentrations in the experimental diets. It is anticipated that catabolism of other lower carbon chain fatty acids such as monounsaturated oleic acid [18:1(*n*-9)] in the tissues of *Moina* species might enable conversion to LA and LnA as shown in some fish species and herbivorous insects (Blomquist et al., 1991; Piedecausa et al., 2007).

5.4 Effects of Effluent Water from a Recirculating Aquaculture System (RAS) on the Population Growth of *Moina macrocopa*

There are several factors which potentially affect the life history of *M. macrocopa* in a culture environment, such as population density, trophic conditions, water quality, and water-borne chemical cues (Rose et al., 2002). In a controlled culture environment, for example in an RAS system, the water quality and other chemical parameters in the water are usually well monitored. The factor that plays an important role in determining survivability and reproduction of *M. macrocopa* in a culture system is most likely the population density, which was the main focus of this study. This study

demonstrated the growth performance of *Moina macrocopa* at different initial stocking densities in a culture environment using wastewater from an RAS system as a culture medium. The age of the wastewater from the RAS system and the fish stocking densities in the tanks were not expected to affect the growing pattern of *M. macrocopa*, since the major factors influencing the water characteristics were basically attributed to the water quality. These factors are the total amount of ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen, which have been discussed in this study.

The initial stocking density of *M. macrocopa* at 4 ind. 40 mL^{-1} (or equivalent to 100 ind. L^{-1}) showed a superior performance in population growth than 20, 40, and 80 ind. in 40 mL, although the density of 20 ind. 40 mL^{-1} shared a similar growth trend as the density of 4 ind. 40 mL^{-1}. However, in terms of time and efficiency, *M. macrocopa* at 4 ind. 40 mL^{-1} reached its peak population on day-7, which was 3 days earlier than the density of 20 ind. 40 mL^{-1}. The population growth rate (r) of *M. macrocopa* at 4 ind. 40 mL^{-1} was 0.374, which was the highest growth rate among the four different initial densities tested in this study. This implied that *M. macrocopa* could be harvested within a shorter period of time, thus allowing a greater number of batches to be cultivated per cycle.

A short production time is important for livefeed producers (Fabiola et al., 2005), because it could reduce time and is less cost consuming. In addition, a shorter cultivation time means a lower risk of cross-contamination since prolonged culture would increase the chances of microbial contamination as

well as competition from other zooplankton. *Rotifer*s were the main competitors for space and food during the cultivation period that resulted in poor quality *M. macrocopa*.

The present study showed that *M. macrocopa* cultivated at 20 ind. 40 mL^{-1} initial population density reached a higher population number (61 individuals) when compared with 4 ind. 40 mL^{-1}, which produced 55 individuals at the population peak. However, the population growth rate (*r*) of *M. macrocopa* cultivated at 20 ind. 40 mL^{-1} was 0.112, was lower when compared to 4 ind. 40 mL^{-1} (0.374) (Table 4.11). In addition, *M. macrocopa* cultivated at 20 ind. 40 mL^{-1} required another 9 days to reach its peak density compared to 4 ind. 40 mL^{-1}. In commercial aquaculture, increases in the duration of cultivation would also increase the cost of production and water usage. *M. macrocopa* at 4 ind. 40 mL^{-1} and 20 ind.40 mL^{-1} starting density showed a significant difference ($p \leq 0.05$) on day-11 and day-15. Therefore, instead of using a higher initial population density ($\geq$ 20 ind. 40 mL^{-1}), a 4 ind. 40 mL^{-1} initial population density is preferable in terms of cost and time.

The *Moina macrocopa* was found to be highly competitive in density adaptations to the culture environment when compared to other cladoceran species e.g. *M. micrura*. According to Jana and Pal (1985), the growth performance of *M. micrura* was found to reach a limit at the population density of 75 ind. L^{-1} as the results were related to the competition for space, food, and confinement. However, *M. macrocopa* showed optimal performance in a high density of 100 to 500 ind. L^{-1} (or equivalent to 4 ind. 40 mL^{-1} and 20

ind. 40 mL^{-1} in the study). The present study also suggested that the initial stocking density of *Moina* should not exceed 500 ind. L^{-1}. An intensive culture of over 500 ind. L^{-1} could possibly lead to a population collapse due to insufficient space, competition for food, breakdown of energy reserves, sexual transformation, and allelopathic effects (Goulden and Hornig, 1980; Stross, 1987; Hobaek and Larsson, 1990; Kleiven et al., 1992; Slusarczyk, 1995; Burns, 1995; Pagano et al., 2000; Innes and Singleton, 2000; He et al., 2001; Zadereev, 2003; Begon et al., 2006; Fernando et al., 2007).

A high population density or overcrowding will result in space limitations, food deprivation, and accumulation of excretory products. The culture of *M. macrocopa* started at 40 and 80 ind. 40 mL^{-1} with RAS wastewater effluent and de-chlorinated water showed the same trend as in Figures 4.14 to 4.18. An increase in population density was rare in these cultures. No significant increase ($p > 0.05$) was observed throughout the cultivation. This phenomenon indicated that the higher densities of 40 and 80 ind. 40 mL^{-1} were unsuitable as the starting density for cultivating *M. macrocopa*. The decreasing trend in the number of individuals could be explained by the low levels of resources that are inadequate for maintaining a population (Benider et al., 2002), and the increase of metabolic waste products. The food supply of *Moina* embryos are closely related to the energy reserves of the females where the embryos are fed by the placentas (Goulden and Hornig, 1980; Burak, 1997). The survival of neonates is dependent upon the level of reserves which remain after the completion of the embryonic development in the female's brood pouch. Therefore, high population densities cause the zooplankton to experience

limitation in food supplies, hence reducing the population growth rate (Rose et al., 2002).

Population density is associated with sexual reproduction, which occurs when a density peak of parthenogenetic reproducing individuals has been reached (Pagano et al., 2000). When environmental conditions are unfavourable, high population density would shift the *Moina* reproduction mode from asexual to sexual (gamogenesis). Unfavourable conditions would induce the production of males and sexual females, leading to the formation of ephippia (resistant eggs) which would hatch into parthenogenetic females when the environmental conditions become favourable again (Innes and Singleton, 2000; Fernando et al., 2007). At high population densities, the reproduction mode will shift from parthenogenesis to gamogenesis and causes the number of newborns to decrease, reducing the overall of population number (Rose et al., 2002).

At high population densities, organisms tend to modify their feeding behaviour by releasing and accumulating chemical substances (Zadereev, 2003). These chemical substances are not the products of food metabolism. These effects are termed as 'negative interference' or 'allelopathy'. The chemical substances will inhibit the population growth and produce smaller sized animals as reported in the population study on *Daphnia* (Burns, 1995).

Moina cultivation with crude wastewater from treatment plants and animal waste products has been reported by Nandini et al. (2004) and Golder et al.

(2007). The risks of pathogenic contamination or toxicant pollution were found to be considerably high, which may cause bio-security issues on livefeed production to be raised by consumers (Siebe and Cifuentes, 1995). Consequently, bioaccumulation of toxicants may affect the overall performance of fish fry after long term feeding with contaminated *Moina*. The aquaculture effluents from RAS systems could be an alternative way to propagate *M. macrocopa* in a safe and effective manner. Under good water quality control and monitoring, the effluents discharged in the RAS system may be considerably safer than untreated crude wastewater from treatment plants or terrestrial animals' waste products as a potential culture medium for *M. macrocopa*.

5.5 Future Studies

These studies covered various aspects from the growth of *Moina macrocopa* using fish wastes and oil emulsions, to the potential of using these culture materials on fatty acids improvement, also to the optimal culture density of the species in a captive environment. These comprehensive datasets provided a basic, informative guideline for hatchery producers on *Moina* enrichment and utilization for freshwater fish and shrimp larval feeding. To better understand the application of enriched *Moina* in larval feeding and a nutrient delivery mechanism, it will be of interest to focus on nutrient improvement, survival rate, growth performance and especially the growth rate of the fed fish or shrimp larvae. Understanding how nutrients can be delivered, and the nutrient

content of the target species may be a key to enhancing the production and performance of a hatchery.

CHAPTER 6.0

CONCLUSION

The application of fish faeces for cultivating *Moina macrocopa* appears to show a promising result for the larviculture industry, especially for freshwater recirculating aquaculture systems (RAS) where fish wastes can be easily harvested and recycled as a natural food source.

Life table experiments of *Moina macrocopa* were carried out using a range of concentrations for different potential diets which allowed the elucidation of their effects on the life history variables of this species. In this study, fish wastes were used for the cultivation of *M. macrocopa* instead of conventional feeds such as yeast and green algae. This helped to promote sustainable aquaculture practices through the recycling of aquaculture effluents. In addition, these results also provided some useful information on the optimal range of enrichment concentrations, which can be used as a reference for the enrichment of *M. macrocopa* in the aquaculture industry.

Enrichment time, concentration, and a variety of lipid emulsions have been investigated in order to better understand the fatty acid profile of *Moina macrocopa* enrichment. It is suggested that fatty acids in *M. macrocopa* could be improved with the addition of lipid emulsions as has already been demonstrated for *Artemia*. Animal- and plant- based oil emulsions, such as squid and canola oil, and aquaculture effluents offer a competitive advantage

by enhancing different fatty acid distributions as well as increasing the *n*-3 and *n*-6 lipid ratios of the livefeed. The use of aquaculture effluents also leads to a zero cost investment, thus making it, along with the supplementation of oil emulsions, a viable alternative for *M. macrocopa* enrichment.

In conclusion, the *M. macrocopa* population growth experiment using RAS effluent water as a culture medium demonstrated that *Moina macrocopa* could be cultivated by the wastewater discharged from the commercial aquaculture system. This experiment showed that the initial stocking density of *M. macrocopa* that started with 4 ind. 40 mL^{-1} or equivalent to 100 *Moina* per liter, resulted in a more superior performance in population growth compared to 20, 40 and 80 ind. in 40 mL (or equivalent to 500, 1000 and 2000 *Moina* per liter).

LIST OF REFERENCES

Agh, N. and Sorgeloos, P. (2005). Handbook of protocols and guidelines for culture and enrichment of live food for use in larviculture. (pp. 8 - 40) Artemia and Aquatic Animals Research Center, Urmia University, Iran.

Ahlgren, G., Carlstein, M. and Gustafsson, I.-B. (1999). Effects of natural and commercial diets on the fatty acid content of European grayling. *Journal of Fish Biology,* 55, 1142-1155.

Ahmad, I. and Ahmad, B.M. (1992). Freshwater Ecology. Department of Botany, Faculty of Life Science, Universiti Kebangsaan Malaysia, Selangor.

Alam, M.J., Cheah, S.H. and Ang, K.J. (1991). Possible use of *Moina* spp. as a live feed substitute in larval rearing of the freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture and Fisheries Management,* 22, 531-535.

Alam, J. (1992). *Moina micrura (Kurz) as a live substitute for Artemia sp. in larval rearing of Macrobrachium rosenbergii (De Man).* Ph.D. Thesis. Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia.

Alam, M.J., Ang, K.J. and Cheah, S.H. (1993a). Use of *Moina micrura* (Kurz) as *Artemia* substitute in the production of *Macrobranchium rosenbergii* (de Man) post-larvae. *Aquaculture*, 109, 337-349.

Alam, M.J., Ang, K.J., Cheah, S.H., Ambak, M.A. and Saad, C.R. (1993b). Effects of *Moina micrura* (Kurz) from two different culture sources as a replacement of *Artemia* sp. in production of *Macrobrachium rosenbergii* (de Man) post-larvae. *Aquaculture and Fisheries Management,* 24, 47-56.

American Public Health Association (APHA) (1985). Standard methods for the examination of water and wastewater, 16th Edition. American Public Health Association, Washington, DC.

Armitage, K.B., Saxena, B. and Angino, E.E. (1973). Population dynamics of pond zooplankton, I. *Diaptomus pallidus* Herrick. *Hydrobiologia*, 42, 295-333.

Association of Official Analytical Chemists (AOAC) (1995). In: Cunniff, P.A. (Ed.) 16th Edition, *AOAC International* vol. 1., Arlington, USA.

Balasubramaniam P.R. and Kasturi Bai R. (1994). Utilization of an aerobically digested cattle dung slurry for the culture of zooplankton, *Daphniasimilis* Claus (Crustacea: Cladocera). *Asian Fishery Society,* 7, 67-76.

Begon, M., Townsend, C.R. and Harper, J.L. (2006). *Ecology from individuals to ecosystems.* 4th Edition. Blackwell Publishing Ltd., UK.

Benider, A., Tifnouti, A. and Pourriot, R. (2002). Growth of *Moina macrocopa* (Straus 1820) (Crustacea, Cladocera): influence of trophic conditions, population density and temperature. *Hydrobiologia,* 468,1-11.

Berdanier, C.D. and Zempleni, J. (2009). Advanced nutrition-macronutrients, micronutrients and metabolism (529 p.). CRC press, U.S.A.

Bessonart, M., Izquierdo, M.S., Salhi, M., Hernandez-Cruz, C.M., Gonzalez, M.M. and Fernandez-Palacios, H. (1999). Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. *Aquaculture*, 179, 265-275.

Beveridge, M. (1987). Cage Aquaculture (351 p.). *Farnham*, England.

Bisbal, G.A. and Bengtson, D.A. (1991). Effect of dietary n-3 HUFA on survival and growth of summer flounder *Paralichthys dentatus* larvae. In: P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier (Eds), *Larvi'91-Fish and Crustacean Larviculture Symposium* (pp 56 – 67)*,* Ghent, Belgium: European Aquaculture Society.

Blancheton, J.P. (2000). Developments in recirculation systems for Mediterranean fish species. *Aquacultural Engineering*, 22, 17-31.

Blomquist, G.J., Borgeson, C.E. and Vundla, M. (1991). Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochemistry*, 21, 99-106.

Bottrell, H.H. (1975). The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the River Thames, with a discussion of temperature functions. *Oecologia*, 18, 63-84.

Bransden, M.P., Butterfield, G.M., Walden, J., McEvoy, L.A. and Bell, J.G. (2005). Tank colour and dietary arachidonic acid affects pigmentation, eicosanoid production and tissue fatty acid profile of larval Atlantic cod (*Gadus morhua*). *Aquaculture*, 250, 328-340.

Brett, M.T. and Müller-Navarra, D.C. (1997). The role of highly unsaturated fatty acids in aquatic foodweb process*.* *Freshwater Biology*, 38, 483-499.

Burak, E.S. (1997). Life tables of *Moina macrocopa* (Straus) in successive generations under food and temperature adaptation. *Hydrobiologia*, 360, 101-108.

Burns C.W. (1968). The relationship between body sizes of filter feeding Cladocerans and the maximum size of particle ingested. *Limnology and Oceanography,* 13, 675-678.

Burns, C.W. (1995). Effects of crowding and different food levels on growth and reproductive investment of *Daphnia*. *Oecologia*, 101, 234-244.

Cahu, C., Zambonino Infante, J. and Takeuchi, T. (2003). Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, 227, 245-258.

Carver, R.H. and Nash, J.G. (2005). Analysis of variance (I) and analysis of variance (II). In: Carver, R.H. and Nash, J.G. (Eds.), *Doing data analysis with SPSS®*, (pp 135-161). Singapore: Thomson Brooks/cole.

Cary, T.L., Chandler, G.T., Volz, D.C., Walse, S.S. and Ferry, J.L. (2004). Phenylpyrazole insecticide fipronil induces male infertility in the estuarine meiobenthic crustacean *Amphiacus tenuiremis*. *Environmental Science Technology*, 38, 522-528.

Cauchie H.M., Hoffmann L., Jasper-Versali M.F., Salvia M. and Thomé J.P. (1995). *Daphnia magna* Straus living in an aerated sewage lagoon as a source of chitin: ecological aspects. *Belgian Journal of Zoology,* 125, 67-78.

Cheah, S.H., Ang, K.J. and Abidin, A.Z. (1988). Functions of a stream unit on the growth of phytoplankton, zooplankton and fish fry in a new culture tank system. *Aquacultural Engineering*, 7, 295-308.

Chu, K.H., Wong, C.K. and Chiu, K.C. (1997). Effects of the insect growth regulator(S)-Methoprene on survival and reproduction of the freshwater cladoceran *Moina macrocopa*. *Environmental Pollution,* 96, 173-178.

Chuah, T.S., Loh, J.Y. and Hii, Y.S. (2007). Acute and chronic effects of the insecticide-endosulfan on freshwater cladoceran, *Moina macrocopa* Straus. *Bulletin of Environmental Contamination and Toxicology*, 79, 557-561.

Conceição, L.E.C., Morais, S. and Rønnestad, I. (2007). Tracers in fish larvae nutrition: A review of methods and applications. *Aquaculture*, 267, 62-75.

Corraze, G. (2001). Lipid nutrition. In: Guillaume, J., Kaushik, S., Bergot, P. and Metailler, R. (Eds.), *Nutrition and feeding of fish and crustaceans* (pp. 111-129). Springer-Praxis, UK.

Coutteau, P. and Sorgeloos, P. (1997). Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures. *Freshwater Biology*, 38, 501-512.

Das, S.K., Tiwari, V.K., Venkateshwarlu, G., Reddy, A.K. and Parhi, J. (2007). Growth, survival and fatty acid composition of *Macrobrachium rosenbergii* (de Man, 1879) post larvae fed HUFA-enriched *Moina micrura*. *Aquaculture*, 269, 464-475.

Deng, D.G. and Xie, P. (2003). Effect of food and temperature on the growth and development of *Moina irrasa* (Cladocera: Moinidae). *Journal of Freshwater Ecology*, 18, 503-513.

De Pauw, N., Laureys, P. and Morales, J. (1981). Mass cultivation of *Daphnia magna* Straus on rice bran. *Aquaculture*, 25, 141-152.

Dhert, P. (1996). Rotifers. In: Lavens, P., Sorgeloos, P. (Eds.), *Manual on the production and the use of live food for aquaculture* (362, 49-78), FAO: Fisheries Technical Paper.

Dobson, G. (2002). Analysis of fatty acids in functional foods with emphasis on ω3 fatty acids and conjugated linoleic acid. In: Hurst, W.J. (Ed.), *Methods of analysis for functional foods and nutraceuticals* (pp. 65-99), Washington, DC: Functional Foods and Nutraceuticals Series.

Dodson, S.I. and Frey, D.G. (2000). Cladocera and other Branchiopoda. In: Thorp J.H. and Covich A.P. (Eds), *Ecology and Classification of North America Freshwater Invertebrates* (pp 850-914), San Diego, USA: Academic Press.

DOF (2009). *Annual Fisheries Statistics 2007. Ministry of Agriculture and Agro-based Industries*. URL: http:// dof.gov.my Accessed on 25 September 2009

Estévez, A., McEvoy, L.A., Bell, J.G. and Sargent, J.R. (1998). Effects of temperature and starvation time on the pattern and rate of loss of essential fatty acids in *Artemia* nauplii previously enriched using arachidonic acid and eicosapentaenoic acid-rich emulsions. *Aquaculture*, 165, 295-311.

FAO (Food and Agriculture Organization) (1996). Manual on the production and use of live food for aquaculture. (pp. 295). Rome, FAO: Fisheries Technical Paper.

FAO (Food and Agriculture Organization) (2003). Review of the state of world aquaculture. (pp. 95). Rome, FAO: Fisheries Circular No. 886, Review 2.

Fabiola, P.A., Nandini, S. and Sarma, S.S.S. (2005). Differences in population growth of rotifers and cladocerans raised on algal diets supplemented with yeast. *Limnologica*, 35, 298-303.

Faulk, C.K. and Holt, G.J. (2005). Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture system: live prey enrichment and greenwater culture. *Aquaculture*, 249, 231-243.

Fernando, M.J., Jesus, R.E. and Rafael, V.C. (2007). Effect of culture density and volume on *Moina micrura* (Kurz, 1874) reproduction, and sex ration in the progeny. *Hydrobiologia,* 594, 69-73.

Fivlstad S., Thomassen J.M., Smith M.J., Kjartansson H. and Anderson A.B. (1990). Metabolic production rates from Atlantic salmon (*Salmo salar* L.) and Arctic char (*Salvelinus alpinus* L.) reared in single pass land based brackish water and sea water system. *Aquacultural Engineering*, 9, 1-21.

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

Fukusho, K. (1991). Review of the research status of zooplankton production in Japan. In: Fulks, W. and Main, K.L. (Eds.), *Rotifer and Microalgae Culture System*. (pp. 55-60). Honolulu, Hawaii: Proceedings of a United State – Asia Workshop.

Fulks, W and Main, K.L. (1991). Rotifer and microalgae culture system. *Proceedings of a U.S.-Asia workshop*. Honolulu, Hawaii.

Ghioni, C., Tocher, D.R., Bell, M.V., Dick, J.R. and Sargent, J.R. (1999). Low C_{18} to C_{20} fatty acid elongase activity and limited conversion of stearidonic acid, 18:4(n-3), to eicosapentaenoic acid, 20:5(n-3), in a cell line from the turbot, *Scophthalmus maximus*. *Biochemistry and Biophysical Acta*, 1437, 170-181.

Golder, D., Rana, S., Sarkar, P. D. and Jana, B.B. (2007). Human urine is an excellent liquid waste for the culture of fish food organism, *Moina micrura*. *Ecological Engineering*, 30, 326-332.

Goulden, C.E. and Hornig, L.L. (1980). Population oscillations and energy reservers in planktonic Cladocera and their consequences to competition. *Proceeding of National Academy of Science*, 77, 1716-1720.

Guary, J.C., Kayama, M., Murakami, Y. and Ceccaldi, H.J. (1976). The effects of a fat-free diet and compounded diets supplemented with various oils on molt, growth and fatty acid composition of prawn, *Penaeus japonicus* Bate. *Aquaculture,* 7, 245-254.

Gulati, R.D. and DeMott, W.R. (1997). The role of food quality for zooplankton. *Freshwater Biology,* 38, 447–771.

Hach, (2005). Hach DR/890 spectrophotometer procedures manual (616 p). Loveland, USA: Hach Ltd.

Hakanson L., Ervik A., Makinen T. and Moller B. (1988). Basic concepts concerning assessments of environmental effects of marine fish farms. (103 p.). Copenhagen: Nordic Council of Ministers.

Hamre, K., Moren, M., Solbakken, J., Opstad, I. and Pittman, K. (2005). The impact of nutrition on metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, 250, 555-565.

Han, K.M., Geurden, I. and Sorgeloos, P. (2000). Enrichment strategies for *Artemia* using emulsions providing different levels of *n*-3 highly unsaturated fatty acids. *Aquaculture*, 183, 335-347.

Hanaee, J., Agh, N., Hanaee, M., Delazar, A. and Sarker, S.D. (2004). Studies on the enrichment of *Artemia urmiana* cysts for improving fish food value. *Animal Feed Science and Technology,* 120, 107-112.

He, Z.H., Yan, L.G. and Zhang, Y. (1998a). Effect of food on the growth, reproduction and intrinsic growth rate of *Moina mongolica*. *Journal of Dalian Fishery University*, 11, 21-27.

He, Z.H., Wang, Y., Cui, H., Guo, L.Z. and Qian, H. (1998b). Studies on the mass culture of *Moina mongolica* in seawater. *Journal of Fishery in China*, 22,17-23.

He, Z.H., Qin, J.G., Wang, Y., Jiang, H. and Wen, Z. (2001). Biology of *Moina Mongolia* (Moinidae, Cladocera) and perspective as live food for marine fish larvae: review. *Hydrobiologia*, 457, 25-37.

Henderson, R.J., Forrest, D.A.M., Black, K.D. and Park, M.T. (1997). The lipid composition of sealoch sediments underlying salmon cages. *Aquaculture*, 158, 69-83.

Hobaek, A. and Larsson, P. (1990). Sex determination in *Daphnia magna*. *Ecology*, 71, 2255-2268.

Innes, D.J. (1997). Sexual reproduction of *Daphnia pulex* in a temporary habitat. *Oecologia,* 111, 53-60.

Innes, D.J. and Singleton, D.R. (2000). Variation in allocation to sexual and asexual reproduction among clones of cyclically parthenogenetic *Daphnia pulex* (Crustacea: Cladocera). *Biological Journal of Linnean Society*, 71, 771-787.

Izquierdo, M.S., Socorro, J., Arantzamendi, L. and Hernández-Cruz, C.M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry*, 22, 97-107.

Jahn, G.C., Almazan, L.P., and Pacia, J. (2005). Effect of nitrogen fertilizer on the intrinsic rate of increase of the rusty plum aphid, *Hysteroneura setariae* (Thomas) (Homoptera: Aphididae) on rice (*Oryza sativa* L.). *Environmental Entomology*, 34, 938-943.

Jana, B.B. and Pal, G.P. (1985). Effects of inoculum density on growth, reproductive potential and population size in *Moina micrura* (Kurz). *Limnologica*, 16, 315-324.

Jhingran V.G. (1991). *Fish and Fisheries of India*. New Delhi, India: Hindustan Pub. Corp.

Johnson, D.S. (1962). Water-fleas. *Malay Natural Journal,* 16, 126-144.

Kamler, E., Wolnicki, J., Kamiński, R. and Sikorska, J. (2008). Fatty acid composition, growth and morphological deformities in juvenile cyprinid, *Scardinius erythrophthalmus* fed formulated diet supplemented with natural food. *Aquaculture*, 278, 69-76.

Kang, C.K., Park, H.Y., Kim, M.C. and Lee, W.J. (2006). Use of marine yeasts as an available diet for mass cultures of *Moina macrocopa*. *Aquaculture Research*, 37, 1227-1237.

Kanazawa, A., Teshima, S. and Tokiwa, S. (1977). Nutritional requirements of prawn: VII. Effect of dietary lipids on growth. *Bulletin of Japan Society Science of Fish*, 43, 849-856.

Kanazawa, A., Teshima, S., Ono, K. and Chalayondeja, K. (1979). Biosynthesis of fatty acids from acetate in the prawn, *Penaeus monodon* and *Penaeus merguensis. Bulletin of Japan Society Science of Fish,* 28, 21-26.

Kanazawa, A. (1993a). Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. *Journal of World Aquaculture Society,* 24, 162-166.

Kanazawa, A. (1993b). Important of dietary docosahexaenoic acid on growth and survival of fish larvae. In: Lee, C.S., Su, M.S., Liao, I.C. (Eds.), *Finfish Hatchery in Asia: Proceeding of Finfish Hatchery in Asia 91*. (3, 87-95). TML: Conference Proceeding

Kibria, G., Nugegoda, D., Fairclough, R. and Lam, P. (1997). The nutrient content and the release of nutrients from fish food and faeces. *Hydrobiologia*, 357, 165-171.

Kim, S.K., Kong, I., Lee, B.H., Kang, L., Lee, M.G. and Suh, K.H. (2000). Removal of ammonium-N from a recirculation aquacultural system using an immobilized nitrifier. *Aquacultural Engineering,* 21, 139-150.

Kim D., Kim T.S., Ryu H.D. and Lee S.I. (2008). Treatment of low carbon-to-nitrogen wastewater using two-stage sequencing batch reactor with independent nitrification. *Process Biochemistry,* 43, 406-413.

Kleiven, O.T., Larsson, P. and Hobaek, A. (1992). Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos*, 65, 197-206.

Kobayashi, M. (1981). Respiratory function of haemoglobin in *Moina macrocopa*. *Comparative Biochemistry and Physiology*, 70, 381-385.

Koven, W., van Anholt, R., Lutzky, S., Ben-Atia, I., Nixon, O., Ron, B. and Tandler, A. (2003). The effect of dietary arachidonic acid on growth, survival and cortisol levels in different age gilthead seabream larvae (*Sparus auratus*) exposed to handling or daily salinity change. *Aquaculture*, 228, 307-320.

Koven, W. (2003). Key factors influencing juvenile quality in mariculture: review. *Israel Journal of Aquaculture*, 4, 283-297.

Kraul, S., Brittain, K., Cantrell, R., Nagao, T., Ako, H., Ogasawara, A. and Kitagawa, H. (1993). Nutritional factors affecting stress resistance in larval mahimahi *Coryphaena hippurus*. *Journal of World Aquaculture Society*, 24, 186-193.

Krebs, C.J. (1985). Ecology. In: Harper and Row (Eds.), *The experimental analysis of distribution and abundance*. (pp. 789). New York.

Krohne, D.T. (2001). General ecology, 2nd Edition. (512 p.). USA: Brooks.

Lampert, W. And Sommer, U. (1997). Limnoecology : the ecology of lakes and streams. Oxford University Press.

Lampert, W. and Schoeber, U. (1980). The importance of 'threshold' food concentrations. In : Kerfoot, W.C. (Ed.), *Evaluation and Ecology of Zooplankton Communities.* (pp. 264-267). New Hampshire, Hanover: University Press of New England.

Langis R., Proulx D., Noüe D.J. and Couture P. (1988). Effects of a bacterial biofilm on intensive *Daphnia* culture. *Aquacultural Engineering,* 7, 21-38.

Lechevallier, M.W., Schulz ,W. and Lee, R.G. (1991). Bacterial nutrients in drinking-water. *Applied and Environmental Microbiology*, 57, 857-862.

Lee, H.L., Chen, T.W., Tea, L.H. and Shim, K.F. (1985). Culture of *Moina sp*. with waste materials. In: *Proceedings of the second workshop on technology of animals feed production utilizing food waste materials*. (pp. 207-222). China: Dalian Fishery University.

Léger, Ph., Naessens-Foucquaert, E. and Sorgeloos, P. (1987). International study on *Artemia* XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M). In: Sorgeloos, P., Bengston, D.A., Decleir, W. and Jaspers, E. (Eds.), *Artemia research and its application*, *ecology, culturing and use in Aquaculture*. (3, 411-424). Wetteren, Belgium: Universa Press.

Lim, L.C., Dhert, P. and Sorgeloos, P. (2003). Recent developments in the application of live feeds in the freshwater ornamental fish culture. *Aquaculture*, 227, 319-331.

Logue, J.A., Howell, B.R., Bell, J.G. and Cossins, A.R. (2000). Dietary *n*-3 long-chain polyunsaturated fatty acid deprivation, tissue lipid composition, *ex vivo* prostaglandin production, and stress tolerance in juvenile *Dover Sole* (*Solea solea* L.). *Lipids*, 35, 745-755.

Loh, J.Y., How, C.W, Hii, Y.S., Khoo, G. and Alan Ong, H.K. (2009). Fish faeces as a potential food source for *Moina macrocopa* Straus cultivation. *Journal of Science and Technology in Tropics*, 5, 5-9.

Lotka, A.J. (1913). A natural population norm. *Journal of Washington Academic Science*, 3, 241-293.

Lund, I., Steenfeldt, S.J. and Hansen, B.W. (2007). Effect of dietary arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid on survival, growth and pigmentation in larvae of common sole (*Solea solea* L.). *Aquaculture*, 273, 532-544.

Mayer, C.M. and Wahl, D.H. (1997). The relationship between prey selectivity and growth and survival in larval fish. *Canadian journal of Fishery Aquatic Society*, 54, 1504-1512.

Merchie, G. (1996). Use of nauplii and meta-nauplii. In: Lavens, P. and Sorgeloos, P. (Eds.), *Manual on the production and use of live food for aquaculture*. (361, 137-163). Rome, FAO: FAO Fisheries Technical Paper.

Metcalfe, M. R. (1995). Investing in aquacultural wastewater techniques for improved water quality: a coastal community case study. *Coastal Management*, 23, 327-335.

Millar, J.S. and Zammuto, R.M. (1983). Life histories of mammals: an analysis of life tables. *Ecology*, 64, 631-635.

Molles, M.C.Jr. (2005). Ecology: concepts and applications, 3rd Edition. (622 p.). USA: McGraw-Hill.

Morais, S., Narciso, L., Dores, E. and Pousao-Ferreira, P. (2004). Lipid enrichment for Senegalese sole (*Solea senegalensis*) larvae: effect on larval growth, survival and fatty acid profile. *Aquaculture International*, 12, 281-298.

Mourente, G. and Tocher, D.R. (1993). Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in wild-caught juveniles of golden grey mullet, *Liza aurata*, in vivo. *Fish Physiology Biochemistry*, 12, 119-130.

Müller-Navarra, D.C. (1995). Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Archiv för Hydrobiologie*, 132, 97-307.

Murthy, H.S. (1998). Effect of enriched *Artemia* on metamorphosis and survival of larvae of giant freshwater prawn, *Macrobranchium rosenbergii. Journal of Aquaculture Tropical,* 12, 215-222.

Naas, K., Naess, T. and Harboe, T. (1992). Enhance first feeding of halibut larvae (*Hippoglossus hippoglossus*) in green water. *Aquaculture,* 105, 143-156.

Nandini, S. and Sarma, S.S.S. (2000). Lifetable demography of four cladoceran species in relation to algal food (*Chlorella vulgaris*) density. *Hydrobiologia*, 435, 117-126.

Nandini, S. and Sarma, S.S.S. (2003). Population growth of some genera of Cladocerans (Cladocera) in relation to algal food (*Chlorella vulgaris*) levels. *Hydrobiologia*, 491, 211-219.

Nandini, S., Lara, D.A., Sarma, S.S.S. and Garcia, P.R. (2004). The ability of selected cladoceran species to utilize domestic wastewaters in Mexico City. *Journal of Environmental Management*, 71, 59-65.

National Research Council. (NRC) (1992). Marine aquaculture opportunities for growth. Washington, DC: National Academy Press.

Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V. and Amat, F. (1999). Lipid conversion during enrichment of *Artemia*. *Aquaculture*, 174, 155-166.

Nelson, A. and Fernando, G. (2005). Population dynamics and adaptative strategies of *Ceriodaphnia pulchella* (Crustacea, Cladocera) presented at the *VII*th *International Symposium on Cladocera*, Herzberg, Switzerland.

Ng, W.K., Lim, P.K. and Boey, P.L. (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle a-tocopherol concentration of African catfish, *Clarias gariepinus*. *Aquaculture*, 215, 229-243.

Ng, W.K. (2009). The current status and future prospects for the aquaculture industry in Malaysia. In: Stickney, R.R. (Ed.), *World Aquaculture*. (40, 26-30) US: World Aquaculture Society.

Nichols, P.D., Mooney, B.D. and Elliott, N.G. (2004). Value-adding to Australian marine oil. In: Sakaguchi, M. (ed.), *More Efficient Utilization of Fish and Fisheries Products*. (pp. 115-130). USA: Elsevier Ltd.

Nicolaides, N. and Woodall, A.H. (1962). Impaired pigmentation in chinook salmon fed diets deficient in essential fatty acids. *Journal of Nutrition,* 78, 429-438.

Nigawaba C., Nalubega M., Vinnerås B., Sundberg C. and Jönsson H. (2008). Bench-scale composting of source-separated human faeces for sanitation. *Waste Management,* 29, 585-589.

Oda, S., Tatarazako, N., Watanabem H., Morita M. and Iguchi, T. (2004). Production of male neonates in four cladocerans species exposed to a juvenile hormone analog, fenoxycarb. *Chemosphere,* 22, 123-130.

Pagano, M., Jean, S.L., Arfi, R., Bouvy, M. and Shep, H. (2000). Population growth capacities and factors in monospecific cultures of the cladocerans *Moina micrura* and *Diaphanosoma excisum* and the copepod *Thermocyclops decipiens* from Coˆte d'Ivoire (West Africa). *Aquatic Living Resources*, 13, 163-172.

Parker, R. (2002). Aquaculture Science, 2nd Edition. (621 p.). USA: Delmar Thomson Learning.

Papandroulakis, N., Divanach, P. and Kentouri, M. (2002). Enhanced biological performance of intensive sea bream (*Sparus aurata*) larviculture in the presence of phytoplankton with long photophase. *Aquaculture*, 204, 45-63.

Patil, S.S., Ward, A.J., Kumar, M.S. and Ball, A.S. (2010). Utilising bacterial communities associated with digested piggery effluent as a primary food source for the batch culture of *Moina australiensis*. *Bioresearch Technology*, 101, 3371-3378.

Pedersen, B.H., Nilssen, E.M. and Hjelmeland, K. (1987). Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus*) digesting copepod nauplii. *Marine Biology*, 94, 171-181.

Pedersen, B.H. and Hjelmeland, K. (1988). Fate of typsin and assimilation efficiency in larval herring (*Clupea harengus*) following digestion of copepods. *Marine Biology*, 97, 467-476.

Peña-Aquado. F., Nandini, S. and Sarma, S.S.S. (2005). Differences in population growth of rotifers and cladocerans raised on algal diets supplemented with yeast. *Limonologica*, 35, 298-303.

Penczak, T., Galicka, W., Molinski, M., Kusto, E. and Zalewski, M. (1982). The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairdneri*. *Journal of Apply Ecology*, 19, 371-393.

Pickova, J., Brännäs, E. and Andersson, T. (2007). Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. *Aquaculture International*, 15, 305-311.

Piedecause, M.A., Mazón, M.J., García García, B. and Hernández, M.D. (2007). Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). *Aquaculture*, 263, 211-219.

Porter, K.G., Gerritsen, J. and Orcutt, J.D.Jr. (1982). The effect of food concentration on swimming patterns, feeding behaviour, ingestion, assimilation and respiration by *Daphnia*. *Limnology and Oceanography*, 27, 935-949.

Proulx, D. and Noüe, S.E.L. (1985). Harvesting *Daphnia magna* grown on urban tertiarily treated effluent. *Water Research*, 19, 1319-1324.

Qin, J.G. and Culver, D.A. (1996). Effect of larval fish and nutritional enrichment on plankton dynamics in experimental ponds. *Hydrobiologia*, 321, 109-118.

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.

Read, G.H.L. (1981). The response of *Penaeus indicus* (Crustacea: Penaeidea) to purified and compound diets of varying fatty acid composition. *Aquaculture,* 24, 245-256.

Reitan, K.I., Rainuzzo, J.R. and Olsen, Y. (1994). Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. *Aquaculture International,* 2, 33-34.

Roche K.F. (1995). Growth of the rotifer *Brachionus calyciflorus* Pallas in dairy waste stabilization ponds. *Water Research,* 29, 2255-2260.

Rodgers, L.J. and Barlow, C.G. (1987). Better nutrition enhances survival of barramundi larvae. *Australian Fisheries*, 46, 30-32.

Rodolfo, F.V. and Edmundo, M.E. (1980). Preliminary studies on *Moina* sp. production in freshwater tanks. *Aquaculture,* 21, pp. 93-96.

Rose, R.M., Warne, M.S.J. and Lim, R.P. (2002). Some life history responses of the cladoceran *Ceriodaphnia cf. dubia* to variations in population density at two different food concentrations. *Hydrobiologia*, 481, 157-164.

Rottmann, R.W., Scott Graves, J., Watson, C. and Yanong, R.P.E. (1992). *Culture Techniques of Moina: The Ideal Daphnia for Feeding Freshwater Fish Fry* URL: http://edis.ifas.ufl.edu/pdffiles/FA/FA02400.pdf Accessed on 25 July 2007

Sáenz Y., Zarazaga M., Briñas L., Lantero M., Larrea F.R. and Torres C. (2001). Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *International Journal of Antimicrobial Agents,* 18, 353-358.

Sargent, J.R., Bell, J.G. and McEvoy, L.A. (1997). Requirements, presentation and sources of poly-unsaturated fatty acids in marine fish larval feeds. *Aquaculture,* 155, 117-127.

Sargent, J.R., McEvoy, L., Estévez, A., Bell, G., Bell, M., Henderson, J. and Tocher, D. (1999a). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, 179, 217-229.

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

Sargent, J.R., McEvoy, L.A., Estevez, A., Bell, M., Henderson, J. and Tocher, D. (1999c). Lipid nutrition of marine fish during early development: current status and future direction. *Aquaculture*, 179, 217-229.

Schipp, G.R., Bosmans, J.M.P. and Marshall, A.J. (1999). A method for hatchery culture of tropical calanoid copepods *Acartia* spp. *Aquaculture*, 174, 81-88.

Schneider, O., Sereti, V., Eding, E. and Verreth, J.A.J. (2007). Heterotrophic bacterial production on solid fish waste: TAN and nitrate as nitrogen source under practical RAS conditions. *Bioresource Technology*, 98, 1924-1930.

Shim, K.F. and Bajrai, J.R. (1982). Growth rates and food conversion in young guppy *Poecilia reticulate* fed on artificial and natural foods. *Singapore Journal of Primary Industrial*, 10, 26-38.

Shim, K.F. (1988). By-product utilization in live food culture for tropical aquarium fish. In: De Silva, S. S. (Ed.), *Finfish Nutrition Research in Asia*. (pp. 42-47). Singapore: Proceedings of 2nd Asian Fish Nutrition Network Meeting.

Sick, K.P. (2003). *Pengkulturan Moina sp. dengan menggunakan yis dan air hijau*. Msc. Thesis. Faculty of Agroteknologi and Food Science. Kolej Universiti Sains dan Teknologi Malaysia.

Siebe, C. and Cifuentes, E. (1995). Environmental impact of wastewater irrigation in central Mexico: an overview. *International Journal of Environmental Health Research,* 5, 161–173.

Slusarczyk, M. (1995). Predator-induced diapause in *Daphnia*. *Ecology*, 76, 1008-1013.

Smith, R.L. and Smith, T.M. (2003). Elements of ecology, 5th Edition. (627 p.). USA: Benjamin Cummings.

Sorgeloos, P. and Lavens, P. (1996). Manual on the production and use of live food for aquaculture. In: Sorgeloos, P. and Lavens, P. (Eds.), *FAO Fisheries Technical Paper 361*, (295 p). University of Ghent, Belgium: Laboratory of Aquaculture and *Artemia* Reference Center.

Sorgeloos, P. and Agh, N. (2005). Handbook of protocols and guidelines for culture and enrichment of live food for use in larviculture. (42 p.). Urmia University, Iran: Artemia and Aquatic Animals Research Center.

Sorgeloos, P. and Lavens, P. (1996). Manual on the production and use of live food for aquaculture. (295 p.). University of Ghent, Belgium: FAO Fisheries Technical Paper 361.

Stansby, M.E. (1981). Reliability of fatty acid values purporting to represent composition of oil from different species of fish. *Journal of America Oil Chemistry Society*, 58, 13.

Stearns, S.C. (1976). Life history tactics: a review of ideas. *Quarterly Reviews of Biology*, 51, 37-47.

Stottrup, J.G. and Norsker, N.M. (1997). Production and use of copepods in marine fish larviculture. *Aquaculture,* 155, 231-247.

Stross, R.G. (1987). Photoperiodism and phased growth in *Daphnia* populations: coactions in perspective. In: Peters, R.H. and Bernardi, R.D. (Eds), *Daphnia*, 45, 413-437.

Sui, L.Y., Mathieu, W., Cheng, Y.X. and Patrick, S. (2007). The effect of dietary *n*-3 HUFA levels and DHA/EPA ratios on growth, survival and osmotic stress tolerance of chinese mitten crab *Eriocheir sinensis* larvae. *Aquaculture*, 273, 139-150.

Tacon, A.G.J. (1990). Standard methods for the nutrition and feeding of farmed fish and shrimp. (pp 208). Washington, USA: Argent Laboratories Press, Redmond,

Tamaru, C.S., Lee, C.S. and Ako, H. (1991). Improving the larval rearing of striped mullet (*Mugil cephalus*) by manipulating quantity and quality of the *Rotifer*, *Branchionus plicatilis*. In: Fulks, W. and Main, K. L. (Eds.), *Rotifer and Microalgae culture system.* (pp. 89-103). Honolulu, Hawaii: Proceedings of a U.S. - Asia Workshop.

Tamaru, C.S., Murashige, R., Lee, C.S., Ako, H. and Sato, V. (1993). Rotifers fed various diets of backer's yeast and/or *Nannochloropsis oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) larvae. *Aquaculture*, 110, 361-372.

Tamaru, C.S., Ako, H., Paguirigan, Jr., R. and Pang, L. (2003). *Enrichment of Artemia for Use in Freshwater Ornamental Fish Production. Center for Tropical and Subtropical Aquaculture.* URL: http://aqua.ucdavis.edu/dbweb/outreach/aqua/CTSA133.PDF Accessed on 22 April 2008.

Tocher, D.R. and Harvie, D.G. (1988). Fatty acid composition of the major phosphoglycerides from fish neural tissues: (*n*-3) and (*n*-6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. *Fish Physiology and Biochemistry,* 5, 229-239.

Tocher, D.R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews of Fisheries Science*, 11, 107-184.

Tong, S.Y., Liu, C.H. and Wang, X.T. (1988). Appraisement and analysis of nutrient composition for *Moina mongolica* Daddy. *Journal of Dalian Fishery University*, 11, 29-33.

USDA/ERS. (1999). Changes and consequences. Agriculture Information Bulletin In: Frazo, E. (Ed.), *America's Eating Habits.* (142, 1412–1418). Washington, DC. USDA.

US-Environmental Protection Agency (EPA) (1980). Water Quality Criteria Documents. (520 p.). Wahington, D.C. USA: US-EPA Technical Report.

Van Leeuwen, C.J., Luttmer, W.J. and Griffieon, P.S. (1985). The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. *Ecotoxicology and Environmental Safety*, 9, 26-39.

Van Rijn, J., Tal, Y. and Schreier, H.J. (2006). Denitrification in recirculating systems: theory and applications. *Aquacultural Engineering*, 34, 364-376.

Van Stappen, G. (1996). *Introduction, biology and ecology of Artemia,* URL: http://www.fao.org/DOCREP/003/W3732E/w3732e0m.htm. Accessed on 9 July 2007

Ventura, R. and Enderez, E. (1980). Preliminary studies on *Moina* sp. production in freshwater tank. *Aquaculture*, 21, 93-96.

Vetter, R.D., Houdson, R.E. and Arnold, C. (1983). Energy metabolism in a rapidly developing marine fish egg the red drum *Sciaenops ocellata*. *Canadian Journal of Fisheries and Aquaculture Science*, 40, 627-634.

Vijverberg, J. (1980). Effect of temperature in laboratory studies on development and growth of Cladocera and copepoda from Tjeukemeer, The Netherlands. *Freshwater Biology*, 10, 317-340.

Vijverberg, J. (1989). Culture technique for studies on the growth, development and reproduction of *copepod* and cladoceran under laboratory and *in situ* condition. *Freshwater Biology*, 21, 317-373.

Villeneuve, L., Gisbert, E., Zambonion-Infante, J.L., Quazuguel, P. and Cahu, C.L. (2005). Effect of nature of dietary lipids on European sea bass morphogenesis: implication of retinoid receptors. *British Journal of Nutrition*, 94, 877-884.

Ward, O.P. and Singh, A. (2005). Omega-3/6 fatty acids: alternative sources of production. *Process Biochemistry*, 40, 3627-3652.

Wardlaw, G.M., Hampl, J.S. and Disilvestro, R.A. (2004). Lipids. In: Wardlaw, G.M., Hampl, J.S. and Disilvestro, R.A. (Eds.), *Perspectives in Nutrition, 6th Ed*. (pp. 177-222). New York, USA: Colin H. Wheatley.

Watanabe, T., Kitajima, C. and Fujita, S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34, 115-143.

Watanabe, T. (1993). Importance of docosahexaenoic acid in marine larval fish. *Journal of World Aquaculture Society,* 24, 152-161.

Watanabe, T and Kiron, V. (1994). Review: prospects in larval fish dietetics. *Aquaculture*, 124, 223-251.

Whelan, J. (1996). Antagonistic effects of dietary arachidonic acid and *n*-3 polyunsaturated fatty acids. *Biological Effects of Dietary Arachidonic Acid Symposium,* 1, 1086-1091.

Yamasaki, S. and Uchiyama, H. (2001). Neonate production potential of *Moina macrocopa* females hatched from parthenogenesis eggs and resting eggs. *Fisheries Science*, 67, 758-760.

Zadereev, S.Y. (2003). Maternal effects, conspefic chemical cues, and switching from parthenogenesis to gametogenesis in cladoceran *Moina macrocopa*. *Aquatic Ecology*, 37, 251-225.

Zadereev, E.S., Prokopkin, I.G., Gubanov, V.G. and Gubanov, M.V. (2003). Individual-based model of the reproduction cycle of *Moina macrocopa* (Crustacea: Cladocera). *Ecological Modeling*, 162, 15-31.

Appendix A

Water Quality Examination Protocols

a) Dissolved oxygen (DO) determination

Dissolved oxygen level (DO) was determined by YSI DO meter (YSI model 200) from UTAR engineering department laboratory. Approximately 500 mL water samplers were collected from the sampling points for DO measurement.

b) Water pH determination

Water pH was determined by using electrometric method based on standard methods for the examination of water and wastewater (APHA, 1998). Delta 320 desktop pH meter (Mettler-Toledo Ltd., China) was calibrated prior pH determination.

c) Water temperature determination

Water temperature from the sampling points was measured by using a mercury thermometer (Alla France, France).

d) Total ammonia nitrogen (TAN) determination

Method 8155

NITROGEN, AMMONIA (0 to 0.50 mg/L NH_3-N) For water, wastewater, seawater

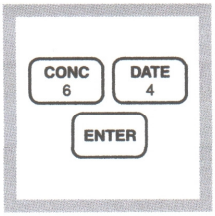
Salicylate Method*

1. Enter the stored program number for ammonia nitrogen (NH_3-N).

Press: **PRGM**

The display will show:

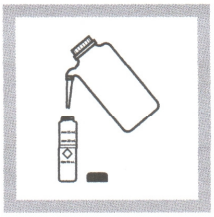
PRGM ?



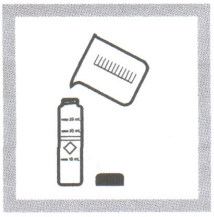
2. Press: 64 ENTER

The display will show **mg/L, NH3-N** and the **ZERO** icon.

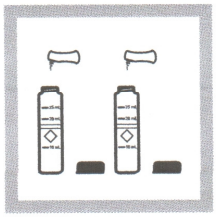
Note: For alternate forms (NH_3, NH_4), press the **CONC** *key.*



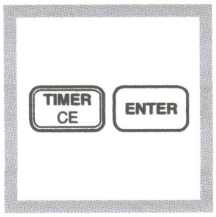
3. Fill a sample cell with 10 mL of deionized water (the blank).



4. Fill a second sample cell with 10 mL of the sample.



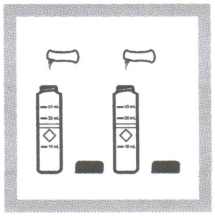
5. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to each sample cell. Cap both cells and shake to dissolve.



6. Press:

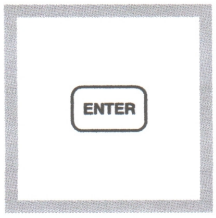
TIMER ENTER

A three-minute reaction period will begin.



7. After the timer beeps add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each sample cell. Cap the cells and shake to dissolve the reagent.

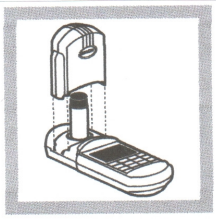
Note: *A green color will develop if ammonia nitrogen is present.*



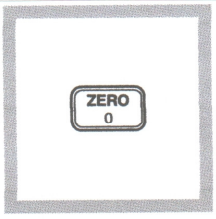
8. The display will show: **15:00 TIMER 2**

Press: **ENTER**

A 15-minute reaction period will begin.



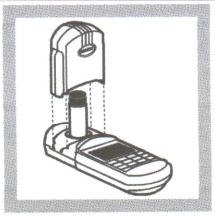
9. After the timer beeps, place the blank into the cell holder. Tightly cover the sample cell with the instrument cap.



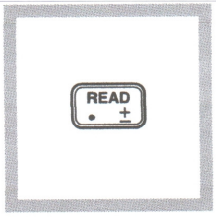
10. Press: **ZERO**

The cursor will move to the right, then the display will show:

0.00 mg/L NH3-N



11. Place the prepared sample into the cell holder. Tightly cover the sample cell with the instrument cap.



12. Press: **READ**

The cursor will move to the right, then the result in mg/L ammonia nitrogen will be displayed.

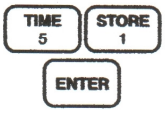
* Adopted from Hach manual (Hach, 2005).

e) Nitrate concentration determination

Method 8039

NITRATE, High Range (0 to 30.0 mg/L NO_3^--N) For water, wastewater, and seawater*

Cadmium Reduction Method *(Using Powder Pillows or AccuVac Ampuls)*
Using Powder Pillows



1. Enter the stored program number for high range nitrate nitrogen (NO_3^-–N) powder pillows.

Press: **PRGM**

The display will show:

PRGM ?

Note: *For most accurate results, perform a Reagent Blank Correction using deionized water (see Section 1).*

2. Press: **51 ENTER**

The display will show **mg/L, NO3-N** and the **ZERO** icon.

Note: *For alternate forms (NO_3), press the* **CONC** *key.*

3. Fill a sample cell with 10 mL of sample.

Note: *Adjust the pH of stored samples before analysis.*

4. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to the sample cell (the prepared sample). Cap the sample cell.

Note: *It is important to remove all of the powder from the foil pillow. Tap the pillow until no more powder pours out.*

* Adopted from Hach manual (Hach, 2005).

5. Press:
TIMER ENTER

A one-minute reaction period will begin. Shake the sample cell vigorously until the timer beeps.

Note: *It is important to shake the cell vigorously. Shaking time and technique influence color development. For most accurate results, do successive tests on a standard solution and adjust the shaking time to obtain the correct result.*

6. After the timer beeps, the display will show:
5:00 TIMER 2

Press: **ENTER**

A five-minute reaction period will begin.

Note: *A deposit will remain after the reagent dissolves and will not affect test results.*

Note: *An amber color will develop if nitrate nitrogen is present.*

7. Fill another cell with 10 mL of sample (the blank). Wipe off any fingerprints or liquid.

8. Place the blank into the cell holder. Tightly cover the sample cell with the instrument cap.

9. When the timer beeps, press **ZERO.**

The cursor will move to the right, then the display will show:

0.0 mg/L NO3-N

Note: *If Reagent Blank Correction is on, the display may flash "limit". See Section 1.*

10. Place the prepared sample into the cell holder. Tightly cover the sample cell with the instrument cap.

11. Press: **READ**

The cursor will move to the right, then the result in mg/L NO_3-N (or alternate form) will be displayed.

Note: *Use of the Standard Adjust feature for each new lot of reagent is highly recommended. See* Accuracy Check.

Note: *Rinse the sample cell immediately after use to remove all cadmium particles. Save the spent sample for proper hazardous waste disposal for cadmium.*

* Adopted from Hach manual (Hach, 2005).

f) Nitrite concentration determination

Method 8507

NITRITE, Low Range (0 to 0.350 mg/L NO_2^--N) **For water, wastewater, seawater**

Diazotization Method* (Powder Pillows or AccuVac Ampuls);
USEPA approved for reporting wastewater and drinking water analyses.

1. Enter the stored program number for nitrite nitrogen (NO_2^--N), powder pillows.

Press: **PRGM**

The display will show:

PRGM ?

Note: *For most accurate results, perform a Reagent Blank Correction using deionized water (see*

2. Press: **60 ENTER**

The display will show **mg/L, NO2-N** and the **ZERO** icon.

Note: *For alternate forms (NO_2^-, $NaNO_2$), press the* **CONC** *key.*

3. Fill a sample cell with 10 mL of sample.

4. Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow to the sample cell. Cap the cell and shake to dissolve.

Note: *Accuracy is not affected by undissolved powder.*

5. Press:
TIMER ENTER

A 15-minute reaction period will begin.

Note: *A pink color will develop if nitrite is present.*

6. When the timer beeps, fill an empty sample cell with 10 mL of sample (the blank).

7. Wipe the outside of the sample cell with a towel. Place the blank into the cell holder. Tightly cover the sample cell with the instrument cap.

Note: *Wiping with a damp cloth, followed by a dry pne, removes fingerprints and other marks.*

8. Press: **ZERO**

The cursor will move to the right, then the display will show:

0.000 mg/L NO2-N

Note: *If Reagent Blank Correction is on, the display may flash "limit." See Section 1.*

9. Place the prepared sample into the cell holder. Tightly cover the sample cell with the instrument cap.

10. Press: **READ**

The cursor will move to the right, then the result in mg/L nitrite nitrogen (or an alternate form) will be displayed.

* Adopted from Hach manual (Hach, 2005).

Appendix B

Exp. 3: Life History Processed Data

Examples of data:

Fecundity (m_x)

A1 DHA Selco							
Diet:	A1 DHA Selco						
Conc.:	1.0 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	0	0	0	0	0	0.00	0.0000
2	0	0	0	0	0	0.00	0.0000
Total	0	0	0	0	0		

Diet:	A1 DHA Selco						
Conc.:	0.5 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	0	0	0	0	0	0.00	0.0000
2	0	0	0	0	0	0.00	0.0000
3	0	0	0	0	0	0.00	0.0000
Total	0	0	0	0	0		

Diet:	A1 DHA Selco						
Conc.:	0.25 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	0	0	0	0	0	0.00	0.0000
2	0	0	0	0	0	0.00	0.0000
3	0	0	0	0	0	0.00	0.0000
Total	0	0	0	0	0		

Diet:	A1 DHA Selco						
Conc.:	0.125 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	0	0	0	0	0	0.00	0.0000
2	0	0	0	0	0	0.00	0.0000
3	6	7	5	8	26	6.50	1.2910
4	0	0	0	0	0	0.00	0.0000
Total	6	7	5	8	26		

Diet:	A1 DHA Selco						
Conc.:	0.0625 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	0	0	0	0	0	0.00	0.0000
2	2	4	4	6	16	4.00	1.6330
3	8	9	6	7	30	7.50	1.2910
4	0	0	0	0	0	0.00	0.0000
5	0	0	0	0	0	0.00	0.0000
6	0	0	0	0	0	0.00	0.0000
7	0	0	0	0	0	0.00	0.0000
8	0	0	0	0	0	0.00	0.0000
9	0	0	0	0	0	0.00	0.0000
10	0	0	0	0	0	0.00	0.0000
Total	10	13	10	13	46		

Survivorship (I_x)

A1 DHA Selco

Diet:	A1 DHA Selco						
Conc.:	1.0 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	1	1	1	1	4	1.00	0.0000
2	0	0	0	0	0	0.00	0.0000
Total	1	1	1	1	4		

Diet:	A1 DHA Selco						
Conc.:	0.5 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	1	1	1	1	4	1.00	0.0000
2	1	1	1	1	4	1.00	0.0000
3	0	0	0	0	0	0.00	0.0000
Total	2	2	2	2	8		

Diet:	A1 DHA Selco						
Conc.:	0.25 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	1	1	1	1	4	1.00	0.0000
2	1	0	1	1	3	0.75	0.5000
3	0	0	0	0	0	0.00	0.0000
Total	2	1	2	2	7		

Diet:	A1 DHA Selco						
Conc.:	0.125 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	1	1	1	1	4	1.00	0.0000
2	1	1	1	1	4	1.00	0.0000
3	0	0	1	1	2	0.50	0.5774
4	0	0	0	0	0	0.00	0.0000
Total	2	2	3	3	10		

Diet:	A1 DHA Selco						
Conc.:	0.0625 g L^{-1}						
Day	R1	R2	R3	R4	SUM	Mean	SD
1	1	1	1	1	4	1.00	0.0000
2	1	1	1	1	4	1.00	0.0000
3	1	1	1	1	4	1.00	0.0000
4	1	1	0	0	2	0.50	0.5774
5	1	1	0	0	2	0.50	0.5774
6	1	1	0	0	2	0.50	0.5774
7	1	1	0	0	2	0.50	0.5774
8	1	1	0	0	2	0.50	0.5774
9	1	1	0	0	2	0.50	0.5774
10	0	0	0	0	0	0.00	0.0000
Total	9	9	3	3	24		

Net reproduction (R_0)

A1 DHA Selco

Diet:	A1 DHA Selco							
Conc.:	1.0 g L^{-1}							
Day(x)	R1	R2	R3	R4	n_x	l_x	m_x	l_xm_x
1	1	1	1	1	4	1.00	0.00	0.00
2	0	0	0	0	0	0.00	0.00	0.00
Total	1	1	1	1	4			

net reproduction (R_0), $\sum l_xm_x$ = 0.00

Diet:	A1 DHA Selco							
Conc.:	0.5 g L^{-1}							
Day(x)	R1	R2	R3	R4	n_x	l_x	m_x	l_xm_x
1	1	1	1	1	4	1.00	0.00	0.00
2	1	1	1	1	4	1.00	0.00	0.00
3	0	0	0	0	0	0.00	0.00	0.00
Total	2	2	2	2	8			

net reproduction (R_0), $\sum l_xm_x$ = 0.00

Diet:	A1 DHA Selco							
Conc.:	0.25 g L^{-1}							
Day(x)	R1	R2	R3	R4	n_x	l_x	m_x	l_xm_x
1	1	1	1	1	4	1.00	0.00	0.00
2	1	0	1	1	3	0.75	0.00	0.00
3	0	0	0	0	0	0.00	0.00	0.00
Total	2	1	2	2	7			

net reproduction (R_0), $\sum l_xm_x$ = 0.00

Diet:	A1 DHA Selco							
Conc.:	0.125 g L^{-1}							
Day(x)	R1	R2	R3	R4	n_x	l_x	m_x	l_xm_x
1	1	1	1	1	4	1.00	0.00	0.00
2	1	1	1	1	4	1.00	0.00	0.00
3	0	0	1	1	2	0.50	6.50	3.25
4	0	0	0	0	0	0.00	0.00	0.00
Total	2	2	3	3	10			

net reproduction (R_0), $\sum l_xm_x$ = 3.25

Diet:	A1 DHA Selco							
Conc.:	0.0625 g L^{-1}							
Day(x)	R1	R2	R3	R4	n_x	l_x	m_x	l_xm_x
1	1	1	1	1	4	1.00	0.00	0.00
2	1	1	1	1	4	1.00	4.00	4.00
3	1	1	1	1	4	1.00	7.50	7.50
4	1	1	0	0	2	0.50	0.00	0.00
5	1	1	0	0	2	0.50	0.00	0.00
6	1	1	0	0	2	0.50	0.00	0.00
7	1	1	0	0	2	0.50	0.00	0.00
8	1	1	0	0	2	0.50	0.00	0.00
9	1	1	0	0	2	0.50	0.00	0.00
10	0	0	0	0	0	0.00	0.00	0.00
Total	9	9	3	3	24			

net reproduction (R_0), $\sum l_xm_x$ = 11.50

Generation time (*T*)

A1 DHA Selco					
Diet:	A1 DHA Selco				
Conc.:	1.0 g L^{-1}				
Day(x)	n_x	l_x	m_x	l_xm_x	xl_xm_x
1	4	1.00	0.00	0.00	0.00
2	0	0.00	0.00	0.00	0.00

Ro =	0.00				
$\sum x l_x m_x$ =	0.00		T = $\sum x l_x m_x / Ro$ =		NV

Diet:	A1 DHA Selco				
Conc.:	0.5 g L^{-1}				
Day(x)	n_x	l_x	m_x	$l_x m_x$	$x l_x m_x$
1	4	1.00	0.00	0.00	0.00
2	4	1.00	0.00	0.00	0.00
3	0	0.00	0.00	0.00	0.00

Ro =	0.00				
$\sum x l_x m_x$ =	0.00		T = $\sum x l_x m_x / Ro$ =		NV

Diet:	A1 DHA Selco				
Conc.:	0.25 g L^{-1}				
Day(x)	n_x	l_x	m_x	$l_x m_x$	$x l_x m_x$
1	4	1.00	0.00	0.00	0.00
2	3	0.75	0.00	0.00	0.00
3	0	0.00	0.00	0.00	0.00

Ro =	0.00				
$\sum x l_x m_x$ =	0.00		T = $\sum x l_x m_x / Ro$ =		NV

Diet:	A1 DHA Selco				
Conc.:	0.125 g L^{-1}				
Day(x)	n_x	l_x	m_x	$l_x m_x$	$x l_x m_x$
1	4	1.00	0.00	0.00	0.00
2	4	1.00	0.00	0.00	0.00
3	2	0.50	6.50	3.25	9.75
4	0	0.00	0.00	0.00	0.00

Ro =	3.25				
$\sum x l_x m_x$ =	9.75		T = $\sum x l_x m_x / Ro$ =		3.00

Diet:	A1 DHA Selco				
Conc.:	0.0625 g L^{-1}				
Day(x)	n_x	l_x	m_x	$l_x m_x$	$x l_x m_x$
1	4	1.00	0.00	0.00	0.00
2	4	1.00	4.00	4.00	8.00
3	4	1.00	7.50	7.50	22.50
4	2	0.50	0.00	0.00	0.00
5	2	0.50	0.00	0.00	0.00
6	2	0.50	0.00	0.00	0.00
7	2	0.50	0.00	0.00	0.00
8	2	0.50	0.00	0.00	0.00
9	2	0.50	0.00	0.00	0.00
10	0	0.00	0.00	0.00	0.00

Ro =	11.50				
$\sum x l_x m_x$ =	30.50		T = $\sum x l_x m_x / Ro$ =		2.65

Intrinsic rate of natural increase (*r*)

A1 DHA Selco	
Diet:	A1 DHA Selco
Conc.:	1.0 g L^{-1}
net reproduction (Ro), $\sum l_x m_x$ =	0.00
Generation time *(T)*, $\sum x l_x m_x / Ro$ =	NV
Per capita rate of increase (*r*), ln R_0 / T =	NV
Diet:	A1 DHA Selco
Conc.:	0.5 g L^{-1}
net reproduction (Ro), $\sum l_x m_x$ =	0.00
Generation time *(T)*, $\sum x l_x m_x / Ro$ =	NV
Per capita rate of increase (*r*), ln R_0 / T =	NV
Diet:	A1 DHA Selco
Conc.:	0.25 g L^{-1}
net reproduction (Ro), $\sum l_x m_x$ =	0.00
Generation time *(T)*, $\sum x l_x m_x / Ro$ =	NV
Per capita rate of increase (*r*), ln R_0 / T =	NV
Diet:	A1 DHA Selco
Conc.:	0.125 g L^{-1}
net reproduction (Ro), $\sum l_x m_x$ =	3.25
Generation time *(T)*, $\sum x l_x m_x / Ro$ =	3.00
Per capita rate of increase (*r*), ln R_0 / T =	0.39
Diet:	A1 DHA Selco
Conc.:	0.0625 g L^{-1}
net reproduction (Ro), $\sum l_x m_x$ =	11.50
Generation time *(T)*, $\sum x l_x m_x / Ro$ =	2.65
Per capita rate of increase (*r*), ln R_0 / T =	0.92

Life expectancy (e_x)

A1 DHA Selco								
Diet:	A1 DHA Selco							
Conc.:	1.0 g L^{-1}							
Day(x)	n_x	l_x	d_x	q_x	L_x	T_x	e_x	SD
1	4	1.00	1.00	1.00	0.500	0.500	0.50	0.0000
2	0	0.00	0.00	0.00	0.000	0.000	0.00	0.0000

Diet:	A1 DHA Selco							
Conc.:	0.5 g L^{-1}							
Day(x)	n_x	l_x	d_x	q_x	L_x	T_x	e_x	SD
1	4	1.00	0.00	0.00	1.000	1.500	1.50	0.0000
2	4	1.00	1.00	1.00	0.500	0.500	0.50	0.0000
3	0	0.00	0.00	0.00	0.000	0.000	0.00	0.0000

Diet:	A1 DHA Selco							
Conc.:	0.25 g L^{-1}							
Day(x)	n_x	l_x	d_x	q_x	L_x	T_x	e_x	SD
1	4	1.00	0.25	0.25	0.875	1.250	1.25	0.0000
2	3	0.75	0.75	1.00	0.375	0.375	0.50	0.5000
3	0	0.00	0.00	0.00	0.000	0.000	0.00	0.0000

Diet:	A1 DHA Selco							
Conc.:	0.125 g L^{-1}							
Day(x)	n_x	l_x	d_x	q_x	L_x	T_x	e_x	SD
1	4	1.00	0.00	0.00	1.000	2.000	2.00	0.0000
2	4	1.00	0.50	0.50	0.750	1.000	1.00	0.0000
3	2	0.50	0.50	1.00	0.250	0.250	0.50	0.5774
4	0	0.00	0.00	0.00	0.000	0.000	0.00	0.0000

Diet:	A1 DHA Selco							
Conc.:	0.0625 g L^{-1}							
Day(x)	n_x	l_x	d_x	q_x	L_x	T_x	e_x	SD
1	4	1.00	0.00	0.00	1.000	5.500	5.50	0.0000
2	4	1.00	0.00	0.00	1.000	4.500	4.50	0.0000
3	4	1.00	0.50	0.50	0.750	3.500	3.50	0.0000
4	2	0.50	0.00	0.00	0.500	2.750	5.50	0.5774
5	2	0.50	0.00	0.00	0.500	2.250	4.50	0.5774
6	2	0.50	0.00	0.00	0.500	1.750	3.50	0.5774
7	2	0.50	0.00	0.00	0.500	1.250	2.50	0.5774
8	2	0.50	0.00	0.00	0.500	0.750	1.50	0.5774
9	2	0.50	0.50	1.00	0.250	0.250	0.50	0.5774
10	0	0.00	0.00	0.00	0.000	0.000	0.00	0.0000

Cumulative birth

		A1 DHA Selco			

Diet:	A1 DHA Selco				
Conc.:	1.0 g L-1				
Day	R1	R2	R3	R4	Cum.
1	0	0	0	0	0
2	0	0	0	0	0
Total	0	0	0	0	0

Diet:	A1 DHA Selco				
Conc.:	0.5 g L-1				
Day	R1	R2	R3	R4	Cum.
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
Total	0	0	0	0	0

Diet:	A1 DHA Selco				
Conc.:	0.25 g L-1				
Day	R1	R2	R3	R4	Cum.
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
Total	0	0	0	0	0

Diet:	A1 DHA Selco				
Conc.:	0.125 g L-1				
Day	R1	R2	R3	R4	Cum.
1	0	0	0	0	0
2	0	0	0	0	0
3	6	7	5	8	26
4	0	0	0	0	26
Total	6	7	5	8	52

Diet:	A1 DHA Selco				
Conc.:	0.0625 g L-1				
Day	R1	R2	R3	R4	Cum.
1	0	0	0	0	0
2	2	4	4	6	16
3	8	9	6	7	46
4	0	0	0	0	46
5	0	0	0	0	46
6	0	0	0	0	46
7	0	0	0	0	46
8	0	0	0	0	46
9	0	0	0	0	46
10	0	0	0	0	46
Total	10	13	10	13	384

Appendix C

2 way ANOVA Followed by *Post Hoc* Comparison (Tukey Test)

a)Average Longevity

Tukey Simultaneous Tests
Response Variable Average
All Pairwise Comparisons among Levels of Conc.

Conc. = 0.0000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.0625	0.950	0.6538	1.453	0.6947
0.1250	-0.300	0.6538	-0.459	0.9974
0.2500	-0.350	0.6538	-0.535	0.9946
0.5000	-0.100	0.6538	-0.153	1.0000
1.0000	-2.750	0.6538	-4.206	**0.0009**

Conc. = 0.0625 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.1250	-1.250	0.6538	-1.912	0.4018
0.2500	-1.300	0.6538	-1.988	0.3572
0.5000	-1.050	0.6538	-1.606	0.5969
1.0000	-3.700	0.6538	-5.659	**0.0000**

Conc. = 0.1250 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.2500	-0.050	0.6538	-0.076	1.0000
0.5000	0.200	0.6538	0.306	0.9996
1.0000	-2.450	0.6538	-3.747	**0.0042**

Conc. = 0.2500 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.5000	0.250	0.6538	0.382	0.9989
1.0000	-2.400	0.6538	-3.671	**0.0053**

Conc. = 0.5000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1.0000	-2.650	0.6538	-4.053	**0.0015**

Tukey Simultaneous Tests
Response Variable Average
All Pairwise Comparisons among Levels of Diet*Conc.

Remark (diet): 1=ADS; 2=SO; 3=CO; 4=FP; 5=FF

Diet = 1
Conc. = 0.0625 subtracted from:

Level Diet*Conc.		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.1250	-3.500	1.462	-2.394	0.8337
1	0.2500	-4.250	1.462	-2.907	0.4717
1	0.5000	-4.000	1.462	-2.736	0.6010
1	1.0000	-5.000	1.462	-3.420	0.1694
2	0.0000	-1.500	1.462	-1.026	1.0000
2	0.0625	-2.250	1.462	-1.539	0.9622
2	0.2500	-3.250	1.462	-2.223	0.9130
2	0.5000	-3.750	1.462	-2.565	0.7264
2	1.0000	-5.000	1.462	-3.420	0.1694
3	0.0000	-1.500	1.462	-1.026	1.0000
3	0.0625	-1.000	1.462	-0.684	1.0000
3	0.1250	-1.250	1.462	-0.855	1.0000
3	0.2500	-2.250	1.462	-1.539	0.9994
3	0.5000	-3.000	1.462	-2.052	0.9622
3	1.0000	-4.500	1.462	-3.078	0.3515
4	0.0000	-1.500	1.462	-1.026	1.0000
4	0.0625	-1.250	1.462	-0.855	1.0000
4	0.1250	-1.000	1.462	-0.684	1.0000
4	0.2500	-1.250	1.462	-0.855	1.0000
4	0.5000	-2.500	1.462	-1.710	0.9966
4	1.0000	-3.250	1.462	-2.223	0.9130
5	0.0000	-1.500	1.462	-1.026	1.0000
5	0.0625	1.750	1.462	1.197	1.0000
5	0.1250	-0.250	1.462	-0.171	1.0000
5	0.2500	1.750	1.462	1.197	1.0000
5	0.5000	5.250	1.462	3.591	0.1103
5	1.0000	-3.500	1.462	-2.394	0.8337

b)Gross reproduction rate

Tukey Simultaneous Tests
Response Variable Gross re
All Pairwise Comparisons among Levels of Conc.

Conc. = 0.0000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.0625	11.150	0.9580	11.639	**0.0000**
0.1250	8.450	0.9580	8.820	**0.0000**
0.2500	-0.750	0.9580	-0.783	0.9698
0.5000	-0.400	0.9580	-0.418	0.9983
1.0000	-5.650	0.9580	-5.898	**0.0000**

Conc. = 0.0625 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.1250	-2.70	0.9580	-2.82	0.0636
0.2500	-11.90	0.9580	-12.42	**0.0000**
0.5000	-11.55	0.9580	-12.06	**0.0000**
1.0000	-16.80	0.9580	-17.54	**0.0000**

Conc. = 0.1250 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.2500	-9.20	0.9580	9.60	**0.0000**
0.5000	-8.85	0.9580	9.24	**0.0000**
1.0000	-14.10	0.9580	14.72	**0.0000**

Conc. = 0.2500 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.5000	0.350	0.9580	0.365	0.9991
1.0000	-4.900	0.9580	-5.115	**0.0000**

Conc. = 0.5000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1.0000	-5.250	0.9580	-5.480	**0.0000**

Tukey Simultaneous Tests
Response Variable Gross re
All Pairwise Comparisons among Levels of Diet*Conc.

Remark (diet): 1=ADS; 2=SO; 3=CO; 4=FP; 5=FF

Diet = 1
Conc. = 0.0625 subtracted from:

Level Diet*Conc.		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.1250	-5.00	2.142	-2.334	0.8650
1	0.2500	-11.50	2.142	-5.368	**0.0003**
1	0.5000	-11.50	2.142	-5.368	**0.0003**
1	1.0000	-11.50	2.142	-5.368	**0.0003**
2	0.0000	-4.25	2.142	-1.984	0.9745
2	0.0625	0.25	2.142	0.117	1.0000
2	0.1250	-1.25	2.142	-0.584	1.0000
2	0.2500	-9.50	2.142	4.435	**0.0082**
2	0.5000	-11.50	2.142	-5.368	**0.0003**
2	1.0000	-11.50	2.142	-5.368	**0.0003**
3	0.0000	-4.25	2.142	1.984	0.9745
3	0.0625	10.25	2.142	4.785	**0.0024**
3	0.1250	3.75	2.142	1.751	0.9951
3	0.2500	-9.50	2.142	4.435	**0.0082**
3	0.5000	-11.00	2.142	-5.135	**0.0007**
3	1.0000	-11.50	2.142	-5.368	**0.0003**
4	0.0000	-4.25	2.142	-1.984	0.9745
4	0.0625	-8.25	2.142	-3.851	0.0535

4	0.1250	8.50	2.142	3.968	**0.0377**
4	0.2500	5.25	2.142	2.451	0.8008
4	0.5000	9.25	2.142	4.318	**0.0122**
4	1.0000	-6.25	2.142	2.918	0.4638
5	0.0000	-4.25	2.142	-1.984	0.9745
5	0.0625	32.25	2.142	15.055	**0.0001**
5	0.1250	15.00	2.142	7.002	**0.0001**
5	0.2500	0.25	2.142	0.117	1.0000
5	0.5000	1.50	2.142	0.700	1.0000
5	1.0000	-8.75	2.142	-4.085	**0.0262**

c)Initial age of reproduction

Tukey Simultaneous Tests
Response Variable Initial
All Pairwise Comparisons among Levels of Conc.

Conc. = 0.0000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.0625	-0.600	0.2173	-2.761	0.0734
0.1250	-0.600	0.2173	-2.761	0.0734
0.2500	-1.350	0.2173	-6.212	**0.0000**
0.5000	-1.550	0.2173	-7.133	**0.0000**
1.0000	-1.500	0.2173	-6.903	**0.0000**

Conc. = 0.0625 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.1250	0.0000	0.2173	0.000	1.0000
0.2500	-0.7500	0.2173	-3.451	**0.0107**
0.5000	-0.9500	0.2173	-4.372	**0.0005**
1.0000	-0.9000	0.2173	-4.142	**0.0011**

Conc. = 0.1250 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.2500	-0.7500	0.2173	-3.451	**0.0107**
0.5000	-0.9500	0.2173	-4.372	**0.0005**
1.0000	-0.9000	0.2173	-4.142	**0.0011**

Conc. = 0.2500 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0.5000	-0.2000	0.2173	-0.9204	0.9403
1.0000	-0.1500	0.2173	-0.6903	0.9826

Conc. = 0.5000 subtracted from:

Level Conc.	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1.0000	0.05000	0.2173	0.2301	0.9999

Tukey Simultaneous Tests
Response Variable Initial
All Pairwise Comparisons among Levels of Diet*Conc.

Remark (diet): 1=ADS; 2=SO; 3=CO; 4=FP; 5=FF

Diet = 1
Conc. = 0.0625 subtracted from:

Level Diet	*Conc.	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
1	0.1250	0.000	0.4859	0.000	1.0000
1	0.2500	-2.000	0.4859	-4.116	**0.0237**
1	0.5000	-2.000	0.4859	-4.116	**0.0237**
1	1.0000	1.000	0.4859	2.058	0.9609
2	0.0000	1.000	0.4859	2.058	0.9609
2	0.0625	-0.000	0.4859	-0.000	1.0000
2	0.1250	0.000	0.4859	0.000	1.0000
2	0.2500	-1.000	0.4859	-2.058	0.9609
2	0.5000	-2.000	0.4859	-4.116	**0.0237**
2	1.0000	-2.000	0.4859	-4.116	**0.0237**
3	0.0000	1.000	0.4859	2.058	0.9609
3	0.0625	0.000	0.4859	0.000	1.0000
3	0.1250	1.000	0.4859	2.058	0.9609
3	0.2500	0.250	0.4859	0.514	1.0000
3	0.5000	-0.000	0.4859	-0.000	1.0000
3	1.0000	-2.000	0.4859	-4.116	**0.0237**
4	0.0000	1.000	0.4859	2.058	0.9609
4	0.0625	1.000	0.4859	2.058	0.9609
4	0.1250	0.000	0.4859	0.000	1.0000
4	0.2500	0.000	0.4859	0.000	1.0000
4	0.5000	0.250	0.4859	0.514	1.0000
4	1.0000	1.000	0.4859	2.058	0.9609
5	0.0000	1.000	0.4859	2.058	0.9609
5	0.0625	1.000	0.4859	2.058	0.9609
5	0.1250	1.000	0.4859	2.058	0.9609
5	0.2500	1.000	0.4859	2.058	0.9609
5	0.5000	1.000	0.4859	2.058	0.9609
5	1.0000	-0.500	0.4859	-1.029	1.0000

Appendix D

Exp 2: Lipid Extraction Protocols

Determination of Saturated Fat

Standard Preparation	Sample Preparation	Internal Quality Control
↓	(Solid sample blend into liquid sample)	
	↓	1.Standard Check
Standard stock solution (FAME standard) for standard	Weigh ~1g samples (liquid) into beaker (250ml)	Direct Inject 1µl of FAME
37 components of fatty acid purchased from Approved Vendor. criteria:±5%	↓ + 10ml H_2O + 10ml conc. HCl Close with wash glass	2.Duplicate analysis acceptance

↓

Put on the water bath
95°C for 1 hour

↓ cool

Pour into separator funnel

↓ + 50ml Pet.Ether / Pet.Benzene
40°C-60°C (2x)
+ 50ml absolute ethanol (2x)

upper layer collect into bottom flask
bottom layer pour back into separator funnel

↓ + 30ml Pet. Ether
+ 30ml absolute ethanol

After filter, rinse filter paper with absolute ethanol & pet. Ether

↓

Evaporate all solvent by distillation at water bath 95°C

↓

Heat it at oven 105°C for 1 hour

↓

Weigh final reading for bottom flask

↓

Saponification

↓

Add methanolic NaOH (refer table) solution and boiling chip

↓

Attach condenser and reflux until fat globules disappear
(usually 5-10 minutes)

↓

Add BF3 solution & continue boiling for 2 minutes

↓

Add 2-5ml heptane & boil 1 minute longer

↓

Remove heat then add several ml (~5 ml) saturated NaCl solution

↓

Perform the GC analysis

Figure a. Lipid extraction protocols

GC condition

Column : (Length) 100m x (diameter) 0.25mm x (layer thickness) 0.2µm,
Temperature limits : 50-250 deg C
(Agilent J&W Scientific HP 88)

Column carrier gas & flow rate :29.6 psi Helium

Injector :70^{o}C

Detector :FID at 240^{o}C

Oven temperature:70^{o}C at 0 min hold (Initial Temp), ramp at 5^{o}C/min to 240^{o}C
for 18.00 minutes.

Max. temp. in oven : 300 deg C.

Holding time : 999.0 minutes

Injection :Manual, 1.0µl, normal injection speed

Attenuation :4

Gases :H_2, 45.0ml/min ; Air, 450.0ml/min

Range :1

Integration and software model : TotalChrom Navigator – Autosystem XL.

EQUIPMENT

i) GC-MS (Autosystem XL Gas Chromatography) Perkin Elmer
ii) Analytical Balance, readable at 0.1mg
iii) Water bath
iv) Heating mantle
v) Filter paper (Whatman 42 Ashless circle 150mm)

APPARATUS

i) Round bottom flask 250ml
ii) Biker 250ml
iii) Wash glass
iv) Cylinder 100ml
v) Separator funnel 250 ml
vi) Condenser
vii) Syringe,10µl & 100µl

REAGENT & MATERIALS

i) Petroleum Ether, 40°C-60°C
ii) Petroleum Benzene, 40°C-60°C
iii) Absolute Ethanol
iv) Methanolic NaOH
v) Boron Trifluoride Methanol
vi) Heptane
vii) Saturated NaCl
viii) Lipid Internal standard, FAME Reference Std, Accustandard, 3ul.

Table a. Volume of Methanolic NaOH solution and Boron Trifluoride-Methanol Complex added in FAME preparation

Sample,mg	0.5N NaOH, ml	BF_3 Reagent, ml
100-250	4	5
250-500	6	7
500-750	8	9
750-1000	10	12

Appendix E

Fatty Acid Methyl Ester (FAME) Preparation and Analysis

I) FAME preparation

Approximately 1.5 g of *Moina* was weighed for each sample. Samples were homogenized into semi-solid and liquid form. Samples were mixed with 10 ml distilled water and 10 ml concentrated hydrochloric acid. The mixture was heated in a water bath at 95 ^{o}C for 1 hour. After heating, the sample mixture was brought down to room temperature and poured into a pre-weighed separator funnel. The sample mixture was added with 50 ml petroleum ether (40-60 ^{o}C) and 50 ml absolute ethanol. Sample mixture was shaken vigorously in the separator funnel for a few minutes to mix evenly. The lipid-water phase was held for a few minutes to allow separation. The upper layer (lipid content) was collected in a round bottom flask. The separation steps were repeated for a second time as described above, only 30 ml petroleum ether (40-60 ^{o}C) and 30 ml absolute ethanol were used to ensure the lipid residue was completely eluted from the solvent mixture at the final stage. The lipid content was filtered through the filter paper (Whatman 42 Ashless circle 1150 mm) and collected in a round bottom flask. The filter paper was rinsed with several drops (≈5 ml) of petroleum ether and absolute ethanol in order to remove any residual lipids from the filter paper. At the process of distillation, lipids were evaporated in the water bath at 95 ^{o}C until only a trace amount of solvent remained in the flask. The whole flask containing lipids was dried in the oven at 105 ^{o}C for 1 hour. Final weight of the dried flask was recorded to calculate total lipid contains based on the formula as below:

$$[1] \quad \text{Total Fat, \%} = \frac{(\text{final flask weight} - \text{initial flask weight}) \times 100}{\text{Sample weight}}$$

$$[2] \quad \text{Fatty Acid Composition, \%} = \frac{\text{Gas chromatograph reading} \times \text{Total Fat \%}}{100}$$

In the same flask, 4 ml methanolic NaOH (0.5 N) was added to saponify the lipids. The solution was refluxed in the soxhlet apparatus until the fat globules disappeared, with the process taking 5-10 minutes depending on the solution volume. Afterwards, 5 ml of Boron Trifluoride Methanol (BF_3) was added to the flask and boiled for 2 minutes, followed by additional of 2 ml heptane and boiled for another (1) minute. The flask was then cooled to room temperature. In the final step, 3 ml of saturated NaCl was added to the solution. The solution was kept at 4^{o}C for GC qualitative and quantitative analysis.

II) FAME Analysis

Qualitative and quantitative determination was carried out by using Autosystem XL gas chromatography/GC (PerkinElmer INC., USA). GC

analysis was performed by injected solution (1 μL) into GC polar capillary column (Agilent J&W Scientific HP 88) with 100 m length, a diameter of 0.25 mm and a layer thickness of 0.2 μm. Gas helium as the carrier gas with flow rate of 29.6 psi, Flame Ionized Detector (FID) as detector mode with the temperature set at 240^{o}C, and injector temperature at 70^{o}C. FAME were separated at a constant pressure with a split ration equal to 4 at the following oven condition: a) Initial temperature was raised up to 70^{o}C; b) 70^{o}C to 240^{o}C was increased at a rate of 5^{o}C per min; c) 240^{o}C was increased to 300^{o}C at the same rate and the holding time was 999 minutes. Identification of fatty acids was based on lipid internal standard (FAME reference standard, Accustandard, 3μL). Integration and calculations were performed by a computer software program (TotalChrom Navigator – Autosystem XL).

a) Fatty acids profile of control *Moina macrocopa*

Fatty acid	12 h						24 h						36 h					
	R1(%)	R2(%)	R3(%)	R4(%)	Ave(mg/l)	SD	R1(%)	R2(%)	R3(%)	R4(%)	Ave(mg/l)	SD	R1(%)	R2(%)	R3(%)	R4(%)	Ave(mg/l)	SD
12:0	0.0007	0.0007	0.0000	0.0000	0.00	0.00	0.0000	0.0000	0.0002	0.0002	0.00	0.00	0.0000	0.0000	0.0004	0.0004	0.00	0.00
14:0	0.0021	0.0020	0.0012	0.0010	0.02	0.00	0.0000	0.0000	0.0007	0.0007	0.00	0.00	0.0000	0.0000	0.0006	0.0006	0.00	0.00
14:1	0.0012	0.0011	0.0008	0.0009	0.01	0.00	0.0000	0.0000	0.0001	0.0001	0.00	0.00	0.0000	0.0000	0.0005	0.0004	0.00	0.00
15:0	0.0126	0.0132	0.0071	0.0074	0.10	0.00	0.0159	0.0167	0.0001	0.0001	0.08	0.01	0.0083	0.0085	0.0044	0.0046	0.06	0.00
16:0	0.0020	0.0020	0.0051	0.0060	0.04	0.00	0.0034	0.0000	0.0061	0.0063	0.04	0.00	0.0030	0.0033	0.0051	0.0052	0.04	0.00
17:0	0.0005	0.0006	0.0767	0.0820	0.40	0.05	0.1271	0.1329	0.0008	0.0007	0.65	0.07	0.2149	0.2154	0.0714	0.0747	1.44	0.08
18:0	0.0531	0.0537	0.0095	0.0093	0.31	0.03	0.0765	0.0765	0.0112	0.0107	0.44	0.04	0.0522	0.0506	0.0085	0.0081	0.30	0.02
18:1*n*-9	0.0124	0.0124	0.0017	0.0012	0.07	0.01	0.0000	0.0000	0.0510	0.0492	0.25	0.03	0.0000	0.0000	0.0003	0.0003	0.00	0.00
18:2*n*-6	0.0402	0.0398	0.0066	0.0065	0.23	0.02	0.0000	0.0000	0.1613	0.0162	0.44	0.08	0.0103	0.0106	0.0187	0.0184	0.15	0.00
20:0	0.0046	0.0045	0.0009	0.0009	0.03	0.00	0.0045	0.0052	0.0829	0.0800	0.43	0.04	0.0030	0.0055	0.0003	0.0003	0.02	0.00
18:3*cis*-6	0.0405	0.0404	0.0549	0.0524	0.47	0.01	0.0256	0.0253	0.0099	0.0091	0.17	0.01	0.0222	0.0208	0.0013	0.0012	0.11	0.01
18:3*n*-3	0.0244	0.0241	0.0039	0.0037	0.14	0.01	0.0000	0.0000	0.0003	0.0001	0.00	0.00	0.0000	0.0000	0.0003	0.0004	0.00	0.00
22:0	0.0008	0.0008	0.0006	0.0006	0.01	0.00	0.0000	0.0000	0.0007	0.0006	0.00	0.00	0.0000	0.0000	0.0010	0.0010	0.01	0.00
20:3*n*-6	0.0004	0.0004	0.0007	0.0003	0.00	0.00	0.0000	0.0000	0.0006	0.0006	0.00	0.00	0.0000	0.0000	0.0027	0.0026	0.01	0.00
20:3*n*-3	0.0077	0.0079	0.0011	0.0012	0.04	0.00	0.0000	0.0000	0.0016	0.0025	0.01	0.00	0.0000	0.0000	0.0000	0.0000	0.00	0.00
20:4*n*-6	0.0003	0.0002	0.0000	0.0000	0.00	0.00	0.0000	0.0000	0.0014	0.0013	0.01	0.00	0.0000	0.0000	0.0000	0.0000	0.00	0.00
20:5*n*-3	0.0031	0.0030	0.0278	0.0277	0.15	0.01	0.0156	0.0168	0.0013	0.0026	0.09	0.01	0.0159	0.0164	0.0027	0.0030	0.10	0.01
22:6*n*-3	0.0004	0.0004	0.0000	0.0000	0.00	0.00	0.0000	0.0000	0.0006	0.0005	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.00	0.00
∑ fat																		
∑ SAFA	23.3023	23.7198	31.583	32.5612	277.92	4.96	54.163	54.6479	18.999	19.5588	368.42	20.28	64.554	64.246	52.342	54.037	587.95	6.51
∑ MUFA	38.8271	38.6120	23.031	22.9741	308.61	9.07	33.091	32.2588	41.482	40.9330	369.41	4.94	19.092	18.850	27.372	26.062	228.44	4.51
∑ PUFA	37.8706	37.6681	45.387	44.4646	413.47	4.15	12.746	13.0934	39.520	39.5083	262.17	15.35	16.355	16.905	20.287	19.901	183.62	2.02
∑ n-3 PUFA	10.3584	10.3043	7.7133	7.6785	90.14	1.52	2.0245	2.1751	0.4174	0.6115	13.07	0.92	2.4256	2.5112	1.6902	1.8889	21.29	0.40
∑ n-6 PUFA	0.1787	0.1895	0.1645	0.0731	1.51	0.05	0.0000	0.0000	0.2114	0.2040	1.04	0.12	0.0000	0.0000	1.4799	1.4672	7.37	0.85
AA/EPA	0.0968	0.0667	0.0000	0.0000	0.41	0.05	0.0000	0.0000	1.0769	0.5000	3.94	0.51	0.0000	0.0000	0.0000	0.0000	0.00	0.00
AA/DHA	0.7500	0.5000	NV	NV	3.13	0.18	NV	NV	2.3333	2.6000	12.33	0.19	NV	NV	NV	NV	0.00	0.00
DHA/EPA	0.1290	0.1333	0.0000	0.0000	0.66	0.08	0.0000	0.0000	0.4615	0.1923	1.63	0.22	0.0000	0.0000	0.0000	0.0000	0.00	0.00

Appendix G

2 Way ANOVA followed by *Post Hoc* Comparison (Tukey Test)

a) Fatty acids comparison of the diets at various concentrations
Test sample: A1 DHA selco® (conc. vs. hour)
0: control; 1: 1g l^{-1}; 2: 2 g l^{-1}

Factor	Type	Levels	Values
Conc.	fixed	3	0 1 2
Hour	fixed	3	12 24 36

Analysis of Variance for C12:0, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Conc.	2	0.0000028	0.0000028	0.0000014	1.17	0.326
Hour	2	0.0000107	0.0000107	0.0000053	4.44	0.021
Conc. *Hour	4	0.0000091	0.0000091	0.0000023	1.89	0.141
Error	27	0.0000324	0.0000324	0.0000012		
Total	35	0.0000549				

Example of fatty acud-C12: 0

Tukey Simultaneous Tests
Response Variable C12:0
All Pairwise Comparisons among Levels of Conc.*Hour

Conc. = 0
Hour = 12 subtracted from:

Level Conc.*Hour		Difference of Means	SE of Difference	Adjusted T-Value	P-Value
0	24	-0.000250	0.000774	-0.3228	1.0000
0	36	-0.000150	0.000774	-0.1937	1.0000
1	12	-0.000350	0.000774	-0.4520	0.9999
1	24	0.002150	0.000774	2.7763	0.1690
1	36	-0.000175	0.000774	-0.2260	1.0000
2	12	-0.000250	0.000774	-0.3228	1.0000
2	24	0.000925	0.000774	1.1944	0.9509
2	6	-0.000350	0.000774	-0.4520	0.9999

Example of AA/DHA ratio

Tukey Simultaneous Tests
Response Variable AA/DHA
All Pairwise Comparisons among Levels of Conc.*Hour

Conc. = 0
Hour = 12 subtracted from:

Level Conc.*Hour		Difference of Means	SE of Difference	Adjusted T-Value	P-Value
0	24	0.9208	5.452	0.16890	1.0000
0	36	-0.3125	5.452	-0.05732	1.0000
1	12	0.1149	5.452	0.02107	1.0000
1	24	-0.3125	5.452	-0.05732	1.0000
1	36	-0.3125	5.452	-0.05732	1.0000
2	12	0.7714	5.452	0.14149	1.0000
2	24	-0.0491	5.452	-0.00900	1.0000
2	36	26.7947	5.452	4.91481	0.0011

Appendix H

Population Density Study Processed Data

a) Results of *Moina macrocopa* at different population densities

4 Ind./ 40 mL

Day(s)	Control							
	1	**2**	**3**	**4**	**5**	**6**	**Ave.**	**SD**
0	4	4	4	4	4	4	4	0
1	4	3	4	11	8	11	7	3.656
2	2	3	3	11	6	10	6	3.869
3	1	1	3	9	3	2	3	2.994
4	1	0	7	5	4	4	4	2.588
7	0	0	3	7	5	5	3	2.875
8	0	0	4	6	5	3	3	2.530
9	0	0	3	4	4	3	2	1.862
10	0	0	12	-	-	-	4	6.928
11	0	0	16	13	14	12	9	7.223
15	-	-	-	1	2	2	2	0.577
16	-	-	-	0	0	0	0	0.000

Remark:
> Dash indicates dead *M. macrocopa* at the particular time.

Appendix I

1 Way ANOVA Followed by *Post Hoc* Comparison (Tukey Test)

a) Comparison of population density between control and treatment cultures with 4 ind./40ml initial density for each day

Day		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	0.750	1	0.750	0.063	0.806
	Within Groups	118.167	10	11.817		
	Total	118.917	11			
2	Between Groups	0.333	1	0.333	0.022	0.886
	Within Groups	153.667	10	15.367		
	Total	154.000	11			
3	Between Groups	147.000	1	147.000	8.368	0.016*
	Within Groups	175.667	10	17.567		
	Total	322.667	11			
4	Between Groups	363.000	1	363.000	18.060	0.002**
	Within Groups	201.000	10	20.100		
	Total	564.000	11			
7	Between Groups	7905.333	1	7905.333	49.574	0.000***
	Within Groups	1594.667	10	159.467		
	Total	9500.000	11			
8	Between Groups	5334.083	1	5334.083	21.076	0.001***
	Within Groups	2530.833	10	253.083		
	Total	7864.917	11			
9	Between Groups	4446.750	1	4446.750	28.212	0.000***
	Within Groups	1576.167	10	157.617		
	Total	6022.917	11			
10	Between Groups	2948.167	1	2948.167	10.485	0.031*
	Within Groups	1124.667	4	281.167		
	Total	4072.833	5			
11	Between Groups	2610.750	1	2610.750	48.332	0.000***
	Within Groups	540.167	10	54.017		
	Total	3150.917	11			
15	Between Groups	42.667	1	42.667	19.692	0.011*
	Within Groups	8.667	4	2.167		
	Total	51.333	5			
16	Between Groups	32.667	1	32.667	49.000	0.002**
	Within Groups	2.667	4	0.667		
	Total	35.333	5			

b) Comparison of population density among different days at different initial densities in control culture tubes

Density		Sum of Squares	df	Mean Square	F	Sig.
4	Between Groups	321.548	11	29.232	2.231	0.027*
	Within Groups	668.167	51	13.101		
	Total	989.714	62			
20	Between Groups	4335.889	11	394.172	8.149	0.000***
	Within Groups	2467.000	51	48.373		
	Total	6802.889	62			
40	Between Groups	16434.437	11	1494.040	32.148	0.000***
	Within Groups	2370.167	51	46.474		
	Total	18804.603	62			
80	Between Groups	67043.857	11	6094.896	30.382	0.000***
	Within Groups	10231.000	51	200.608		
	Total	77274.857	62			

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001

c) Comparison of population density among different days at different initial densities in treatment culture tubes

Density		Sum of Squares	df	Mean Square	F	Sig.
4	Between Groups	22955.548	11	2086.868	14.464	0.000***
	Within Groups	7358.167	51	144.278		
	Total	30313.714	62			
20	Between Groups	17060.762	11	1550.978	7.388	0.000***
	Within Groups	10706.667	51	209.935		
	Total	27767.429	62			
40	Between Groups	6219.389	11	565.399	2.208	0.028*
	Within Groups	13062.167	51	256.121		
	Total	19281.556	62			
80	Between Groups	74641.437	11	6785.585	7.743	0.000***
	Within Groups	44695.167	51	876.376		
	Total	119336.603	62			

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001