Controlling the Fatty Acid Content of Live Food for Cultured Larval Sablefish

By

Paul Callow
B.Sc., University of British Columbia, 1996

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

Supervisor: Dr. Louis A. Hobson

Abstract

Sablefish (*Anoplopoma fimbria*) has potential as a cultured species in British Columbia. However, high mortality during the larval stage makes the rearing of sablefish in captivity impractical. To address this problem, techniques have been developed to improve the nutritional quality of the live food which is fed to larval sablefish. This study examined how different growth techniques and enrichment protocols affect the amount of essential fatty acids in rotifers (*Brachionus plicatilis*), Brine Shrimp (*Artemia* spp.) and the copepod *Tisbe* sp. Fatty Acid content was determined by gas-liquid chromatography. The fatty acid information was used to determine standard procedures for preparing these three species of zooplankton to ensure they are nutritionally suitable when fed to larval fish. This study has contributed to the first survival of cultured sablefish larvae to the juvenile stage.

Table of Contents

List of Tables

List of Figures

Acknowledgments

I thank my supervisor, Dr. Louis Hobson, for his support throughout this work. I also thank the members of my committee for reviewing this thesis, and Dr. Jim Powell for his guidance through the BC Science Council's GREAT Award application process.

Particular thanks are due to Dr. Don Noakes and the members of his staff at the Pacific Biological Station in Nanaimo: Drs. Ian Whyte and Craig Clarke; John Jensen and John Blackburn. I recognize the contribution of my colleagues on the sablefish project team: Spino Pakula, Joe Klimek, Peter Anderson, John Westby, Sean McConnachie, Christy Falkenberg, Melinda Jacobs and Brad Overbye.

I gratefully acknowledge the financial assistance provided by the Science Council of BC through the GREAT Award program. Finally, my thanks are due to Northern Marine Farms Ltd., Aquametrix Research Ltd., and Dr. Brad Hicks of Pacific Aquafoods Ltd.

Controlling the Fatty Acid Content of Live Food for Cultured Larval Sablefish

Chapter 1: Introduction

Northern Marine Farms and Pacific Aqua Salmon Farming Partners, with the support of Aquametrix Research Ltd. and in collaboration with the Department of Fisheries and Oceans and the Ministry of Fisheries, Government of BC, are developing techniques and practices to establish a viable sablefish *Anoplopoma fimbria* (also known as black cod) aquaculture industry in British Columbia. The University of Victoria has assisted them in this project. This thesis addresses one aspect of the work: supplying essential nutrients to larval sablefish.

Sablefish are considered good candidates for netpen culture. They have a high market value, and adapt well to captivity (Kennedy, 1972, McFarlane, 1989). Sablefish are also a local species, so questions of adapting to the local environment and the introduction of exotic species do not arise. However, cultured juveniles for out-growing on farms are not yet available. To this end, the partnership has sought to complete the sablefish life cycle in captivity by investigating the husbandry of adult sablefish in netpens, production of a constant supply of eggs through induced maturation of adult sablefish, rearing of larvae and eggs, and rearing of juvenile sablefish.

As part of this project, juvenile sablefish have been caught in Bute Inlet, B.C., transferred to netcages and successfully grown to adult fish. The suitability of growing sablefish from juveniles to adults in captivity has been investigated at Pacific Aqua Foods' Atlantic salmon farm near Thurlow Island, British Columbia. Sablefish in pens have a high growth rate, a wide tolerance to various diets, and appear to be resistant to most diseases that effect farmed salmon. Also, sablefish appear to have no effect on Atlantic salmon in neighbouring pens. Sablefish culture has required no modifications to existing fish farms.

The major challenge in culturing sablefish is mortality during the larval stage. The larvae grow to a length of 12 mm, but mortality increases as yolk sac reserves are depleted. The inability of sablefish to grow larger than 12mm was in part attributed to insufficient amounts of two essential fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in their diet (Watanabe, 1993; Whyte *et al* 1994). Similar studies on turbot (*Scophthalmus maximus*) lingcod (*Ophidiodon elongatus*) and Japanese flounder (*Paralichthys olivaceus*) have shown that it is necessary to have adequate amounts of essential fatty acids in their diets because juvenile fish lack the ability to synthesize these fatty acids *de novo* (Stottrup and Attramadal, 1992; Furuita *et al*, 1998). Sablefish larvae larger than 20mm have been caught in the wild and successfully grown to juvenile fish (McFarlane, 1989). This may be due to the presence of essential fatty acids in wild feeds, which are critical in ensuring the survival of the wild larvae from the 12 to 20 mm size (Watanabe, 1993). It may therefore be postulated that larval survival of captive sablefish may be possible if they are supplied with adequate amounts of exogenous essential fatty acids.

Objective

The objective of this project was to examine how the live feed diet of larval sablefish can be manipulated to improve nutritional quality and thus improve the survival of larval sablefish. Success of this project is a vital step towards completing the sablefish life cycle in captivity. It means that sablefish eggs can be hatched, larval sablefish grown to marketable sizes and brood stock produced without depending on wild stocks.

Rationale

Prior to the sablefish project at the Pacific Biological Station, sablefish larvae grown in the laboratory failed to survive past yolk sac absorption. It is likely that this was due to an inadequate supply of the essential fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the larval feed (Watanabe, 1993; Whyte *et al.*, 1994; Bell and Sargent, 1996).

The roles of DHA and EPA in fish development has been extensively investigated (reviewed by Rainuzzo *et al*, 1997). Cold-water marine fish contain a high amount of these essential fatty acids in their tissues. Fish cannot synthesize these fatty acids, and so have a dietary requirement for them as soon as they begin to rely on an exogenous food supply. Lipids, in general, are a source of metabolic energy for developing larval fish (Sargent *et al*, 1999). DHA appears to be the more important fatty acid for energy purposes, as it is more quickly consumed during larval development than EPA.

EPA and DHA are also important for the growth of neural tissues during this developmental stage in fish. It has been shown that a diet with low amounts of DHA hinders the growth of the brain and cerebellum in the Japanese Flounder (Furuita *et al*, 1998). DHA has also been shown to collect in brain tissues of developing fish, where it has a structural role (Mourente *et al*, 1991).

Essential fatty acid content in larval fish diets has also been shown to affect pigmentation in turbot *Scopthalmus maximus* (Estévez *et al*, 1999) and in Atlantic halibut *Hippoglossus hippoglossus* (McEvoy *et al*, 1998). In general, inadequate amounts of these essential fatty acids cause malpigmentation in these species. While pigmentation does not necessarily affect survival rate in an aquaculture setting, it does affect the market value of the fish, and therefore is a major concern to aquaculturists (McEvoy *et al*, 1998).

Larval marine fish raised in captivity are typically fed cultured rotifers (*Brachionus plicatilis* Mueller), copepods (*Tisbe* sp.) or, during later larval development, brine shrimp (*Artemia* spp.). These metazoans are mainly used because they are relatively easy to grow in laboratory conditions (Walford and Lam, 1992). While these species are not the normal natural food of most larval fish, they are used extensively in mariculture because the wild zooplankton available to larval fish cannot be grown efficiently in laboratory conditions. *Artemia* and rotifers not raised with special consideration of their fatty acid content are not nutritionally adequate to sustain larval marine fish in a cold water environment (Tocher, *et al.*, 1997; Whyte *et al*, 1994). *Tisbe sp.* do potentially have

enough essential fatty acids, but it is difficult to raise these copepods in sufficient numbers to sustain a large batch of larval fish. They are therefore impractical to use as food for larval fish on a production scale (Støttrup *et al*, 1998). The essential fatty acid content of rotifers, *Artemia* and copepods can be increased by varying their diet of microalgae, or supplementing their diet with commercially available enrichment products such as Super Selco (INVE Aquaculture) (Bell and Sargent, 1996).

Initial research on the fatty acid content of rotifers had been conducted at Pacific Biological Station (PBS) in Nanaimo (Whyte *et al.*, 1994). More recently, work by the study team at the PBS has shown that enrichment diets can increase the essential fatty acid content of rotifers. However, some diets increase both the essential and nonessential fatty acids, resulting in a very high overall fatty acid content of rotifers. High fatty acid content comes at the expense of other nutrients such as carbohydrates and protein, and rotifers fed on these diets may then have enough essential fatty acid to sustain larval sablefish, but simultaneously may be too low in other nutrients. An ideal enriched diet should contain increased amounts of essential fatty acid, without any increase of total fatty acid.

Fatty acid analysis done as part of this graduate project indicates that copepods (*Tisbe sp.*) have inherently higher essential fatty acid content than do rotifers. Tests have not yet been done to see the effects of enrichment diets on copepod essential fatty acid content. Analysis of larval sablefish caught in the wild suggests that calanoid copepods are the favoured food of larval sablefish (Grover and Olla, 1987, 1990). Calanoid copepods

cannot be grown efficiently in captivity, and are available from plankton tows only inconsistently during the spring months. They are therefore unsuitable as feed for captive larval sablefish

This study indicates which microalgal species should be used to feed to rotifers and copepods before they are fed to larval fish. An attempt was made to use a local species of cultured microalgae, *Tetraselmis gracile* in order to emulate the natural diet of larval sablefish. This study characterizes essential fatty acid profiles for rotifers, brine shrimp, copepods and larval sablefish. The analysis was conducted on zooplankton fed various single and combined algal diets and enriched with commercially available enrichment products.

There were several suitable species of microalgae available: *Thalassiosira pseudonana* (Hustedt clone 3H), *Isochrysis galbana* (Green clone T-Iso, termed Tahitian *Isochrysis)*, *Pavlova lutheri* Droop, *Chaetoceros gracilis* Schutt, and a local species of *Tetraselmis gracilis* Butcher. The rotifer used for all experiments was *Brachionus plicatilis* Mueller. The brine shrimp were either *Artemia franciscana* (Argent, platinum grade) or Russian *Artemia* (INVE Aquaculture). The copepod was a locally isolated species of an harpacticoid copepod, *Tisbe* sp. Sablefish (*Anoplopoma fimbria* Pallas) were fed diets that were deemed suitable by the fatty acid analysis and this, in part, contributed to the first ever survival of larval sablefish to the juvenile stage in captivity. There are many factors which contributed and will continue to contribute to larval survival: tank design,

broodstock and egg quality, husbandry techniques, water quality live food quality and stocking density.

During the course of this study, other members of the sablefish research team addressed these factors. This study does not specifically examine how diets of varying nutritional quality affect larval sablefish, but focuses instead on producing large quantities of nutritious live food for larval sablefish in an efficient and economical manner.

Chapter 2

Materials and Methods

Part 1: Zooplankton culture techniques

A large portion of my Masters work has been to participate in developing techniques to produce large numbers of zooplankton for feeding to larval fish. Because of the importance of these techniques to my thesis and to the sablefish aquaculture project as a whole, the culture and preparation techniques for rotifers, copepods, and *Artemia* will be discussed in detail. For rotifers and *Artemia*, some existing protocols have been modified to suit the demands and available equipment of this project. Rotifer and *Artemia* culture protocols are based on procedures described by Lavens and Sorgeloos (1996). The protocols for raising *Tisbe* were developed by members of the sablefish research team and are not based on previous work.

Rotifer culture techniques

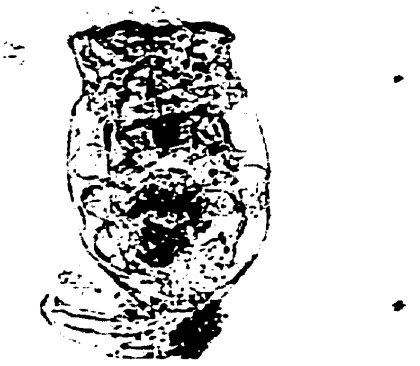

Figure 1: The rotifer, ***Brachionus plicatilis***, about 120μm long and 70μm diameter. For feeding to larval fish, width, rather than length of the prey organism is a critical measurement, as the size of a particle that can be consumed by a larval fish is determined by the mouth gap width of an individual larvae.

1. Starter Culture

The first stage of rotifer culture is a small population which is used to inoculate and restore a second, larger, population. The small backup culture of rotifers is started from rotifer resting eggs. To start the large culture, 1ml of resting eggs are added to a mixture of 10 liters of filtered sea water and 10 liters of algal culture, with a density of at least 2 million cells/ml. The species of microalgae is not critical for growout purposes, but *Isochrysis galbana* was typically used in this study because it was readily available from the algae production facility. Rotifer resting eggs are maintained at a temperature between 18 and 25 °C for about 4 days, when rotifers can be seen in the culture container. Rotifers are then removed from this container by siphoning them into a 40-μm sieve, washing them very gently with 20 °C filtered seawater, and then flushing them from the

sieve into a 4-liter beaker. The container is filled to three liters with filtered seawater. The rotifers are then enumerated by evenly suspending them in the container, and taking a 0.1-ml sample for counting under a dissecting microscope. If there is at least 1 million rotifers total, they are used to inoculate a bag culture.

2. Bag Culture

Rotifer bag culture is the secondary stage of rotifer production. Rotifers produced in this stage can be enriched with essential fatty acids and fed to larval fish, used to produce resting eggs, or inoculated into larger batch culture tanks. Rotifer bags are clear high-grade plastic and can contain a volume of water up to 200 liters. They are suspended in a metal frame in front of a bank of fluorescent lights. Bag culture is maintained under bright lighting at all times. At least 1 million rotifers are added to 50 liters of filtered salt water in a rotifer bag, and are then immediately fed 30 liters of algal culture ranging in denity form 2 to 5 million cells/ml. Twenty liters of high-density algae are added every other day until the bag is full. Then, one third to half of its volume can be harvested on a daily basis, provided the volume of algae is replaced every day. In this way, about 3 million rotifers can be harvested from a rotifer bag every day. Rotifer bag cultures are maintained at room temperature. Rotifers are harvested by siphoning them into a 40μm sieve which is placed in a wide flat pan so that the fabric of the sieve is below the water line. This allows rotifers to be harvested from the bag without drying out on the sieve. Once the bag has been harvested, the rotifers must be fresh-water rinsed to remove any contaminating ciliates. A freshwater rinse is done by placing the sieve in a pan and running fresh water either into the pan or gently into the sieve. The rotifers should be

completely submerged in 23°C fresh water for at least five minutes. Fresh water rinsing for too long can destroy the rotifers, so they should not be submerged in fresh water for longer than 10 minutes. Once they are rinsed, the rotifers are flushed with salt water into a small container for counting.

3. Rotifer Resting Egg Production

Resting eggs are the dormant form of rotifers. They are kept on hand in a fish hatchery in case a problem arises with the main rotifer culture. Rotifers are induced to form resting eggs when their growth environment has a low salinity (Hagiwawara *et al.* 1993). To produce resting eggs, a tank is prepared with a mixture of fresh and salt water to produce a final salinity of 15ppt. The rotifers are lightly fed over several days with algae, but the salinity should be maintained at 15ppt. An equal volume of fresh water thus should be added for each volume of algae added. After 5 days, detrital matter is removed from the tank and is washed in fresh water. This detrital matter will be rich in resting eggs. Under a dissecting microscope (500X), they appear as dense particles about 40μm in diameter surrounded by a clear envelope giving a total diameter of about 60μm. Resting eggs can be stored and are viable for up to 3 years if kept cool and in the dark.

4. Batch Culture

Batch culture is a procedure designed to produce a large number of rotifers easily and cleanly on a daily basis. It is designed for the production of rotifers on a hatchery scale, where daily husbandry of large tanks is not practical. To reduce the amount of husbandry

needed, batch cultures are never allowed to get very old, so build-up of detrital matter is minimal. Batch culture requires at least 7 tanks, one for each day of a seven-day cycle. Two more tanks are also useful as back-ups. Batch tanks are usually inoculated with a large quantity of rotifers: usually half the number that is required for feeding to fish every day. Up to 50 million rotifers can be inoculated into a 400-liter tank. A constant drip of a yeast solution is pumped into each tank, which receives 10 grams of yeast per 100 liters of growth media per day. Each tank also receives 10 percent of its volume per day of high-density (at least 2 million cells/ml) microalgae. After rotifers have been fed in this way for 7 days, they are harvested. To harvest, the contents of the tank are drained into a collection sieve (40μm mesh size). A collection sieve is specifically designed to gently concentrate a large number of rotifers or *Artemia*. The sieve fabric is attached to a long V-shaped frame which is placed inside a large container. The interior of the V-shaped sieve drains through a short pipe directly to the outside of the container in which it is placed. The contents of a tank containing zooplankton is drained into the outside container. Growth medium passes into the v-shaped sieve and is drained to the outside of the container, leaving concentrated zooplankton outside the sieve and inside the container. (See figure 2)

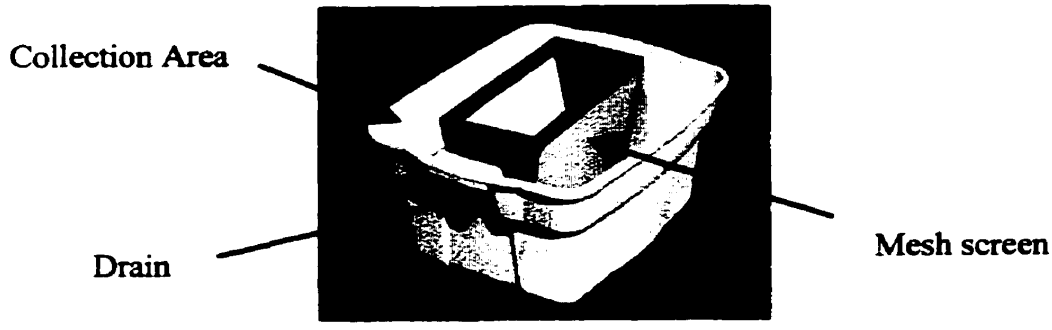

Figure 2: a collection sieve. Water from a tank is drained into the collection area. The water then passes through the mesh screen and out the drain, leaving the zooplankton concentrated in the collection area.

The rotifers are rinsed in salt water in the collection sieve until the effluent runs clear. They are then rinsed with fresh water for five minutes. One half of the harvested rotifers are then used to inoculate a new tank. The other half is prepared for feeding to larval fish. Harvest timing of the batch culture tanks is arranged so that one tank is ready every day. The batch culture system takes about two weeks to reach full production capacity, and so should be started before live feed is needed.

5. Rotifer Enrichment

Rotifers have to be enriched before they can be fed to larval fish. Fatty acid analyses have shown that unenriched rotifers lack adequate essential fatty acids to be nutritionally suitable for feeding to larval marine fish (Tocher, *et al*, 1997). Rotifer enrichment is usually carried out in tanks similar to batch culture tanks. There are several

commercially available products designed to enrich their essential fatty acid content prior to feeding to larval fish. Each enrichment product works best under specific conditions, and different procedures may have to be used for different enrichments. In general, an enrichment product is an emulsified oil that is added to the rotifer growth medium. The rotifers remain in the enrichment for a period of time determined by the enrichment product. Once rotifers have been enriched, they must be handled with extra care. Rough handling, such as vigorous spraying or stirring causes enriched rotifers to form clumps which cannot be fed to fish. Clumps form because of the high oil content of the enrichment, in conjunction with vigorous handling, causing rotifers to stick together. To harvest enriched rotifers, they are drained into a collection sieve with a prefilter with a 250-μm mesh size. The prefilter is used to catch any clumps of rotifers and prevent them from getting into the larval rearing tanks. Once the enrichment tank is drained, the rotifers are rinsed with salt water in the collection sieve until the effluent runs clear. They are then poured into a suitable container, counted, and fed to the larval fish.

Tisbe culture techniques

Several generations of the harpactacoid copepod *Tisbe sp.* were cultured at the Pacific Biological Station as part of the sablefish project. Techniques have been developed which ensure a clean, reliable supply of copepods of a size class suitable for feeding to larval fish. In this study, copepods have been fed numerous algal diets, and the effect of these diets on their fatty acid profile have been determined. *Tisbe* has substantially higher amounts of the essential fatty acids DHA and EPA than rotifers fed the same diets. *Tisbe* is therefore more competent to provide larval finfish their nutritional requirement of essential fatty acids. Although *Tisbe* has a higher nutritional quality, it is difficult to produce in adequate numbers due to its long life cycle. Current research on copepods is therefore focused on the use of *Tisbe* as a supplement for other larval feeds, and on increasing yield through intensive production systems.

A major portion of the copepod study was focused on the development of methods for cultivating *Tisbe* in large quantities. Over the four years *Tisbe* has been cultivated at the Pacific Biological Station, methods have been developed which result in high-density production of copepods, and in a clean and simple procedure.

The protocols to culture *Tisbe* in captivity are designed to maintain a clean, dense population and to allow easy removal of specific size classes for feeding to larval fish. Stringent adherence to the protocols for *Tisbe* care can in some instances result in a smaller population than may otherwise be possible, but these protocols are needed to

maintain the culture over several generations. Careful evaluation of the health, population, and developmental stage of *Tisbe* is critical to successfully culturing a large population of copepods over several generations.

The *Tisbe* life cycle starts when nauplii hatch from the egg sac of a gravid adult copepod. At this stage, they have a rounded shape, and are about 80 μm in diameter. These nauplii grow via a series of molts to the copepodite stage. *Tisbe* copepodites range in size from about 150 to 350μm, and 60μm to 140μm in diameter. This size class is typically fed to larval fish. Adult *Tisbe* are over 350 μm in length. Male *Tisbe* can reach 1 mm in length and 400μm diameter, and gravid females can reach 1.3 mm and 550μm in diameter.

Figure 3: *Tisbe* nauplii hatching from the yolk sac of a gravid adult

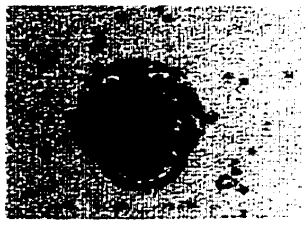

Figure 4: Free swimming *Tisbe* nauplii, about 120μm long

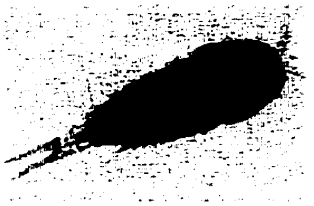

Figure 5: *Tisbe* copepodite. About 500μm long, 200 μm in diameter

Figure 6: Adult *Tisbe*. Female (lower) and male (top) are about 1 mm long.

Tisbe cultivation is typically carried out in 150-liter fiberglass tanks. Other sizes of tanks can be used, but it is important to maintain a high surface area to volume ratio for large copepods. Large tanks, around 300 liters, are not suitable for *Tisbe* culture unless extra substrate is added. This extra substrate can be gravel, or other high-surface area objects such as plastic honeycomb mesh.

The 150-liter fiberglass tanks have a central removable standpipe, and a central drain connected to a flexible hose about 75 cm. long. This hose is usually directed down the main drain in the culture room, but can be moved to other containers when necessary.

To prepare a tank for *Tisbe* culture, it is cleaned and bleached. Before a tank is used for the first time, it should be filled with fresh water and bleached with 100 ml of 12% bleach overnight. This procedure should be repeated after every *Tisbe* culture, or whenever the tank appears particularly dirty.

Before inoculation with *Tisbe*, roughly 20 liters of algal culture are added to the tank. The temperature of the water in the tank can then be adjusted by adding salt water. *Tisbe* of all size classes are killed if the temperature rises above 20°C. 17°C appears to be the ideal temperature for inoculation.

Tisbe culture usually begins with nauplii. Nauplii are easily separated from a batch of *Tisbe* of mixed sizes by placing a 120-μm sieve inside a 40-μm sieve, and pouring the copepods into the 120-μm sieve. Copepodites and adult copepods will be retained in the 120-μm sieve, and nauplii will be caught in the 40-μm sieve. The copepods can then be washed from the sieves with salt water into separate containers for evaluation and counting. After the nauplii have been counted, 5×10^5 are inoculated into a prepared tank. The *Tisbe* from the 120-μm sieve are returned to the tank they came from, or are fed to larval fish.

A copepod broodstock tank is similar to a nauplii tank, the major difference being temperature control. Adult *Tisbe* appear to be more sensitive to higher temperatures than nauplii. Broodstock tanks should be kept at about 14°C. This can be achieved by

flowing cold water through a coil of hose placed around the standpipe in the middle of the tank.

Because *Tisbe* culture tanks are static, air must be provided via an air-stone. The amount of air is not critical, but should be sufficient to keep the algae in suspension, without causing a boiling action on the surface.

If animals in the culture tank are well fed and maintained between 15 and 20°C for 8 days, most of the nauplii will have developed to the copepodite stage by the end of this time period. On day eight, the contents of the tank should be siphoned out through a 120-μm sieve. This is an effective way of removing ciliates and other contaminating species from the *Tisbe* culture. Anything that passes through the 120- μm sieve can usually be discarded. However, it is a good idea to check if a large population of nauplii remains in the culture after eight days. The presence of nauplii after eight days can be indicative of low temperature, an infestation of ciliates, poor food quality or quantity, or poor water quality.

Once the copepodites are collected in the 120-μm sieve, they are washed into a container and counted. In a healthy culture, there should be very little loss of copepods as they develop from nauplii to copepodites. The copepodites can then be fed to larval fish, or inoculated into a fresh tank. If copepodites are kept in a tank and fed to larval fish over several days, the size of the copepodites must be carefully monitored to ensure that they remain an appropriate size for larval feed.

After about two days, dark detrital matter may begin to appear on the bottom of the copepodite tank. If this detrital matter is left in the tank, hydrogen sulphide producing bacteria will begin to grow, which appears to reduce *Tisbe* growth. Detrital matter should be removed daily by siphoning it through a 120-μm sieve. The copepods in the sieve can then be rinsed thoroughly with salt water and returned to the copepodite tank.

Copepodites can also be harvested from a tank by placing the main drain hose of the tank into a 120-μm sieve, and pulling the standpipe. The advantage is that it is much faster than siphoning. However, adult *Tisbe* cannot survive the high flow rate that occurs when the standpipe is pulled from a full tank. To drain a tank which contains adult *Tisbe*, it should be siphoned until just a few liters are left in the tank. The drain hose can then be placed in a sieve and the standpipe pulled. The remaining copepods are then washed with salt water from the bottom of the tank into the drain hose.

When copepodites have been in a tank for about six days, adult *Tisbe* will be visible in the tank. Adults typically cluster on the sides of the tank near the surface of the water, and will be especially dense on the walls of the tank close to the air-stone. Adult *Tisbe* develop at different rates, so once they begin to appear in the tank there may be a period of about four days when both copepodites and adults are present in the tank. This must be taken into consideration if copepodites are being fed to larval fish. To separate copepodites from adults, they can be siphoned through a 250-μm sieve and caught in a

120-μm sieve. Adults in the 250-μm sieve can be returned to the tank, and the copepodites can be fed to the larval fish.

Three days after adult *Tisbe* are visible, nauplii will begin to hatch. At this point, care must be taken to avoid losing nauplii when detrital matter is siphoned from the tank. When there are adult *Tisbe* in the tank, detrital matter should be siphoned into a 40-μm sieve, washed, and animals returned to the tank.

The contents of the detrital matter should be monitored daily during this stage. Between 16 and 18 days after the original tank was inoculated with nauplii, large numbers of nauplii will be present in the detrital matter of the broodstock tank. Once the nauplii population appears to reach a maximum, all of the water in the broodstock tank should be siphoned through a 40-μm sieve, then washed into a small container. The *Tisbe* from the broodstock tank can then be sequentially sieved through 250, 120 and 40 μm sieves. The nauplii will be present in the 40-μm sieve, and can be immediately used to inoculate a nauplii tank. Copepodites in the 120-μm sieve are added to another copepodite tank. Similarly, any adults present in the 250-μm sieve can be added to another adult *Tisbe* tank.

Tisbe are able to survive and grow to a high density on various microalgal diets. *Thalassiosira pseudonana*, *Isochrysis galbana*, *Chaetoceros gracilis*, and *Tetraselmis gracile* all appear to be appropriate. Mixed diets, especially *Tetraselmis* and *Isochrysis*, yield the highest *Tisbe* densities. To ensure maximum growth of the *Tisbe*, they should

be fed to satiation. Algal density should be maintained in the copepod tanks at around 4 x 10^4 cells/ml. However, feeding more algae than can be consumed by the copepods does not appear to have any detrimental effect on copepods. While a quantitative experiment has not been conducted on how diet or feeding protocol affects growth rate, the culturing procedure described above is based on observations of *Tisbe* behavior and through trial and error. In general, it appears that algal diet has little effect on growth rate, with the exception of *Pavlova lutheri*, which when fed to *Tisbe*, results in poor growth. The temperature of algal cultures should always be matched to that of the copepod culture.

Tisbe can also be fed commercially available diets. Combining *Nannochloropsis oculata* paste and Culture Selco in a 10:1 ratio sustains growth similar to algal diets. Extra measures must be taken when feeding a combined *Nannochloropsis paste* and Culture Selco diet to keep the *Tisbe* culture clean. This diet can cause rapid fouling of the water, and rapid build-up of detrital matter. Feeding this diet at too high a density can cause the *Tisbe* culture to become anoxic. Detrital matter from *Tisbe* fed this diet is more pale than detrital matter from algal diets, and may be difficult to see at the bottom of the tank.

Tisbe culture does not require special lighting conditions, and they grow rapidly in natural daylight conditions. To maximize growth, constant fluorescent lighting can be used. This lighting helps maintain the algae in the copepod tanks, thus making available more food for the copepods.

If the above procedures are followed, *Tisbe* can reach a density of 7 individuals/ml, which is at least one order of magnitude less than the density to which rotifers can be grown. Further research is therefore needed to increase the density to which *Tisbe* can be cultured.

If there is no need to grow a large population of *Tisbe* to feed larval fish, a smaller, slower-growing culture can be used instead. About 100 adult *Tisbe* are removed from the side of a broodstock tank with a pipette, placed in a 4 liter beaker with an air-stone and fed about 500 ml of algae at about 1 million cells/ml per week. A small population of *Tisbe* can be maintained in this way for up to a month. Keeping this small culture at 12°C effectively reduces the growth of contaminating organisms.

Artemia Culture techniques

Figure 7: The brine shrimp *Artemia franciscana*, about 1300μm in length, and about 800μm at the widest point.

Protocols for preparing *Artemia* to be fed to larval fish have been developed in the sablefish and halibut hatchery over three years. The procedure involves tank and container preparation, cyst decapsulation, *Artemia* nauplii hatch-out, nauplii enrichment, and final clean up before feeding to larval fish.

1. Equipment needed for *Artemia* production

Artemia are very tolerant to various growth conditions and environments, but using the correct equipment can greatly increase the yield and nutritional quality of the nauplii. Hatch-out and enrichment can be carried out in the same container. A conical bottom tank with an air source at the bottom is most suitable. This design of the tank allows for easy removal of the *Artemia*. Sloped walls allow for easy collection of unhatched cysts, resulting in cleaner nauplii. The hatch-out and enrichment tanks are held in a frame with

the bottom of the tanks about half a meter above the ground. Suspended above the hatch-out and enrichment tanks is a bank of fluorescent lights. *Artemia* nauplii are continuously illuminated during the hatch-out and enrichment stages. When cysts are being hatched, or when nauplii are being enriched, the water in the tanks must be kept at a temperature of about 28°C. An aquarium heater is used to maintain the temperature in the tank. Up to 80 million *Artemia* can be prepared in a 140-liter conical tank.

2. Decapsulation of Artemia Cysts

In order to increase the hatching efficiency of some brands of *Artemia*, the cysts need to be decapsulated prior to hatch. Some commercially available cysts are pre-decapsulated, so this step is not always necessary. However, most commercially available cysts do require decapsulation. One advantage of decapsulation, along with increasing hatch yield, is to sterilize the cysts. During the course of this study, two species of *Artemia* were used: Russian *Artemia* (INVE Aquaculture) and *Artemia franciscana* (Argent). The Russian *Artemia* cysts were pre-decapsulated and could be added directly to the hatch-out tanks. *Artemia franciscana* had to be decapsulated. The following procedure was used.

First, a required number of cysts are weighed (60 - 70 grams) and poured into a 4l. glass beaker. They amount of cysts used was determined by assuming there are 330,000 cysts per gram, in accordance with the instructions provided with the *Artemia* cysts, for both INVE and Argent products. The cysts are then hydrated by filling the beaker with tap water, and air passed through an air-stone is added to agitate the cysts. After 2 hours,

hydrated cysts are poured onto a 200-μm sieve, and transferred to a clean glass container where decapsulation will take place. 33ml of 40% NaOH and 4.5 ml of 12% bleach is needed for each gram of cysts. In a third container, a volume of 9°C salt water (9ml of salt water is used for each gram of *Artemia* cysts) is prepared.

The foregoing solutions are combined in the beaker containing the cysts. Air passed through an air-stone is added to agitate the cysts during decapsulation. Rigorous aeration and occasional stirring is used throughout the decapsulation process. After about four minutes, individual cysts change from their normal brown to a pale yellow colour. As decapsulation continues, the cysts become more pale as the mixture of solvents becomes brown. It is necessary to examine individual cysts rather than the mixture as a whole to determine when decapsulation is complete. When most of the cysts are pale yellow, decapsulation is halted by pouring the contents of the decapsulation beaker through a sieve (200-μm) and flushing the cysts with copious amounts of cold tap water. Sodium thiosulphate can also be used to neutralize the bleach, but if enough water is used, this is not normally necessary. After rinsing, the cysts are transferred into a small container in preparation for hatch-out.

3. *Artemia* hatch

To hatch *Artemia* cysts, a conical tank is filled with warm (25-30°C) salt water, and a heater is added. The tank is allowed to reach a temperature of about 28°C. Air is bubbled into the tank from the bottom. Sufficient air is required to produce a boiling

action on the surface. Decapsulated cysts are then added to the container. Initially, the cysts may clump up or stick to the sides of the container, but this is of no concern, as the clumps will usually break apart after about 20 minutes. Clumps of *Artemia* cysts can be broken by vigorously stirring the water in the hatch-out tank.

Almost all of the cysts will hatch 18-24 hours after the cysts have been added to the tank. To evaluate hatch success, a small volume is removed from the hatch-out tank with a pipette. By counting the number of cysts and nauplii in the pipette, a good idea of the hatch-out success can be determined. When the newly hatched nauplii are ready to be harvested, the air source to the tank is turned off, and freshly hatched nauplii allowed to settle for about 20 minutes. This allows most of the unhatched cysts and shells to float to the surface. After about 20 minutes, most of the cysts will have separated from the nauplii, and the nauplii are then transferred from the tank to a sieve (200-μm) through the air inlet hose. The hatch-out tank is allowed to drain into the sieve until cysts are visible in the drain hose. Nauplii are then rinsed in freshwater for about two minutes, and transferred to a small container with salt water. Nauplii are then further separated from any residual cysts by pouring them into a large separatory funnel. Nauplii are allowed to settle, and cysts allowed to float to the surface in the funnel for about two minutes. Nauplii are then drained from the separator funnel into a small container, and were ready for enrichment.

4. *Artemia* Enrichment.

Several commercially available enrichment media are available for *Artemia*, and they are all used in a similar way. Similar to rotifer enrichment products, these enrichments contain high levels of essential fatty acids and are used by emulsifying them and adding them to water containing *Artemia* nauplii. The Artemia become enriched as they grow from Instar 1 to Instar 2 nauplii in the enrichment media (Lavens and Sorgeloos, 1996).

The enrichment tank is prepared in the same way as the hatch-out tank. Freshly hatched *Artemia* nauplii are added to 28°C salt water which is agitated vigorously by means of airflow from the bottom of the enrichment tank. Enrichment is continued according to the instructions provided by the manufacturers of the enrichment product. Individual enrichment products have specific requirements for concentration and duration of the enrichment.

After the enrichment is complete, the air to the enrichment tank is turned off, and the *Artemia* are allowed to settle. They are then drained into a 200-μm collector sieve and gently rinsed with warm salt water until no enrichment media can be seen in the collector sieve outflow. Nauplii are then poured from the collector sieve into a smaller sieve, and rinsed with cold salt water into a small container. The enriched nauplii are then ready to be fed to larval fish.

Materials and Methods Part 2: Fatty Acid Analysis

1.Fatty acid preparation for gas chromatography

With the exception of one study on the feeding cycle of rotifers, all the fatty acid data presented in this thesis are from experiments that were performed in triplicate. Duplicates only were available for the rotifer feeding cycle experiment. Results are presented as the average of the three (or two, in the case of the rotifer feeding cycle experiment) data points, ± 1 standard deviation.

Statistical analysis was used to determine significant differences between groups in individual experiments, and to compare results between experiments. For all statistical comparisons, Sigmastat 2.0® (Jandel Scientific) software was used to perform one-way ANOVA followed by a Tukey test. When data were determined to be of unequal variance or not normally distributed, a one-way ANOVA on ranks was performed followed by a Student-Newman-Keuls test. Significant differences in results were determined with a p value of 0.05.

All three species of zooplankton were sampled for fatty acid analysis in the same way. A sample of zooplankton was poured through a 45-μm sieve to concentrate the zooplankton on the sieve fabric. The sieve was then blotted dry with paper towel, and soaked in cold 3.2% ammonium formate. The sieve was then blotted dry once again. The zooplankton

were transferred to a centrifuge tube by flushing the sieve with ammonium formate. The tubes were centrifuged, the ammonium formate was poured off, and the samples frozen at –80°C. The frozen samples were then freeze-dried for at least 18 hours before they were processed for chromatographic analysis.

The fatty acids in the freeze-dried samples were converted to fatty acid methyl esters before they could be injected into a gas chromatograph column. Reacti-Vials containing a known amount of heneicosanoic acid (C:21) as an internal standard were used to prepare the methyl esters. C:21 is a saturated fatty acid containing 21 carbon atoms, and is not normally found in marine lipids (Whyte *et al.*, 1987). Dichloromethane containing a known concentration of dissolved C:21 was added to each reaction vial. The dichloromethane was then evaporated, and the vials sealed and stored in a refrigerator until required. Each freeze-dried sample (20-50 milligrams) was weighed into the 5 ml Reacti-Vial (Pierce Chemical Co.) containing a Reacti-vial magnetic stirrer and capped with a loose fitting Mininert valve (Pierce Chemical Co.) and evacuated in a vacuum oven at 25°C. The oven was vented with nitrogen and the vial sealed tightly with the valve. Methanolic potassium hydroxide (1ml, 0.5*M*, purged with nitrogen) was added through the valve septum using a 2ml. Luer-Lok glass syringe and the contents stirred with a vortex mixer. Subsequent additions and withdrawals from the vials were made using syringes. The mixture was stirred at 85°C for 30 minutes in a Reacti-Therm module (Pierce Chemical Co.) and when cooled, hexane (1ml) was added and stirred. The hexane containing unsaponified material was withdrawn and discarded.

Esterification reagent, boron trifluoride-methanol (2ml, 12% from sealed ampoules) was added and the mixture stirred at 85°C for 15 min. When the mixture was tepid, hexane (1ml) and saturated aqueous sodium chloride (0.5ml) were added and stirred. The lower aqueous layer was withdrawn, the resultant organic layer was washed once with saturated aqueous sodium bicarbonate and the washings were discarded. The sample vials were then unsealed and the hexane containing fatty acid methyl esters was removed to a 1ml chromatography vial and evaporated in a vacuum oven. After evaporation, the oily residue left in the chromatography vials was dissolved in 0.1 ml hexane, and transferred to a preweighed 0.1 ml chromatography vial. The hexane was then evaporated in a vacuum oven, and the chromatography vials were sealed under nitrogen. The vials were then weighed, and the weight of fatty acid methyl ester in each vial was determined. Finally, the fatty acid methyl esters were dissolved by the addition of 100µl ethyl acetate for each milligram of fatty acid methyl esters present.

The analyte (1 - 2 µl) was injected into a Hewlett-Packard 5890 gas-liquid chromatograph, fitted with a capillary inlet system, a flame ionization detector and connected to a HP 3393A Integrator, was used for analysis. The fatty acid methyl esters were separated on a Supelcowax 10 fused silica capillary column (30m X 0.32mm ID, 0.25 µm film) operating at 180°C for 35 minutes then programmed to 240°C at 2°C/min and held at 240°C for 16 min. Helium carrier gas was purified through a high capacity heated gas purifier (Supelco Inc.) and controlled at a linear velocity of 10 cm/s with a split ratio of 5:1. This method of fatty acid methyl ester preparation allows quantification

by weight of individual fatty acids (Whyte, 1998; Whyte and Nagata, 1990; Nagata and Whyte, 1992).

Individual fatty acids were then identified according to their retention times and equivalent chain lengths. All peaks that constituted less than 0.2% of all fatty acid methyl esters and all peaks for components that emerged before myristic acid (14:0) and after nervonic acid (24:1n9), and the peak corresponding to the internal standard were removed from the chromatogram. The total area of all the peaks in the chromatogram was used to determine the amount of each fatty acid as a proportion of all fatty acids. Finally, the ratio of the known amount of the internal standard to the area of the peak of the internal standard was used to determine the amount of each fatty acid as a proportion of dry weight. Because of the large number of calculations for each chromatogram, and the large number of fatty acid analyses in this study, a program was written in Visual Basic to identify all the fatty acids in a chromatogram, perform the necessary calculations, and report the information in a standard format. This allowed easy comparison with other fatty acid samples. After the calculations for a chromatogram have been done, there are two pieces of information available for each identified fatty acid. First is the area percent, which is the area under a peak for a fatty acid as a percentage of the total area of all the peaks on the chromatogram. Second is the dry weight amount of each fatty acid as a proportion of total dry weight. It is expressed as milligrams fatty acid per gram of sample.

2. Fatty Acid Analysis of Algae

Several species of algae that were used in my thesis were analyzed for fatty acid content. All samples of algae were taken from cultures which were growing in the exponential phase and appeared to be free of contaminating organisms upon microscopic examination. Though the samples were not all taken at the same time, all the algae were grown in the same laboratory with the same lighting and temperature conditions. Three samples of each species of algae were taken for fatty acid analysis. Each sample was filtered onto preweighed fiberglass filters with a pore size of 0.2 μm. The filter papers were then washed with 3.2% ammonium formate, and freeze-dried in preparation for fatty acid analysis.

3. Rotifer Fatty Acid Analysis

Experiments were carried out on rotifers to determine the effect of different algal diets, growth conditions, and enrichments on the essential fatty acid content of rotifers.

3a. Essential Fatty Acid Retention in Enriched Rotifers

This experiment was designed to emulate the conditions of a larval fish-rearing tank. In such a tank, rotifers are added to the water and the larvae consume them. After 12 hours

in the tank, most of the rotifers will have been flushed out or eaten by the larvae. The concern that this experiment was designed to address was how long enriched rotifers should be allowed to remain in a larval rearing tank before they are no longer nutritionally advantageous for the fish larvae to consume, and must therefore be flushed out of the tank. Rotifers were enriched according to the manufacturers' instructions, except for the Algamac 3010 Flake (Aquafauna Biomarine) enrichment, in which 1/6 of the recommended amount was used, and for only 2/3 of the recommended enrichment time. Rotifers were enriched with Algamac 3010 Flake at a concentration of 1 gram/liter, for 4 hours. For the Super Selco enrichment, 0.1 grams of enrichment material was used for each liter of enrichment media divided into two doses: one at the start of enrichment, and the second 3 hours later. The rotifers were harvested after 6 hours of enrichment. All the enrichments were carried out at 26°C. The enriched rotifers were rinsed, counted, and divided equally into nine 4-liter beakers; 3 replicates were used for each of three treatments. Each beaker was started with 1 million rotifers, which is a much higher density than would be found in a larval rearing tank. However, for the purposes of fatty acid analysis, a lower density would not have been practical. At the end of the enrichment process, the entire contents of the enrichment beakers were used for fatty acid analysis.

3b. Super Selco and DHA Selco Enrichments

The next rotifer experiment was designed to determine the effect of enriching rotifers with Super Selco and DHA Selco (INVE Aquaculture) over a standard 6-hour period at different temperatures. The procedure for enriching rotifers with DHA Selco and Super

Selco is identical to the protocol described for experiment 3a. For both the enrichments and at all the temperatures tested except for 11.8°C in the Super Selco experiment, rotifers were separated into two size fractions after the enrichment but prior to sampling. To do this, rotifers were passed through a 120-μm sieve and concentrated in a 45-μm sieve, resulting in a 120+ μm fraction and a 45-199μm fraction. These sizes do not actually represent the size of the rotifers, since the sieve sizes are measured on the edges of individual squares in the screen fabric, and a rotifer larger than 120 μm in length could pass through a 120 μm sieve. All screens used in this study were made of a square mesh, meaning the diagonal distance for individual squares in the mesh can be use to give an indication of the width of a particle that can pass through it. The squares in the 120 μm screen have a diagonal distance of 170 μm, and the squares in the 45 μm screen have a diagonal distance 64 μm. The size fractions, 120+ and 45-119, thus indicate the mesh size of the sieves used, not the size of the rotifers. Sieves of these two mesh sizes were chosen because they effectively separate fecund rotifers from non-fecund rotifers. This was done to determine how essential fatty acid enrichments affect different size classes of rotifers. Specifically, the 120 μm fraction contained mostly fecund rotifers, and the 45 to 199 μm fraction contained all the others. The first part of this experiment dealt with rotifers enriched with Super Selco. A parallel experiment was conducted to determine the effects of different temperatures on the effectiveness of a DHA Selco enrichment.

3c. Fatty Acid Content of Rotifers During a 24-hour Feeding Cycle

An experiment was carried out to determine how the essential fatty acid profile of rotifers is affected by a daily feeding routine of 3H and yeast. Rotifers were sampled from bag cultures at 24 hours post feeding, and immediately fed to satiation with a mixture of 3H and yeast. They were then sampled for fatty acid analysis at 3, 6,12, 18 and 24 hours. The rotifers used for this experiment were growing in a continuous bag culture. Approximately 300,000 rotifers were removed from the bags for each sample. It should be noted that the results of this experiment are from duplicates of all the samples, rather than triplicates that have been used in all other data presented in this study.

3d. Effects of Algae and Enrichment Products on the Essential Fatty acid Content of Rotifers

The next experiment on rotifers was designed to examine the effects of several different brands of rotifer enrichment products, as well as some effects of rotifer algal diet prior to enrichment. The standard algal diet for the rotifers prior to enrichment was a combination of 3H and yeast. For two of the enrichment experiments a combination of *T. gracile, P.lutheri*, and 3H supplemented with yeast was fed to the rotifers in equal proportions prior to enrichment. Enrichments were carried out according to the enrichment manufacturer's recommendations. Algamac 2000 enrichments were done with

0.1 grams of Algamac for each liter of enrichment media, in a manner similar to the procedure described earlier for Algamac 3010 Flake. Ratio HUFA Enrich and Protein HUFA Enrich (INVE Aquaculture) were used in the same way. O.1 grams of enrichment product was used for each liter of enrichment media. Ratio HUFA Enrich and Protein HUFA Enrich were weighed and then emulsified in one liter of salt water. Half the solution was used at the start of the enrichment, and the other half was used 3 hours later. Rotifers here harvested at 6 hours. All enrichments for this experiment took place at 26°C

3e. Effect of Algal Diets on the Essential Fatty Acid Profiles of Rotifers

An experiment was carried out to determine how different algal diets affect the fatty acid profiles of rotifers. Rotifers were fed to satiation with different species of algae for a four-day period, and then sampled for fatty acids. During the experiment, the rotifers were maintained at a constant temperature of 24°C.

4. *Tisbe* Fatty Acid Analysis

4a. Effect of algal diet on the essential fatty acid content of *Tisbe*

This experiment was designed to determine how the *Tisbe* fatty acid profile is affected by algal diet. For each of 5 feeding trials, 1 million *Tisbe* nauplii were removed from a culture tank, washed, and separated between 54 and 120 μm screens. Prior to the feeding trials, animals in the culture tank had been fed a standard diet of T-Iso. The nauplii were then divided equally into 3 30L containers each containing 10 liters of filtered seawater.

The algal density of the water was determined for each of the containers. They were then given 3 liters of algae of a known density, and the algal density of the culture containers was determined again. Animals in the experimental containers were fed in this way every day for 7 days. Temperature was maintained at 18°C. *Tisbe* copepodites were sampled for fatty acid analysis on day 8.

4b. Fatty acid profiles of *Tisbe* produced in mass culture

The second experiment on copepods was designed to determine how culture protocol affects *Tisbe* fatty acid content. The first experiment determined how algal diet affected copepod essential fatty acid content. However, that experiment was done on a small scale, and so it was necessary to determine if there is a difference in the nutritional quality of *Tisbe* produced by a large-scale hatchery protocol compared to a smaller laboratory scale. *Tisbe* were raised in 140 liter tanks according to several feeding and culture protocols. All the feeding protocols were tested on cultures started with 16, 000 nauplii, graded between 54 and 120μm sieves. Unless otherwise indicated, all the cultures were maintained between 17 and 19°C. Each protocol was carried out in triplicate. The following feeding regimes were examined:

1) Standard feed of T-Iso for 14 days.
2) Standard feed of T-Iso for 7 days
3) T-Iso feed for 7 days, then removed from the culture media, washed, placed in 2 liters of filtered salt water, and stored at 4°C for 7 days.
4) *Tetraselmis gracile* for 14 days.

5) *Pavlova lutheri* for 14 days

6) Culture Selco for 14 days

7) T-Iso and constant temperature of 12°C

8) *Nannochloropsis oculata* paste for 14 days

9) *Nannochloropsis oculata* paste and culture Selco (10:1 mixture) for 14 days.

10) T-Iso fed for 14 days, then graded between 120 and 200μm sieves prior to sampling.

The paste used in this experiment was provided by Innovative Aquaculture (Lasquetti Island, BC) and contained concentrated *Nannochloropsis oculata* and a preservative.

5. *Artemia* Fatty Acid Analysis

5a. *Artemia* essential fatty acid retention

This experiment determined how essential fatty acid levels change in enriched and unenriched *Artemia* over a 12-hour period in cold salt water. The experiment was designed to emulate conditions of a larval fish-rearing tank. The goal was to determine how long *Artemia* can remain in the larval rearing tank before they are no longer sufficiently nutritious for larval fish. *Artemia* (Russian strain) were hatched from cysts in the previously described manner, using 140-liter conical tanks. 9 million *Artemia* were prepared in a total volume of 3 liters. Three 30-liter containers were filled with 25 liters of saltwater and placed in a 28°C water bath. Air stones were added to circulate the water, and the tanks were allowed to reach 28 °C. 3 million *Artemia* were then added to

each container. The containers received the following treatments: 1) No enrichment, 2) Algamac 3010 Flake, 3) Aquagrow. The enrichments lasted for 18 hours, at which point each group of animals was rinsed through a 200 μm sieve, flushed into a beaker and made up to 3 liters. One quarter of the volume of each treatment group was immediately sampled for fatty acid analysis. The remaining *Artemia* were made up to a total volume of 3 liters.

Nine beakers were each filled with 2 liters of salt water and allowed to acclimatize in a 9°C water bath. This temperature was used because it is the same temperature at which sablefish larval rearing tanks were maintained. One million enriched *Artemia* were added to each beaker. The *Artemia* were then sampled for fatty acid analysis at 4, 8 and 12 hours post enrichment.

5b. DC DHA Selco, Algamac 3010 Flake, and Aquagrow enrichments for *Artemia*

This experiment examined how three enrichments affect the essential fatty acid content of *Artemia* produced on a large hatchery scale. Russian *Artemia* (INVE aquaculture) were hatched in 140 liter conical tanks according to the process earlier described. They were then rinsed and placed in clean 140 liter conical tanks containing 28°C salt water. They were enriched with DC DHA Selco, Algamac 3010 Flake, or Aquagrow. The duration of each enrichment was determined by the manufacturer's instructions for each enrichment product. For each treatment, 0.6 grams of enrichment product was used for each liter of water in the container where enrichment would take place. Each enrichment was

suspended in 1 liter of salt water in a blender, and half of this suspension was added to the *Artemia* at the start of enrichment. For Algamac 3010 flake and Aquagrow, the remaining half of the enrichment was added 6 hours later, and enrichment was complete at 13 hours. For the DC DHA Selco enrichment, the remaining half of the enrichment suspension was added 12 hours after the start of enrichment, and the enrichment process was complete after 24 hours. It should be noted that these procedures resulted in the Aquagrow and Algamac 3010 flake–enriched *Artemia* being 12 hours younger than DC DHA Selco-enriched *Artemia* at the time of sampling. After each enrichment, the *Artemia* were sampled for fatty acid analysis. Each enrichment experiment was repeated twice, to provide triplicate samples of *Artemia* nauplii.

5c. *Artemia* Enriched with *Nannochloropsis oculata/Schyzochytrium* Paste Followed by Super Selco

This experiment tested the effectiveness of commercially available nutrient paste consisting of a mixture of *Nannochloropsis oculata* and *Schyzochytrium*. The paste was a product of Innovative Aquaculture. This experiment also looked at the nutritional quality of two day-old *Artemia*. Two-day-old *Artemia* were studied because they have the potential to be fed to older larval fish, which are larger and therefore require a larger size of prey. Freshly hatched nauplii contain no DHA, which is the main reason *Artemia* have to be enriched (Verreth, *et al.* 1994).

Artemia franciscana were decapsulated and hatched in a 20-liter container in salt water at 28°C. After hatch they were rinsed, counted, and a sample was taken for fatty acid analysis. 1.2 million nauplii were added to a 30-liter flat-bottom beaker containing 20 liters of salt water at 28°C for enrichment with *Nannochloropsis oculata/Schyzochytrium* paste. For each liter of enrichment medium, 0.2 grams of the paste were used. The paste was dissolved in salt water with a blender prior to adding to the enrichment tank. After 18 hours of enrichment with *Nannochloropsis ocualta* paste, the nauplii were removed from the enrichment by siphoning into a 200-μm sieve. They were rinsed gently in salt water, and half of the *Artemia* were sampled for fatty acid analysis. The remaining half was placed in another 30-liter container under the same conditions as the paste enrichment. The *Artemia* were then enriched for another 18 hours with Super Selco, according to the manufacturer's instructions. 0.6 grams of Super Selco per liter of enrichment media was emulsified in one liter of salt water. Half of this suspension was added to the *Artemia* at the start of the enrichment, the other half 12 hours later, and the Artemia were sampled for fatty acid analysis 12 hours after the second addition of enrichment media. After the Super Selco enrichment, the *Artemia* nauplii were sampled for fatty acid analysis. The experiment was repeated three times to produce three fatty acid samples each for unenriched, paste enriched, and paste and Super Selco enriched *Artemia*.

6. Sablefish egg and whole larvae analysis

In order to determine the suitability of foods for larval fish, it is useful to compare the essential fatty acid content of live feeds to the essential fatty acid content of larval

sablefish and sablefish eggs. Sablefish eggs were collected from captive broodstock at the Pacific Biological Station, fertilized, and immediately sampled for fatty acid analysis by washing them with 3.2 % ammonium formate followed by freezing at -80 °C. They were then analyzed for fatty acid content in a procedure identical to that for zooplankton. Sablefish larvae were collected one day before they were ready to be fed. They were prepared for fatty acid analysis in a procedure identical to that for sablefish eggs.

Chapter 3

Results

1. Whole fatty acid profiles of Zooplankton

Whole fatty acid profiles from myristic acid (14:0) to nervonic acid (24:1n9) for some species of algae, rotifers, *Artemia* and *Tisbe* used in the preparation of live food for larval sablefish are shown in Tables 1a and 1b. Fatty acid profiles representative of species or treatments were selected. None of the samples shown here contain any nervonic acid. Also, any peak in the fatty acid spectra that amounted to less than 0.2% of the total area of all the peaks was eliminated, and the area% (table 1a) for each fatty acid was adjusted to reflect this. Of particular interest are the amounts of the essential fatty acids DHA (22:6n3) and EPA (20:5n3).

In Table 1b, the values reflect the weight of each fatty acid in milligrams divided by the total weight in grams of the sample. The sum of all these values in any fatty acid spectrum gives the total mass of fatty acids in milligrams per gram of dry weight of the organism. This total mass of all fatty acids can be use as an indicator of the total fat content of the sample. However, it should be noted that the total fatty acid content is not a measure of the total lipid content, as other lipids, such as sterols, are not detected in the fatty acid analysis.

Another aspect of whole fatty acid spectra is that they readily identify the species from which the fatty acid sample came. In general, algal species contain less variety of fatty

acids than zooplankton. In Table 1a and 1b, both *P. lutheri* and *C. gracilis* have more simple spectra than the other species. The dominant feature of any spectrum of a sample of *Artemia* is a large amount of linoleic acid (18:3n3). This high content of 18:3n3 is very characteristic of this species and was seen in all the *Artemia* analyses done in this study.

The most difficult animals to differentiate based on fatty acid spectra alone are *Tisbe* and rotifers. In general, rotifer spectra are more evenly spread than copepod spectra. Unlike those for rotifers, copepod fatty acid spectra tend to have high amounts of short-chain fatty acids, such as 14:0 and 16:1n9, and high levels of polyunsaturated fatty acids such as DHA. However, these differences are not always distinctive, so whole fatty acid profiles are not a good way to differentiate rotifers and copepods.

Because of the importance of the essential fatty acids DHA and EPA to the nutrition of larval sablefish, most of this thesis will discuss these two fatty acids only. While arachidonic acid (20:4n6) has also been identified as an essential fatty acid, it appears that live feeds contain adequate amounts of this fatty acid, and do not normally need to be enriched (reviewed by Sargent, *et al*, 1997).

Table 1a: Fatty Acid profiles of selected algal species and zooplankton (area%)

	C. gracilis	*P.lutheri*	Unenriched *Artemia*	*Tisbe* fed *C. gracilis*	Unenriched Rotifers Fed T-Iso	Super Selco Enriched Rotifers
14:0	10.7 ± 0.8	9.8 ± 0.4	0.8 ± 0.1	3.9 ± 1.4	5.5 ± 0.8	5.6 ± 0.1
16:0	12.0 ± 1.7	9.8 ± 0.4	11.2 ± 2.1	16.3 ± 1.5	10.1 ± 1.0	9.4 ± 0.3
16:1n7	34.5 ± 1.8	16.4 ± 0.4	3.5 ± 0.9	21.3 ± 0.7	8.4 ± 0.9	8.2 ± 0.3
16:2n7	4.2 ± 0.3	-	-	2.9 ± 0.4	-	-
16:3n6	-	-	-	0.8 ± 0.4	-	-
16:3n4	5.5 ± 0.7	1.8 ± 0.1	-	3.1 ± 0.7	-	-
16:4n3	-	-	-	-	-	-
18:0	0.5 ± 0.1	-	5.0 ± 1.1	3.9 ± 0.5	4.4 ± 0.4	4.3 ± 0.2
18:1n11	-	-	-	-	-	-
18:1n9	0.8 ± 0.3	2.5 ± 0.1	20.2 ± 2.2	1.6 ± 0.3	9.4 ± 1.0	9.0 ± 0.4
18:1n7	2.2 ± 0.8	-	7.6 ± 1.1	3.6 ± 0.3	3.7 ± 0.3	3.6 ± 0.1
18:2n7	-	-	-	0.2 ± 0.0	-	-
18:2n6	0.9 ± 0.1	-	5.6 ± 0.1	0.8 ± 0.1	9.6 ± 0.8	9.4 ± 0.4
18:3n6	-	-	-	-	-	4.8 ± 0.2
18:3n3	-	1.6 ± 0.1	26.8 ± 2.3	-	5.1 ± 0.3	-
18:4n3	0.5 ± 0.2	7.2 ± 0.2	3.5 ± 0.1	0.3 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
20:1n11	-	-	-	-	1.4 ± 0.2	1.8 ± 0.3
20:1n9	-	-	0.6 ± 0.1	-	2.8 ± 0.2	3.1 ± 0.3
20:1n7	-	-	-	0.5 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
20:4n6(ARA)	5.8 ± 1.0	-	1.1 ± 0.1	3.5 ± 0.9	2.9 ± 0.2	2.8 ± 0.1
20:3n3	-	-	0.6 ± 0.1	-	-	-
20:4n3	-	-	0.6 ± 0.1	0.3 ± 0.1	6.1 ± 0.6	5.8 ± 0.3
20:5n3(EPA)	16.4 ± 0.7	29.9 ± 0.4	3.7 ± 0.3	16.8 ± 1.9	4.0 ± 0.5	3.6 ± 0.2
22:5n6	-	-	-	0.5 ± 0.2	1.3 ± 0.1	1.2 ± 0.2
22:5n3	-	-	-	1.7 ± 0.3	2.0 ± 0.2	1.9 ± 0.1
22:6n3(DHA)	1.4 ± 0.2	8.9 ± 0.1	-	11.1 ± 3.3	4.8 ± 0.4	4.0 ± 0.1

Results are expressed as percentages of all fatty acids ± 1 standard deviation.

Table 1b: Fatty Acid profiles of selected algal species and zooplankton (mg/g)

	C. gracilis	*P.lutheri*	Unenriched *Artemia*	*Tisbe* fed *C. gracilis*	Unenriched Rotifers Fed T-Iso	Super Selco Enriched Rotifers
14:0	12.8 ± 1.4	7.1 ± 1.2	1.1 ± 0.1	2.5 ± 1.1	1.9 ± 0.1	2.4 ± 0.1
16:0	14.4 ± 2.5	7.1 ± 1.2	15.1 ± 0.4	10.3 ± 3.2	3.2 ± 0.2	7.7 ± 0.1
16:1n7	41.4 ± 3.9	11.9 ± 2.0	4.8 ± 0.1	13.4 ± 3.6	2.8 ± 0.2	4.1 ± 0.3
16:2n7	2.4 ± 0.1		-	2.4 ± 0.7	-	-
16:3n6		-	-	0.2 ± 0.2	-	-
16:3n4	6.6 ± 0.9	1.3 ± 0.2	-	1.5 ± 0.1	-	-
16:4n3	-	-	-	-	-	-
18:0	0.6 ± 0.2	-	6.7 ± 0.2	2.4 ± 0.5	1.5 ± 0.1	4.7 ± 0.4
18:1n11	-	-	-	-	-	-
18:1n9	0.9 ± 0.4	1.8 ± 0.3	27.2 ± 0.8	1.0 ± 0.4	3.1 ± 0.2	12.9 ± 0.5
18:1n7	2.6 ± 1.0	-	10.3 ± 0.3	2.2 ± 0.4	1.2 ± 0.1	3.7 ± 0.1
18:2n7	-	-	-	0.2 ± 0.1	-	-
18:2n6	1.0 ± 0.2	-	7.6 ± 0.2	0.5 ± 0.1	3.2 ± 0.2	9.8 ± 0.3
18:3n6	-	-	-	-	-	2.9 ± 0.1
18:3n3	-	1.1 ± 0.2	36.5 ± 1.5	-	1.7 ± 0.1	-
18:4n3	0.6 ± 0.1	5.2 ± 0.9	4.8 ± 0.2	0.2 ± 0.1	0.6 ± 0.1	1.7 ± 0.1
20:1n11	-	-	-	-	0.6 ± 0.1	0.9 ± 0.1
20:1n9	-	-	0.8 ± 0.1	-	1.1 ± 0.1	2.5 ± 0.2
20:1n7	-	-	-	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.1
20:4n6 (ARA)	6.3 ± 0.7	-	1.5 ± 0.1	2.1 ± 0.2	1.0 ± 0.1	2.3 ± 0.1
20:3n3	-	-	0.9 ± 0.1	-	-	-
20:4n3	-	-	0.9 ± 0.1	0.2 ± 0.1	2.0 ± 0.1	3.0 ± 0.1
20:5n3 (EPA)	18.2 ± 3.0	21.6 ± 3.8	5.1 ± 0.2	10.4 ± 1.7	1.2 ± 0.1	22.0 ± 2.3
22:5n6	-	-	0.0 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	1.0 ± 0.2
22:5n3	-	-	-	1.1 ± 0.2	0.6 ± 0.1	3.2 ± 0.3
22:6n3 (DHA)	1.5 ± 0.5	6.5 ± 1.3	0.3 ± 0.2	6.8 ± 1.8	1.4 ± 0.1	15.2 ± 1.1
Total Fatty Acid Dry Weight	119.9 ± 7.4	65.6 ± 11.4	135.6 ± 4.4	62.6 ± 14.5	34.6 ± 2.6	107.0 4.4

Results are expressed as milligrams of each fatty acid per gram of sample ± 1 standard deviation.

2. Essential Fatty Acid Retention by Rotifers

The first experiment for fatty acid retention was done on unenriched, T-Iso fed rotifers over a 12-hour starvation period in 9°C salt water (Table 2). There were no significant differences in the essential fatty acid levels over the 12-hour period. These results indicate that when rotifers were not enriched, there was no significant loss of essential fatty acids. Also, unenriched rotifers, if they are fed to larval fish, do not need to be removed from the larval rearing tank in less than 12 hours.

The second experiment for fatty acid retention was done on rotifers enriched with Algamac 3010 Flake. As with the unenriched rotifers, there were no significant changes in the amount of essential fatty acids over the 12-hour starvation period in 9°C water (Table 2). This suggests that Algamac is absorbed by the rotifers, and it is neither washed off nor degraded over the 12-hour starvation period.

The final experiment for fatty acid retention was with Super Selco (INVE Aquaculture) enriched rotifers. After 12 hours of starvation, there was a significant difference in the EPA content as a proportion of dry weight (Table 2). After only 4 hours, there was a significant difference in the EPA content as a proportion of all fatty acids. This means that EPA is lost from the rotifers faster than other fatty acids.

Because there are changes in EPA both as a proportion of all fatty acids and as a proportion of dry weight, it cannot be determined if this loss in fatty acids is due to the rotifers metabolizing the fatty acids, or due to the fatty acids being washed off the

rotifers. Because Super Selco enriched rotifers lose their enrichment over a 12-hour period they cannot be allowed to remain in a larval fish rearing tank for as long as Algamac enriched rotifers. The differences in the lengths of time rotifers enriched with Super Selco and Algamac 3010 Flake retain their enrichments may be due to the physical attributes of the enrichments, rather than their fatty acid content. Super Selco is a concentrated oil which is likely to coat the outside of rotifers. In contrast, Algamac 3010 Flake contains *Schyzochytrium* cells, which are small particles rather than an oil, and may approximate the natural algal diet of rotifers better than an oil product. Therefore, rotifers may be able to adsorb and store nutrients from Algamac 3010 Flake more efficiently than from Super Selco. A simple experiment could be done to determine how easily enrichments are washed off rotifers. Groups of rotifers could be enriched with various enrichment products, and immediately sampled for sampled for fatty acid analysis. The remaining rotifers could then be washed several times with salt water, and then sampled again for fatty acid analysis. By comparing the fatty acid profiles of enriched rotifers before and after washing, the effect of washing rotifers after enrichment can be determined.

Table 2: Enriched Rotifer Essential Fatty Acid Retention

Time	DHA mg/g	EPA mg/g
Unenriched		
0	1.4 ± 0.1	1.2 ± 0.1
4	1.6 ± 0.1	1.3 ± 0.1
8	1.7 ± 0.7	2.0 ± 1.5
12	1.2 ± 0.8	1.1 ± 0.4
Algamac		
0	19.9 ± 4.2	3.1 ± 0.7
4	20.7 ± 2.5	3.6 ± 0.6
8	19.4 ± 1.8	3.3 ± 0.2
12	19.8 ± 2.4	3.4 ± 0.4
Super Selco		
0	15.2 ± 1.1	22.0 ± 2.3 [d]
4	13.5 ± 5.3	18.7 ± 7.8
8	11.5 ± 1.6	15.5 ± 1.8
12	8.4 ± 2.0	10.9 ± 2.2 [a]

Time	DHA area %	EPA area %	Ratio DHA:EPA
Unenriched			
0	3.9 ± 0.2	3.5 ± 0.3	1.1 ± 0.5
4	4.8 ± 0.4	4.0 ± 0.5	1.2 ± 1.0
8	3.8 ± 0.3	4.0 ± 1.1	1.0 ± 1.4
12	3.9 ± 1.9	3.6 ± 0.9	1.0 ± 2.8
Algamac			
0	25.7 ± 1.1	4.0 ± 0.1	6.4 ± 1.0
4	24.6 ± 0.5	4.3 ± 0.3	5.8 ± 1.2
8	23.9 ± 1.0	4.0 ± 0.3	6.0 ± 1.8
12	23.6 ± 2.1	4.1 ± 0.4	5.8 ± 2.5
Super Selco			
0	14.2 ± 0.6 [d]	20.6 ± 1.4 [b,c,d]	0.7 ± 1.9
4	11.0 ± 0.9	15.1 ± 0.8 [a]	0.7 ± 1.7
8	10.3 ± 3.2	13.5 ± 2.5 [a]	0.8 ± 5.8
12	10.0 ± 0.5 [a]	13.0 ± 0.2 [a]	0.8 ± 0.8

Superscripts: within each enrichment trial, a = significant difference from time 0, b = significant difference from 4 hours, c= significant difference from 8 hours, and d = significant difference form 12 hours.

3. Effect of Temperature on the enrichment Super Selco of different size classes of rotifers

Temperature at which rotifers were enriched significantly affected their content of essential fatty acids (Table 3). Within each fatty acid measurement, there were also significant differences between the 120+ and 45-119μm size fractions at 24.5 and 5.5 °C. There were no significant differences between the size fractions when the rotifers were enriched at 18.5°C.

When measured as a proportion of dry weight, there was no difference in EPA and DHA content in rotifers when enriched at 24.5 and 18.5°C. However, there were significant differences in the essential fatty acid levels when measured as a proportion of all fatty acids. This indicates that varying the temperature during the enrichment does not alter the total amount of essential fatty acids absorbed by the rotifers, but can affect how other fatty acids are adsorbed during enrichment, which in turns affects the content of essential fatty acids in relation to other fatty acids.

The ratio of DHA to EPA for all the samples in this experiment always remained below one, indicating that a Super Selco enrichment results in a rotifer that has more DHA than EPA. This result agrees with the results of the rotifer essential fatty acid retention experiment.

At all temperatures examined, the variance for values DHA and EPA were higher in rotifers from the larger (120+) size class. This higher variance may be due to the

fecundity of the rotifers. A sieve with a 120μm screen was used to separate fecund rotifers from non-fecund ones. However, the screen did not allow for a complete separation, and some large non-fecund rotifers were present in this size fraction. As this experiment was conducted over several days, it is possible that there was some variability in fecundity of the rotifers. When the 120+ size class was prepared after enrichment, this variability in fecundity would have resulted in a variable ratio of fecund to non-fecund rotifers in the this size class, which in turn would be reflected in the variability of the amounts of essential fatty acids in this size class.

Table 3: Rotifers Enriched with Super Selco: Comparison of Enrichment Temperature and size classes

Temperature	Size class	DHA mg/g		EPA mg/g	
24.5°C	120+	20.6 ± 2.4	b,f	30.1 ± 2.7	f
24.5°C	45-119	26.6 ± 2.8	a,e,f,g	35.0 ± 2.4	e,f,g
18.5°C	120+	23.8 ± 2.3	e,f,g	35.1 ± 2.1	e,f,g
18.5°C	45-119	22.4 ± 1.0	e,f,g	32.9 ± 1.5	f
11.8°C	120+	17.5 ± 1.5	b,c,d,f	28.3 ± 2.2	b,c,f
5.5°C	120+	10.7 ± 0.6	a,b,c,d,e,g	19.8 ± 1.0	a,b,c,d,e,g
5.5°C	45-119	19.0 ± 0.4	b,c,f	28.9 ± 0.8	b,c,f

Temperature	Size class	DHA Area %		EPA Area %		Ratio DHA:EPA
24.5°C	120+	12.8 ± 0.9	b,c,d,f	18.7 ± 0.7	b,c,d,e,g	0.7 ± 1.7
24.5°C	45-119	15.7 ± 0.0	a,e,f,g	20.7 ± 0.7	a,c,d,f	0.8 ± 0.7
18.5°C	120+	15.3 ± 0.8	a,e,f,g	22.6 ± 0.3	a,b,e,f	0.7 ± 1.3
18.5°C	45-119	15.2 ± 0.2	a,e,f,g	22.4 ± 0.6	a,b,e,f	0.7 ± 0.8
11.8°C	120+	12.4 ± 0.2	b,c,d,f,g	20.4 ± 0.2	a,c,d,f	0.6 ± 0.5
5.5°C	120+	9.6 ± 0.3	a,b,c,d,e,g	17.9 ± 0.5	b,c,d,e,g	0.5 ± 0.8
5.5°C	45-119	14.1 ± 0.1	b,e,f	21.5 ± 0.3	a,f	0.7 ± 0.4

Size classes: 120+: Rotifers which were retained in a sieve with a 120-µm mesh. All fecund rotifers were in this size fraction. 45-119: Rotifers which passed through the sieve with 120-µm mesh and were retained in a 45-µm mesh.

Superscripts: a= significantly different from 24.5°C, 120+, b= significantly different from 24.5°C, 45-119, c= significantly different from 18.5°C, 120+, d= significantly different from 18.5°C, 45-119, e= significantly different from 11.8°C, 120+, f= significantly different from 5.5°C, 120+, g= significantly different from 5.5°C, 45-119.

4. Effect of Temperature on enrichment with DHA Selco of different size classes of rotifers

No significant differences in the amount of essential fatty acids as a proportion of dry weight were found among rotifers enriched with DHA Selco at different temperatures (Table 4). There were some differences in essential fatty acids as a proportion of all fatty acids. These differences mean that temperature can have an effect on how fatty acids are absorbed by rotifers enriched with DHA Selco. However, since essential fatty acid content as a proportion of all fatty acids was not affected, it appears that the efficiency of enrichment with DHA Selco is less susceptible to variation in temperature than is enrichment with Super Selco.

In general, DHA Selco caused problems when enriching rotifers, and this is reflected in the high variability of the fatty acid results. The appeared much oilier and did not emulsify as well as Super Selco. Clumping of rotifers was seen on the surface of the water in the enrichment tank, and vigorous rinsing was required to produce a sample of live rotifers suitable for fatty acid analysis. Since clumps of rotifers are not fed to larval fish, the clumps had to be removed from the enrichment in order for the fatty acid analysis to reflect a diet that could be fed to larval fish. This may explain why the results are lower than other researchers' findings for DHA Selco enriched rotifers (Blair *et al.* 1998).

Table 4: Rotifer Enriched with DHA Selco: Comparison of Enrichment Temperature and size classes

Temperature	Size Class	DHA mg/g	EPA mg/g
5.5 °C	120+	36.2 ± 17.2	25.3 ± 10.8
5.5 °C	45-119	25.3 ± 2.3	16.6 ± 0.8
12 °C	120+	41.0 ± 16.2	26.6 ± 11.8
12 °C	45-119	33.3 ± 2.8	20.5 ± 1.8
18 °C	120+	25.9 ± 8.0	19.7 ± 4.2
18 °C	45-119	23.9 ± 3.0	16.2 ± 1.3

Temperature	Size Class	DHA Area %	EPA Area %	Ratio DHA:EPA
5.5 °C	120+	14.1 ± 0.9	10.0 ± 0.2 [f]	1.4 ± 1.0
5.5 °C	45-119	14.1 ± 0.2	9.3 ± 0.2	1.5 ± 0.5
12 °C	120+	14.7 ± 0.7	9.4 ± 0.3	1.6 ± 1.0
12 °C	45-119	15.2 ± 0.4 [e]	9.4 ± 0.2	1.6 ± 0.7
18 °C	120+	12.9 ± 1.3 [d]	10.0 ± 0.6 [f]	1.3 ± 1.8
18 °C	45-119	13.3 ± 0.6	9.0 ± 0.2 [a,e]	1.5 ± 0.8

Size classes: 120+: Rotifers which were retained in a sieve with a 120-μm mesh. All fecund rotifers were in this size fraction. 45-119: Rotifers which passed through the sieve with 120-μm mesh and were retained in a 45-μm mesh.

Superscripts: a= significantly different from 5.5°C, 120+, b= significantly different from 5.5°C, 45-119, c= significantly different from12°C, 120+, d= significantly different from 12°C, 45-119, e= significantly different from 18°C, 120+, f= significantly different from 18°C, 120+.

5. Essential fatty Acid content of rotifers during one 24-hour feeding cycle.

Results show that the DHA content of rotifers does not vary over a 24 hour feeding period, but there are significant differences in the EPA content of the rotifers during the same feeding cycle (Table 5). There were two sets of samples taken of rotifers that had been starved for 24 hours, and they had a significant difference in EPA content. This is probably due to variability in the rotifer feed (in this case, 3H and yeast). The source of the feed for the rotifers in this experiment was the same for both feedings, but it is possible that the fatty acid content of the algae can vary in a 24 hour period.

Table 5: Essential Fatty Acid Changes in Rotifers During one Feeding Cycle of *Thalassiosira pseudonana* (clone 3H) and yeast

Time post feeding (hours)	DHA mg/g	EPA mg/g
24 (since previous feeding)	2.3 ± 0.1	10.2 ± 0.4 [e,f]
3	2.4 ± 0.0	9.7 ± 0.6 [e,f]
6	2.2 ± 0.2	9.5 ± 0.6 [e,f]
12	3.3 ± 1.5	9.5 ± 0.1 [e,f]
18	2.2 ± 0.1	7.6 ± 0.5 [a,b,c,d]
24	1.6 ± 0.1	6.4 ± 0.3 [a,b,c,d]

Time post feeding (hours)	DHA FA%	EPA FA%	Ratio DHA:EPA
24 (since previous feeding)	3.0 ± 0.1	13.6 ± 0.6 [e,f]	0.2 ± 0.6
3	3.3 ± 0.3	13.2 ± 0.3 [e,f]	0.2 ± 0.8
6	2.9 ± 0.1	12.3 ± 0.3 [f]	0.2 ± 0.4
12	3.7 ± 1.3	11.2 ± 1.6	0.3 ± 3.7
18	2.7 ± 0.1	9.3 ± 0.9 [a,b]	0.3 ± 0.7
24	2.2 ± 0.1	9.1 ± 0.1 [a,b,c]	0.2 ± 0.2

Superscripts: a= significantly different from first 24 hour samples, b= significantly different from 3 hours, c= significantly different from 6 hours, d= significantly different from 12 hours, e= significantly different from 18 hours, significantly different from second 24 hour sample.

6. Essential Fatty Acid content of rotifers for different size classes, enrichment conditions, and enrichment media

The treatment which resulted in the highest levels of DHA as a proportion of all fatty acids was the diet consisting of 3 algal species supplemented with yeast followed by enrichment with Algamac 2000 (Aquafauna Biomarine), in the 45-119 μm size class (Table 6). Rotifers that had been fed the mixed algal diet followed by enrichment with Algamac 2000 resulted in the highest ratio of DHA to EPA. The results of this experiment show that the algal diet of the rotifers prior to enrichment plays a role in conjunction with the enrichment in determining the final fatty acid profile of the rotifers. For example, the 120+ fraction of rotifers fed 3H and yeast and enriched with Algamac 2000 has a significantly lower DHA content than the 120+ fraction of rotifers fed the mixed algal diet and treated with the same enrichment. The different algal diets also result in differences in the EPA content of the rotifers enriched with Ratio HUFA Enrich (INVE Aquaculture), both as a proportion of all fatty acids and as a proportion of dry weight. The Ratio HUFA Enrich enrichment resulted in the highest dry weight of essential fatty acids. However, this enrichment, in a similar manner as DHA Selco, resulted in clumping of the rotifers, making it difficult to obtain a sample suitable for feeding to larval fish or for fatty acid analysis. Therefore, feeding rotifers a mixed algal diet, followed by enrichment with Algamac 2000 appears to be the technique which results in the diet most suitable for feeding to larval fish in this experiment. Algmac is made from *Schyzochytrium*, which is a heterotrophically grown unicellular fungus. Whole cells of *Schyzochytrium* are used for this enrichment rather than oils, as the case with the HUFA Enrich products. Algamac may be closer in structure to the natural algal

diet of rotifers, and may thus enrich rotifers by causing them to absorb essential fatty acids through their gut tract, rather than simply coating the outside of the animal. (McEvoy and Sargent, 1998)

Table 6: Rotifer Essential Fatty Acid Content after various Enrichments, and for different size classes

Rotifer Diet Prior to Enrichment	Enrichment	Size Class	DHA mg/g	EPA mg/g
3H+Yeast	Algamac 2000	120+	14.9 ± 1.0 [c,d,e,f,g,h,i,j]	14.2 ± 0.6 [d,g,h,i,j]
3H+Yeast	Algamac 2000	45-119	16.2 ± 1.2 [c,d,e,f,g,h,i,j]	11.7 ± 0.8 [e,g,h,i,j]
Mixed Algal Diet	Algamac 2000	120+	18.2 ± 0.3 [a,b,e,f,g,h,i,j]	12.2 ± 0.3 [g,h,i,j]
Mixed Algal Diet	Algamac 2000	45-119	20.2 ± 1.6 [a,b,e,f,g,h,i,j]	9.3 ± 0.9 [a,e,f,g,h,i,j]
3H+Yeast	Ratio HUFA Enrich	120+	28.3 ± 1.9 [a,b,c,d,i,j]	15.5 ± 3.0 [b,d,g,h,i,j]
3H+Yeast	Ratio HUFA Enrich	45-119	25.1 ± 1.8 [a,b,c,d,i,j]	13.4 ± 0.8 [d,g,h,i,j]
Mixed Algal Diet	Ratio HUFA Enrich	120+	33.2 ± 5.5 [a,b,c,d,i,j]	25.3 ± 0.7 [a,b,c,d,e,f,h,i,j]
Mixed Algal Diet	Ratio HUFA Enrich	45-119	28.8 ± 2.1 [a,b,c,d,i,j]	21.6 ± 1.4 [a,b,c,d,e,f,g]
3H+Yeast	Protein HUFA Enrich	120+	10.3 ± 1.3 [a,b,c,d,e,f,g,h,i]	19.3 ± 0.8 [a,b,c,d,e,f,g]
3H+Yeast	Protein HUFA Enrich	45-119	12.9 ± 0.4 [a,b,c,d,e,f,g,h,i]	20.8 ± 0.6 [a,b,c,d,e,f,g]

Rotifer Diet Prior to Enrichment	Enrichment	Size Class	DHA %FA	EPA %FA	Ratio
3H+Yeast	Algamac 2000	120+	12.3 ± 0.2 [d,e,f,i]	11.7 ± 0.2 [b,c,d,e,f,i,j]	1.1 ± 0.3 [d]
3H+Yeast	Algamac 2000	45-119	13.9 ± 0.3 [d,e,f,i,j]	10.0 ± 0.1 [a,d,e,f,i,j]	1.4 ± 0.4 [d]
Mixed Algal Diet	Algamac 2000	120+	14.2 ± 0.3 [d,i,j]	9.5 ± 0.5 [a,d,e,f,i,j]	1.5 ± 0.9
Mixed Algal Diet	Algamac 2000	45-119	17.3 ± 0.2 [a,b,c,g,h,i,j]	8.0 ± 0.3 [a,b,c,e,g,h,i,j]	2.2 ± 0.6 [a,b,h,j]
3H+Yeast	Ratio HUFA Enrich	120+	16.5 ± 1.6 [a,b,i,j]	8.9 ± 0.4 [a,b,c,d,g,h,i,j]	1.9 ± 1.9
3H+Yeast	Ratio HUFA Enrich	45-119	16.3 ± 0.1 [a,b,i,j]	8.7 ± 0.1 [a,b,c,g,h,i,j]	1.9 ± 0.2 [i]
Mixed Algal Diet	Ratio HUFA Enrich	120+	14.3 ± 1.1 [d,i,j]	11.0 ± 0.8 [d,e,f,i,j]	1.3 ± 1.9
Mixed Algal Diet	Ratio HUFA Enrich	45-119	14.5 ± 0.1 [d,i,j]	10.9 ± 0.1 [d,e,f,i,j]	1.3 ± 0.2 [d]
3H+Yeast	Protein HUFA Enrich	120+	9.1 ± 1.5 [a,b,c,d,e,f,g,h]	17.0 ± 1.6 [a,b,c,d,e,f,g]	0.5 ± 3.4
3H+Yeast	Protein HUFA Enrich	45-119	11.1 ± 0.4 [b,c,d,e,f,g,h]	17.8 ± 0.2 [a,b,c,d,e,f,g]	0.6 ± 0.7 [d,f]

Size classes: 120+: Rotifers which were retained in a sieve with a 120-µm mesh. All fecund rotifers were in this size fraction. 45-119: Rotifers which passed through the sieve with 120-µm mesh and were retained in a 45-µm mesh.

Mixed Algal Diet = 1/3 *T.gracile*, 1/3 *P.lutheri*, and 1/3 3H+Yeast.

Superscripts: a= significantly different from 3H +Yeast, Algamac 2000, 120+, b= significantly different from 3H +Yeast, Algamac 2000,45-119, c= significantly different from mixed algal diet, Algamac 2000, 120+, d= significantly different from mixed algal diet, Algamac 2000, 45-119, e= significantly different from 3H+ Yeast, Ratio HUFA Enrich, 120+, f= significantly different from 3H+ Yeast, Ratio HUFA Enrich, 45-119, f= mixed algal diet, Ratio HUFA Enrich, 120+, g= significantly different from mixed algal diet, Ratio HUFA Enrich, 120+, h= significantly different from mixed algal diet, Ratio HUFA Enrich, 45-119, i= significantly different 3H+Yeast, Protein HUFA Enrich, 120+, 3H+Yeast, Protein HUFA Enrich, 45-119.

7. Effect of Algal diet on the Essential Fatty Acid profiles of Rotifers

Diet significantly affected the essential fatty acid profile of rotifers (Table 7). T-Iso resulted in the highest level of DHA as a proportion of dry weight, and was significantly different from all other algal species tried. *Chaetoceros gracilis* resulted in the highest EPA content as a proportion of dry weight, and was significantly different from all other species tried. T-Iso resulted in the highest level of DHA as a proportion of all fatty acids, and was significantly different from all other trials except the unfed control. *Pavlova lutheri* resulted in the highest EPA content as proportion of all fatty acids of the algal species tried, and was significantly different from all algal species other than *Chaetoceros gracilis*. The high level of DHA in the unfed rotifers is due to the final feeding of T-Iso they received prior to the 4-day water regimen undergone before sampling. This indicates even after a 4-day starvation period, rotifers do not preferentially metabolize DHA. This result agrees with the results of rotifer essential fatty acid retention experiment, which showed that over a 12-hour period DHA content in unenriched rotifers does not change significantly. The ratio of DHA to EPA was highest in T-Iso fed rotifers, and was significantly different from all other species tried. This experiment showed that T-Iso is the best diet for producing rotifers that have a high DHA content and a high ratio of DHA to EPA. This experiment did not include an evaluation of dietary effects on rotifer growth rates, but previous studies have shown that different algal species have little effect on growth rate, and that culture temperature is much more critical in determining growth rate (Scott and Baynes, 1978).

Table 7: Effect of Algae on Rotifer Essential Fatty Acids

	DHA mg/g	EPA mg/g
Chaetoceros gracilis	11.0 ± 0.7 [b,c,d,e]	11.2 ± 0.7 [b,c,d,e]
Tahitian *Isochrysis*	14.4 ± 0.9 [a,c,d,e]	3.5 ± 0.5 [a,e]
Tetraselmis gracile	4.6 ± 0.0 [a,b]	8.0 ± 0.4 [a,d,e]
Pavlova lutherii	3.3 ± 0.3 [a,b,e]	4.1 ± 0.2 [a,c,e]
Unfed	5.9 ± 0.5 [a,b,d]	2.6 ± 0.2 [a,b,c,d]

	DHA Area %	EPA Area %	Ratio DHA:EPA
Chaetoceros gracilis	9.5 ± 0.4 [a,b,c,e]	9.7 ± 0.5 [b,c,e]	1.0 ± 0.4 [b,e]
Tahitian *Isochrysis*	13.3 ± 0.2 [a,.c,d]	3.2 ± 0.2 [a,c,d,e]	4.2 ± 0.2 [a,c,d,e]
Tetraselmis gracile	3.9 ± 0.2 [a,b,d,e]	6.7 ± 0.0 [a,b,d]	0.6 ± 0.2 [b,e]
Pavlova lutherii	8.5 ± 1.4 [b,c,e]	10.5 ± 1.3 [b,c,e]	0.8 ± 1.7 [b,e]
Unfed	13.0 ± 0.4 [a,c,d]	5.6 ± 0.2 [a,b,d]	2.3 ± 0.2 [a,b,c,d]

Superscripts: a= significantly different from *Chaetoceros gracilis*, b= significantly different from Tahitian *Isochrysis galbana*, c= significantly different from *Tetraselmis gracile*, d= significantly different from *Pavlova lutheri*, e= significantly different from water control

8. Effect of Algal diet on the essential fatty acid content of *Tisbe*

As with rotifers, algal diet can affect copepod fatty acid content (Table 8). *Tisbe* unfed for one week have a large decrease in total fatty acid content, and are significantly different in dry weight content of DHA and EPA when compared to copepods fed any species of algae. Similarly, starved copepods show a large reduction of essential fatty acids as a percentage of total fatty acids when compared to fed copepods. This indicates that copepods either preferentially metabolize essential fatty acids when they are in short supply, or they do not synthesize them during starvation. The presence of DHA in copepods fed *T. gracile* means that copepods have the ability to synthesize DHA *de novo*, since *Tetraselmis* has very little or no DHA (Volkman *et al.*, 1989, Reitan *et al.*, 1997)

Tisbe with the highest essential fatty acid content are of the greatest interest in aquaculture. *C. gracilis, Pavlova lutheri*, and T-Iso all produce copepods with high amounts of essential fatty acids. However, as noted earlier, *Tisbe* fed *Pavlova lutheri* do not grow rapidly or reach a high population. *C. gracilis* and T-Iso both resulted in dense populations of copepods, but T-Iso always resulted in less detrital matter accumulating in the tank, and therefore produced less work in cleaning tanks. Further, the ratio of DHA to EPA in the *C. gracilis* fed copepods is low, and therefore T-Iso seems to be the best algal species to feed to copepods which will be used as fish food.

Table 8: Effect of Algal Diet on Essential Fatty Acids of *Tisbe*

	DHA mg/g		EPA mg/g	
Chaetoceros gracilis	19.0 ± 4.9	c,e	28.7 ± 4.6	b,c,d,e
Tahitian *Isochrysis*	14.5 ± 5.6	e	4.5 ± 1.9	a,c,d,e
Tetraselmis gracile	8.6 ± 1.6	a,d	8.2 ± 1.0	a,b,d,e
Pavlova lutherii	19.6 ± 3.6	a,b,d	11.8 ± 0.8	a,b,c,d
Unfed	0.5 ± 0.3	c,e	0.2 ± 0.1	a,b,c,e

	DHA Area %		EPA Area %		Ratio DHA:EPA
Chaetoceros gracilis	11.1 ± 3.3	b,d,e	16.8 ± 1.9	b,c,e	0.7 ± 5.7
Tahitian *Isochrysis*	28.1 ± 1.4	a,c,e	8.6 ± 1.3	a,d,e	3.3 ± 3.7
Tetraselmis gracile	7.5 ± 0.2	b,d	7.1 ± 0.4	a,d,e	1.1 ± 0.6
Pavlova lutherii	30.7 ± 2.2	a,b,d	18.6 ± 0.9	a,b,c,d	1.7 ± 3.0
Unfed	4.6 ± 3.3	a,c,e	2.2 ± 1.6	b,c,e	2.1 ± 5.0

Superscripts: a = significantly different from *Chaetoceros gracilis*, b = significantly different from T-Iso. c= significantly different from *Tetraselmis gracile*, d = significantly different from *P.lutheri*, e = significantly different from water control.

9. Effect of growth conditions and algal diet on the essential fatty acid content of *Tisbe*

As with the algal diet experiment on copepods, the results of the mass culture experiment show that essential fatty acid content is dependent on algal diet (Table 9). All but one of the copepod trials in the mass culture experiment were sampled after 2 weeks. After two weeks, a copepod population contains all size classes, including adults. The gravid females contribute most of the dry weight to the samples, and therefore cause the essential fatty acid content of the entire population to be higher than a population of copepodites only. The diet which resulted in copepods with the highest essential fatty acid content was a mixture of *Nannochloropsis* paste and Culture Selco. This diet is also the easiest to prepare, because it does not require live algae. However, this diet can cause rapid fouling of the tank, and extra husbandry procedures may be required. The mixture of Culture Selco and *Nannochloropsis oculata* paste contains no live cells, and it tends to settle in the tank rapidly. This causes detrital material to accumulate much more rapidly in *Tisbe* cultures fed *Nannochloropsis oculata* paste and Culture Selco than cultures fed a live algal diet.

The experiment on the mass culture of copepods included four comparisons of copepods fed T-Iso under different conditions. The four T-Iso groups were: 14 days at 18°C, 7 days at 18°C, 14 days at 12°C and 14 days at 18°C followed by grading between 120 and 200μm sieves. Copepods fed T-Iso for 14 days and for 7 days were not found to be different in essential fatty acid content in any of the values measured. This indicates that

a population of mixed nauplii and copepodites (the 7 day old culture) has the same fatty acid profile as a population containing all size classes (the 14 day old culture). However, copepods graded between 120 and 200μm had consistently lower DHA content, indicating that most of the essential fatty acids in the *Tisbe* population are contained either in the adults or in nauplii, not copepodites (Table 9). The *Tisbe* culture grown at 12°C had consistently lower essential fatty acid content than those grown at 18-20°C in all the values measured. *Tisbe* fed T-Iso for7 days, followed by a starvation period of 7 days at 4^0C had high values DHA and EPA. It is possible that at low temperatures *Tisbe* do not metabolize long-chain fatty acids, and instead use them for structural purposes in order to maintain membrane fluidity in their cells.

Another useful comparison is copepods fed T-Iso over 7 days in this experiment to copepods fed T-Iso for 7 days in the copepod algal diet experiment. The major difference in these groups was culture conditions, as algal type and density were the same. A one-way ANOVA was done to compare all the measurements for a 7 day old culture of T-Iso fed copepods, and there was no significant difference ($p=0.05$) between the copepods in the this and the algae experiment.

One can also compare the three groups of copepods fed T-Iso, *P.lutheri*, and *T. gracile* for 14 days in this experiment to copepods fed the same algae for 7 days in the algae experiment. A one-way ANOVA was done to compare the samples, and no significant difference was found between groups in the protocol experiment and groups fed the same

algae in the algae experiment. These results indicate that the fatty acid profile of the copepods is determined by the algae they graze upon, not their growth conditions.

Table 9: Essential Fatty Acid Content of *Tisbe* Grown on Production Scale

	DHA mg/g		EPA mg/g	
Standard feed of T-Iso for 14 days.	15.0 ± 1.0	[j]	4.7 ± 0.5	c,d,e,g,h,i
Standard feed of T-Iso for 7 days	15.5 ± 0.8	[j]	5.6 ± 0.5	c,d,e,f,g,i
7 day feed T-Iso, starved 7 day 4 °C	22.8 ± 1.5	d,f,g,i,j	7.7 ± 0.6	a,b,f,g,h,i,j
Tetraselmis gracile for 14 days.	13.3 ± 0.3	c,e,g,i	11.7 ± 0.2	a,b,f,g,i,j
Pavlova lutheri for 14 days	22.6 ± 5.3	d,f,g,i,j	11.6 ± 2.1	a,b,f,g,i
Culture Selco for 14 days	9.5 ± 2.3	c,e,i	5.2 ± 1.0	c,d,e,g,h,i
T-Iso and constant temperature of 12°C	7.0 ± 0.8	c,d,e,i	3.3 ± 0.4	a,b,c,d,e,f,h,i,j
Nannochloropsis oculata paste for 14 days	8.1 ± 3.0	i	8.3 ± 0.9	a,c,f,g,i
Nannochloropsis oculata paste and Culture Selco	24.2 ± 0.3	a,b,c,d,e,f,g,h,j	10.1 ± 0.2	a,b,c,d,f,g,i
T-Iso graded 120-200 µm	4.9 ± 0.9	a,b,c,e,i	4.0 ± 0.7	c,d,e,g,h,i

	DHA Area %		EPA Area %		Ratio DHA:EPA	
Standard feed of T-Iso for 14 days.	29.0 ± 2.6	c,d,f,g,h,i,j	9.3 ± 0.0	c,e,h,i,j	3.1 ±	1.8
Standard feed of T-Iso for 7 days	24.7 ± 2.0	c,d,f,g,h,i,j	8.9 ± 1.2	c,e,h,i,j	2.8 ±	3.7
7 day feed T-Iso, starved 7 days at 4 °C	37.7 ± 0.6	a,b,d,f,g,i,j	12.8 ± 0.2	a,b,d,e,f,i	3.0 ±	0.8
Tetraselmis gracile for 14 days.	9.6 ± 0.8	a,b,c,e,f,i,j	8.5 ± 0.5	c,e,g,h,i,j	1.1 ±	1.2
Pavlova lutheri for 14 days	31.8 ± 3.5	d,f,g,i,j	16.4 ± 0.9	a,b,c,d,f,g	1.9 ±	3.9
Culture Selco for 14 days	17.2 ± 3.1	a,b,c,d,e,i	9.4 ± 1.1	c,e,h,i,j	1.8 ±	4.0
T-Iso and constant temperature of 12°C	14.7 ± 0.9	a,b,c,e,i	7.0 ± 0.3	c,e,h,i,j	2.1 ±	1.1
Nannochloropsis oculata paste for 14 days	14.0 ± 4.4	a,b,i	14.3 ± 1.0	a,b,d,f,g	1.0 ±	5.4
Nannochloropsis oculata paste and Culture Selco	39.1 ± 3.3	a,b,c,d,e,f,g,h,j	16.2 ± 1.2	a,b,c,d,f,g	2.4 ±	4.4
T-Iso graded 120-200 µm	18.4 ± 1.2	a,b,c,d,e,i	14.8 ± 1.2	a,b,d,f,g	1.2 ±	2.4

Superscripts: a = significantly different from Standard feed of T-Iso for 14 days. b = significantly different from standard feed of T-Iso for 7 days. c = Significantly different from 7 day feed, 7-day starve at 4°C. d = significantly different from *Tetraselmis gracile* for 14 days. e = significantly different from *Pavlova lutheri* for 14 days. f = Significantly different from Culture Selco for 14 days. g = Significantly different from T-Iso at 12°C. h = Significantly different from *Nannochloropsis oculata* paste for 14 days. i = significantly different from *Nannochloropsis oculata* paste and Culture Selco for 14 days. j = significantly different from T-Iso graded between 120 and 200µm sieves.

10. Essential Fatty Acid Retention of *Artemia*

The results of the *Artemia* fatty acid retention experiment show that unenriched *Artemia* change little in essential fatty acid content over a 12- hour period in 9°C salt water (Table 10). The only significant difference noted was in dry weight content of EPA between the 0 and the 12-hour samples. Unenriched *Artemia* had no DHA, and so the ratio of DHA to EPA remained at 0 throughout the 12-hour starvation period.

For Algamac enriched *Artemia*, significant differences over time were noted only in EPA as a percentage of all fatty acids. After 8 hours there was a significant increase in the amount of EPA as a proportion of all fatty acids. Since there is no significant change in the dry weight content of the *Artemia* over this same period, it means that fatty acids other than EPA and DHA are metabolized preferentially during a starvation period of 12 hours. There are no significant changes in the ratio of DHA to EPA for the first 8 hours of starvation, but there is a significant drop in the ratio between 8 and 12 hours. This indicates that after 8 hours, Algamac-enriched *Artemia* begin to lose DHA at a slightly faster rate than EPA. However, even after 12 hours post-enrichment with Algamac, the ratio of DHA to EPA is still 1.44:1, which is much higher than *Artemia* enriched with paste or paste and Super Selco, even before starvation.

Over time, *Artemia* enriched with Aquagrow also changed significantly in essential fatty acid content. After 8 hours, there is a significant decrease in the amount of DHA as a proportion of dry weight. Over this same period, there is no change in DHA as a proportion of all fatty acids. These two results indicate that DHA is not preferentially

metabolized over EPA in Aquagrow enriched *Artemia*. Conversely, the EPA level as proportion of dry weight is significantly different after only 4 hours of starvation, and there are no significant differences in EPA as a proportion of all fatty acids over the entire 12-hour starvation period. As with the Aquagrow enrichment, the amount of EPA as a proportion of all fatty acids increases over time. Finally, the ratio of DHA to EPA decreases significantly after only 4 hours of starvation, due to the increasing proportion of EPA and the decreasing proportion of DHA.

Table 10: Enriched *Artemia* Essential Fatty Acid Loss

Time	DHA mg/g	EPA mg/g
Unenriched		
0	0.1 ± 0.2	5.1 ± 0.2 [d]
4	0.0 ± 0.0	3.8 ± 0.4
8	0.0 ± 0.0	4.2 ± 0.9
12	0.0 ± 0.0	3.7 ± 1.0 [a]
Algamac		
0	14.6 ± 1.1	8.0 ± 0.7
4	12.1 ± 3.6	6.9 ± 1.7
8	11.3 ± 1.4	6.8 ± 1.1
12	9.9 ± 1.2	6.9 ± 1.0
Aquagrow		
0	25.4 ± 2.6 [c,d]	8.0 ± 0.6
4	20.6 ± 1.6	7.0 ± 0.5
8	18.0 ± 3.5 [a]	6.5 ± 1.3
12	16.6 ± 1.1 [a]	6.4 ± 0.6

Time	DHA area %	EPA area %	Ratio DHA:EPA
Unenriched			
0	0.1 ± 0.2	3.7 ± 0.1	0.0 ± 3.3
4	0.0 ± 0.0	4.2 ± 0.3	0.0 ± 0.0
8	0.0 ± 0.0	4.4 ± 0.6	0.0 ± 0.0
12	0.0 ± 0.0	3.9 ± 0.1	0.0 ± 0.0
Algamac			
0	8.3 ± 0.1	4.6 ± 0.1 [d,c]	1.8 ± 0.2
4	8.8 ± 0.7	5.0 ± 0.2	1.8 ± 0.9
8	8.7 ± 0.5	5.2 ± 0.2 [a]	1.7 ± 0.7 [d]
12	7.6 ± 0.1	5.2 ± 0.1 [a]	1.4 ± 0.3 [c]
Aquagrow			
0	12.9 ± 0.6	4.1 ± 0.1 [b,c,d]	3.2 ± 0.5 [a]
4	12.9 ± 0.3	4.4 ± 0.0 [a,d]	2.9 ± 0.3 [b]
8	12.7 ± 0.3	4.6 ± 0.1 [a]	2.8 ± 0.4
12	12.1 ± 0.4	4.7 ± 0.1 [a,b]	2.6 ± 0.4

Superscripts: within each enrichment trial, a = significant difference from time 0, b = significant difference from 4 hours, c= significant difference from 8 hours, and d = significant difference from 12 hours.

Effect of enrichments on *Artemia* Essential Fatty Acid Content

None of the results showed any significant difference between Algamac 3010 Flake and Aquagrow. *Artemia* prepared with these two enrichments were significantly higher in DHA content (both as percentage of total fatty acids and as a proportion of dry weight) compared to DC DHA Selco enriched *Artemia* (Table 11*)*. Only Algamac 3010 Flake enriched *Artemia* were significantly different in EPA content than DC DHA Selco enriched *Artemia*, and only as a percentage of total fatty acids. The ratio of DHA to EPA was significantly different in both the Aquagrow and Algamac Flake 3010 enriched *Artemia* compared to DC DHA Selco enriched. These results indicate that the enrichments mostly affect the DHA content of *Artemia*. Also, because of low DHA content and a low ratio of DHA to EPA, DC DHA Selco enriched *Artemia* are not a nutritious diet for larval sablefish. In a similar manner as rotifers enriched with Algamac, the higher retention of DHA in *Artemia* enriched with Aquagrow and Algamac 3010 Flake when compared to the DC DHA Selco enrichment may be due to the content of whole cells of *Schyzochytrium* in both Aquagrow and Algamac 3010 Flake. It is possible that presenting whole cells to *Artemia* rather than emulsified oil allows them to absorb and retain essential fatty acids more efficiently.

Table 11: Effect of Enrichments on *Artemia* Essential Fatty Acid Content

	DHA mg/g	EPA Mg/g
DC DHA Selco	2.5 ± 0.7 [b,c]	3.5 ± 0.9
Algamac 3010 Flake	21.0 ± 3.5 [a]	9.2 ± 1.9
Aquagrow	22.6 ± 2.9 [a]	6.6 ± 1.9

	DHA Area %	EPA Area %	Ratio DHA:EPA
DC DHA Selco	1.9 ± 0.2 [b,c]	2.7 ± 0.1 [b]	0.7 ± 0.3 [b,c]
Algamac 3010 Flake	12.7 ± 0.5 [a]	5.8 ± 0.6 [a]	2.4 ± 1.3 [a]
Aquagrow	12.3 ± 0.5 [a]	3.6 ± 0.8	3.6 ± 2.0 [a]

Superscripts: a significant difference from DC DHA Selco, b = significant difference from Algamac 3010 Flake, c = significant difference from Aquagrow.

12. Essential Fatty Acid Content of *Artemia* Enriched with *Nannochloropsis oculata*/*Schizochytrium* Paste Followed by Super Selco

After one day of enrichment with *Nannochloropsis oculata* and *Schyzochytrium* paste alone, *Artemia* have significantly higher levels of DHA, both as a proportion of dry weight and as a percentage of total fatty acid (Table 12). The one-day enrichment with the paste has no significant effect on the EPA content of *Artemia*. A second day of enrichment with Super Selco results in *Artemia* with significantly higher levels of DHA when compared to both unenriched and one-day paste-enriched *Artemia*. EPA levels are also significantly increased after the Super Selco enrichment. It is possible that the high levels of EPA in the two-day-old *Artemia* may be due to the higher level of digestive tract development in these animals, in comparison to one-day-old Artemia used in all other experiments presented in this thesis.

Table 12: Essential Fatty Acid Content of *Artemia* Enriched with *Nannochloropsis oculata/Schizochytrium* Paste Followed by Super Selco

	DHA mg/g	EPA mg/g
Freshly hatched Nauplii	0.0 ± 0.0 [b,c]	4.9 ± 0.6 [c]
1 Day paste Enriched	1.3 ± 0.3 [a,c]	5.4 ± 0.5 [c]
1 Day paste Enriched +1 Day DC Super Selco	20.0 ± 5.1 [a,b]	36.4 ± 8.3 [a,b]

	DHA % FA	EPA %FA	Ratio DHA :EPA
Freshly hatched Nauplii	0.0 ± 0.0 [b,c]	3.2 ± 0.6 [c]	0.0 ± 0.0
1 Day paste Enriched	1.2 ± 0.3 [a,c]	5.2 ± 0.5 [c]	0.2 ± 1.1
1 Day paste Enriched +1 Day DC Super Selco	10.3 ± 1.3 [a,b]	18.7 ± 1.8 [a,b]	0.5 ± 3.1

Superscripts: a = significant difference from freshly hatched nauplii, b = significant difference from 1 day paste enriched, c = significant difference form 1 day paste enriched + 1 day DC Super Selco.

13. Comparison of Total Fatty Acid Contents in Live feed

In order to compare the total fat content of various live feeds for larval sablefish, the individual weights of each fatty acid in a whole fatty acid profile were added. The result is the total mass of all fatty acids per gram of sample. Though this value is not a true measure of total lipid content, it can be used to determine how much fat is delivered to a larval fish for each prey item it consumes.

Table 13: Comparison of Total Fatty Acid Contents in Live feed

Diet	Total weight of fatty acids (mg/g)
Artemia 1	174.8 ± 13.2 ^{c,d,e,f}
Artemia 2	182.9 ± 16.9 ^{c,d,e,f}
Rotifers 1	77.8 ± 19.3 ^{a,b}
Rotifers 2	107.0 ± 4.4 ^{a,b,e,f}
Tisbe 1	50.8 ± 5.9 ^{a,b,d}
Tisbe 2	63.2 ± 1.6 ^{a,b,d}

Results are expressed as averages of triplicates ± standard deviation.
Artemia 1: Enriched with Algamac 3030 Flake (fatty acid retention experiment, time 0)
Artemia 2: Enriched with Aquagrow (fatty acid retention experiment, time 0)
Rotifer 1: Enriched with Algamac 3010 Flake (fatty acid retention experiment, time 0)
Rotifer 2: Enriched with Super Selco (fatty acid retention experiment, time 0)
Copepod 1: Fed 14 days with T-Iso (Copepod production experiment)
Copepod 2: Fed 7 days with T-Iso (Copepod algal diet experiment)
Superscripts: a= significant difference from *Artemia* 1, b= Significant difference from *Artemia* 2, c= significant difference from Rotifer 1, d= significant difference from Rotifer 2, e= significant difference from Copepods 1, f= significant difference from Copepods 2.

14. Essential Fatty Acid Content of Sablefish Eggs and Larval Sablefish

Whole sablefish larvae and eggs were analysed for essential fatty acid content (Table 14). The eggs were sampled immediately after fertilization, and the larvae were sampled one day before their first feeding. Significant differences were found in the EPA content of sablefish larvae and eggs in both dry weight and as a proportion of all fatty acids. DHA content was only significant in sablefish eggs and larvae when measured as a proportion of all fatty acids. As sablefish eggs develop into larvae, there is a reduction in the amount of EPA present when the whole animal is sampled, and a relative increase in the amount of DHA. These changes may be due to an increased reliance on DHA for structural, rather than energy, purposes, and a concurrent increased reliance on EPA for energy purposes.

Table 14: Essential Fatty Acid Content of Sablefish Eggs and Larval Sablefish

	DHA mg/g	EPA mg/g	
Eggs	23.0 ± 5.5	11.8 ± 2.8 [a]	
Sablefish Larvae	24.4 ± 1.7	6.9 ± 0.9 [b]	
	DHA %FA	EPA %FA	Ratio DHA:EPA
Eggs	28.1 ± 0.1	14.4 ± 0.1 [a]	2.0 ± 0.2
Sablefish Larvae	31.9 ± 1.3	9.0 ± 0.4 [b]	3.5 ± 1.7

Results are expressed as averages of triplicates ± standard deviation.

Superscripts: a= significant difference from sablefish eggs, b= significant difference from larval sablefish.

Chapter 4: Discussion

The most challenging aspect of using fatty acid analysis to determine the nutritional quality of larval feeds is the interpretation of which aspects of the fatty acid results are most important. The procedures and enrichments studied in this work were all designed to increase the level of two essential fatty acids in larval feeds: docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Adequate amounts of these two essential fatty acids have long been known to be needed in larval fish diets (Watanabe, 1993). More recently, arachidonic acid (20:4n6, ARA) has also been determined to be an essential fatty acid for larval marine fish. Arachidonic acid, as well as EPA, is a known precursor of eicosanoid hormones such as prostaglandins and leukotrienes, in larval fish, but the dietary requirements of ARA in larval feed is not as well understood as those for DHA and EPA (reviewed by Sargent, McEvoy, and Bell, 1997; Estéves, *et al* 1998, Sargent *et al.*, 1999). Commonly used live feeds for larval fish do not normally have enough of these essential fatty acids to sustain the larvae, which is why techniques and products have been developed to improve the fatty acid content of live feeds (Harel and Place, 1998; McEvoy, and Sargent, 1998).

The natural diet of larval sablefish cannot be reproduced in an aquaculture setting. To solve this problem larvae are fed cultured live prey that have been manipulated in some way to enhance their fatty acid content. Efficient ways of growing live food and delivering essential fatty acids to larval fish in the correct amounts is the main goal of live feed production. However, different larval fish diets deliver these essential fatty acids

to larval fish in different ways, and determining which routes are most important to the larval fish is critical in choosing the right diet to ensure maximum survival and growth.

Fatty acid data are presented in this work in two ways: individual essential fatty acids as a proportion of all fatty acids (FA%), and individual fatty acids as a proportion of dry weight (mg fatty acid/gram dry weight). The first measure indicates the quality of the fatty acids present in the feeds. In general, the goal in the production of live feeds for marine finfish is to maximize this amount (Harel and Place, 1998; McEvoy and Sargent 1998; Rosenlund, *et al* 1997; Gapasin *et al,* 1998). The second measure indicates how much of a fatty acid is delivered to a larval fish for each food particle or prey it consumes; it is a measure of the efficiency of the food in delivering essential fatty acids to larvae. When producing live feed for larval fish, maximizing these values is important, but the effects on food quality as a whole must be considered. Some enrichment procedures increase the total dry weight fat content in zooplankton. An increase in fat as a proportion of dry weight indicates that the proportion of other nutrients, such as essential amino acids, has decreased.

When deciding which diet to feed to larval fish, one must consider not only the dry weight of essential fatty acids, but also the total fat content. Ideally, a zooplankton enrichment should increase only the essential fatty acid dry weight content, not the total dry weight fat content. Such a diet would result in food that has a high content of essential fatty acids as a proportion of all fatty acids (%FA), high dry weight content of

essential fatty acids (mg/g), and low overall dry weight of all fatty acids. Designing a procedure that results in a diet to meet these criteria was one of the goals of this study.

This work did not include an analysis of the total lipid content of live feeds. However, all the fatty acid analyses permitted calculation of a dry weight amount of each individual fatty acid. For the sake of simplicity, only the dry weights of essential fatty acids have been discussed. However, by adding the dry weight of each fatty acid in any given sample, an indication of total lipid can be determined. This measure (total dry weight of fatty acids) can then be used to compare total fat content between live feeds. In Table 13, the total dry weight of all fatty acids was presented for three live feeds studied in this work.

Table 13 shows that in general, enriched *Artemia* have the highest dry weight of total fatty acids, followed by rotifers and then *Tisbe*. However, there were no significant differences between rotifers enriched with Algamac 3010 Flake and either of the copepod diets. Enriched *Artemia* deliver more fatty acid to larval fish than do copepods or rotifers. What effect this may have on larval sablefish is undetermined, but the high fatty acid content of *Artemia* must be taken into consideration when determining when sablefish should be weaned from rotifers onto *Artemia*, and what role a supplementary diet of *Tisbe* can have during the weaning process. Copepods, because they are unenriched, may be considered a more natural diet than *Artemia* for larval fish. The significant difference in total fat content between *Artemia* and copepods indicates that enriched *Artemia* may deliver more than optimal levels of fat to larval fish.

The best way to determine the ideal diet for larval fish is to run an experiment on a series of larval fish fed different diets, and then correlate survival rates to different diets. However, this is not easily done. There are many factors which contribute to the survival of larval fish; diet is only one of them. A feeding trial experiment was attempted in this study, but failed to yield useful data. The larvae were kept in an array of nine 4-liter beakers, three for each of three diets being evaluated. However, the small containers rapidly become infected with ciliates, and all larval fish died before useful data could be obtained. A valid feeding trial on sablefish cannot be done with small containers. To perform a structured feed-comparison, an array of full-sized larval rearing tanks (up to 4000l) should be used. Large tanks appear to be much better suited for rearing larval sablefish. This may be due to the higher volume to surface area ratio of larger tanks, or because of better stability of temperature and water quality, in comparison to smaller containers. However, an experiment involving feeding various diets to larval fish in large tanks was beyond the scope of this study.

An alternative to using feeding trials to determine the ideal diet for larval sablefish is to examine what larval fish eat in the wild, and then attempt to manipulate cultured live feeds to emulate the wild diet fatty acid profile. In the wild, larval sablefish are known to eat calanoid copepod nauplii (Grover and Olla, 1990). At the time of this study, a local species of calanoid copepod nauplii was not available, but studies have examined the possibility of wild-caught zooplankton as potential food for cultured marine larvae (Næss *et al*, 1995; McEvoy *et al*, 1998). Also, because of the seasonal variability of the nutritional quality of wild *calanoid* copepods (Norrbin *et al*, 1990), it would be difficult

to determine if a wild-caught *calanoid* was really the same species a sablefish larvae would select in natural conditions. Hatchery-grown sablefish larvae are usually feeding on copepod-sized particles in May and June, and wild sablefish larvae on the coast of British Columbia are usually feeding on the same particle size in April (Mason *et al.* 1983; Shenker and Olla, 1986; Grover and Olla, 1987).

Harpacticoid copepods are a potential alternative to calanoid copepods, and can be grown in captivity. The copepod used in this study was a local species of the harpacticoid *Tisbe sp.* This species has been cultured at the Pacific Biological Station for over 4 years, and has over that time has shown signs of becoming domesticated. In general, cultured *Tisbe* appear to be becoming more pelagic in nature over successive generations. This is significant for two reasons: first, pelagic behavior means the *Tisbe* do not necessarily need high levels of substrate in order to reach a high population, and in general are easier to culture. Secondly, the pelagic behavior in *Tisbe* means that larval fish do not have to approach the sides of a larval rearing tank in order to prey upon *Tisbe*, which closely emulates the sablefish larvae's natural, pelagic environment. While empirical evidence was found that larval sablefish would consume *Tisbe*, no data were obtained to suggest how *Tisbe* affected larval sablefish survival. It has been shown, however, that *Tisbe* can improve the growth rate of haddock and plaice, although some detrimental effects on survival have also been observed (Nanton and Castell, 1998). It is also known that wild zooplankton consisting mostly of copepods can be beneficial by improving fish pigmentation (McEvoy *et al*, 1998). *Tisbe* are benthic in nature: they do not stay in the water column for very long, and may therefore not be available to fish larvae in the larval

rearing tank (Nanton and Castell, 1998). This underscores one of the main problems of using copepods as feed for larval fish: making them available for larval consumption. One potential solution is to place them in a mesh-bottom tray which floats in the larval rearing tanks, allowing a slow, constant supply of small nauplii to the larvae (Kahan *et al*, 1982).

The fatty acid content of cultured harpacticoids is variable. Both salinity and diet can affect the final size and fecundity of female *Tisbe holothuriae*, which in turn will affect how much essential fatty acid is delivered to larval fish for each copepod consumed (Miliou, 1996; Norsker and Støttrup, 1994).This study showed algal diet has a direct influence on the fatty acid profile of copepods, and this result is similar to the conclusions of Nanton and Castell (1999). These factors indicate the need to have a consistent procedure when growing *Tisbe* in a hatchery for feeding to marine finfish larvae. This study has demonstrated that either T-Iso or a dry diet consisting of Culture Selco and *Nannochloropsis* paste are suitable for the culture of *Tisbe* for feeding to larval fish.

There is considerable evidence to show that copepods and specifically *Tisbe* have potential use in aquaculture (Kahan *et al*, 1982; Nanton *et al*, 1996; Støttrup, and Norsker, 1997; Nanton and Castell, 1998; Nanton, and Castell 1999). One of the main challenges to incorporating them into a larval feeding regime is that it is currently impractical to grow them in the quantities needed by most hatcheries (Kahan *et al*, 1982). At current production levels, it is not practical to use copepods as the only food for marine fish larvae. However, if a time frame can be identified when a highly nutritious diet is most critical to larval development, *Tisbe* may then be offered. (Støttrup *et al*,

1998). Determining this time frame will be a matter of experimentation and will probably be different for different species of fish. *Tisbe* has the potential to be a practical highly nutritious dietary supplement for larval sablefish, once the appropriate time frame for offering this species to larvae is determined.

Another way to determine what dietary essential fatty acid content a larval fish requires is to examine the fatty acid profiles of eggs and yolk-sac larvae. One may speculate that the ideal diet for a larval fish as its first exogenous nutrient supply should closely match its endogenous food supply. To investigate this, fertilized eggs and whole larvae were analyzed for fatty acid content, and the results summarized in Table 14.

The larvae were sampled two days after ponding, one day before they were fed for the first time. It has been shown with gilthead seabream larvae that essential fatty acids, especially DHA, are needed at this stage for structural rather than energy purposes (Rodriguez *et al*, 1994). One would therefore expect the fatty acid profile of whole larvae to reflect the actual amount required in their diet, as the effect of larvae metabolizing essential fatty acids for energy purposes would be minimal. For the larval sablefish sampled here, there are significant differences in the fatty acid profiles of larval sablefish and sablefish eggs. EPA content of sablefish is reduced as they develop from eggs to larvae, while DHA content remains the same when measured as a proportion of dry weight. This preferential retention of DHA also indicates that it is the more critical essential fatty acid for structural purposes as larvae develop from eggs to larvae (Koven, *et al.* 1993). Ideally, the yolk sacs alone would have been sampled, as this would have

been a good indication of the correct larval fish nutritional requirements at the start of feeding. However, these results from whole larvae samples, combined with wild calanoid copepod nauplii data, can be used to indicate the composition of the natural larval sablefish diet.

The results of the enrichment experiments in this study indicate that Aquagrow and Algamac enrichments result in rotifers that are best suited for feeding to larval fish. It should be noted, however, that the enrichment that produced the rotifer with the highest DHA content, Algamac 3010 Flake, caused mortality of rotifers when used according to the manufacturer's instructions.

Procedures for growing rotifers are well established. The standard diet for rotifers is either yeast alone, or a combination of yeast and algae (Walford and Lam, 1992). Yeast does not contribute to the essential fatty acid content of rotifers, but it can greatly increase the population density (Nagata and Whyte, 1992). There are some problems when using yeast, such as higher level of detritus accumulating in culture tanks. Addition of yeast to a semi-continuous rotifer culture causes clumps of yeast and algae to form in the water, and these are difficult to remove as they are similar in particle size to rotifers. For this reason a batch approach is used for the production of large quantities of rotifers that are fed yeast (Walford and Lam, 1992).

As larval sablefish grow, they are weaned from a diet of rotifers to a diet of *Artemia*. Sablefish larvae are weaned in this way because they require a larger food particle size as

they grow, and because *Artemia* are much easier to produce than rotifers. In general, *Artemia* have a higher fat content than rotifers, which is why it is important to not wean the larvae onto *Artemia* before they are able to survive on this diet. In general, enriched *Artemia* are a poorer diet for larval fish than enriched rotifers because of higher total fat content, and a high content of indigestible material (reviewed by Rainuzzo *et al*, 1997). Conversely, *Artemia* have potential to be a vehicle for delivering therapeutics to larval fish (Sureyya and Chu, 1994; Hontoria *et al*., 1994)

Artemia, when introduced to a larval fish rearing tank, also have the potential to affect the bacterial ecosystem of the tank (reviewed by Planas and Cunha, 1999). *Artemia* cultures typically contain a large number of *Vibrio* spp., which bloom during hatching because of glycerol released when the *Artemia* cyst breaks (Lavens and Sorgeloos, 1996). *Vibrio* spp. has been identified as a pathogen in larval fish (Grisez *et al*, 1996). To counteract this, some *Artemia* enrichments contain disinfectants which reduce the bacterial population. In this Project, the only enrichment to contain such a disinfectant was DC DHA Selco.

Furthermore, during the stages when rotifers and *Artemia* are being fed to larval fish, the larvae themselves are developing their own gut flora. The feed which they consume during this stage is critical in determining the make-up of this gut flora (Ringø and Gatesoupe, 1998). It may therefore be possible to use rotifers and *Artemia* as a vehicle not only to deliver essential nutrients to larval fish, but also to deliver probiotic bacterial

strains which could then contribute to the gut flora of the larvae, resulting in healthier fish (Skjermo and Vadstein, 1999).

This study has examined the fatty acid profiles of live feeds that can be fed to larval fish. As previously discussed, there are several criteria that can be applied to fatty acid data to determine an ideal diet. These data include a high DHA content, low total fat content, and a close match to the fatty acid profiles of larval fish and species of zooplankton similar to those consumed by larvae in the wild. While it is difficult to determine how critical the dietary ratio of DHA to EPA is to the survival of larval fish, it is known that larval marine fish do require more DHA than EPA, and if this ratio is ever less than one, as in the case of Super Selco-enriched rotifers (Table 3), larval fish survival will be low (Ibeas *et al.* 1997; Rodríguez *et al* 1997). However, the ratio of DHA to EPA cannot be used as the only criteria for determining the suitability of food for larval fish. While ratio can give an indication of the quality of the fat content of the diet, it does not give an indication of the actual amounts of essential fatty acids being delivered to larval fish.

Using the previously described criteria, this study has shown that rotifers enriched with either Algamac 2000, Algamac 3010 Flake or Aquagrow after being grown on a diet of yeast and T-Iso are the most suitable for initial feeding of larval sablefish. As the larval sablefish grow, they are weaned onto a diet of *Artemia* that have been enriched with either Algamac 3010 Flake or Aquagrow. During a critical period which occurs at the end of the yolk sac-stage, when the fish are in transition from endogenous to exogenous feed, they should be fed as many small copepods as possible. The experiments to

determine how long enrichments last in rotifers (Table 2) and *Artemia* (Table 10) indicate that zooplankton enriched with Algamac flake or Aquagrow retain much of their essential fatty acids up to 12 hours after they have been removed from the enrichment media. In *Artemia,* EPA appears to be lost at a faster rate than DHA, and this agrees with results of similar experiments with other *Artemia* enrichments. (Estévez *et al.*, 1998, Evjemo *et al*, 1997)

Though this study has determined ideal diets for larval sablefish, more research is indicated. One of the important determinations to be made is how diets of different fatty acid content will effect larval fish. An experiment involving different diets fed to separate groups of larvae in large larval rearing tanks will be required.

In 1998, the first sablefish were reared to the juvenile stage by feeding them a diet of rotifers enriched on alternate days with Super Selco and DHA Selco. They were then weaned onto *Artemia* enriched with the same products. In 1999, a much higher survival of sablefish larvae was achieved using more rigorous cleaning procedures in both live feed preparation and in fish husbandry, and by using some of the diets found to be suitable during the course of this study.

Live food nutritional quality is only one aspect of ongoing research to develop sablefish aquaculture. Broodstock and egg quality, egg incubation procedures, larval tank management, and live feed preparation will all contribute to increasing the survival of larval sablefish.

Literature Cited

Bell, M.V. and J.R. Sargent. Lipid nutrition and fish recruitment. 1996. Marine Ecology Progress Series 134:315-316.

Blair, T., J. Batt, R. Melanson, S. Kirk, and J. Castell. 1998. Evaluation of several commercial enrichment media for enhancing the nutritional value of Rotifers fed to winter flounder (*Pleuronectes americanus*). Bull. Aquacul. Assoc. Canada. 98-4: 35-37.

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

Evjemo, J.O., P. Coutteau, Y. Olsen, and P. Sorgeloos. 1997. The stability of docosahexaenoic acid in two *Artemia* species following enrichment and subsequent starvation. Aquaculture 155: 135-148.

Furuita, H.I, T. Takeuchi, and K. Uematsu. 1998. Effects of eicosapentaenoic and docosahexaenoic acids on growth, survival and brain development of Japanese flounder (*Paralichthys olivaceus*) Aquaculture 161:269-279.

Gapasin, R.S.J., R.Bombeo, P.Lavens, P. Sorgeloos, and H. Nelis. 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish (*Chanos Chanos*) larval performance. Aquaculture 162:269-286.

Grisez, L. M. Chair, P. Sorgeloos, and F. Ollevier. 1996. Mode of infection and spread of *Vibrio anguillarum* in turbot *Scopthalmus maximus* larvae after oral challenge through live feed. Diseases of Aquatic Organisms 26:181-187.

Grover, J.J. and B.L. Olla. 1990. Food habits of larval sablefish from the Bering Sea. Fishery Bulletin 88(4) 811-813.

Grover, J.J. and B.L. Olla. 1987. Effects of an el Niño event on the food habits of larval sablefish, *Anoplopoma fimbria*, off Oregon and Washington. Fishery Bulletin 85(1):71-79.

Hagiwawara, A., Kenichiro H., A. Nishi, K. Imaizumi, and K. Hirayama. 1993. Mass production of rotifer *Brachionus plicatilis* Resting eggs in 50 m^3 tanks. Nippon Suisan Gakkaishi, 59(1): 93-98.

Harel, M., and A.R. Place 1998. The nutritional quality of live feeds for larval fish. Bull. Aquacul. Assoc.Canada. 98-4:6-11.

Hontoria, F., J. H. Crowe, L. M. Crowe, and F. Amat. 1994. Potential use of liposomes in larviculture as a delivery system through *Artemia* nauplii. Aquaculture 127:255-264.

Ibeas, C., J.R.Cejas, R. Fores, P.Badía, T. Gómez, A. Lorenzo Hernández. 1997. Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream *(Sparus aurata*) juveniles. Aquaculture 150:91-102.

Kahan, D., G. Uhlig, D. Schwenzer, and L. Horowitz. 1982 A simple method for cultivating harpactacoid copepods and offering them to fish larvae. Aquaculture 26: 303-310.

Kennedy, W.A. 1972. Preliminary study of sablefish culture, a potential new industry. J. Fish Res. Board Can. 29: 207-210.

Koven, W.M., A. Tandler, D. Sklan, G.W. Kissil. 1993. The association of eicsapentaenoic acid and doacoshexaenoic acid in the main phospholipids of differnt-age *Sparrus aurata* larvae. Aquaculture 162: 269-286.

Lavens, P. and P. Sorgeloos 1996. Manual on the production and use of live food for aquaculture. P. Lavens and P. Sorgeloos eds. Laboratory of Aquaculture and Artemia reference center. Ghent: Belgium, FAO.

Mason, J. C., R. J. Beamish, and G.A. MacFarlane. 1983. Sexual maturity, fecundity, spawning, and early life history of sablefish (*Anoplopoma fimbria*) off the Pacific Coast of Canada. Can. J,. Fish. Aquat. Sci. 40: 2126-2134.

McEvoy, L., T.Naess, J.G. Bell, and Ø. Lie. 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163: 237-250.

McEvoy, L, and J.R. Sargent. 1998. Problems and techniques in live prey enrichment. Bull. Aquacul. Assoc. Canada. 98-4:12-16.

McFarlane, G.A. Sablefish mariculture. 1989. Aquaculture Association of Canada Bulletin 89-2:25-29.

Miliou, H. 1996. The effect of temperature, salinity, and diet on final size of female *Tisbe holothuriae* (Copepoda, Harpactacoida). Crustaceana 69(6) 742-754.

Mourente,G., D. R. Tocher, and J.R. Sargent. 1991. Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids of juvenile turbot *Scopthalmus maximus* L. Lipids 26:871-877.

Næss, T., M. Germain-Henry, and K.E. Naas. 1995. First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of *Artemia* and wild zooplankton. Aquaculture 130:235-250.

Nagata, W.D. and J.N.C. Whyte. 1992 Effects of yeast and algal diets on the growth and biochemical composition of the rotifer *Brachionus plicatilis*.. Aquacul. Fish.Manage. 23:13-21.

Nanton, D.A. and J.D. Castell. 1998. Preliminary trials using a harpactacoid copepod, *Tisbe* sp., as a diet for larval haddock and American plaice. Bull Aquacul. Assoc. Canada. 98(4) 38-40.

Nanton, D.A. and J.D. Castell. 1999. The effects of temperature and dietary fatty acids on the fatty acid composition of harpactacoid copepods, for use as a live food for marine fish larvae. Aquaculture 175: 167-181.

Nanton, D.A., J.D. Castell, and I.A. McLaren. 1996. Fatty acid composition and nutritional value of a marine harpactacoid copepod fed various algal diets. Bull. Aquacult. Assoc. Canada. 96-1:38-40.

Norrbin, M.F., R.-E. Olsen, and K.S. Tande. 1990. Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, Northern Norway. Marine Biology 105, 205-211.

Norsker, N. and J.G. Støttrup. 1994. The importance of dietary HUFAs for fecundity and HUFA content in the harpactacoid, *Tisbe holothuriae* Humes. Aquaculture 125: 155-166.

Planas, M. and I Cunha. 1999. Larviculture of marine fish: problems and perspectives. Aquaculture 177:171-190.

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

Reitan, Kjell Inge, Jose R. Rainuzzo, Gunvor Øie and Yngvaar Olsen. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155:207-221.

Ringø, E., and F. Gatesoupe. 1998. Lactic acid bacteria in fish: a review. Aquaculture 160: 177-203.

Rodríguez, C, J.A. Pérez, M. Díaz, M.S. Izquierdo, H Fernández-Palacios and A. Lorenzo. 1997 Influence of the EPA/DHA ratio on gilthead seabream (*Sparus aurata*) larval development. Aquaculture 150:77-89.

Rodriguez, C. J. A. Perez, A. Lorenzo, M.S. Izquierdo and J.R. Cejas. 1994. N-3 HUFA requirement of larval gilthead seabream *Sparus aurata* when using high levels of eicosapentaenoic acid. Comp. Biochem. Physiol. 107A(4) 693-698.

Rosenlund, G., L. Joergensen, R. Waagboe, K. Sandnes. 1997. Effects of different dietary levels of ascorbic acid in plaice. Comparative Biochemistry and Physiology 96: 395-398.

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

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

Scott, A. and S.M. Baynes. 1978. The effect of algal diet and temperature on the chemical composition of the rotifer *Brachionus plicatilis*. Aquaculture 14: 247-260.

Shenker, J.M. and B.L, Olla. 1986. Laboratory feeding and growth of juvenile sablefish, *Anoplopoma fimbria*. Can J. Fish. Aquat. Sci. 43: 930-937.

Skjermo, J and O. Vadstein 1999. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 177:333-343.

Støttrup, J.G. and Y. Attramadal. 1992. The influence of different rotifer and *Artemia* enrichment diets on growth, survival, and pigmentation in turbot (*Scophthalmus maximus* L.) Larvae. Journal of the World Aquaculture Society 23, 4.

Støttrup, J.G., and N.H. Norsker. 1997. Production and use of copepods in marine fish larviculture. Aquaculture 155:231-247.

Støttrup, J.G., R.Shields, M. Gillespie, M.B. Gara, J.R. Sargent, J.G.Bell, R.J. Henderson, D.R. Tocher, R. Sutherland, T. Næss, A. Mangor Jensen, K. Naas, T. van der Meeren, T. Harboe, F.J. Sanchez, P.Sogeloos, P. Dhert and R. Fitzgerald. 1998. The production and use of copepods in larval rearing of halibut, turbot, and cod. Bull Aquacul. Assoc. Canada 98(4) 41-45.

Sureyya O., and F.E. Chu. 1994. Uptake and Metabolism of Liposomes by *Artemia* nauplii. Aquaculture 128:131-141.

Tocher, D.R., G. Mourente, and J.R. Sargent. 1997. The use of silages prepared from fish neural tissues as enrichers for rotifers (*Brachionus plicatilis*) and *Artemia* in the nutrition of larval marine fish. Aquaculture 213-231.

Verreth, J., J. Coppoose, and H. Segner. 1994. The effect of low HUFA- and high HUFA-enriched *Artemia*, fed at different feeding levels, on growth, survival, tissue fatty acids and liver histology of *Clarias gariepinus* larvae. Aquaculture 126:137-150.

Volkman, J.K., S.W. Jeffrey, P.D. Nichols, G.I Rogers, and C.D. Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128: 219-240.

Walford, J and T.J. Lam. 1992. High density production of rotifers (*Brachionus plicatilis*) using baker's yeast (*saccharomyces cerevisiae*) and their n-3 highly unsaturated fatty acid content. J.Aqua.Trop., 7:287-300.

Watanabe, T. 1993. Importance of Docosahexaenoic acid in marine larval fish. Journal of the World Aquaculture Society. 24(2):152-161.

Whyte, J. N. C. 1988. Fatty acid profiles from direct methanolysis of lipids in tissue of cultured species. Aquaculture 75:193-203.

Whyte, J.N.C., W.C. Clarke, N.G. Ginther, J.O.T. Jensen and L.D. Townsend. 1994. Influence of composition of *Brachionus Plicatilis* and *Artemia* on growth of larval sablefish (*Anoplopoma fimbria* Pallas). Aquaculture 119: 47-61.

Whyte, J.N.C., and Nagata, W.D., 1990. Carbohydrate and fatty acid composition of the rotifer, *Brachionus plicatilis*, fed monospecific diets of yeast or phytoplankton. Aquaculture 89:263-272.