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The Effect of Highly Unsaturated Fatty Acids (HUFA) on Lake Trout (*Salvelinus namaycush*) Alevins Using *Artemia* nauplii Enriched with Commercial Emulsions and Dry Diets

By

Blake Jason Snyder

A thesis submitted to the Department of Environmental Science and Biology of The College at Brockport State University of New York in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science and Biology

October 12, 2011

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By

Blake J. Snyder

2011

The Effect of Highly Unsaturated Fatty Acids (HUFA) on Lake Trout (*Salvelinus namaycush*) Alevins Using *Artemia* nauplii Enriched with Commercial Emulsions and Dry Diets

Department of Environmental Science and Biology
Thesis Defense by

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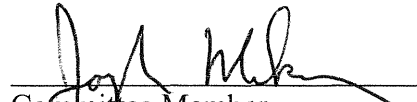
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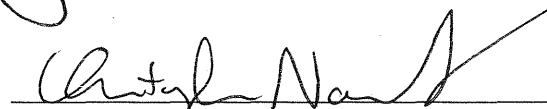
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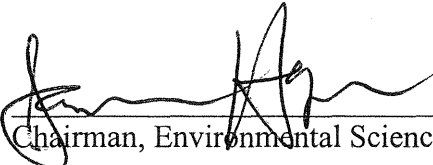


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ABSTRACT:

Highly unsaturated fatty acids (HUFA) are important nutrients for fish survival, development, and reproduction. Fish oil (FO), rich in HUFA, is the dominant lipid source for first feeds in salmonid aquaculture. To determine if other lipid sources would influence survival, growth, and fatty acid profiles in lake trout (*Salvelinus namaycush*) alevins, two 8-week feeding experiments were performed. Diets used in the *Artemia* Experiment included: diet 1, non-enriched *Artemia*; diet 2, SELCO-enriched *Artemia*; diet 3, Super SELCO-enriched *Artemia*; and diet 4, BioVita #0, all of which had significantly different fatty acid compositions. The Fish Oil Replacement Experiment used diets that differed solely in lipid source and fatty acid composition: diet 1, oleic acid (OA); diet 2, linseed oil (LO); diet 3, cod liver oil (CLO); and diet 4, lecithin (LE).

Results from both experiments show that dietary lipid source and fatty acid composition can significantly influence survival, growth, and fatty acid composition of lake trout alevins. In the *Artemia* Experiment, lake trout fed a non-enriched *Artemia* diet lacking in HUFA displayed lower growth than fish fed enriched *Artemia* diets that included HUFA although survival was not significantly different among treatments. Lake trout fed Super SELCO-enriched *Artemia*, which had the highest concentration of HUFA, did not differ statistically to lake trout fed SELCO-enriched *Artemia* for any growth parameter. In the Fish Oil Replacement Experiment, lake trout fed the OA diet, which was lacking in essential fatty acids (linolenic acid (18:3n-3) and linoleic acid (18:2n-6)) and HUFA, had significantly lower survival

and growth. Fish fed CLO had significantly higher final length and mass but were statistically similar to fish fed the LE diet in regards to mass gain, SGR, FCR, and K. In both experiments, neutral and phospho-lipid fatty acid profiles of whole body lake trout were reflective of dietary fatty acids. These experiments suggest lipid source and dietary fatty acids can greatly affect the survival, growth, and fatty acid composition of lake trout alevins but alternatives to fish oil, such as vegetable oils, may be a suitable substitute in the first feed of lake trout.

1. Introduction

Lipids play a significant role in the survival, growth, development, and reproduction of fish. Two major categories of lipids are triacylglycerols (TAG) or neutral lipids, and phospholipids. Triacylglycerols are used as immediate energy or can be stored for later use. Phospholipids are important in tissue and membrane structure, proper renal and neural development, and serve as precursors to eicosanoids. Eicosanoids are a group of hormone-like compounds produced by cells to act in their immediate areas and consist of prostaglandins, prostacyclins, thromboxanes, and leukotrienes (Tocher 2003, Arts and Kohler 2009). These are important for renal and neural function, cardiovascular tone, blood clotting, and inducing immune and inflammatory responses (Bell *et al.* 1997, Kanazawa 1997, Sargent *et al.* 1999a, Tocher *et al.* 2008).

A critical component of both triacylglycerols and phospholipids are fatty acids (FA). Triacylglycerols contain three fatty acids (Figure 1) while phospholipids contain two fatty acids (Figure 2). Fatty acids are carboxylic acids with a hydrocarbon chain that can be either saturated or unsaturated. The nomenclature of a fatty acid, as designated by IUPAC (International Union of Pure and Applied Chemistry), uses the number of carbons and double bonds in its carbon chain. Saturated fatty acids (SAFA) contain no double bonds in their hydrocarbon chain. Saturated fatty acids include lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0). Saturated fatty acids are often used as a source of energy. High melting points are characteristic of SAFA, generally making them solid

at room temperature. This contributes to membrane rigidity. Unlike SAFA, unsaturated fatty acids contain one or more double bonds in the hydrocarbon chain. Monounsaturated fatty acids (MUFA) contain one double bond while polyunsaturated fatty acids (PUFA) contain two or more double bonds in their hydrocarbon chain. Monounsaturated fatty acids, such as oleic acid (18:1 n -9), are important as a structural component in lipids. Highly unsaturated fatty acids (HUFA) are a subset of fatty acids within PUFA that contain twenty or more carbons and two or more double bonds. Arachidonic acid (ARA, 20:4 n -6), eicosapentaenoic acid (EPA, 20:5 n -3), and docosahexaenoic acid (DHA, 22:6 n -3) are included in this group and are of particular interest in fish nutrition.

Like all vertebrates, fish are not capable of synthesizing certain fatty acids *de novo*, requiring them to be obtained from their diet (Wallis *et al.* 2002). These fatty acids are termed essential fatty acids (EFA). Essential fatty acids vary by species and are influenced by the environment of the organism. Fish living in freshwater ecosystems have different EFA requirements than fish living in marine ecosystems. Linolenic (18:3 n -3) and linoleic acid (18:2 n -6) are EFAs in freshwater fish (Watanabe 1982, Sargent *et al.* 2002). Freshwater fish are capable of synthesizing EPA and DHA, members of the n -3 HUFA, family if given linolenic acid; whereas ARA, a member of the n -6 HUFA family, can be synthesized using linoleic acid (Owen *et al.* 1975, Kanazawa *et al.* 1979, Sargent *et al.* 2002, Tocher 2003). The n -3 and n -6 HUFA are synthesized by means of elongase and desaturase enzymes (Figure 3). Marine species, however, do not possess these enzymes, thus requiring dietary

HUFA in addition to linolenic and linoleic acids (Tocher 2003). Although most freshwater fish are capable of synthesizing HUFA if given the proper precursors, freshwater piscivores may have reduced enzymatic activity or lack the necessary enzymes to elongate and desaturate the precursors, ergo requiring dietary HUFA (Schwalme 1994, Henderson *et al.* 1995, Desvillettes *et al.* 1997).

Essential fatty acids are required for normal growth and development in marine and freshwater fish. Castell *et al.* (1972) determined that, regardless of size or first feeding, rainbow trout (*Oncorhynchus mykiss*) fed diets deficient in linolenic acid and PUFA displayed fin erosion, heart myopathy, and shock syndrome. Owen *et al.* (1975) showed that nine-month old rainbow trout used linolenic acid to synthesize DHA. It has since been determined that EPA and DHA are synthesized from diets high in linolenic acid. Conversion of EPA and DHA from linolenic acid has been observed in Atlantic salmon (*Salmo salar*) (Bell *et al.* 2002, Sargent *et al.* 2002), as well as in other freshwater fish, such as Murray cod (*Maccullochella peelii peelii*) (Francis *et al.* 2006) and Nile tilapia (*Oreochromis niloticus*) (Karapanagiotidis *et al.* 2007). These results suggest that lake trout (*Salvelinus namaycush*), a freshwater species, should be able to synthesize EPA and DHA if given linolenic acid and ARA if given linoleic acid. Although freshwater fish are capable of synthesizing EPA and DHA, larval fish seem to be more dependent on dietary HUFA than adult fish due to high somatic growth rates that may not be satisfied solely by their conversion abilities (Brett and Müller-Navarra 1997). Therefore, although freshwater larval fish fed diets

including linolenic and linoleic acid will be able to convert these precursors to HUFA, performance may be enhanced if HUFA are included in their diet.

Fish oil (FO), which includes high concentrations of HUFA of the *n*-3 family, particularly DHA and EPA, is commonly used in the formulation of dry diets for aquaculture (Bell *et al.* 2001). The primary source of FO is small pelagic fish that feed at lower trophic levels in marine food webs. These species can include, but are not limited to, anchovy (Engraulidae), mackerel (Scombridae), herring (Clupeidae), capelin (*Mallotus villosus*), menhaden (Clupeidae), and sardines (Clupeidae) (Naylor *et al.* 2000). Although FO has high nutritional properties and supports good survival and growth when fed to farm raised fish, many problems are associated with it.

One such problem is the unsustainable harvesting practices needed to produce fish oil. It is estimated that one third of the total world catches are used in aquaculture feeds (Drakeford and Pascoe 2010), with 20.2 million tons being used in 2006 (FAO 2009). From 1950 to 2004, the aquaculture industry has grown at an average annual rate of 8.8% (Turchini *et al.* 2009), making it the fastest growing food production industry. Aquaculture production during the 1950s was less than one million tons annually but has increased significantly to reach 52.5 million tons in 2008 (FAO 2010). With the expansion of aquaculture practices more feed is needed. In 2006, 843,000 thousand tons of fish oil were used for aquaculture purposes, which accounts for 88.5% of the global fish oil output (Tacon and Metian 2008), representing an increase of nearly 50% since 1995. As the aquaculture sector continues to grow, the amount of feed needed to meet demands increases, which puts

more pressure on ocean fisheries. This can lead to a decrease in biodiversity, which in turn may have effects ecosystem sustainability (Worm *et al.* 2006).

Feed is the most expensive production cost when operating an aquaculture facility; small increases in ingredient price can have a large overall influence on market prices of aquaculture products (Naylor *et al.* 2000). A decrease in fish oil supply in 2005-2006 due to poor environmental conditions caused by El Niño events and subsequently reduced fishing quotas led to a sharp price hike (Jackson 2006), demonstrating that fish oil could be a major limiting factor in aquaculture production.

Another problem associated with fish oil as a lipid source in fish feeds is its potential contamination with compounds such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzo-*p*-furans (PCDF), polybrominated diphenyl ethers (PBDE), organochlorine pesticides (OCP), arsenic, mercury, and lead. Fish used to produce fish oil can accumulate contaminants through diet and store them in their lipid reserves (Jacobs *et al.* 1998). When farm raised fish are fed diets that include contaminated fish oil, they can also accumulate the contaminants. European and North American farm raised Atlantic salmon were compared to wild caught ones with respect to 14 common contaminants (Hites *et al.* 2004); 13 were significantly higher in farmed salmon when compared to the wild caught, including PCB and dichlorodiphenyltrichloroethane (DDT). When the two locations of the farmed salmon were compared, it was determined that salmon from Europe had higher concentrations of all 14 contaminants, indicating that contamination concentrations vary by location.

Dewailly *et al.* (2007) compared levels of total PCB, total PCDD/PCDF, and mercury in farm raised and wild caught rainbow trout and Atlantic salmon. Total mercury was statistically higher in both wild caught rainbow trout and Atlantic salmon when compared to the farm raised counterparts but total PCDD/PCDF did not differ statistically for either species. Total PCB did not differ statistically between farm raised and wild rainbow trout but were statistically higher in farm raised Atlantic salmon when compared to wild caught.

Problems with sustainability, price, and contaminants call for alternatives to fish oil. One alternative is plant-based oils. Some plant oils that have been used as total or partial FO replacement in diets are barley, canola, corn, cottonseed, rapeseed, soybean, and wheat (Gatlin *et al.* 2007), but they are not without problems. Plant oils are deficient in *n*-3 HUFA, although trace amounts of linolenic acid, the precursor to *n*-3 HUFA, are sometimes present (Turchini *et al.* 2009b). To ensure that *n*-3 HUFA deficiency does not occur in marine fish, a full substitution of fish oil with plant oils is not done and a portion of fish oil is still added to meet EFA requirements. Replacement of fish oil by plant oil in the diets of freshwater fish is more achievable, as the EFA for freshwater fish, linolenic and linoleic acids, are included in plant oils.

Plant-based oils can, however, contain high concentrations of *n*-6 PUFA, particularly linoleic acid and ARA. This can greatly influence the *n*-3 to *n*-6 ratio in fatty acid profiles of the cultured species, which in turn affects the EPA to ARA ratio. Eicosapentaenoic acid and ARA give rise to two distinct series of eicosanoids, which are biologically active compounds responsible for immune and inflammatory

responses, neural and renal function, and hematological and cardiovascular activity (Tocher 2003). Arachidonic acid derived eicosanoids are known to be more biologically active and promote inflammation when compared to the anti-inflammatory EPA series (Arts and Kohler 2009). An increase in dietary *n*-3 fatty acids such as EPA can result in modified physiological responses by inhibiting ARA-derived eicosanoids. These modified responses can be done by a higher ratio of *n*-3 displacing *n*-6 in phospholipids, a greater competition for the eicosanoid forming enzymes cyclooxygenases and lipoxygenase, or EPA derived eicosanoids counteracting or blocking the effects of ARA derived eicosanoids (Bell *et al.* 1994).

Salmonids account for only 3% of global aquaculture production but use 51% of fish oil supplies (FAO 2009) and the diet composition can contain 9 to 35% fish oil (Tacon and Metian 2008). Even though these carnivorous species account for a relatively small amount of production in the aquaculture sector, they use a huge amount of the total fish oil supply, so, there is a great need to reduce the amount of fish oil used in salmonid diets. In 1995, the average amount of fish oil in salmon diets was 25% but decreased to 16% by 2007 (Naylor *et al.* 2009). However, the total amount of feed used more than doubled from 806 thousand tons in 1995 to 1,923 thousand tons in 2007, due to an increase in aquaculture production.

The effect of partial or total replacement of fish oil by plant-based oils on physical characteristics and whole body fatty acid composition of fish has been researched in numerous species. In a study by Rinchar *et al.* (2007), juvenile rainbow trout (initial weight 182 ± 51 mg) were fed one of four diets with different

lipid sources: oleic acid (18:1 n -9), olive and linseed oil, cod liver oil, or refined soybean lecithin. Growth performance with regards to final weight, weight gain, specific growth rate (SGR), and food conversion ratio (FCR) were significantly higher in fish fed the plant based soybean lecithin diet. Whole body fatty acid composition of rainbow trout also reflected dietary fatty acids, where fish fed the soybean lecithin diet had significantly higher concentrations of linoleic acid and ARA in both neutral and phospho-lipid fractions. This suggests that although whole body fatty acids of fish are significantly altered, soybean lecithin may be a suitable replacement for fish oil in juvenile rainbow trout.

Menoyo *et al.* (2007) investigated the effects of replacing fish oil and linseed oil (LO) with varying amounts of sunflower oil (SO) on Atlantic salmon post-smolt growth performance (initial weight 220 g). The eight experimental diets included a blend of FO or LO with SO in ratios of 100:0, 25:75, 50:50, and 75:25. After the end of the 12-week feeding experiment, Atlantic salmon displayed no significant difference in survival, final weight, SGR, or FCR. The highest concentrations of the sum of n -3 HUFA in the neutral lipid fraction of fillets was observed in fish fed 100% LO. Moreover, the concentrations of the sum of n -3 HUFA decreased with increasing inclusion levels of SO in both the FO and LO based diets. The lipid source itself, either FO or LO, also affected concentrations of n -3 HUFA, with lower levels observed in fish fed the LO diet. Although fatty acid composition in the muscle and liver of Atlantic salmon were altered based on diet, no negative effects on survival, health, growth, or feed efficiency were observed, suggesting that vegetable oils such

linseed or a graded blend of linseed and sunflower oil may be an acceptable fish oil replacement.

Soybean oil (SBO) has also been used as a fish oil replacement for Atlantic salmon. Ruyter *et al.* (2006) fed fish 100% FO, 50:50% FO/SBO, or 100% SBO for 950 degree days. Fatty acid profiles were reflective of the dietary treatment. At the end of the experiment fish fed the FO/SBO blend or 100% SBO diets had significantly higher levels of linoleic acid in intestine and liver neutral and phospho-lipid fractions when compared to fish fed 100% FO. Highest concentrations of EPA and DHA in both neutral and phospho-lipid fractions were observed in fish fed 100% FO and were again reflective of the dietary treatment suggesting that diet has plays a large role in fatty composition.

As an alternative to dry aquafeeds containing FO, live diets can be used, particularly during first feeding. Some live diets used include *Daphnia*, copepods, rotifers, and algae or green water. *Artemia* nauplii, commonly referred to as brine shrimp, are also used in both marine and freshwater aquaculture practices. They account for 40% of early larval feed in many cultured fishes as they are readily accepted as a first feed after yolk absorption (Sorgeloos *et al.* 2001). A prominent issue that plagues the use of brine shrimp as first feeds for fish is their poor nutritional quality. Brine shrimp naturally lack sufficient nutritional value to provide specific essential nutrients needed for optimal fish growth and development. They are naturally low in HUFA, containing approximately 5% EPA, low values of ARA, and no DHA, but are rich in the HUFA precursors linolenic acid (11-15%; Estevez and

Kanazawa 1995) and, to a lesser extent, linoleic acid (Sargent *et al.* 1993, Czesny *et al.* 1999, Sargent *et al.* 1999b, Han *et al.* 2000).

To improve nutritional value in *Artemia*, particularly *n*-3 HUFA, enrichments are available. Being passive filter feeders, nauplii placed in enrichment incorporate this medium in their digestive tract, serving as live vehicles of enrichment. This process of enrichment incorporation in *Artemia* is termed bioencapsulation (Navarro *et al.* 1999, Sorgeloos *et al.* 2001). Enrichment sources vary and have included unicellular algae (Watanabe *et al.* 1980), yeast (Watanabe *et al.* 1980), microencapsulated diets (Sakamoto *et al.* 1982), and fish oil emulsions (Sargent *et al.* 1999b, Han *et al.* 2000). Commercial emulsion supplements, such as SELCO (Self-Emulsifying Lipid Concentrate) (INVE Aquaculture, Salt Lake City, UT), are readily available to fortify *Artemia* by increasing concentrations of HUFA, specifically in the *n*-3 family. Evjemo and Olsen (1997) have reported an increase in *n*-3 HUFA from 7% to 38% in *Artemia* nauplii when using Super SELCO. The dominant fatty acids in this enrichment were DHA and EPA, which constituted for 15% and 22% of the fatty acid profile, respectively.

Little to no dietary research has been done on lake trout (*Salvelinus namaycush*), a species widely cultured for stocking in North America. This study aimed to determine the effects different lipid sources have on lake trout alevins, a species within the Great Lakes. Before suffering a stock crash due to overfishing, environmental changes, and sea lamprey (*Petromyzon marinus*) predation, lake trout were an important top predator in the Great Lakes food web (Christie 1974). In an

effort to preserve ecological diversity, stabilize populations, and create a sport fishery in the Great Lakes, lake trout are commonly cultured for stocking programs. Although stocking programs are in place, current lake trout populations are not self-sustaining, with the exception of Lake Superior (Hansen *et al.* 1995). Possible impediments to successful recruitment of lake trout include insufficient broodstock (Lawrie and MacCallum 1980), diminished spawning habitat (Sly 1988), predation on eggs by alewives (*Alosa pseudoharengus*) (Krueger *et al.* 1995), contaminants (Hickey *et al.* 2006), and thiamine (vitamin B₁) deficiency (Fitzsimons *et al.* 1999).

Objectives

This study used diets with different lipid sources as the first feed of lake trout alevins to determine if growth or survival would be compromised and how this would affect whole body fatty acid profiles. This was accomplished through two 8-week feeding experiments. In the *Artemia* Experiment, lake trout alevins were offered one of four diets: non-enriched *Artemia*, *Artemia* enriched with SELCO, *Artemia* enriched with Super SELCO, or a dry diet, BioVita #0 (Bio-Oregon, Westbrook, ME). Diets had significantly different fatty acid profiles. Non-enriched *Artemia* had high concentrations of the HUFA precursors, whereas the remaining diets had different concentrations of HUFA. This had an influence on survival, growth, and fatty acid composition of lake trout alevins.

The Fish Oil Replacement Experiment also aimed to determine if replacing fish oil in the first feed of lake trout alevins affects survival, growth, and fatty acid

composition. A concurrent objective for the Fish Oil Replacement Experiment was to determine whether plant oil (linseed or soy-refined lecithin) as the sole lipid source would be a suitable replacement for fish oil in the diet of lake trout alevins. In the Fish Oil Replacement Experiment, lake trout were offered one of four diets: oleic acid methyl esters (18:1 n -9) (OA), linseed oil (LO), cod liver oil (CLO), or soy-refined lecithin (LE). Like the *Artemia* Experiment, diets from the Fish Oil Replacement Experiment had drastically different fatty acid compositions. Specifically, the OA diet was deficient in HUFA and HUFA precursors, whereas the LO and LE diet contained only HUFA precursors. The CLO diet contained the precursors to HUFA and intact HUFA. These various dietary fatty acid compositions influenced survival, growth, and fatty acid composition of lake trout alevins.

2. Materials and Methods

2.1. Experiment location

Feeding trials were conducted in the aquaculture laboratory at The College at Brockport State University of New York in Brockport, New York. The 8-week feeding trials began on March 20, 2009 and March 7, 2010 for the *Artemia* Experiment and the Fish Oil Replacement Experiment, respectively.

2.2. Diets used in the *Artemia* Experiment

Diets in the *Artemia* Experiment consisted of non-enriched *Artemia*, *Artemia* enriched with SELCO (INVE Aquaculture, Salt Lake City, UT), *Artemia* enriched

with Super SELCO (INVE Aquaculture), or BioVita #0, a floating dry starter diet formulated for salmonid species. According to the manufacturer, SELCO and Super SELCO contained 200 mg/g and 400 mg/g *n*-3 HUFA, respectively (Table 1).

2.2.1. *Artemia* preparation

Artemia cysts (Argent, Redmond, WA) were decapsulated with a hypochlorite solution before enrichment according to the procedure developed by Sorgeloos *et al.* (1977). During this process, the hard indigestible outer-shell, known as the chorion, surrounding the *Artemia* eggs was removed, aiding in the hatching process by reducing the chance of bacterial infections and poor water quality. Briefly, dried *Artemia* cysts (1 can ~ 430 g) were hydrated in a bucket containing 6 L of freshwater with aeration for 1.5 h at room temperature before decapsulation. Cysts were filtered using a 125- μ m mesh sieve and transferred to a bucket containing a solution of 2.2 L of salt water (12% or 12 ppt), 150 mL of sodium hydroxide (67% NaOH) and 4.54 L of bleach. The bucket was kept on ice and constantly stirred until cysts turned from brown to orange. When the cysts were 90% orange, the reaction was quickly stopped by filtering the cysts (125- μ m mesh) and rinsing them well with freshwater. To neutralize residual chlorine, cysts were rinsed with a solution of sodium thiosulfate (1%). Cysts were then dried of excess water using a vacuum filter and refrigerated at 4°C in a plastic container until needed.

2.2.2. *Artemia* hatching and enrichment

Stock *Artemia* were hatched daily in a 5-L McDonald hatching jar with 30% (30 ppt) salt water under vigorous aeration for 24 h. Water temperature was kept at 28°C using a water heater during hatching. Initially, 30 g of *Artemia* were hatched daily, but was increased to 50 g on day 40. After the 24 h hatching period, *Artemia* were sieved (125 µm), rinsed with fresh seawater (30 ppt) and unhatched cysts were removed. *Artemia* nauplii were distributed equally into three 5-L tanks with the same conditions as the stock tank (30% salt water, aeration, and 28°C) (Figure 4). Enrichments (SELCO or Super SELCO) were added to the designated tanks at a concentration of 0.6 g/L, as recommended by the manufacturer. *Artemia* were enriched for 24 h. The control diet, non-enriched *Artemia*, received no enrichment emulsion but was also placed in a tank for 24 h under the same conditions. After the 24 h of enrichment, *Artemia* were sieved and rinsed with fresh seawater (30 ppt) to remove excess emulsion. The *Artemia* treatments were placed in three separate 1-L beakers with fresh seawater and aeration and used as the daily food supply. *Artemia* treatments were stirred to ensure a homogenized mixture and rinsed with freshwater using a 125-µm mesh before being fed to the respective tank. The batch of *Artemia* was used within 9 h of enrichment.

2.2.3. Diet sampling

Artemia were sampled prior to enrichment and after enrichments for lipid and fatty acid composition. The dry diet (BioVita # 0) as well as both SELCO and Super

SELCO emulsions were also sampled. Samples were stored in a bio-freezer (So-Low, Cincinnati, OH) at -80°C prior to biochemical analysis.

2.3. Diets used in the Fish Oil Replacement Experiment

2.3.1. Diet composition

Four semi-purified casein-gelatin diets containing different lipid sources were used in the Fish Oil Replacement Experiment. Diets were formulated to be isolipidic (14%) and isonitrogenous (60%) (Table 2). The first diet contained only oleic acid methyl esters (18:1*n*-9) as the lipid source and was used as the essential fatty acid deficient diet (OA). Linseed oil (LO), which is high in the precursors to HUFA, linoleic acid (18:2*n*-6) and linolenic acid (18:3*n*-3), was the lipid source in the second diet. Cod liver oil (CLO) served as the lipid source for the third diet and is high in HUFA and its precursors. The fourth diet used soy-refined lecithin (LE) as the lipid source and contained high concentrations of linoleic acid (18:2*n*-6) in the form of phospholipids.

2.3.2. Diet preparation

Diets were prepared in the laboratory at The College at Brockport - State University of New York. All ingredients were purchased from MP Biomedicals (Solon, OH), with the exception of wheat meal (The King Arthur Flour Company, Inc., Norwich, VT), L-arginine, L-lysine, and L-methionine (Sigma-Aldrich Company, St. Louis, MO), and the vitamin and mineral mixtures (Dyets Inc., Bethlehem, PA). Proper proportions of ingredients were mixed together in a clean

mixing bowl using a tilt-head stand mixer (KitchenAid, Shelton, CT). A small amount of water was added until a dough-like consistency was achieved. The dough was then made into spaghetti-like strands using a food grinder attachment for the tilt-head mixer. Diets were placed in clean plastic trays, covered with tinfoil, and refrigerated. To remove excess water, diets were freeze-dried for 24 h (Ward's Natural Science, Rochester, NY). A pestle and sieves (Fisher Scientific, Pittsburgh, PA) were used to grind and separate the diet into 400 μm and 600 μm pellet sizes. Diets were kept in a freezer until needed. A sample of each diet was stored at -80°C for lipid and fatty acid analysis.

2.4. Lake trout embryo origin

Ripe lake trout females from Lake Michigan were stripped of their eggs and ripe males were stripped of milt by employees of the Illinois Natural History Survey – Lake Michigan Biological Station (INHS-LMBS) in October and November of 2008 and 2009 near Waukegan, Illinois for Experiments 1 and 2, respectively. Nineteen and ten ripe females were used for Experiments 1 and 2, respectively. Unfertilized eggs and milt were transported on ice to the INHS-LMBS. Milt was analyzed for sperm motility and sperm with a motility of 80% or higher was pooled and used to fertilize eggs. Using the dry spawning method, 100 μL of milt fertilized approximately 100 eggs from each female. Eggs were then immersed in an allithiamine (Ecological Formulas, Concord, CA) solution (1,000 mg/L) for 1 h at 4°C to water harden and reverse the potential effects of early mortality syndrome (EMS; thiamine deficiency) (Brown *et al.* 2005). Eggs were removed from the allithiamine

solution and rinsed with lake water. Eggs were then transported in a cooler with water at a temperature of approximately 4°C to the laboratory at The College at Brockport - State University of New York and placed in hatching trays (MariSource, Fife, WA). A recirculating system was used to supply water to the hatching trays and additional water was added as needed. Water temperature was kept constant using a water chiller (Frigid Units, Inc, Toledo, OH) and averaged $4.9 \pm 1.4^\circ\text{C}$ and $5.8 \pm 1.0^\circ\text{C}$ for Experiments 1 and 2, respectively until hatching. Once alevins hatched, they were transferred to a flow-through system in a plastic bin (53cm x 43cm x 10cm) until swim-up stage. Between hatching and swim-up stage, water temperatures ranged from 6.1 to 6.7°C and 5.0 to 8.2°C, for Experiments 1 and 2, respectively.

2.5. Feeding experiments

A flow-through system using municipal water with a flow rate of 1.3 L/min was used in both experiments. Municipal water was dechlorinated using a carbon filter (Siemens Water Technologies, Warrendale, PA). Aquaria were randomly assigned to one of four dietary treatments for both experiments. Fish for both experiments were fed three times daily. Daily, mortality and water temperature were recorded and waste was removed. Water temperature ranged from 6.6 to 12.0°C and 5.9 to 12.3°C, in the *Artemia* Experiment and the Fish Oil Replacement Experiment, respectively. The duration of each experiment was eight weeks.

At swim-up stage, fish were randomly distributed in 12 38-L aquaria. In the *Artemia* Experiment, 40 lake trout [average (avg.) weight: 94.3 ± 21.4 mg, avg. length: 26 ± 1.5 mm] per aquarium were used. Prior to the start of the *Artemia*

Experiment, fish were fed non-enriched *Artemia* for two days. The Fish Oil Replacement Experiment used 50 lake trout (avg. weight: 94.1 ± 18.8 mg, avg. length: 26 ± 1.3 mm) were used per aquarium. Lake trout from the Fish Oil Replacement Experiment were fed with non-enriched *Artemia* three times prior to the start of the experiment.

In the *Artemia* Experiment, fish in each tank were fed 300 mL of *Artemia* per day at a concentration of 550,000 or 1,000,000 *Artemia*/L, depending on amount of stock *Artemia* used (30 or 50 g, respectively). Fish fed BioVita # 0 were fed 5% of their average body weight. This was adjusted every two weeks after weighing. Fish in the Fish Oil Replacement Experiment were fed daily at a rate of 5% of their average body weight and feeding rates were adjusted biweekly. Initially, the size of the pellets offered to the fish was 400 μ m but it increased to 600 μ m at the start of the fourth week.

2.6. Sampling procedures, lipid and fatty acid analysis

Before lake trout were distributed in the *Artemia* Experiment, 50 fish were individually measured for total length and weight. These fish were then stored at -80°C for analysis of lipids and fatty acid profile. This process was repeated with 30 fish in the Fish Oil Replacement Experiment.

Every two weeks after the start of both experiments, bulk weight of lake trout in each aquarium was measured. Flow was temporarily turned off and water levels for each tank were lowered. Fish were netted and placed in a beaker with water. They were then poured into a net, patted dry with paper towels, and placed in a tared beaker

with water on a scale (Mettler Toledo, Columbus, OH). Fish were then individually counted and returned to their assigned aquarium. Individual fish weight was calculated by dividing bulk weight by the number of fish in that tank. Average individual fish weight for all tanks was then calculated.

At the end of the experiments, fish were overdosed with one g of MS-222/L (Tricaine-S (Tricaine Methanesulfonate) Western Chemical Inc., Ferndale, WA). In the *Artemia* Experiment, bulk weight of each tank and number of fish were recorded. In addition, 10 fish from each tank were individually measured for total length (mm) and weight (g). Fish were then placed in vials and stored at -80°C for lipid and fatty acid analysis. This was repeated at the end of the Fish Oil Replacement Experiment except each individual fish from every aquarium was measured for total length and weight. Fish sampled from the Fish Oil Replacement Experiment were shipped on dry ice to the United States Geological Survey - Great Lakes Science Center in Ann Arbor, Michigan where they were freeze dried for 48 h. Samples were then shipped back to the laboratory at The College at Brockport - State University of New York and stored at -80°C until biochemical analysis.

Parameters measured for both experiments included survival, total length, mass gain ($MG = (\text{final weight} - \text{initial weight}) * 100 / \text{initial weight}$), specific growth rate ($SGR = (\log \text{final weight} - \log \text{initial weight}) * 100 / \text{duration of experiment in days}$), condition factor ($K = (\text{weight}/\text{length}^3) * 100,000$). Food conversion ratio (FCR = average amount of food used per fish/ average individual weight gain) was also determined in the Fish Oil Replacement Experiment.

Lipids were extracted using the gravimetric method developed by Folch *et al.* (1957). When wet tissue was used, as was the case for the *Artemia* diets and lake trout from the *Artemia* Experiment, one gram was weighed and placed in a homogenization tube. For freeze-dried samples, such as the dry diets and lake trout from the Fish Oil Replacement Experiment, 0.3 g was used for lipid extraction. Whole body lake trout from the *Artemia* Experiment were placed in homogenization tubes. In the *Artemia* Experiment, fish were pooled from the respective treatment for lipid analysis. Fish from the Fish Oil Replacement Experiment were also pooled based on tank but were homogenized into a powder prior to lipid extraction with a pestle and mortar. Initial lake trout were pooled for each experiment for lipid analysis. Twenty mL of solvent comprising of chloroform-methanol (2:1, v/v) and 0.01% of butylated hydroxytoluene (BHT) as an antioxidant was added to each tube. Tubes were capped and placed on ice. While kept on ice, samples were homogenized for one min using a Power Gen 500 homogenizer (Fisher Scientific, Pittsburgh, PA). After each sample, the probe was rinsed twice with deionized water, twice with chloroform-methanol solvent, and wiped dry. Tubes containing the homogenized samples were capped and kept on ice. Samples were then vacuum-filtered. Filter paper (Whatman, Piscataway, NJ) was wetted with solvent and the sample poured onto the filter. The tube was rinsed twice with chloroform-methanol solvent and poured onto the filter. The filtered extract was transferred to a clean test tube with 4 mL of 6% magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), filled with nitrogen, and capped. Tubes containing the samples were vortexed for one minute, refilled with nitrogen,

and stored at room temperature for 24 h. The bottom layer was then extracted using a Pasteur pipette and transferred to a clean glass tube. Samples were placed in a warm water bath and put under nitrogen to evaporate solvent. Once samples had a small amount of solvent left, they were transferred to pre-weighed test tubes. Evaporation under nitrogen continued and samples were weighed until a stable weight was reached. This weight was recorded and represented the amount of lipid in the sample. A small amount of chloroform was added and samples were capped under nitrogen before storage at -80°C . Percent of lipid content ($(\text{weight of lipid}/\text{weight of tissue}) * 100$) was then calculated.

Whole body lipids of fish were separated into neutral lipid and phospholipid fractions using the method developed by Juaneda and Rocquelin (1985). Sep-Pak columns (Waters Corporation, Milford, MA) were attached to 20 mL syringes and total lipids were placed onto the columns using Pasteur pipettes. Total lipid vials were rinsed with a small amount of chloroform and placed on the column. Twenty mL of chloroform was added to the column to elude the neutral lipids into a tube. After neutral lipids were separated, phospholipids were separated using 20 mL of methanol. Both neutral lipid and phospholipid fractions were evaporated under nitrogen. Once the majority of neutral or phospholipid had been evaporated, they were transferred to pre-weighed glass tubes and evaporation continued. Samples were weighed until weight was stable. Recorded weight represented the percentage of neutral lipid or phospholipid in the total lipid. A small amount of chloroform was added to the neutral or phospho-lipids before being capped under nitrogen and stored

at -80°C. Percentage of neutral lipid and phospholipid fractions ((weight of neutral lipids or phospholipids/weight of total lipids)*100) were then calculated. The total amount of neutral lipid or phospholipid in the total lipid was also determined ((weight of total lipid*weight of neutral lipid or phospholipid)/100).

Fatty acid profiles of whole body lake trout neutral and phospho-lipid fractions were determined for each dietary treatment. Total lipid fatty acid profiles of *Artemia* enrichments, *Artemia* diets, and dry diets were also determined. Transmethylation of fatty acids were done according to the method described by Metcalfe and Schmitz (1969). Chloroform from samples was evaporated under nitrogen. A proportional amount of internal reference stock solution, composed of eight mg nonadecanoic acid (19:0) per one mL of hexane, was added to the total, neutral, or phospho-lipid sample and evaporated under nitrogen. Neutral lipids were capped under nitrogen and incubated at 80°C for one h after the addition of 1.5 mL sodium hydroxide (0.5M NaOH). This step, known as saponification, cleaves the fatty acid from the glycerol and adds a hydroxyl group. After incubation, tubes were cooled to room temperature. Two mL of borontrifluoride methanol (Sigma-Aldrich Company, St. Louis, MO) was added to neutral lipid and phospholipid samples. This step cleaves the hydroxyl group from the fatty acids and replaces it with a methyl group, making a fatty acid methyl ester (FAME) that is detectable by the gas chromatography/mass spectroscopy (GC/MS). Samples were capped under nitrogen, incubated at 80°C for 0.5 h, and cooled to room temperature. One mL of hexane was added to the samples, which were then capped and vortexed. This step was repeated

with one mL of water. The hexane layer was transferred using a Pasteur pipette to a clean test tube containing a small spoonful of anhydrous sodium sulfate to remove any excess water. Another one mL of hexane was added to the original sample and was capped and vortexed. This hexane layer was also transferred to the vial with sodium sulfate, which was capped and vortexed. Samples were transferred to a 4-mL vial, filled with nitrogen, and capped. Samples were stored at -80°C until being injected into the GC/MS.

The analysis of fatty acid methyl esters was performed with an Agilent 7890A gas chromatograph equipped with a G4513A series injector interfaced to an Agilent 5975C inert XL EI/CL mass selective detector (Agilent Technologies, Santa Clara, CA). FAMES were separated using an Omegawax 250 capillary column with a length of 30 m and a diameter of 25 μm (Supelco, Bellefonte, PA). Initial oven temperature used in the fatty acid methyl ester method was 70°C. Temperature was ramped up after two minutes to 230°C and increased to 240 and 270°C throughout the run. Total run time of the method was 72 min and 30 s. Helium was used as the carrier gas at a rate of 20 ml per min. The source and analyzer temperature of the MS was set at 230°C. Individual fatty acids were identified by comparing the retention times of authentic standard mixtures (FAME 37 components, Supelco, Bellefonte, PA) and with known spectrographic patterns of fatty acid methyl esters. Fatty acid methyl esters were quantified by comparing their peak areas with that of the internal standard. All fatty acids are expressed in percent of total fatty acids detected in each fraction.

2.7. Statistical analysis

Diets were not subjected to statistical analysis, as sample size ($n=2$) was too small to detect significance. Individual aquaria were used as the statistical test unit for all analyses. When appropriate, a one-way analysis of variance (ANOVA) was used to detect statistical differences among groups. Data was checked for normality and homogeneity of variance prior to analysis. Percent data was arcsine transformed before analysis, with the exception of mass gain data, which was \log_{10} transformed since this parameter included values over 100%. When statistical differences were observed using ANOVA, a *post hoc* Tukey's test was performed to determine which groups differed.

The Bonferroni correction factor (BCF), which accounts for a large number of comparisons by reducing the alpha level as to decrease the probability of obtaining a type I error, was used when analyzing fatty acid profiles among groups. The Bonferroni correction factor was calculated by dividing the alpha level (0.05) by the number of fatty acids analyzed. Since 28 individual fatty acids were analyzed, the new alpha value used for individual fatty acids was 0.002 ($\alpha = 0.05/28$). A new alpha level of 0.006 ($\alpha = 0.05/8$) was also calculated for the sum of saturated, sum of $n-6$, sum of MUFA, sum of $n-3$, sum of PUFA, DHA/EPA, ARA/EPA, and $n-3/n-6$.

If data failed to meet the requirements of ANOVA, the non-parametric Kruskal-Wallis test was used. When statistical differences were observed, an ANOVA was then used with a *post hoc* Tamhane's test, which does not assume equal variances. Linear regression analyses were performed between dietary fatty acids and

whole body lake trout fatty acid profiles in neutral and phospholipids fractions. Differences were considered statistically significant if $p < 0.05$, except for fatty acids, which used the alpha values calculated from the Bonferroni correction. Superscript letters indicate statistical significance in all tables and figures.

3. Results

3.1. Results: *Artemia* Experiment

3.1.1. Lipid content and fatty acid profiles of SELCO and Super SELCO-enrichments and stock *Artemia*

Lipid content between SELCO and Super SELCO-enrichments were similar (Table 3). The sum of SAFA was similar in both enrichments, with 16:0 being the dominant fatty acid. The SELCO-enrichment had considerably higher concentrations of 18:1 n -9 and, to a lesser extent, 20:1 n -9. The sum of PUFA was higher in the Super SELCO-enrichment. The sum of n -6 was similar in both enrichments but the SELCO-enrichment had nearly twice the amount of linoleic acid (18:2 n -6) in comparison to the Super SELCO-enrichment. However, the Super SELCO-enrichment had two times the amount of ARA. The Super SELCO-enrichment also had higher concentrations of the sum of n -3 fatty acids, which were composed of mainly EPA and DHA, with both being twice as high in the Super SELCO-enrichment. Interestingly, the ratios of DHA/EPA and ARA/EPA were similar between the SELCO and Super SELCO-enrichments.

Total lipids in stock *Artemia* were extremely low (2.1%; Table 3). The main constituent of the SAFA was palmitic acid (16:0). The MUFA consisted mainly of

18:1 n -9 and 18:1 n -7. The concentration of n -3 PUFA was significantly higher than the concentration of the n -6 PUFA. Linoleic acid was the dominant fatty acid in the n -6 PUFA, whereas linolenic acid (18:3 n -3) was the primary constituent in the n -3 PUFA. ARA and EPA were present at low levels in the stock *Artemia*, but DHA was not detected.

3.1.2. Lipid and fatty acid composition of diets: non-enriched *Artemia*, SELCO-enriched *Artemia*, Super SELCO-enriched *Artemia*, and BioVita #0

The highest lipid content was observed in the commercial BioVita #0 diet (Table 4), while the lowest was in non-enriched *Artemia*. SELCO-enriched *Artemia* had slightly higher lipid content when compared to Super SELCO-enriched *Artemia*, although both diets were considerably lower when compared to BioVita #0.

Fatty acid composition differences among the four diets were observed. The total concentration of SAFA in diets was highest in BioVita #0 and consisted mainly of palmitic acid, which was approximately double the concentration observed in the *Artemia* diets (Table 4). Super SELCO-enriched *Artemia* had the lowest concentration of SAFA. The dominant fatty acid in all *Artemia* diets was palmitic acid, reflecting the enrichment composition. Non-enriched *Artemia* did, however, have a high concentration of stearic acid (18:0).

The highest concentration of MUFA was observed in SELCO-enriched *Artemia*, followed closely by non-enriched *Artemia* (Table 4). Super SELCO-enriched *Artemia* and BioVita #0 had similar concentrations of MUFA, while BioVita #0 had the lowest overall concentration. The main constituent of MUFA for each diet

was oleic acid (18:1 n -9) but non-enriched *Artemia* had the highest concentrations of this fatty acid when compared to the other diets (Table 4).

Super SELCO-enriched *Artemia* had the highest concentration of PUFA. Super SELCO-enriched *Artemia* had the highest concentrations of n -3 PUFA and moderate concentrations of n -6 PUFA. Remaining diets had similar concentrations of n -3 PUFA but non-enriched *Artemia* had the lowest concentration of n -6 PUFA.

Of the n -6 fatty acid family, linoleic acid was the dominant fatty acid in each diet, with SELCO-enriched *Artemia* having the highest concentration (Table 4). Arachidonic acid was detected in each diet at low concentrations but showed an increasing trend from the SELCO to Super SELCO-enrichment.

The concentration of linolenic acid was nearly double in non-enriched *Artemia* when compared to Super SELCO-enriched *Artemia*, which had the second highest concentration. BioVita #0 had considerably lower concentrations of linolenic acid compared to all other diets. The concentrations of EPA and DHA increased with the addition of SELCO and Super SELCO-enrichments. Non-enriched *Artemia* had the lowest concentrations of EPA and non-detectable levels of DHA while Super SELCO-enriched *Artemia* had the highest concentrations of both fatty acids. Super SELCO-enriched *Artemia* had double the concentration of EPA when compared to SELCO-enriched *Artemia*. BioVita #0, however, did have the highest concentration of DHA.

3.1.3. Survival and growth of lake trout

After eight weeks of feeding, survival and growth parameters of lake trout were analyzed. Survival of lake trout alevins did not statistically differ among the four dietary treatments (Kruskal-Wallis, Chi-square = 7.460, $df = 3$, $p = 0.059$) (Table 5). Statistically significant differences were observed in lake trout final length and mass (ANOVA, $F = 46.015$, $df = 3$, $p < 0.001$ and ANOVA, $F = 71.738$, $df = 3$, $p < 0.001$ for length and mass, respectively). Length and mass were significantly higher in fish fed BioVita #0 (Tukey's *post hoc*, $p < 0.002$ and Tukey's *post hoc*, $p < 0.001$ for length and mass, respectively); lake trout fed the non-enriched *Artemia* treatment were significantly smaller (Tukey's *post hoc*, $p < 0.002$ and Tukey's *post hoc*, $p < 0.006$, for length and mass, respectively). Lake trout fed SELCO or Super SELCO-enriched *Artemia* did not statistically differ from one another in final length or mass (length: Tukey's *post hoc*, $p = 0.981$; mass: Tukey's *post hoc*, $p = 0.989$) (Table 5).

Significant differences (ANOVA, $F = 12.614$, $df = 3$, $p = 0.002$) in average mass were already observed after four weeks of feeding. Fish fed non-enriched *Artemia* had significantly lower mass (Tukey's *post hoc*, $p < 0.05$) than fish fed remaining diets (Figure 5). After six weeks of feeding, significant differences were observed in fish mass among dietary treatments (ANOVA, $F = 62.737$, $df = 3$, $p < 0.001$). Fish fed BioVita #0 had significantly higher mass (Tukey's *post hoc*, $p < 0.002$) than fish fed the remaining treatments while fish fed non-enriched *Artemia* had significantly lower mass (Tukey's *post hoc*, $p < 0.001$). Differences observed in fish

mass among dietary treatments at week six were similar to those observed at week eight, although mass of fish fed BioVita #0 showed the largest increase at this time.

The final mass of lake trout followed a unimodal distribution. The final mass modes were similar for fish fed the SELCO and Super SELCO-enriched *Artemia* diets at 0.51 to 0.60 g (Figure 6). The most abundant range for lake trout fed non-enriched *Artemia* was 0.41 to 0.50 g. Fish fed BioVita #0 had the most widespread distribution of final mass but the mass mode, 0.81 to 0.90 g, was the highest among treatments.

The final length mode of lake trout followed a similar trend to mass. Fish fed SELCO or Super SELCO-enriched *Artemia* had a length mode between 48 to 49 mm (Figure 7). Fish fed non-enriched *Artemia* were shortest, with the majority ranging from 42 to 43 mm. Length mode of BioVita #0 fed lake trout had the widest distribution among treatments, with the mode being 52 to 53 mm.

Mass gain (Kruskal-Wallis, Chi-square = 9.462, df = 3, $p = 0.024$), specific growth rate (Kruskal-Wallis, Chi-square = 9.462, df = 3, $p = 0.024$), and condition factor (ANOVA, $F = 19.581$, df = 3, $p < 0.001$) followed similar trends to those observed for final mass and length (Table 5). All three parameters were highest in fish fed BioVita #0. Mass gain and SGR were lowest in fish fed non-enriched *Artemia*. Condition factor, however, was similar in fish fed *Artemia* diets (Tukey's *post hoc*, $p > 0.459$). Lake trout fed SELCO or Super SELCO-enriched *Artemia* did not differ from each other in mass gain (Tukey's *post hoc*, $p > 0.989$), SGR (Tukey's *post hoc*, $p > 0.993$), or condition factor (Tukey's *post hoc*, $p = 1.000$).

3.1.4. Total lipid, phospholipid, and neutral lipid in whole body lake trout

Statistically significant differences were observed in whole body total lipids among fish fed the four dietary treatments (Kruskal-Wallis, Chi-square = 8.746, $df = 3$, $p = 0.033$). The concentration of total lipids were statistically lowest (Tamhane's *post hoc*, $p < 0.047$) in fish fed the non-enriched *Artemia* diet (Table 6). Total lipid concentration was highest in lake trout fed the SELCO-enriched *Artemia* diet, however it was not statistically different (Tamhane's *post hoc*, $p > 0.246$) from fish fed Super SELCO-enriched *Artemia* or BioVita #0.

Statistically significant differences were observed in lake trout neutral and phospho-lipid concentrations (ANOVA, $F = 88.292$, $df = 3$, $p < 0.001$ and ANOVA, $F = 78.240$, $df = 3$, $p < 0.001$ for neutral and phospho-lipids, respectively). The concentration of neutral lipids in fish fed non-enriched *Artemia* was significantly lowest (Tukey's *post hoc*, $p < 0.001$) (Table 6). The opposite trend was observed for the concentration of phospholipids (Tamhane's *post hoc*, $p < 0.001$) (Table 6). The concentration of both lipid fractions did not differ statistically among fish fed SELCO-enriched *Artemia*, Super SELCO-enriched *Artemia*, or BioVita #0 (Tukey's *post hoc*, $p > 0.321$ and $p > 0.101$ for neutral and phospho-lipids, respectively).

3.1.5. Fatty acid composition in whole body lake trout neutral lipid

Dietary fatty acid composition was largely reflected in the neutral lipid fraction of whole body lake trout at the end of the eight-week feeding experiment. The concentration of SAFA (ANOVA, $F = 357.143$, $df = 3$, $p < 0.001$), MUFA

(ANOVA, $F = 13.786$, $df = 3$, $p = 0.002$) and PUFA (ANOVA, $F = 94.565$, $df = 3$, $p < 0.001$) in whole body lake trout differed significantly among dietary treatments (Table 7). Fish fed BioVita #0 and non-enriched *Artemia* had the highest concentrations of SAFA, which was double of what was reported at the start of the experiment. Fish fed SELCO-enriched *Artemia* displayed the lowest concentration of SAFA (Bonferroni correction factor (BCF) $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$). Regardless of diet, 16:0 was the dominant SAFA in the neutral lipid fraction, however this fatty acid was statistically highest in non-enriched *Artemia* and BioVita #0 fed lake trout (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). In fish fed non-enriched *Artemia*, 18:0 was statistically higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) when compared to fish fed all other dietary treatments.

Although SELCO-enriched *Artemia* fed lake trout had the highest concentrations of MUFA, they were not statistically different from lake trout fed non-enriched *Artemia* (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 0.100$) or BioVita #0 (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 0.242$) (Table 7). Oleic acid, the dominant MUFA in the neutral lipid fraction of whole body lake trout alevins at the start and end of the *Artemia* Experiment, was significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed SELCO-enriched *Artemia* (Table 7). Lake trout fed the other dietary treatments were not statistically different (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.005$).

The sum of PUFA followed the same trend that was observed in dietary fatty acids and statistical differences were observed among fish fed the different dietary

treatments (ANOVA, $F = 94.565$, $df = 3$, $p < 0.001$). The concentration of PUFA was significantly higher (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) in lake trout fed Super SELCO-enriched *Artemia*, although non-enriched *Artemia* fed lake trout did not differ statistically (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p > 0.064$) from any dietary treatment due to high variance. The sum of fatty acids from the *n*-6 family was significantly higher (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) in fish fed SELCO-enriched *Artemia* when compared to Super SELCO-enriched *Artemia* and BioVita #0 fed lake trout. Non-enriched *Artemia* fed lake trout did not differ statistically (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p > 0.101$) from any dietary treatment in regards to the sum of *n*-6. Of the *n*-6 family, linoleic acid was the dominant fatty acid in all treatments, followed by ARA. Lake trout fed SELCO-enriched *Artemia* had significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentrations of linoleic acid. On the other hand, non-enriched *Artemia* fed lake trout had significantly lower (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentrations of linoleic acid. Arachidonic acid was significantly lower (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p = 0.001$) in both SELCO-enriched *Artemia* and BioVita #0 fed lake trout. Even though the non-enriched *Artemia* diet had the lowest concentrations of ARA, non-enriched *Artemia* fed lake trout had the highest concentrations of ARA, which were not statistically different (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p > 0.410$) from fish fed other diets.

The sum of *n*-3 was significantly higher in fish fed Super SELCO-enriched *Artemia* (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). SELCO-enriched *Artemia*

and non-enriched *Artemia* fed lake trout had statistically similar concentrations of the sum of *n*-3 (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 0.602$), while the BioVita #0 treatment had statistically lower concentrations (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$).

Linolenic acid, EPA, and DHA were the dominant fatty acids of the *n*-3 family. Even though the concentration of linolenic acid was the highest in the non-enriched *Artemia* diet, lake trout fed this diet did not differ statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 1.000$) when compared to SELCO or Super SELCO-enriched *Artemia* fed lake trout. Fish fed BioVita #0 had statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) lower concentrations of linolenic acid. Linolenic acid increased by nearly a factor of four in fish fed the *Artemia* diets when compared to concentrations at the start of the experiment. Fish fed BioVita #0 had less than half the concentration of linolenic acid when compared to the start of the experiment. Eicosapentaenoic acid was statistically different (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) among dietary treatments, with the exception of lake trout fed SELCO-enriched *Artemia* or BioVita #0, which did not differ significantly (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 0.088$). Docosahexaenoic acid was significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) for Super SELCO-enriched *Artemia* and BioVita #0 fed lake trout in comparison to fish fed the other dietary treatments. Concentrations of DHA in fish fed the *Artemia* diets were slightly lower than concentrations observed in lake trout at the start of the experiment. As observed in the fatty acid composition of the diets, EPA and DHA in whole body lake trout

increased from non-enriched *Artemia*, SELCO-enriched *Artemia*, to Super SELCO-enriched *Artemia*.

The dietary ratio $n-3/n-6$ was also reflected in the neutral lipid fraction of whole body lake trout. Statistical differences were observed among treatments (ANOVA, $F = 82.921$, $df = 3$, $p < 0.001$). The highest $n-3/n-6$ ratio was in *Artemia* and Super SELCO-enriched *Artemia* fed lake trout. The $n-3/n-6$ ratio of non-enriched *Artemia* fed lake trout, however, did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.122$) when compared among dietary treatments.

3.1.6. Fatty acid composition in whole body lake trout phospholipid

The sum of SAFA in the phospholipid fraction of whole body lake trout was statistically significant (ANOVA, $F = 34.598$, $df = 3$, $p < 0.001$) among dietary treatments. The percentage of SAFA was statistically higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.002$) in lake trout fed BioVita #0 (Table 8). Palmitic acid was the dominant fatty acid in the saturated portion of the phospholipid fraction in fish from all dietary treatments, followed by 18:0. The concentration of palmitic acid did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.057$) in lake trout fed the three *Artemia* diets. Moreover, these concentrations were similar to those observed in lake trout at the start of the experiment.

The sum of MUFA was statistically different (ANOVA, $F = 227.265$, $df = 3$, $p < 0.001$) among fish fed all dietary treatments, with the highest concentration observed in non-enriched *Artemia* fed lake trout (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). Oleic acid was the main fatty acid of the MUFA (Table 8). Oleic acid was

statistically similar in lake trout fed non-enriched *Artemia* and SELCO-enriched *Artemia* (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 0.020$). The concentration of oleic acid decreased in the phospholipid fraction of lake trout with the addition of *Artemia* enrichment, with lake trout fed the Super SELCO-enriched *Artemia* diet having the lowest concentration of oleic acid of fish fed the *Artemia* diets. Fish fed BioVita #0 had the lowest overall concentration of oleic acid.

Statistical differences in the phospholipid fraction of whole body lake trout were observed in the sum of PUFA (ANOVA, $F = 36.739$, $p < 0.001$). Lake trout fed non-enriched *Artemia* had a statistically lower concentration of the sum of PUFA (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). Fish fed other treatments did not statistically differ (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.119$). The sum of *n*-6 was statistically highest (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) in lake trout fed non-enriched *Artemia*, whereas Super SELCO-enriched *Artemia* fed lake trout had statistically lower concentrations (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). Non-enriched *Artemia* fed lake trout had the statistically lowest (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentration of fatty acids from the *n*-3 family. Fish fed other diets did not differ among each other (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.017$).

Similar to what was observed in the neutral lipid fraction, linoleic acid and ARA were the dominant fatty acids of the *n*-6 family in the phospholipid fraction of whole body lake trout. High concentrations of linoleic acid were observed in lake trout fed non-enriched *Artemia*, SELCO-enriched *Artemia*, and BioVita #0. Super

SELCO-enriched *Artemia* fed lake trout had significantly lower (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentrations of linoleic acid. Arachidonic acid was statistically highest (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in non-enriched *Artemia* fed lake trout and lowest in BioVita #0 fed lake trout. SELCO-enriched *Artemia* fed lake trout did not differ statistically from lake trout fed Super SELCO-enriched *Artemia* (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 0.076$) or BioVita #0 (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 0.013$). When compared to initial lake trout concentrations, ARA decreased in fish by a factor of two for each dietary treatment.

Linolenic acid was statistically highest (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed non-enriched *Artemia* and increased by a factor of ten when compared to initial concentrations in lake trout (Table 8). Fish fed BioVita #0 had the statistically lowest concentration of linolenic acid (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). In regards to linolenic acid in the phospholipid fraction, SELCO and Super SELCO-enriched *Artemia* fed lake trout did not differ statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 1.000$).

Eicosapentaenoic acid and DHA concentrations in the phospholipids of whole body lake trout ranked from low to high as follows: non-enriched *Artemia*, SELCO-enriched *Artemia*, and Super SELCO-enriched *Artemia*. Eicosapentaenoic acid had significantly higher concentrations in fish fed the Super SELCO-enriched diet than in fish from other *Artemia* treatments (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). Fish fed BioVita #0 were statistically similar (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 1.000$) to SELCO-enriched *Artemia* fed lake trout. The concentration of DHA was

statistically similar in whole body lake trout fed the SELCO, Super SELCO-enriched *Artemia*, and BioVita #0 diets (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.051$) but was statistically lower in lake trout fed non-enriched *Artemia* (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). Regardless of dietary treatment, DHA was the main constituent of the *n*-3 family in lake trout.

The ratio *n*-3/*n*-6 increased in lake trout with the addition of *Artemia* enrichments (ANOVA, $F = 82.921$, $df = 3$, $p < 0.001$). Super SELCO-enriched *Artemia* fed lake trout had the statistically highest (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.002$) *n*-3/*n*-6 ratio while fish fed non-enriched *Artemia* had the lowest ratio (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). The ratio of DHA to EPA was highest in fish fed BioVita #0 and SELCO-enriched *Artemia*. The ratio of ARA to EPA was not significantly different (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.125$) among dietary treatments with the exception of fish fed non-enriched *Artemia*, which had double the ratio of ARA/EPA and were statistically higher than fish fed other treatments (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$).

3.1.7. Relationship between dietary and whole body fatty acids

The majority of dietary fatty acids were not significantly correlated with whole body lake trout fatty acids (Table 9). In the neutral lipid fraction, exceptions to this were linoleic acid (ANOVA, $F = 19.272$, $df = 3$, $p = 0.048$) and the sum of *n*-6 (ANOVA, $F = 29.488$, $df = 3$, $p = 0.032$). The phospholipid fraction had significant linear correlations of dietary fatty acids to whole body lake trout fatty acids with

regards to linolenic acid (ANOVA, $F = 43.742$, $df = 3$, $p = 0.022$), EPA (ANOVA, $F = 17.512$, $df = 3$, $p = 0.050$), DHA (ANOVA, $F = 20,172.721$, $df = 3$, $p < 0.001$), and the sum of SAFA (ANOVA, $F = 48.213$, $df = 3$, $p = 0.020$).

3.2. Results: Fish Oil Replacement Experiment

3.2.1. Lipid and fatty acid composition of oleic acid, linseed oil, cod liver oil, and lecithin diets

Diets were formulated to be isolipidic with a lipid content of 14% (Table 2). Similar lipid content was observed in the diets and ranged from 15.6 to 16.5%, with the exception of the LE diet, which had a lower lipid content of 13.4% (Table 10).

As expected, the OA diet had the highest concentration of oleic acid in comparison to the other diets (Table 10). Oleic acid accounted for 70% of the total detected fatty acids in the OA diet. Although this diet was formulated to be deficient in linoleic acid and linolenic acid, the precursors to HUFA, fatty acid composition showed that these fatty acids in the diet. Linoleic and linolenic acid were detected at 9.3 and 0.3%, respectively in the OA diet. The HUFA, ARA, EPA, and DHA, however, were not detected. This diet also had the lowest concentration of PUFA.

The LO diet, which was formulated to contain linoleic and linolenic acid, the precursors to HUFA, did so. Linoleic acid and linolenic acid were detected at 17.9 and 46.9%, respectively (Table 10). As formulated, this diet was deficient in HUFA, as ARA, EPA, and DHA were not detected.

The CLO diet was formulated to be the most complete and included the precursors to HUFA and HUFA. The diet contained both linoleic and linolenic acids, although both were in low concentrations in comparison to the LO and LE diets (Table 10). Arachidonic acid was present in the diet but in low concentration. The dominant *n*-3 HUFA in this diet were EPA and DHA.

Like the LO diet, the LE diet contained only the precursors to HUFA but in the form of phospholipids. Opposite of what was observed in the LO diet, the LE diet had high concentrations of linoleic acid and low concentrations of linolenic acid (Table 10). The sum of PUFA were similar for both LO and LE diets.

3.2.2. Survival and growth of lake trout

Significant differences in survival were observed among lake trout based on dietary treatment (Kruskal-Wallis, Chi-square = 9.756, $df = 3$, $p = 0.021$). Lake trout survival was poor for fish fed the OA diet and was significantly lower (Tamhane's *post hoc*, $p < 0.006$) than all other dietary treatments (Table 11). Linseed oil, CLO, and LE fed lake trout had over 93% survival and were not statistically different among each other (Tamhane's *post hoc*, $p > 0.256$).

At the end of the experiment, length and mass were significantly different for fish among dietary treatments (length: ANOVA, $F = 539.299$, $df = 3$, $p < 0.001$; mass: ANOVA, $F = 588.984$, $df = 3$, $p < 0.001$). Final length and mass were statistically highest in fish fed the CLO diet (length: Tukey's *post hoc*, $p < 0.010$; mass: Tukey's *post hoc*, $p < 0.001$). Lake trout fed the OA diet displayed significantly lower final length and mass (length: Tukey's *post hoc*, $p < 0.001$; mass:

Tukey's *post hoc*, $p < 0.001$) (Table 11). Fish fed the LO or LE diets were not statistically different (Tukey's *post hoc*, $p = 0.138$) in regards to final length. Final mass was statistically higher for lake trout fed the LE diet (Tukey's *post hoc*, $p = 0.007$) when compared to LO fed fish.

Statistical differences (ANOVA, $F = 24.444$, $df = 3$, $p < 0.001$) in fish mass were observed after two weeks of feeding (Figure 8). Fish fed the OA diet had significantly lower mass after two weeks (Tukey's *post hoc*, $p < 0.001$) and remained significantly lower throughout the duration of the experiment. After four weeks of feeding, statistical differences were still observed (ANOVA, $F = 203.778$, $df = 3$, $p < 0.001$) and fish fed the CLO diet had significantly higher mass than the LE and OA diet fed lake trout (Tukey's *post hoc*, $p < 0.002$). Mass of fish fed the LO diet was statistically similar to fish fed CLO and LE diets (Tukey's *post hoc*, $p = 0.078$). After six weeks of feeding, significant differences in mass were still observed (ANOVA, $F = 330.738$, $df = 3$, $p < 0.001$). Lake trout fed the CLO diet had significantly higher mass after six weeks of feeding when compared to fish from all other dietary treatments (Tukey's *post hoc*, $p < 0.002$) and remained significantly higher until the end of the experiment (Tukey's *post hoc*, $p < 0.001$). Linseed oil and LE diet fed lake trout had statistically similar masses at week six (Tukey's *post hoc*, $p = 0.872$), but at the end of the experiment, LE diet fed lake trout were significantly higher than fish fed the LO diet (Tukey's *post hoc*, $p = 0.026$).

Mass displayed a unimodal distribution. Mass mode was similar for the LO and LE diet lake trout, 0.25 to 0.71 g and 0.20 to 0.77 g, respectively (Figure 9).

Mass mode distribution of OA diet fed lake trout, 0.08 to 0.33 g, was lower when compared with the other dietary treatments and little overlap with other treatments was observed. Lake trout fed the CLO diet had a higher range of mass mode than that of the other diets, 0.28 to 0.96 g.

Length also displayed a unimodal distribution. Fish fed the OA diet ranged in length from 22 to 40 mm and was lower when compared to fish fed other treatments (Figure 10). As was observed with mass mode, lake trout fed LO and LE diets had similar length modes, 35 to 49 mm and 31 to 50 mm, respectively. Highest average length mode, 37 to 53 mm, was observed in fish fed CLO.

Statistical differences in fish mass gain were observed among dietary treatments (ANOVA, $F = 89.356$, $df = 3$, $p < 0.001$). Mass gain was statistically similar (Tukey's *post hoc*, $p > 0.201$) among treatments, with the exception of fish fed the OA diet, which was statistically lower (Tukey's *post hoc*, $p < 0.001$) than fish from other dietary treatments (Table 11). Specific growth rate also showed statistical differences among dietary treatments (Kruskal-Wallis, Chi-square = 10.385, $p = 0.016$). The CLO diet fed lake trout had the highest SGR but were statistically similar to fish fed the LE diet (Tamhane's *post hoc*, $p = 0.052$). Oleic acid diet fed lake trout had a significantly lower (Tamhane's *post hoc*, $p < 0.023$) SGR when compared among dietary treatments.

Food conversion ratio of fish differed significantly among dietary treatments (ANOVA, $F = 10.215$, $df = 3$, $p = 0.004$). Fish fed the CLO diet had significantly lower FCR (Tukey's *post hoc*, $p < 0.005$) when compared with fish fed other dietary

treatments, with the exception of fish fed the LE diet, which was statistically similar to CLO fed fish (Tukey's *post hoc*, $p = 0.064$). Oleic acid diet fed lake trout had significantly higher (Tukey's *post hoc*, $p < 0.001$) FCR among dietary treatments.

Condition factor of fish fed different diets were statistically different (ANOVA, $F = 56.015$, $df = 3$, $p < 0.001$). Condition factor was significantly lower (Tukey's *post hoc*, $p < 0.001$) in OA diet fed lake trout (Table 11). As was the case with SGR and FCR, fish fed the LE diet were statistically similar (Tukey's *post hoc*, $p > 0.074$) to fish fed the LO and CLO dietary treatments in regards to K. The highest K was observed in CLO diet fed lake trout but did not differ statistically to fish fed the LE diet (Tukey's *post hoc*, $p = 0.074$).

3.2.3. Total lipid, phospholipid, and neutral lipid in whole body lake trout

Statistical difference in total whole body lipids of lake trout was observed among treatments (Kruskal-Wallis, Chi-square = 9.464, $p = 0.024$) (Table 12). Total whole body lipids were statistically similar (Tamhane's *post hoc*, $p = 0.061$) in lake trout fed LO and CLO diets. Total lipids were significantly lower in fish fed OA or LE diets than fish fed the LO or CLO diets (Tamhane's *post hoc*, $p < 0.012$). Lake trout fed OA or LE diets had similar total lipid concentrations (Tamhane's *post hoc*, $p = 0.991$) and suggest these fish were more lean than LO or CLO diets fed fish.

All dietary treatments were significantly different (ANOVA, $F = 42.675$, $df = 3$, $p < 0.05$) from each other in regards to both the neutral and phospho-lipid fractions of total lipids. The energy dense neutral lipids were significantly higher (Tukey's *post hoc*, $p < 0.05$) in fish fed the CLO diet, followed by LO diet and then LE diet

(Table 12). Fish fed the OA diet had the lowest percent of neutral lipids. Fish fed the OA and LE diets had approximately a 50 - 50% neutral to phospho-lipid ratio, whereas fish fed the LO and CLO diets contained a higher percentage of neutral lipids. Cod liver oil diet fed lake trout had the lowest concentration (Tukey's *post hoc*, $p < 0.05$) of phospholipids and OA diet fed fish had a significantly (Tukey's *post hoc*, $p < 0.05$) higher concentration.

3.2.4. Fatty acid composition in whole body lake trout neutral lipid

Statistical differences in total SAFA were observed in whole body lake trout among dietary treatments (ANOVA, $F = 175.273$, $df = 3$, $p < 0.001$). Total SAFA were statistically highest (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) in lake trout fed the LE diet (Table 13). Fish fed the LO diet had the lowest total sum of SAFA for the neutral lipid fraction, but were not significantly different than the OA diet fed lake trout (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 0.020$). Lake trout fed OA, LO, or CLO diets had similar concentrations of total SAFA when compared to the concentration of initial lake trout at the start of the experiment. Lecithin diet fed lake trout, however, had concentrations of SAFA that were almost double of what was observed in initial lake trout. Palmitic acid was the dominant SAFA for fish regardless of dietary treatment. Fish fed the LE diet had a significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentration of 16:0 compared to fish fed other dietary treatments. In fish fed the LE diet, 16:0 was present in concentrations nearly double of those observed in initial lake trout. This trend was also observed in 18:0.

The sum of MUFA was statistically significant among lake trout fed different dietary treatments (ANOVA, $F = 32.511$, $df = 3$, $p < 0.001$). The sum of MUFA was highest in OA and CLO diet fed lake trout, although fish from fed the OA diet did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 1.000$) among dietary treatments as high variance was observed (Table 13). The high variance was caused by one of the three aquaria consistently having higher concentrations in comparison to the other two. Fish fed the LO and LE diets did not differ statistically (Tukey's *post hoc*, $p = 1.000$) between each other. As expected, OA diet fed lake trout had nearly double the concentration of oleic acid when compared to other dietary treatments. However, due to high variance, the OA diet fed lake trout did not differ statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.004$) with the other dietary treatments. Oleic acid in fish fed LO, CLO, and LE diets were similar to concentrations observed at the start of the experiment. Fish fed LO or LE diets had a considerable decrease in the concentration of 16:1 n -7 when compared to initial lake trout at the start of the experiment. Fish fed these diets had statistically lower concentrations of 16:1 n -7 in comparison to fish fed OA or CLO diets (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). Fish fed the CLO diet had statistically higher concentrations of 20:1 n -9 and 22:1 n -9 in comparison to fish fed other dietary treatments (20:1 n -9: BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$; 22:1 n -9: BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$). Concentrations of these two fatty acids were considerably higher in fish fed the CLO diet than concentrations observed in lake trout at the start of the experiment.

Statistical differences in PUFA were observed in whole body lake trout (ANOVA, $F = 41.061$, $df = 3$, $p < 0.001$). The total sum of PUFA was highest in fish fed the LO and LE diets (Table 13). Lake trout fed CLO and OA diets had the lowest concentrations of PUFA and were approximately half the concentration of lake trout at the start of the experiment.

The sum of $n-6$ displayed significant differences among dietary treatments (ANOVA, $F = 207.911$, $df = 3$, $p < 0.001$). For the sum of $n-6$, significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations were observed in the LE diet fed lake trout, reflecting the high concentrations of $n-6$ included in the LE diet. This was three times the amount of the sum of $n-6$ observed in initial lake trout. The lowest concentration of the sum of $n-6$ was observed in fish fed the CLO diet, although this was not statistically different (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 0.063$) when compared to fish fed the OA diet.

Of the $n-6$ family, linoleic acid and ARA were the dominant fatty acids. Lecithin diet fed lake trout had significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentrations of linoleic acid, reflecting the fatty acid profile of the plant-based diet rich in linoleic acid. This is an increase by a factor of nine in linoleic acid when compared to concentrations in initial lake trout. The OA and CLO diet fed lake trout had the lowest concentrations of linoleic acid and were similar to concentrations observed in initial lake trout. Linseed oil diet fed lake trout had moderate amounts of linoleic acid. High concentrations of ARA were observed in lake trout fed the LE diet but highest concentrations were actually observed in the OA

diet fed lake trout. Oleic acid diet fed lake trout were not, however, statistically significant (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p > 0.002$) from fish fed the other dietary treatments and showed lower concentrations of ARA than those observed in initial lake trout. Lake trout fed LO and CLO diets had low concentrations of ARA and were not statistically different (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p = 1.000$).

Like the sum of $n-6$, significant differences were observed in the sum of $n-3$ in the neutral lipid fraction of whole body lake trout (ANOVA, $F = 86.056$, $df = 3$, $p < 0.001$). For the sum of $n-3$, concentrations were significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) in fish fed the LO diet. Lake trout fed the LE diet had significantly lower values of $n-3$ (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.003$).

Linolenic acid, EPA, and DHA were the three main constituents of the $n-3$ fatty acids, but fish fed the LO diet also had relatively high concentrations of $18:4n-3$ (Table 13). Linolenic acid was significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in the LO diet fed lake trout, reflecting high concentrations of linolenic acid incorporated in the diet and increased by a factor of nine when compared to initial lake trout. All other treatments did not differ statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p = 1.000$) in regards to linolenic acid. Eicosapentaenoic acid and DHA were highest in fish fed the CLO diet but did not differ statistically (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.002$; BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.005$) to fish fed the OA diet, as high variance was observed in this dietary treatment. Eicosapentaenoic acid was lowest in fish fed the LE diet. Fish fed LO or LE diets had the lowest concentrations of DHA but did not differ statistically (BCF $\alpha = 0.002$,

Tukey's *post hoc*, $p = 1.000$) from one another. Interestingly, lake trout fed the OA diet, deficient in the precursors to HUFA and HUFA, showed high concentrations of ARA, EPA, and DHA but were statistically similar (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p > 0.002$) among all dietary treatments. Although ARA, EPA, and DHA were observed in lake trout fed the OA diet, concentrations of these fatty acids were lower when compared to lake trout at the start of the experiment.

Statistical differences were observed among dietary treatments in regards to the ratio of *n*-3/*n*-6 (ANOVA, $F = 826.282$, $df = 3$, $p < 0.001$). Fish fed the CLO diet were significantly higher (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) than fish fed other dietary treatments. This ratio was significantly lower (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.002$) in fish fed the LE diet. Lake trout fed OA and LO diets were not statistically different (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p = 0.123$) between each other. Fish fed the LO diet had the lowest ratio of DHA/EPA (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). The ratio of ARA/EPA was lowest in fish fed the LO and CLO dietary treatments. The LE diet fed lake trout had the highest ratios of both DHA/EPA and ARA/EPA.

3.2.5. Fatty acid composition in whole body lake trout phospholipid

In the phospholipid fraction, the sum of SAFA was statistically different (ANOVA, $F = 42.650$, $df = 3$, $p < 0.001$) (Table 14). Statistically highest concentrations of SAFA were observed in fish fed the LE diet (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$). The major constituent of SAFA for fish from all

dietary treatments was 16:0. This fatty acid was statistically highest (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed the LE diet and was similar to concentrations in lake trout at the start of the experiment. The second most abundant fatty acid in SAFA was 18:0. Fish fed the CLO diet had significantly lower concentrations of 18:0 when compared to fish from other dietary treatments (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$).

Statistical differences in regards to the sum of MUFA were observed in fish among dietary treatments (ANOVA, $F = 27.544$, $df = 3$, $p < 0.001$). The total sum of MUFA was highest in fish fed the OA diet, although fish fed the CLO diet were statistically similar (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.012$) to all treatments (Table 14). Linseed oil and LE diet fed lake trout were significantly lower (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) than fish fed the OA dietary treatment. Of the MUFA, oleic acid was the most abundant in fish from all dietary treatments. As expected, oleic acid was significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed the OA diet when compared among dietary treatments and was more than double the concentration when compared to initial lake trout at the start of the experiment. The fatty acid, 20:1 n -9 was statistically higher in fish fed the CLO diet (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) and was double the concentration of what was observed in fish at the start of the experiment. Fish fed the CLO diet also had concentrations of 22:1 n -11, which was not present in fish from the start of the experiment or any other dietary treatment.

Statistical differences in fish in regards to total PUFA were observed among dietary treatments (ANOVA, $F = 21.996$, $df = 3$, $p < 0.001$). Total PUFA were significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) in the LO and LE diet fed lake trout when compared to fish fed the OA diet. Cod liver oil diet fed lake trout did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p > 0.010$) among treatments. Lecithin diet fed lake trout had significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations of total $n-6$ when compared to fish fed other dietary treatments, but had significantly lower (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations of the sum of $n-3$. Lecithin diet fed fish had triple the concentration of the sum of $n-6$ but decreased by more than half in regards to the sum of $n-3$ when compared to concentrations in initial whole body lake trout. The CLO diet fed lake trout had significantly lower (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations of $n-6$ when compared among dietary treatments. Cod liver oil and LO diet fed lake trout had statistically higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations of total $n-3$ among dietary treatments, but did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 1.000$) from each other. Lecithin diet fed lake trout had the statistically lowest (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) concentrations of total $n-3$ and, when compared to initial lake trout, decreased by a factor of 2.5.

Linoleic acid and ARA were the dominant fatty acids in the $n-6$ family for all dietary treatments in the phospholipid fraction, but were significantly higher (Linoleic: BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$; ARA: BCF $\alpha = 0.002$,

Tukey's *post hoc*, $p < 0.001$) in fish fed the LE diet. Based on the fatty acid composition of lake trout before the start of the experiment, linoleic acid increased nearly 18 fold by the end of the experiment in fish fed the LE dietary treatment. Linoleic acid was significantly lower (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) in the OA and CLO diet fed lake trout. Arachidonic acid was also significantly lower (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed the CLO diet.

Linolenic acid was statistically higher (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) in fish fed the LO diet and increased by a factor of seven from the concentration of linolenic acid observed in initial lake trout fatty acid profiles. Fish from remaining dietary treatments had considerably lower concentrations of linolenic acid than fish fed the LO diet and did not differ statistically (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p > 0.006$) among each other. Eicosapentaenoic acid was significantly higher (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) in fish fed the CLO and LO diets. Lecithin and OA diet fed lake trout had significantly lower (BCF $\alpha = 0.002$, Tukey's *post hoc*, $p < 0.001$) concentrations of EPA. Docosahexaenoic acid was the dominant fatty acid in the *n*-3 family for all dietary treatments and was statistically highest (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) in fish fed the CLO diet. Lake trout fed the LE diet had the lowest concentrations of DHA statistically (BCF $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) and was nearly half the concentration observed in initial lake trout.

Statistical differences in the ratio of the sum of $n-3/n-6$ were observed among dietary treatments (ANOVA, $F = 674.122$, $df = 3$, $p < 0.001$). This ratio was statistically highest in fish fed the CLO diet (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.005$). The lowest ratio was in fish fed the LE diet (BCF $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.004$). Interestingly, a significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) ratio of DHA/EPA was observed in lake trout fed the OA diet. The lowest ratio (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) of DHA to EPA was in the LO diet fed lake trout. A significantly higher (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) ratio of ARA/EPA was detected in lake trout fed the LE diet. Fish fed the LO or CLO diets had a significantly lower (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p < 0.001$) ratio ARA/EPA when compared among treatments but did not differ statistically (BCF $\alpha = 0.006$, Tukey's *post hoc*, $p = 1.000$) between each other.

3.2.6. Relationship between body and dietary fatty acids

The majority of whole body fatty acids in lake trout did not show a correlation with dietary fatty acids in the neutral or phospholipid fractions (Table 15). Exceptions to this included, oleic acid (neutral lipid: ANOVA, $F = 246.991$, $df = 3$, $p = 0.004$; phospholipid: ANOVA, $F = 47.845$, $df = 3$, $p = 0.020$), linoleic acid (neutral lipid: ANOVA, $F = 39.426$, $df = 3$, $p = 0.024$; phospholipid: ANOVA, $F = 1641.259$, $df = 3$, $p = 0.001$), and linolenic acid (neutral lipid: ANOVA, $F = 182.794$, $df = 3$, $p = 0.005$; phospholipid: ANOVA, $F = 184.113$, $df = 3$, $p = 0.005$), which were significantly correlated to their respective concentrations in whole body lake trout in

the neutral or phospholipid fractions. The sum of PUFA also showed a significant correlation (ANOVA, $F = 18.583$, $df = 3$, $p = 0.050$) in the phospholipid fraction. Dietary ARA, EPA, and DHA did not show any correlation with these fatty acids in whole body lake trout. This was likely due to high whole body concentrations of HUFA observed in the oleic acid treatment, even though they were not present in the diet.

4. Discussion

In salmonid aquaculture, lipids, often supplied in form of fish oil, are a major component of first feed diets, accounting for 10-12% of the dietary nutrient levels (Tacon 1990). Problems such as contamination, sustainability, and high prices of fish oil plague its use; therefore, alternatives to fish oil are being investigated. Studies have been conducted with Atlantic salmon (Bell *et al.* 2003b, Bell *et al.* 2004b, Bell *et al.* 2010, Berge *et al.* 2004, Berge *et al.* 2009, Nanton *et al.* 2007), brown trout (*Salmo trutta*) (Turchini *et al.* 2003), brook trout (*Salvelinus fontinalis*) (Guillou *et al.* 1995), chinook salmon (Huang *et al.* 2008), and rainbow trout (Caballero *et al.* 2002, Drew *et al.* 2007, Rinchard *et al.* 2007, Turchini *et al.* 2011) where fish oil has been partially or totally replaced with vegetable oils. The replacement of fish oil with lipids from other sources, however, has not been studied in lake trout, particularly during first feeding. To determine if replacing fish oil with other lipid sources is feasible for the first feeding of lake trout, two feeding experiments were conducted. In the *Artemia* Experiment, fish were offered diets of non-enriched *Artemia*, SELCO-

enriched *Artemia*, Super SELCO-enriched *Artemia*, or Bio Vita #0. Similarly, in the Fish Oil Replacement Experiment, lake trout were offered dry diets of either oleic acid, linseed oil, cod liver oil, or lecithin. Diets used in the two experiments, with the exception of the commercial diet Bio Vita #0, differed solely in lipid source and fatty acid composition, which affected survival, growth performance, lipid content, and fatty acid composition of lake trout alevins.

4.1. *Artemia* Experiment: Growth

Throughout the *Artemia* Experiment, lake trout fed non-enriched *Artemia*, containing low or non-detectable concentrations of HUFA, displayed significantly lower growth in comparison to fish fed SELCO or Super SELCO-enriched *Artemia* or BioVita #0. Survival, however, was not compromised in fish fed the non-enriched *Artemia* diet. The non-enriched *Artemia* diet was not deficient of essential fatty acids, as it contained high concentrations of linolenic acid and, to a lesser extent, linoleic acid. The low to non-detectable concentrations of HUFA in the non-enriched *Artemia* diet may be responsible for the lower growth parameters observed in fish fed this diet; as SELCO and Super SELCO-enriched *Artemia* diets had higher concentrations of HUFA, particularly EPA and DHA and fish fed these diets had significantly higher final length, final mass, mass gain, and SGR. These results suggests that incorporation of these fatty acids in the first feed diets improves growth and development of lake trout alevins.

Enriched *Artemia* diets have also been shown to improve growth in other fish species. Ozkizilcik and Chu (1994) conducted a 21-day feeding trial with striped bass (*Morone saxatilis*) larvae (1.4 ± 0.0 mg). Striped bass were fed a control diet of non-enriched *Artemia* or diets of *Artemia* enriched with either a menhaden/yeast oil emulsion (YMO), gelatin-acacia microcapsules containing menhaden oil (GAC), or *Chlorella sp.* (CHL), a type of green algae. The non-enriched *Artemia* diet had a significantly lower concentration of linolenic acid and EPA when compared to the enriched *Artemia* diets. YMO and GAC enriched *Artemia* diets had a significantly higher concentration of EPA. Fish fed the non-enriched *Artemia* diet had significantly lower wet weight and total length at the end of the experiment when compared to fish fed the enriched *Artemia* diets. Fish fed the different enriched *Artemia* diets, however, did not differ significantly for final wet weight and total length. This suggests that enriching *Artemia* with sources high in EPA can improve growth in striped bass larvae. This is similar to what was observed in the *Artemia* Experiment as lake trout fed *Artemia* enriched with SELCO or Super SELCO, high in EPA and DHA, had significantly higher final length, final mass, mass gain, and SGR when compared to fish fed non-enriched *Artemia*; a diet low in EPA and DHA.

Caution should be used when comparing growth parameters of lake trout fed live or dry feeds. In the *Artemia* Experiment, the diets of SELCO enriched *Artemia*, Super SELCO-enriched *Artemia*, and BioVita #0 had similar concentrations of *n*-3 HUFA, with the exception of the Super SELCO-enriched *Artemia* diet, which had significantly higher EPA concentrations. Although concentrations were similar, if not

higher, in regards to *n*-3 HUFA in the two enriched *Artemia* diets when compared to BioVita #0, all growth parameters were significantly higher in fish fed BioVita #0. This may be due to BioVita #0 being a dry feed, whereas, the *Artemia* diets were a live feed. The weight of dry food administered was 5% body weight of lake trout alevins but since the *Artemia* diets were a wet, live food, weight of *Artemia* fed to each tank cannot be determined. The higher growth rates observed in fish fed BioVita #0 may be due to these fish receiving more food than fish from the *Artemia* treatments, and not dietary composition. However, dietary composition is another explanation for the higher growth parameters observed in fish fed BioVita #0. BioVita #0 had a lipid content of 18.4%, more than three times the lipid content observed in the *Artemia* diets (Table 4). Other ingredients, such as protein, vitamins, or minerals may also be different in BioVita #0 when compared to the *Artemia* diets.

4.2. Fish Oil Replacement Experiment: Growth

The survival and growth rates of lake trout alevins fed the oleic acid diet were significantly lower in comparison to fish fed the remaining diets in the Fish Oil Replacement Experiment. The OA diet was deficient in the essential fatty acids, linolenic and linoleic acids, as well as HUFA. Rinchard *et al.* (2007) observed similar results in juvenile rainbow trout (182 ± 51 mg) fed an OA diet during an 8-week feeding experiment. Rainbow trout had significantly lower survival, final mass, mass gain, SGR, and significantly higher FCR when compared to fish fed diets of cod liver oil, soybean lecithin, or a linseed and olive oil blend. Poor survival and growth

has also been observed in other fish species such as juvenile European sea bass (*Dicentrarchus labrax*) (14.4 ± 0.1 g) (Skalli and Robin 2004) and juvenile turbot (*Scophthalmus maximus*) (1.2 ± 0.3 g) (Bell *et al.* 1999) fed EFA-deficient diets, suggesting that EFA are required for proper fish survival and development. Results from the Fish Oil Replacement Experiment confirm that linolenic and linoleic acids are required for lake trout alevins to survive and undergo proper development, as OA fed lake trout displayed poor survival and growth. Like all vertebrates, lake trout lack the $\Delta 12$ and $\Delta 15$ fatty acyl desaturase enzymes to convert 18:1 n -9 to linoleic and linolenic acids; therefore these fatty acids cannot be produced *de novo* and must be acquired through diet (Torstensen and Tocher 2011). It should be noted that although the OA diet was formulated to be EFA deficient, trace amounts of linolenic and linoleic acids were present. However, the survival and growth of alevins were still significantly lower when compared to fish fed other diets, suggesting that concentrations of these EFA were not adequate for the survival or growth of lake trout alevins.

Lake trout fed the vegetable oil diets of either LO or LE had significantly lower final length and mass when compared to fish fed the CLO diet. This is in contrast to observations in juvenile Chinook salmon fed diets supplemented with canola oil (CO), another vegetable oil. A 30-week feeding experiment with Chinook salmon (0.80 ± 0.03 g) was carried out by Huang *et al.* (2008) where fish were fed diets sprayed with a supplementary lipid source. The lipid source contained canola oil or a 1:1 blend of anchovy oil and poultry fat, which accounted for 0, 33, 67, or

100% in the supplemental lipid source or 0, 25, 49, or 72% of the total determined dietary lipid content in the diet. Survival, final mass, mass gain, and SGR of Chinook salmon fed the diets with different amounts of canola oil were not significantly different, suggesting that Chinook salmon survival and growth was not affected by using canola oil as a supplementary lipid source. There were, however, relatively high concentrations of ARA, EPA, and DHA in all diets used in this experiment, a result of fish and krill meal incorporated in the diets. Similar to the LO and LE diets used in the Fish Oil Replacement Experiment, the 72% CO diet used by Huang *et al.* (2008) had high concentrations of 18:2 n -6 (17.60%) and 18:3 n -3 (5.04%). Their 72% CO diet also contained high concentrations of ARA (0.34%), EPA (2.03%), and DHA (3.32%), which were not present in my LO and LE diets. It is likely that these high concentrations of HUFA are responsible for the similarities in growth in Chinook salmon, as the diets were not HUFA deficient. Whereas the LO and LE diets used in the Fish Oil Replacement Experiment were lacking in HUFA resulting in fish fed the LO or LE diets having significantly lower growth than fish fed the CLO diet.

Fish fed the LO diet displayed lower growth than fish fed CLO, but had a high survival rate (98%). The linolenic rich LO has been used as a FO replacement in other salmonid feeding experiments. One such study carried out by Menoyo *et al.* (2005) with Atlantic salmon (220 g) used LO as a FO replacement. When 100% of FO was replaced by LO, no significant differences were observed in regards to final weight, length, and condition factor (Menoyo *et al.* 2005). Similarly, a 72 day feeding trial with rainbow trout (90 g) fed either a diet of 100% FO or 100% LO

showed no significant differences in final mass, weight gain, FCR, or SGR between rainbow trout fed either the FO or LO diet (Turchini and Francis 2009a). These two experiments are contradictory to observations in the Fish Oil Replacement Experiment, as lake trout alevins fed LO were significantly lower in these parameters when compared to fish fed FO. However, larger Atlantic salmon and rainbow trout were used in Menoyo *et al.* (2005) and Turchini and Francis (2009), respectively, whereas lake trout used in the Fish Oil Replacement Experiment were juveniles. This difference in life stage may account for the contradictory results. The rates of development occurring in lake trout alevins are considerably higher than in adult fish and may require higher concentrations of HUFA may be needed in first feeds than an adult grow-out diet.

The dietary composition of the diets used by Menoyo *et al.* (2005) and Turchini and Francis (2009) also differed from the ones used in the Fish Oil Replacement Experiment, which may account for observed differences. Menoyo *et al.* (2005) and Turchini and Francis (2009) used fish meal, which was absent from diets in the Fish Oil Replacement Experiment. Although LO appears to be a suitable FO replacement in larger Atlantic salmon, results from the Fish Oil Replacement Experiment suggest that while survival rates of lake trout alevins would not be compromised, growth would be negatively impacted if fed a diet with LO as the sole lipid source.

Lake trout fed the LE diet were similar to fish fed CLO in regards to survival, mass gain, SGR, FCR, and condition factor; but lake trout fed CLO had statistically

higher final length and mass. Soybean lecithin was administered in the form of phospholipids, which has been observed to aid in protein and energy digestibility in Atlantic salmon (Hung *et al.* 1997), as well as being important in membrane structure (Tocher *et al.* 2003), and improving long chain fatty acid absorption in freshwater fish, such as the common carp (*Cyprinus carpio*) (Fontagné *et al.* 2000). A 24-day study by Hamza *et al.* (2008) fed 10-days post-hatch pikeperch (*Sander lucioperca*) larvae diets of either CLO or soybean lecithin. The CLO diet contained 1.5% phospholipids (dry diet weight), whereas the soybean lecithin diet contained 9.5% of phospholipids. The soybean lecithin diet showed a 50% increase in final mass when compared to fish fed the CLO diet. Such increases in mass were not observed in lake trout alevins fed soybean lecithin in the Fish Oil Replacement Experiment when compared to CLO fed lake trout.

Results from the Fish Oil Replacement Experiment are also contradictory to ones reported by Rinchard *et al.* (2007) for the growth performance of juvenile rainbow trout (182 ± 51 mg). Rainbow trout fed a soy-refined lecithin diet as the sole lipid source displayed significantly higher final mass, mass gain, and SGR; outperforming fish fed a CLO diet. Results from that 8-week study suggest that feeding juvenile rainbow trout soy-refined lecithin as a first feed significantly improved growth performance and reduced body fat. In comparison to lake trout fed the CLO diet in the Fish Oil Replacement Experiment, fish fed the LE diet as a first feed, however, displayed statistically similar growth parameters, with the exception of final length and mass. It appears that fish oil can be totally replaced by soybean

lecithin, a diet high in linoleic and linolenic acids but HUFA deficient, in the first feed of lake trout without compromising survival and most growth parameters. There was a general trend where fish fed the CLO diet had slightly higher values for all growth parameters. This may suggest that if the duration of this experiment was extended past 8-weeks, differences observed in growth parameters may be more marked.

Fish fed soybean lecithin also had lower lipid concentration than fish fed LO or CLO, suggesting that LE fed lake trout were leaner, whereas fish fed the CLO diet had a higher fraction of neutral lipid. In addition to survival and other growth parameters being similar between fish fed the CLO or LE diets, this suggests that these fish had more available lipids for energy when soy-refined lecithin was used as a FO replacement.

4.3. *Artemia* Experiment: Fatty Acid Composition

Whole body lake trout fatty acid profiles in neutral and phospholipid fractions were significantly influenced by dietary fatty acid profiles in the *Artemia* Experiment, as reported in other salmonid studies (Atlantic salmon: Bell *et al.* 2003b, Bell *et al.* 2004b, Bell *et al.* 2010, Berge *et al.* 2004, Berge *et al.* 2009; rainbow trout: Caballero *et al.* 2002, Drew *et al.* 2007, Rinchard *et al.* 2007, Turchini *et al.* 2011). Rinchard *et al.* (2007) reported that dietary fatty acids had a greater influence on neutral lipids than phospholipids fatty acid concentrations in juvenile rainbow trout (182 ± 51 mg) fed diets that differed solely in lipid source. Skalli and Robin (2004) reported similar

results to that of Rinchard *et al.* (2007) in a 12-week feeding experiment using juvenile European sea bass (14.4 ± 0.1 g) fed diets with varying amounts of fish oil. Dietary fatty acids were reflected in both the neutral and phospho-lipid fractions of lake trout from the *Artemia* Experiment but it did not appear that neutral lipids were more influenced than phospholipids. An example of this was observed in fish fed the *Artemia* diets; enrichments increased the DHA and EPA concentrations in *Artemia* diets, which was then reflected in neutral and phospho-lipid fractions of lake trout alevins.

The majority of correlations between dietary fatty acids and whole body fatty acids were not significant in the *Artemia* Experiment. Significant linear correlations of individual dietary fatty acids to whole body fatty acids have been observed in juvenile rainbow trout (Rinchard *et al.* 2007), juvenile Chinook salmon (Huang *et al.* 2008), and post-smolt Atlantic salmon (Bell *et al.* 2001). In the latter study, linoleic acid, linolenic acid, oleic acid, EPA and DHA had correlation coefficients of 0.95 or better. Although linoleic acid in the neutral lipid fraction and linolenic acid, EPA, DHA, and the sum of saturated in the phospholipid fraction were significant, it would be expected that significant linear correlations would exist as dietary fatty acids were reflected in the neutral and phospho-lipid fractions of whole body lake trout.

4.4. Fish Oil Replacement Experiment: Fatty Acid Composition

Dietary fatty acid compositions were reflected in the neutral and phospho-lipid fractions of whole body lake trout. This was particularly apparent in fish fed the

OA or LE diet in the Fish Oil Replacement Experiment. The OA diet had triple or more the concentration of oleic acid in comparison to other diets. Fish fed the OA diet contained nearly double the concentration of oleic acid in the neutral lipid fraction, as well as significantly higher concentrations in the phospholipid fraction. Similarly, lake trout fed the LE diet had higher concentrations of linoleic acid in the neutral and phospho-lipid fractions, which is reflective of the linoleic acid-dominated diet.

Oleic acid fed lake trout had high concentrations of DHA in the phospholipid fraction and high concentrations of ARA, EPA, and DHA in the neutral lipid fraction that were statistically similar to fish fed other dietary treatments. Although the OA diet was formulated to be HUFA precursor and HUFA deficient, fish fed this diet had high concentrations of these fatty acids. At the beginning of the Fish Oil Replacement Experiment, fish had ARA, EPA, and DHA, suggesting that these fatty acids were retained in fish fed the EFA deficient diet of OA. As lake trout fed the OA diet did not receive HUFA or the EFA linoleic and linolenic acids in their diet, fish retained the ARA, EPA, and DHA present, storing them in membranes. Certain fatty acids, such as 16:0, 18:1 n -9, 20:1 n -9, and 22:1 n -11 are preferentially catabolized for metabolic energy, whereas the long-chain n -3's EPA and DHA (which can also be used for metabolic energy) are more likely retained for eicosanoid synthesis (Tocher 2003). Juvenile gilthead seabream fed an EFA deficient diet of beef tallow as the lipid source for a 15-week period retained DHA in the phospholipid fraction, suggesting that this fatty acid is preferentially stored for eicosanoid synthesis

(Montero *et al.* 2001). Although ARA, EPA, and DHA were retained in the neutral and phospho-lipid fractions of lake trout fed the OA diet, fish fed diets lacking in linoleic and linolenic acids displayed significantly lower survival, final length, and final mass; these EFA are necessary for survival and growth.

Lake trout alevins fed the LO diet displayed significantly lower final length, mass, SGR, FCR, and K when compared to fish fed the CLO diet. Survival of lake trout fed the LO diet was high (98.0 ± 2.0) and did not differ significantly from fish fed the CLO diet; suggesting that this linoleic acid-rich vegetable oil may be used as a first feed for lake trout without compromising survival, although growth may be limited.

The major limitation associated with the replacement of FO by vegetable oils, such as lecithin, is the modification of dietary fatty acid profiles, which influence fatty acid profiles in fish. The decreased concentration of *n*-3 PUFA and the increase of *n*-6 PUFA are of particular concern (Bell *et al.* 2001, Drew *et al.* 2007, Berge *et al.* 2009). A low *n*-3/*n*-6 PUFA ratio can be detrimental to fish health and increase mortality. Rainbow trout (80 g) fed purified diets containing only palmitic acid/linolenic acid or palmitic acid/*n*-3 HUFA for 9-weeks displayed a higher macrophage activity and killing efficiency when infected with the bacteria, *Aeromonas salmonicida*, compared to fish fed a palmitic acid/EFA-deficient or palmitic acid/linoleic acid diet (Kiron *et al.* 1995). Rainbow trout juveniles (0.39 g) were fed these diets for 4-weeks prior to inoculation with the infectious haematopoietic necrosis (IHN) virus. Fish fed palmitic acid/linolenic acid or palmitic

acid/*n*-3 HUFA diets had mortality rates of 10% and 15%, respectively, whereas the mortality rate of fish fed the palmitic acid/linoleic acid diet was 30% (Kiron *et al.* 1995). This suggests that *n*-3 fatty acids may be more important than *n*-6 fatty acids for immunological protection.

Similarly, rainbow trout alevins from a broodstock fed a *n*-3 deficient diet had a shorter yolk-sac absorption period and more morphological defects compared to alevins from a broodstock fed a commercial diet (Leray *et al.* 1985), indicating that *n*-3 fatty acids in the diet of broodstock rainbow trout are crucial for the normal development of rainbow trout alevins. This study also shows the importance of dietary fatty acids fed to female broodstock as fatty acids are transferred to eggs during oogenesis.

Lake trout alevins fed the LE diet, containing a low *n*-3/*n*-6 ratio (0.1%), reflected the fatty acid profile of this diet, with lower concentrations of EPA and DHA and higher concentrations of linoleic acid and ARA in both whole body neutral and phospho-lipid fractions but did not compromise survival or growth. Lake trout alevins at the start of the Fish Oil Replacement Experiment had high ratios of *n*-3/*n*-6, as they were able to absorb these fatty acids from their yolksac. However, the *n*-3/*n*-6 ratio was greatly reduced by the end of the 8-week experiment. This suggests that lake trout at an early stage of development may not be negatively affected by a low dietary ratio of *n*-3 to *n*-6 so long as this ratio was adequately supplied to the alevins before exogenous feeding.

Rainbow trout juveniles (5.3 ± 0.02 g) fed diets with conjugated linoleic acid (inclusion levels of 0%, 0.5%, 0.75%, 1% or 2%) integrated into a diet with fish oil (10%, 9.5%, 9.25%, 9% or 8%, respectively) showed no significant effects among treatments in regard to growth rate, feed intake, or FCR (Figueiredo-Silva *et al.* 2005). Fatty acid profiles of whole body rainbow trout from this study, however, were not reported so the *n-3/n-6* ratio is unclear; but fish performance and feed efficiency were not compromised with the inclusion of linoleic acid. Rinchard *et al.* (2007) fed a LE diet to rainbow trout juveniles as a first feed, which had a *n-3/n-6* ratio of 0.2% (% of total fatty acids detected) and is similar to the 0.1% (% of total fatty acids detected) *n-3/n-6* ratio of the LE diet used in the Fish Oil Replacement Experiment. Juvenile rainbow trout fed the LE diet outperformed fish fed a CLO diet in regards to final weight, weight gain, and SGR; but their fatty acid profiles were dramatically altered by diet as fish fed a LE diet had a significantly lower ratio of *n-3/n-6* in both the neutral and phospho-lipid fractions (Rinchard *et al.* 2007). Although lake trout fed the LE diet in our study did not outperform fish fed a CLO diet, a low ratio of *n-3/n-6* in both the neutral and phospho-lipid fractions was observed. This is similar to observations made by Rinchard *et al.* (2007); the low *n-3/n-6* ratio in the LE diet fed to rainbow trout alevins was not detrimental to survival or development but did alter the fatty acid ratio.

As observed in the *Artemia* Experiment, in contrast to studies with juvenile rainbow trout (Rinchard *et al.* 2007), juvenile Chinook salmon (Huang *et al.* 2008), and post-smolt Atlantic salmon (Bell *et al.* 2001), the majority of correlations

between dietary fatty acids and whole body fatty acids were not significant. Because the neutral and phospho-lipid fractions of lake trout were reflective of the dietary fatty acid composition they were fed, it was expected that significant linear correlations would exist. A possible explanation why dietary fatty acids and whole body fatty acids were not significant is that these fatty acids were being used in the synthesis of other fatty acids or as energy, as opposed to being retained.

4.5. Fatty Acid Synthesis

Both experiments support the assumption that lake trout, a freshwater species, is capable of synthesizing C20 and C22 HUFA using $\Delta 6$ and $\Delta 5$ fatty acyl desaturase and Elovl5 and Elovl2 elongases if given the C18 precursors, linolenic acid and linoleic acid (Henderson and Tocher 1987, Torstensen and Tocher 2011). High rates of survival were displayed in both experiments when lake trout were fed diets high in linolenic acid, such as the non-enriched *Artemia*, linseed, and lecithin diets; which were HUFA deficient. Although lake trout alevins are capable of converting the HUFA precursors to HUFA, this may not be the case as fish mature. It has been observed that juvenile and adult rainbow trout fed diets high in vegetable oil (either 2 or 11%), which contained high concentrations of linolenic acid, had a 10-fold greater ability to synthesize DHA (22:6n-3) than fish fed an 11% fish oil diet (Bell and Dick 2004a). This ability to synthesize DHA when given the linolenic acid precursor also tended to be higher in juvenile rainbow trout (0.5 to 1.5 g) when compared to larger

fish (6 to 8 g). It was suggested that since FO already supplies high concentrations of DHA, its synthesis is suppressed (Bell and Dick 2004a).

Huang *et al.* (2008) demonstrated that juvenile Chinook salmon (0.80 ± 0.03 g) were capable of bioconverting linoleic acid to ARA and synthesizing EPA to DHA. In this 30-week feeding trail, CO was used as a supplementary lipid source in the diet of juvenile Chinook salmon. At the end of the experiment, whole body fatty acid concentrations in Chinook salmon were graphed as a function of dietary fatty acids. A line was also plotted of the relationship of whole body fatty acid concentrations to the dietary fatty acids to determine if fatty acids were being used, retained, or bioconverted based on whether the ratio fell at, above, or below this 1:1 relationship. It was shown that linoleic acid, linolenic acid, and EPA were well conserved in Chinook salmon at low concentrations, as concentrations of fatty acids were just below the 1:1 relationship of whole body to dietary fatty acids. As concentrations of these fatty acids increased among diets, however, retention in Chinook salmon decreased. Concentrations of DHA were considerably higher in whole body Chinook salmon in comparison to what was observed in the diet, suggesting that this fatty acid was being produced within the fish. This general trend was also seen in ARA.

It appears that lake trout alevins, like juvenile rainbow trout and Chinook salmon, are capable of synthesizing HUFA if given the precursors. In the Fish Oil Replacement Experiment, concentrations of ARA in the phospholipids of whole body lake trout fed the LE diet, rich in linoleic acid and devoid of ARA, were higher at the

end of the experiment than at the beginning. Similarly, lake trout fed the LO diet, rich in linolenic acid and devoid of HUFA, had concentrations of EPA in the phospholipid fraction higher at the end of the Fish Oil Replacement Experiment than at the beginning. These results suggest that lake trout alevins are converting the HUFA precursors, linoleic and linolenic acids, to HUFA.

Since lake trout have the ability to synthesize HUFA if given the linoleic or linolenic acid precursors, diets high in these precursors, such as the LE diet, may be acceptable alternatives to FO. It has been shown, however, that salmonid life stage can affect the rate of bioconversion of these precursors to HUFA. Bell and Dick (2004) demonstrated that DHA synthesis from linolenic acid was highest in rainbow trout weighing 0.5 to 1.5 g, but was greatly reduced in rainbow trout larger than 2 g. This suggests replacing FO with a diet high in linoleic and linolenic acids may be feasible for lake trout at first feeding but may not be appropriate as fish increase in mass. Lake trout alevins from both the *Artemia* Experiment and the Fish Oil Replacement Experiment had a mass range of 0.15 to 0.93 g; according to Dick and Bell (2004) these fish would have high rates of bioconversion in comparison to larger lake trout, although further experimentation is needed to confirm this.

High rates of survival can be achieved when the essential fatty acids of linoleic and linolenic acids are included in the first feed of lake trout alevins, however, growth performance improved when dietary HUFA were included. The inclusion of HUFA in diets has been shown to be important for fish development. Docosahexaenoic acid is important in the cell membrane formation of neural tissue in

the brain and eyes of fish (Mourente 2003). This is particularly important in larval and juvenile fish, as rates of development are high during these life stages. Deficiency in DHA may result in poorly developed brain and eyes; negatively impacting foraging ability and, ultimately, causing death. It has been shown that juvenile rainbow trout injected intraperitoneally with 18:3 n -3 in the liver, eyes, and brain had a greater ability to desaturate and elongate this fatty acid into HUFA, with DHA being the most abundant synthesized fatty acid. This ability was greater in rainbow trout (3-5 g) than in the gilthead seabream (*Sparus aurata*) (2-4 g), a marine species (Mourente and Tocher 1998). This not only demonstrates that salmonids are able to synthesize HUFA if given the precursor but that DHA is the most important fatty acid need during development of the neural system in fish.

Ishizaki *et al.* (2000) demonstrated that DHA is important in the brain development of larval yellowtail (*Seriola quinqueradiata*) (8.1 ± 0.7 mm). Fish were fed an *Artemia* diet enriched with either oleic acid, EPA, or DHA. Total brain volume, in addition to tectum opticum and cerebellum volumes, parts of the brain associated with visual acuity and swimming performance in fish, were measured at specified length increments throughout the experiment. Fish fed the oleic acid enriched *Artemia* had lower total brain, tectum opticum, and cerebellum volumes throughout the duration of the experiment. Total brain and cerebellum volumes were significantly higher in 20.1 mm long larval yellowtail fed a DHA enriched *Artemia* diet. The tectum opticum volume in larval yellowtail, however, were statistically similar in fish fed either the DHA or EPA enriched *Artemia* diets. These results

suggest that DHA enriched diets enhance brain development of larval yellowtail. Although brains of lake trout were not evaluated in either the *Artemia* Experiment or the Fish Oil Replacement Experiment, further experimentation could be done to determine if diets high DHA impacted lake trout brain development.

Lake trout are capable of synthesizing DHA from linolenic acid, but data suggest that dietary DHA may aid in the development of the neurological system, particularly in lake trout alevins, when rates of development are high. SELCO and Super SELCO-enriched *Artemia* diets fed to lake trout in the *Artemia* Experiment included DHA, whereas the non-enriched *Artemia* diet contained no DHA. Lake trout fed the enriched *Artemia* diets displayed significantly higher growth parameters when compared to fish fed non-enriched *Artemia*. Similarly, in the Fish Oil Replacement Experiment, the only diet to include DHA was the CLO diet. Fish fed this diet displayed higher final length and mass; suggesting that the inclusion of dietary DHA may aid in growth of lake trout alevins.

Arachidonic acid and EPA are important as precursors to eicosanoids, a biologically active group of compounds associated with stress that aid in immune function, blood clotting, inflammatory response, renal and neural function, and cardiovascular tone. Arachidonic acid derived eicosanoids are known to be more biologically active than EPA, and function in immunological defenses (Tocher 2003). Vegetable oils containing various amounts of either *n*-3 or *n*-6 PUFA can alter the ratio of ARA and EPA in fish and hence, influence eicosanoid production. In an experiment by Good *et al.* (in Bell and Sargent 2003a), juvenile Atlantic salmon were

fed a first feed diet of fish oil or vegetable oil (linseed/rapeseed oil; 1:1), containing no ARA. Vegetable oil-fed salmon displayed significant reductions in immune parameters, such as haematocrit, total white and red blood cell counts, and macrophage respiratory burst; no significant difference in mortality among fish fed experimental diets was observed when fish were challenged with the pathogenic agent *Aeromonas salmonicida*.

In the *Artemia* Experiment, all diets used had low concentrations of ARA but bacterial infection and disease was not observed in fish. All diets used in the Fish Oil Replacement Experiment had non-detectable concentrations of ARA, with the exception of low concentrations in the CLO diet. Although the LE diet lake trout had no ARA, these fish had the highest concentrations of ARA in neutral and phospholipid fractions due to high concentrations of the precursor linoleic acid in the diet being converted to ARA. Arachidonic acid was present in fish fed other dietary treatments, as ARA was present in fish prior to the start of the experiment. No symptoms of disease or bacterial infection were observed in fish fed any of the dietary treatments, suggesting that ARA concentrations in fish were adequate to promote immune functioning. However, Atlantic salmon fed vegetable oil diets low in ARA for extended periods could potentially affect immune function by decreasing membrane ARA concentrations, and thus eicosanoid production (Bell and Sargent 2003a). This suggests that lake trout alevins fed a diet deficient in ARA for long durations could compromise immune system health. Future research could evaluate if lake trout alevins become immunocompromised when fed a vegetable oil diet for an

extended period of time. This could be done by testing survival after inoculation with a pathogen or analyzing immune parameters, such as haematocrit or white and red blood cell counts.

Competitive interactions between elongase and desaturase enzymes that convert linoleic acid to ARA and linolenic acid to EPA and DHA may be inhibited by their end products. Elongase and desaturase enzymes prefer the *n*-3 family to the *n*-6 family so the production of ARA may be suppressed, even if high concentrations of linoleic acid are incorporated within a diet. An example of this is seen in Bell *et al.* (2001). A 17-week feeding experiment with post-smolt Atlantic salmon (80 g) was conducted where diets contained fish oil, rapeseed, or blends of fish oil/rapeseed. As percentage of rapeseed oil increased in the diet, concentrations of linoleic and linolenic acid also increased, with highest concentrations of these fatty acids in the 100% rapeseed oil diet. Although the 100% rapeseed oil diet contained the highest concentrations of linoleic acid and it was expected that high levels of ARA would be present in fish muscle, this was not the case. It was concluded that the high levels of linolenic acid being converted to EPA and DHA were inhibiting the conversion of linoleic acid to ARA, even though this was present in high concentrations. To combat this, the inclusion of ARA in salmonid diets may be beneficial. Lake trout that were fed the LE diet, which had high concentrations of linoleic acid, displayed the highest concentrations of ARA in the neutral and phospho-lipid fractions. Concentrations of linolenic acid in this diet, however, were 5-fold lower than linoleic

acid concentrations. The low concentration of linolenic acid may not have been high enough to inhibit synthesis of ARA from linoleic acid.

4. 6. Conclusion

In conclusion, these studies indicate that replacing lipid sources in the diet of lake trout alevins can significantly affect their survival, growth, and fatty acid composition. Lake trout alevin performance and fatty acid composition were highly influenced by diet, supporting the adage “you are what you eat.” This study also supports the hypothesis that freshwater fish are capable of synthesizing HUFA if the precursors linolenic and linoleic acids are included in the diet. With this knowledge, vegetable oils, such as linseed oil or lecithin that contain little to no amounts of HUFA, can successfully replace the HUFA rich fish oil used in most diets of lake trout without compromising survival. Growth, however, may be reduced when replacing fish oil with alternative lipid sources. Ultimately, with the increase use of vegetable oils in aquafeeds, the dependency on fish oil will be alleviated. Not only will this be a cheaper feed with less contamination, it will reduce stress on natural fisheries as the harvesting of planktivorous fish decrease.

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Tables:

Table 1. Composition of SELCO and Super SELCO (INVE Aquaculture, Salt Lake City, UT). HUFA = highly unsaturated fatty acids

Composition	SELCO	Super SELCO
Moisture	30%	30%
Crude Lipids	67%	67%
Crude Ash	1%	1%
Crude Fiber	1%	1%
Phosphorous	0.2%	0.2%
<i>n</i> -3 HUFA	Min. 200 mg/g dwt	Min. 400 mg/g dwt

Table 2. Composition (%) of the experimental diets used in the Fish Oil Replacement Experiment.

<i>Ingredients</i>	<i>Experimental Diets</i>			
	<i>Oleic Acid</i>	<i>Linseed Oil</i>	<i>Cod Liver Oil</i>	<i>Lecithin</i>
Casein	40	40	40	40
Gelatin	8	8	8	8
Dextrin	6.3	6.3	6.3	6.3
Wheat Meal	20	20	20	20
Oleic Acid	14	0	0	0
Linseed Oil	0	14	0	0
Cod Liver Oil	0	0	14	0
Lecithin	0	0	0	14
Vitamin Mix ^a	4	4	4	4
Mineral Mix ^b	3	3	3	3
Carboxymethylcellulose Sodium Salt	2	2	2	2
L-Arginine	0.5	0.5	0.5	0.5
L-Methionine	0.4	0.4	0.4	0.4
L-Lysine	0.8	0.8	0.8	0.8
Choline Chloride	1	1	1	1

^a Dyet # 390017 Custom Vitamin Mix for Trout Diet (Dyets Inc., Bethlehem, PA), composition of vitamin mix is expressed as g/kg; vitamin D3 (400000 IU/g), 0.21; ascorbic acid, 17.1; inositol, 16.7; vitamin E (50%), 13.3; niacin (98%), 10.2; manadione, 7.3; calcium D-panthothenate, 7; riboflavin (100%), 2; vitamin B12 (0.1%), 3; biotin, 1.7; pyridoxine HCL, 1.65; thiamin HCL, 1.39; folic acid, 0.67; vitamin A palmitate (500000 IU/g), 0.36; choline bitartrate, 200; dextrose, 717.42.

^b Dyet # 200030 Modified Bernhart-Tomarelli Mineral Mix (Dyets Inc., Bethlehem, PA), composition of mineral mix is expressed as g/kg; calcium phosphate, dibasic, 735; calcium carbonate, 21; sodium chloride, 30.6; potassium phosphate, dibasic, 81; potassium sulfate, 68; sodium phosphate, dibasic, 21.4; magnesium oxide, 25; manganous carbonate, 4.212; ferric citrate, U.S.P., 11.64; zinc carbonate, 0.81; cupric carbonate, 0.333; potassium iodide, 0.0072; citric acid, 0.9978.

Table 3. Fatty acid composition (expressed as % of total fatty acids detected) of stock *Artemia* 24 h after hatching and SELCO and Super SELCO-enrichments (INVE Aquaculture, Salt Lake City, UT). n = 1. nd = not detected. na = not available as ratios could not be calculated due to EPA, DHA, or ARA being not detected.

Fatty Acid	Stock <i>Artemia</i>	SELCO	Super SELCO
Lipid (%)	2.1	63.9	62.0
<i>Saturated</i>			
12:0	nd	nd	nd
14:0	0.7	2.9	1.7
15:0	0.2	0.3	0.4
16:0	11.4	11.3	11.6
17:0	0.7	0.2	0.6
18:0	5.2	3.3	4.6
∑ Saturated	18.2	18.0	18.6
<i>Monounsaturated</i>			
16:1n-7	3.4	3.7	3.0
16:1n-9	0.8	0.2	0.2
17:1	0.5	0.3	0.4
18:1n-7	9.0	3.0	2.8
18:1n-9	22.3	24.6	15.9
20:1n-9	0.3	4.4	2.6
22:1n-9	nd	0.4	0.2
22:1n-11	nd	3.3	1.7
∑ Monounsaturated	36.3	39.8	26.8
<i>Polyunsaturated</i>			
<i>n-6</i>			
18:2n-6	6.1	10.0	6.0
20:2n-6	nd	0.4	0.2
20:3n-6	nd	0.1	0.2
20:4n-6	0.2	0.7	1.5
22:4n-6	nd	0.2	0.4
22:5n-6	nd	0.3	0.9
∑ n-6	6.3	11.7	9.2
<i>n-3</i>			
18:3n-3	30.8	2.6	1.2
18:4n-3	5.6	1.2	1.4
20:3n-3	0.5	0.2	0.1
20:4n-3	0.9	1.1	0.8

20:5 n -3	1.5	10.2	17.7
21:5 n -3	nd	0.5	0.8
22:5 n -3	nd	2.9	2.8
22:6 n -3	nd	11.8	20.7
$\sum n$ -3	39.3	30.5	45.5
\sum Polyunsaturated	45.6	42.2	54.7
$\sum n$ -3/ $\sum n$ -6	6.2	2.6	5.0
DHA/EPA	na	1.2	1.2
ARA/EPA	0.1	0.1	0.1

Table 4. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of the four diets used in the *Artemia* Experiment. *Artemia* diets were enriched for a 24 h period before being fed to lake trout. (SELCO and Super SELCO: INVE Aquaculture, Salt Lake City, UT) (BioVita #0: Bio-Oregon, Westbrook, ME). n = 2. nd = not detected. na = not available as ratios could not be calculated due to EPA, DHA, or ARA being not detected.

Fatty Acid	Diet			
	<i>Artemia</i>	SELCO-Enriched <i>Artemia</i>	Super SELCO-Enriched <i>Artemia</i>	BioVita # 0
Lipid (%)	0.9 \pm 0.1	5.1 \pm 0.1	3.2 \pm 0.3	18.4 \pm 0.3
<i>Saturated</i>				
12:0	nd	nd	nd	nd
14:0	0.6 \pm 0.0	1.8 \pm 0.1	0.5 \pm 0.0	5.9 \pm 0.7
15:0	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.4 \pm 0.0
16:0	11.3 \pm 0.2	10.3 \pm 0.0	8.5 \pm 0.2	20.3 \pm 1.7
17:0	0.8 \pm 0.0	0.3 \pm 0.0	0.5 \pm 0.0	0.4 \pm 0.0
18:0	8.6 \pm 0.2	3.8 \pm 0.2	4.9 \pm 0.0	4.3 \pm 0.1
Σ Saturated	21.4 \pm 0.0	16.3 \pm 0.1	14.6 \pm 0.3	31.3 \pm 2.5
<i>Monounsaturated</i>				
16:1 n -7	2.9 \pm 0.3	2.8 \pm 0.0	2.6 \pm 0.1	6.7 \pm 0.4
16:1 n -9	0.6 \pm 0.0	0.4 \pm 0.1	0.5 \pm 0.0	0.2 \pm 0.0
17:1	0.4 \pm 0.0	0.3 \pm 0.0	0.4 \pm 0.0	1.0 \pm 0.1
18:1 n -7	12.2 \pm 0.1	4.8 \pm 0.3	6.2 \pm 0.2	3.2 \pm 0.1
18:1 n -9	24.5 \pm 0.2	27.0 \pm 0.2	20.5 \pm 0.7	15.3 \pm 0.6
20:1 n -9	0.3 \pm 0.0	4.2 \pm 0.2	1.6 \pm 0.1	2.2 \pm 0.2
22:1 n -9	nd	0.4 \pm 0.1	0.1 \pm 0.0	0.3 \pm 0.1
22:1 n -11	nd	3.2 \pm 0.2	0.8 \pm 0.1	2.2 \pm 0.2
Σ Monounsaturated	41.1 \pm 0.4	43.2 \pm 0.3	32.7 \pm 0.7	31.0 \pm 0.6
<i>Polyunsaturated</i>				
<i>n</i> -6				
18:2 n -6	5.2 \pm 0.0	8.6 \pm 0.2	5.5 \pm 0.2	6.6 \pm 0.1
20:2 n -6	nd	0.5 \pm 0.0	0.2 \pm 0.0	0.1 \pm 0.0
20:3 n -6	nd	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0
20:4 n -6	0.4 \pm 0.1	0.7 \pm 0.0	1.3 \pm 0.1	0.7 \pm 0.1
22:4 n -6	nd	nd	0.1 \pm 0.0	nd
22:5 n -6	nd	0.2 \pm 0.0	0.3 \pm 0.1	0.2 \pm 0.1
Σ <i>n</i> -6	5.6 \pm 0.1	10.1 \pm 0.2	7.5 \pm 0.0	7.7 \pm 0.4
<i>n</i> -3				

18:3 <i>n</i> -3	24.7 ± 0.5	7.9 ± 1.1	12.7 ± 0.7	1.3 ± 0.3
18:4 <i>n</i> -3	3.7 ± 0.1	1.2 ± 0.2	2.0 ± 0.0	1.4 ± 0.7
20:3 <i>n</i> -3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	nd
20:4 <i>n</i> -3	0.6 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.6 ± 0.1
20:5 <i>n</i> -3	2.4 ± 0.0	8.8 ± 0.2	17.8 ± 0.1	13.9 ± 0.8
21:5 <i>n</i> -3	nd	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
22:5 <i>n</i> -3	nd	2.4 ± 0.1	1.8 ± 0.1	1.7 ± 0.3
22:6 <i>n</i> -3	nd	8.6 ± 0.5	9.4 ± 0.9	10.5 ± 1.0
∑ <i>n</i> -3	31.9 ± 0.5	30.5 ± 0.4	45.2 ± 0.4	30.0 ± 2.7
∑ Polyunsaturated	37.5 ± 0.4	40.5 ± 0.3	52.7 ± 0.4	37.7 ± 3.1
∑ <i>n</i> -3/∑ <i>n</i> -6	5.7 ± 0.2	3.0 ± 0.1	6.1 ± 0.0	3.9 ± 0.1
DHA/EPA	na	1.0 ± 0.0	0.5 ± 0.1	0.8 ± 0.0
ARA/EPA	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

Table 5. Growth parameters (mean \pm standard deviation) of lake trout juveniles from the *Artemia* Experiment after 8 weeks of being fed four experimental diets. Each tank was a statistical unit and three replicates per treatment were used. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$). SGR = Specific growth rate.

n=3	Dietary Treatment			
	<i>Artemia</i>	SELCO-Enriched <i>Artemia</i>	Super SELCO- Enriched <i>Artemia</i>	BioVita # 0
Survival (%)	96.7 \pm 0.0 ^a	98.9 \pm 1.9 ^a	96.7 \pm 3.3 ^a	90.0 \pm 3.3 ^a
Final length (mm)	42.7 \pm 1.6 ^c	47.6 \pm 0.3 ^b	47.3 \pm 1.1 ^b	52.2 \pm 0.4 ^a
Final mass (g)	0.44 \pm 0.06 ^c	0.62 \pm 0.01 ^b	0.59 \pm 0.06 ^b	0.93 \pm 0.03 ^a
Mass gain (%) ^x	336 \pm 69 ^c	531 \pm 16 ^b	519 \pm 60 ^b	886 \pm 14 ^a
SGR (%) ^y	2.6 \pm 0.3 ^c	3.3 \pm 0.0 ^b	3.2 \pm 0.2 ^b	4.1 \pm 0.0 ^a
Condition factor ^z	0.52 \pm 0.03 ^b	0.55 \pm 0.02 ^b	0.55 \pm 0.02 ^b	0.65 \pm 0.02 ^a

^x Mass gain = (final weight - initial weight)*100/ initial weight

^y SGR = (log final weight - log initial weight)*100/ duration of experiment in days

^z Condition factor = (weight /length³)*100,000

Table 6. Total lipid, neutral lipid, and phospholipid (% of wet weight, mean \pm standard deviation) of whole body lake trout juveniles from the *Artemia* Experiment after 8 weeks of being fed four experimental diets. Each tank was a statistical unit and three replicates per treatment were used. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$).

n=3	Dietary Treatment			
	<i>Artemia</i>	SELCO-Enriched <i>Artemia</i>	Super SELCO-Enriched <i>Artemia</i>	BioVita # 0
Total lipid (%)	2.1 \pm 0.0 ^b	6.4 \pm 0.1 ^a	6.1 \pm 0.4 ^a	5.6 \pm 0.3 ^a
Phospholipid (% of total lipid)	59.7 \pm 5.9 ^a	21.8 \pm 3.6 ^b	17.8 \pm 1.6 ^b	24.0 \pm 1.3 ^b
Neutral lipid (% of total lipid)	40.3 \pm 5.9 ^b	78.2 \pm 3.6 ^a	82.2 \pm 1.6 ^a	76.0 \pm 1.3 ^a

Table 7. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of neutral lipid fraction of whole body lake trout alevins from the *Artemia* Experiment before the start of the experiment (n=1) and after being fed one of four diets for 8 weeks (n=3). Fish were pooled based on tank before lipid and fatty acid analysis. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$). nd = not detected.

Fatty Acid	Dietary Treatment				
	Initial	<i>Artemia</i>	SELCO <i>Artemia</i>	Super SELCO <i>Artemia</i>	BioVita # 0
<i>Saturated</i>					
12:0	nd	nd	nd	nd	nd
14:0	1.5	0.7 \pm 0.1 ^b	0.8 \pm 0.0 ^b	0.5 \pm 0.0 ^b	4.4 \pm 0.1 ^a
15:0	0.2	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.4 \pm 0.0 ^a
16:0	8.2	11.7 \pm 0.9 ^a	7.1 \pm 0.1 ^b	7.8 \pm 0.1 ^b	15.6 \pm 0.2 ^a
17:0	0.2	0.9 \pm 0.0 ^a	0.4 \pm 0.0 ^c	0.6 \pm 0.0 ^b	0.3 \pm 0.0 ^d
18:0	2.6	7.6 \pm 0.1 ^a	3.5 \pm 0.1 ^c	4.5 \pm 0.0 ^b	3.6 \pm 0.1 ^c
Σ Saturated	12.7	21.1 \pm 1.0 ^a	12.0 \pm 0.1 ^c	13.6 \pm 0.1 ^b	24.4 \pm 0.3 ^a
<i>Monounsaturated</i>					
16:1n-7	6.7	2.4 \pm 0.1 ^c	3.0 \pm 0.0 ^b	2.6 \pm 0.1 ^{bc}	6.9 \pm 0.2 ^a
16:1n-9	1.9	0.8 \pm 0.0 ^a	nd	0.5 \pm 0.1 ^b	nd
17:1	0.4	0.4 \pm 0.1 ^b	0.4 \pm 0.0 ^b	0.5 \pm 0.0 ^b	0.7 \pm 0.0 ^a
18:1n-7	6.9	9.1 \pm 1.1 ^a	5.4 \pm 0.1 ^b	5.9 \pm 0.0 ^b	4.2 \pm 0.0 ^b
18:1n-9	22.3	21.8 \pm 1.2 ^b	27.4 \pm 0.1 ^a	20.7 \pm 0.1 ^b	18.7 \pm 0.4 ^b
20:1n-9	2.0	1.7 \pm 0.5 ^b	2.7 \pm 0.1 ^{ab}	1.9 \pm 0.0 ^{ab}	3.2 \pm 0.1 ^a
22:1n-9	0.1	0.2 \pm 0.0 ^c	0.3 \pm 0.0 ^b	0.2 \pm 0.0 ^c	0.4 \pm 0.0 ^a
22:1n-11	nd	nd	1.1 \pm 0.1 ^b	0.6 \pm 0.0 ^c	1.8 \pm 0.0 ^a
Σ Monounsaturated	39.3	36.5 \pm 2.8 ^{ab}	40.3 \pm 0.2 ^a	32.9 \pm 0.2 ^b	35.8 \pm 0.6 ^{ab}
<i>Polyunsaturated</i>					
18:2n-6	3.4	4.4 \pm 0.1 ^d	9.8 \pm 0.1 ^a	6.2 \pm 0.1 ^c	7.8 \pm 0.0 ^b
20:2n-6	1.0	0.4 \pm 0.0 ^d	0.8 \pm 0.0 ^a	0.5 \pm 0.0 ^c	0.7 \pm 0.0 ^b
20:3n-6	0.5	0.6 \pm 0.1 ^{ab}	0.3 \pm 0.0 ^b	0.3 \pm 0.0 ^b	0.4 \pm 0.0 ^b
20:4n-6	6.2	2.4 \pm 0.8 ^{ab}	0.9 \pm 0.0 ^b	1.4 \pm 0.0 ^a	0.9 \pm 0.1 ^b
22:4n-6	1.1	nd	0.1 \pm 0.0 ^b	0.2 \pm 0.0 ^a	0.1 \pm 0.0 ^b
22:5n-6	1.5	0.1 \pm 0.1 ^b	0.2 \pm 0.0 ^{ab}	0.4 \pm 0.0 ^a	0.3 \pm 0.0 ^{ab}
Σ n-6	13.7	7.8 \pm 1.0 ^{abc}	12.1 \pm 0.0 ^a	8.9 \pm 0.0 ^c	10.2 \pm 0.0 ^b
18:3n-3	2.8	11.7 \pm 1.2 ^a	11.7 \pm 0.5 ^a	12.5 \pm 0.2 ^a	1.1 \pm 0.1 ^b
18:4n-3	0.6	5.1 \pm 0.8 ^a	2.1 \pm 0.0 ^b	2.2 \pm 0.0 ^b	1.6 \pm 0.0 ^b
20:3n-3	1.0	1.1 \pm 0.0 ^a	1.0 \pm 0.0 ^b	0.9 \pm 0.0 ^b	0.2 \pm 0.0 ^c
20:4n-3	2.5	2.8 \pm 0.1 ^a	2.0 \pm 0.1 ^b	1.8 \pm 0.0 ^b	1.0 \pm 0.0 ^c

20:5n-3	7.2	5.4 ± 0.9 ^c	7.1 ± 0.1 ^b	11.9 ± 0.2 ^a	8.2 ± 0.2 ^b
21:5n-3	0.2	nd	0.4 ± 0.0 ^c	0.5 ± 0.0 ^b	0.7 ± 0.0 ^a
22:5n-3	6.4	1.9 ± 0.5 ^{abc}	2.9 ± 0.0 ^c	3.9 ± 0.0 ^a	3.2 ± 0.0 ^b
22:6n-3	13.6	6.7 ± 1.8 ^b	8.5 ± 0.3 ^b	11.0 ± 0.2 ^a	13.6 ± 0.2 ^a
∑ n-3	34.3	34.6 ± 1.2 ^b	35.6 ± 0.3 ^b	44.6 ± 0.2 ^a	29.6 ± 0.3 ^c
∑ Polyunsaturated	48.0	42.4 ± 2.2 ^{abc}	47.7 ± 0.3 ^b	53.5 ± 0.2 ^a	39.8 ± 0.3 ^c
∑ n-3/∑ n-6	2.5	4.5 ± 0.4 ^{ab}	2.9 ± 0.0 ^b	5.0 ± 0.0 ^a	2.9 ± 0.0 ^b
DHA/EPA	1.9	1.2 ± 0.2 ^{abc}	1.2 ± 0.0 ^b	0.9 ± 0.0 ^c	1.7 ± 0.0 ^a
ARA/EPA	0.9	0.4 ± 0.1 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b

Table 8. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of phospholipid fraction of whole body lake trout alevins from the *Artemia* Experiment before the start of the experiment (n=1) and after being fed one of four diets for 8 weeks (n=3). Fish were pooled based on tank before lipid and fatty acid analysis. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$). nd = not detected

Fatty Acid	Dietary Treatment				
	Initial	<i>Artemia</i>	SELCO <i>Artemia</i>	Super SELCO <i>Artemia</i>	BioVita # 0
<i>Saturated</i>					
12:0	nd	nd	nd	nd	nd
14:0	0.7	0.3 \pm 0.0 ^b	0.4 \pm 0.0 ^b	0.3 \pm 0.0 ^b	1.5 \pm 0.1 ^a
15:0	0.2	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a
16:0	16.6	16.6 \pm 0.2 ^b	16.3 \pm 0.3 ^b	16.1 \pm 0.3 ^b	20.2 \pm 0.6 ^a
17:0	0.2	0.8 \pm 0.0 ^a	0.5 \pm 0.0 ^c	0.7 \pm 0.0 ^b	0.3 \pm 0.0 ^d
18:0	6.1	7.7 \pm 0.2 ^a	6.9 \pm 0.1 ^b	7.4 \pm 0.1 ^{ab}	5.6 \pm 0.1 ^c
Σ Saturated	23.9	25.5 \pm 0.3 ^b	24.2 \pm 0.2 ^b	24.7 \pm 0.4 ^b	27.7 \pm 0.7 ^a
<i>Monounsaturated</i>					
16:1n-7	1.5	1.1 \pm 0.1 ^b	0.9 \pm 0.0 ^b	0.8 \pm 0.1 ^b	1.6 \pm 0.1 ^a
16:1n-9	0.8	0.6 \pm 0.4 ^a	0.7 \pm 0.0 ^a	0.4 \pm 0.3 ^a	0.4 \pm 0.3 ^a
17:1	0.2	0.3 \pm 0.1 ^a	0.2 \pm 0.0 ^a	0.2 \pm 0.0 ^a	0.1 \pm 0.0 ^a
18:1n-7	5.0	5.1 \pm 0.1 ^a	3.6 \pm 0.1 ^b	3.7 \pm 0.1 ^b	2.5 \pm 0.0 ^c
18:1n-9	9.4	13.6 \pm 0.4 ^a	12.6 \pm 0.2 ^{ab}	11.4 \pm 0.3 ^b	10.0 \pm 0.2 ^c
20:1n-9	1.8	0.4 \pm 0.0 ^b	0.6 \pm 0.1 ^a	0.4 \pm 0.0 ^b	0.7 \pm 0.1 ^a
22:1n-9	nd	nd	nd	nd	nd
22:1n-11	nd	nd	0.1 \pm 0.0 ^a	nd	0.1 \pm 0.0 ^a
Σ Monounsaturated	18.7	21.1 \pm 0.2 ^a	18.7 \pm 0.1 ^b	17.0 \pm 0.4 ^c	15.3 \pm 0.3 ^d
<i>Polyunsaturated</i>					
18:2n-6	0.9	2.7 \pm 0.2 ^a	2.2 \pm 0.0 ^a	1.3 \pm 0.1 ^b	2.5 \pm 0.1 ^a
20:2n-6	0.8	0.2 \pm 0.0 ^b	0.3 \pm 0.0 ^a	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a
20:3n-6	0.2	0.8 \pm 0.1 ^a	0.2 \pm 0.0 ^c	0.1 \pm 0.0 ^c	0.4 \pm 0.0 ^b
20:4n-6	8.6	4.6 \pm 0.2 ^a	3.3 \pm 0.1 ^{bc}	3.6 \pm 0.1 ^b	2.9 \pm 0.1 ^c
22:4n-6	0.6	0.2 \pm 0.0 ^a	0.1 \pm 0.0 ^a	0.1 \pm 0.0 ^a	0.1 \pm 0.0 ^a
22:5n-6	1.7	0.7 \pm 0.0 ^a	0.5 \pm 0.0 ^b	0.6 \pm 0.0 ^{ab}	0.5 \pm 0.0 ^b
Σ n-6	12.8	9.0 \pm 0.1 ^a	6.5 \pm 0.1 ^b	5.8 \pm 0.2 ^c	6.7 \pm 0.1 ^b
18:3n-3	0.5	5.8 \pm 0.6 ^a	2.4 \pm 0.2 ^b	2.3 \pm 0.1 ^b	0.3 \pm 0.0 ^c
18:4n-3	0.1	2.0 \pm 0.3 ^a	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b
20:3n-3	0.6	0.7 \pm 0.0 ^a	0.4 \pm 0.0 ^b	0.3 \pm 0.0 ^b	nd
20:4n-3	0.7	2.6 \pm 0.3 ^a	0.7 \pm 0.0 ^b	0.5 \pm 0.0 ^b	0.4 \pm 0.0 ^b

20:5 <i>n</i> -3	7.0	7.7 ± 0.1 ^c	10.6 ± 0.2 ^b	12.0 ± 0.3 ^a	10.7 ± 0.1 ^b
21:5 <i>n</i> -3	0.1	nd	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.2 ± 0.0 ^a
22:5 <i>n</i> -3	5.2	3.4 ± 0.1 ^a	2.8 ± 0.0 ^b	2.7 ± 0.1 ^b	2.5 ± 0.1 ^b
22:6 <i>n</i> -3	30.5	22.1 ± 1.1 ^b	33.5 ± 0.3 ^a	34.5 ± 1.2 ^a	36.1 ± 0.8 ^a
∑ <i>n</i> -3	44.6	44.4 ± 0.2 ^b	50.6 ± 0.1 ^a	52.6 ± 1.0 ^a	50.3 ± 0.9 ^a
∑ Polyunsaturated	57.4	53.4 ± 0.2 ^b	57.0 ± 0.2 ^a	58.4 ± 0.8 ^a	57.0 ± 0.9 ^a
∑ <i>n</i> -3/∑ <i>n</i> -6	3.5	4.9 ± 0.0 ^c	7.8 ± 0.1 ^b	9.0 ± 0.5 ^a	7.5 ± 0.2 ^b
DHA/EPA	4.4	2.9 ± 0.2 ^b	3.2 ± 0.1 ^{ab}	2.9 ± 0.2 ^b	3.4 ± 0.1 ^a
ARA/EPA	1.2	0.6 ± 0.0 ^a	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b

Table 9. Correlations between dietary fatty acids and fatty acids in whole body lake trout from the *Artemia* Experiment.

Fatty Acids	Neutral Lipids			Phospholipids		
	Slope	r^2	<i>P</i>	Slope	r^2	<i>P</i>
18:1 <i>n</i> -9	0.65	0.78	0.12	0.28	0.81	0.10
18:2 <i>n</i> -6	1.41	0.91	0.05	0.04	0.01	0.90
18:3 <i>n</i> -3	0.38	0.47	0.32	0.23	0.96	0.02
20:4 <i>n</i> -6	-0.67	0.14	0.62	-0.69	0.14	0.63
20:5 <i>n</i> -3	0.39	0.88	0.06	0.26	0.90	0.05
22:6 <i>n</i> -3	0.52	0.68	0.18	1.32	1.00	<0.01
∑Saturated	0.73	0.85	0.08	0.20	0.96	0.02
∑MUFA	0.39	0.61	0.22	0.34	0.70	0.16
∑PUFA	0.78	0.85	0.08	0.20	0.46	0.32
∑ <i>n</i> -3	0.80	0.86	0.07	0.24	0.25	0.50
∑ <i>n</i> -6	0.99	0.94	0.03	-0.52	0.45	0.33

Table 10. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of the four diets used in the Fish Oil Replacement Experiment (n=2). nd = not detected. Na = not available as ratios could not be calculated due to EPA, DHA, or ARA being not detected. OA = Oleic acid, LO = Linseed oil, CLO = Cod liver oil, LE = Lecithin.

Fatty Acid	Diet			
	OA	LO	CLO	LE
Lipid (%)	15.9 \pm 0.3	15.6 \pm 0.2	16.5 \pm 0.3	13.4 \pm 0.0
<i>Saturated</i>				
12:0	nd	nd	nd	nd
14:0	2.3 \pm 0.1	0.1 \pm 0.0	3.0 \pm 0.1	0.1 \pm 0.0
15:0	0.2 \pm 0.0	nd	0.3 \pm 0.0	0.1 \pm 0.0
16:0	4.3 \pm 0.1	5.7 \pm 0.1	9.9 \pm 0.1	18.8 \pm 0.1
17:0	0.1 \pm 0.0	0.1 \pm 0.0	0.2 \pm 0.0	0.1 \pm 0.0
18:0	0.8 \pm 0.0	4.5 \pm 0.0	2.3 \pm 0.0	4.6 \pm 0.1
Σ Saturated	7.8 \pm 0.2	10.5 \pm 0.1	15.6 \pm 0.2	23.8 \pm 0.0
<i>Monounsaturated</i>				
16:1n-7	4.7 \pm 0.1	0.1 \pm 0.0	7.8 \pm 0.1	0.1 \pm 0.0
16:1n-9	0.5 \pm 0.0	nd	0.4 \pm 0.0	nd
17:1	1.2 \pm 0.0	nd	0.3 \pm 0.0	nd
18:1n-7	4.6 \pm 0.1	0.8 \pm 0.0	4.8 \pm 0.0	1.4 \pm 0.0
18:1n-9	70.0 \pm 0.2	23.5 \pm 0.1	17.5 \pm 0.3	9.2 \pm 0.1
20:1n-9	1.0 \pm 0.0	0.2 \pm 0.0	13.8 \pm 0.2	0.1 \pm 0.0
22:1n-9	0.2 \pm 0.0	nd	0.9 \pm 0.0	0.1 \pm 0.0
22:1n-11	0.2 \pm 0.0	nd	6.8 \pm 0.1	0.1 \pm 0.0
Σ Monounsaturated	82.3 \pm 0.1	24.7 \pm 0.1	52.2 \pm 0.1	10.9 \pm 0.1
<i>Polyunsaturated</i>				
18:2n-6	9.3 \pm 0.1	17.9 \pm 0.1	3.2 \pm 0.0	58.2 \pm 0.0
20:2n-6	0.2 \pm 0.0	nd	0.3 \pm 0.0	nd
20:3n-6	nd	nd	0.1 \pm 0.0	nd
20:4n-6	nd	nd	0.4 \pm 0.0	nd
22:4n-6	nd	nd	nd	nd
22:5n-6	nd	nd	0.1 \pm 0.0	nd
Σ n-6	9.6 \pm 0.1	18.0 \pm 0.1	4.1 \pm 0.1	58.2 \pm 0.0
18:3n-3	0.3 \pm 0.0	46.9 \pm 0.1	1.0 \pm 0.0	7.1 \pm 0.0
18:4n-3	nd	nd	2.4 \pm 0.0	nd
20:3n-3	nd	nd	0.1 \pm 0.0	nd
20:4n-3	nd	nd	0.7 \pm 0.0	nd

20:5 <i>n</i> -3	nd	nd	9.5 ± 0.1	nd
21:5 <i>n</i> -3	nd	nd	0.4 ± 0.0	nd
22:5 <i>n</i> -3	nd	nd	1.3 ± 0.0	nd
22:6 <i>n</i> -3	nd	nd	12.6 ± 0.1	nd
∑ <i>n</i> -3	0.3 ± 0.0	46.9 ± 0.1	28.0 ± 0.1	7.1 ± 0.0
∑ Polyunsaturated	9.9 ± 0.1	64.9 ± 0.2	32.2 ± 0.1	65.3 ± 0.0
∑ <i>n</i> -3/∑ <i>n</i> -6	nd	2.6 ± 0.0	6.8 ± 0.1	0.1 ± 0.0
DHA/EPA	na	na	1.3 ± 0.0	na
ARA/EPA	na	na	na	na

Table 11. Growth parameters (mean \pm standard deviation) of lake trout juveniles from the Fish Oil Replacement Experiment after 8 weeks of being fed four experimental diets. Each tank was a statistical unit and three replicates per treatment were used (n=3). Means with different superscript letters in a row indicate statistical significance ($p < 0.05$). SGR = Specific growth rate. FCR = Food conversion ratio. OA = Oleic acid, LO = Linseed oil, CLO = Cod liver oil, LE = Lecithin.

	Dietary Treatment			
	OA	LO	CLO	LE
Survival (%)	33.3 \pm 9.9 ^b	98.0 \pm 2.0 ^a	96.7 \pm 1.2 ^a	92.7 \pm 2.3 ^a
Final length (mm)	31.1 \pm 0.9 ^c	43.7 \pm 0.0 ^b	46.6 \pm 0.3 ^a	44.8 \pm 0.4 ^b
Final mass (g)	0.15 \pm 0.02 ^d	0.48 \pm 0.01 ^c	0.64 \pm 0.02 ^a	0.53 \pm 0.02 ^b
Mass gain (%) ^w	62 \pm 21 ^b	408 \pm 9 ^a	578 \pm 17 ^a	468 \pm 25 ^a
SGR (%/day) ^x	0.9 \pm 0.2 ^c	2.9 \pm 0.0 ^b	3.4 \pm 0.0 ^a	3.1 \pm 0.1 ^{ab}
FCR ^y	5.7 \pm 2.4 ^a	1.4 \pm 0.0 ^b	1.1 \pm 0.0 ^c	1.2 \pm 0.0 ^{bc}
Condition factor ^z	0.49 \pm 0.02 ^c	0.57 \pm 0.01 ^b	0.63 \pm 0.01 ^a	0.60 \pm 0.01 ^{ab}

^w Mass gain = (final weight - initial weight)*100/ initial weight

^x SGR = (log final weight - log initial weight)*100/ duration of experiment in days

^y FCR = (average amount of food used/ average individual weight gain)

^z Condition factor = (weight /length³)*100,000

Table 12. Total lipid, neutral lipid, and phospholipid (expressed as % of dry weight, mean \pm standard deviation) of whole body lake trout fed diets from the Fish Oil Replacement Experiment for 8 weeks. Each tank was a statistical unit and three replicates per treatment were used (n=3). Means with different superscript letters in a row indicate statistical significance ($p < 0.05$). OA = Oleic acid, LO = Linseed oil, CLO = Cod liver oil, LE = Lecithin.

	Dietary Treatment			
	OA	LO	CLO	LE
Total lipid (%)	14.4 \pm 0.3 ^b	18.2 \pm 1.6 ^a	20.6 \pm 1.0 ^a	14.7 \pm 0.7 ^b
Phospholipid (% of total lipid)	54.5 \pm 3.3 ^a	39.5 \pm 3.8 ^c	32.5 \pm 0.6 ^d	48.0 \pm 0.3 ^b
Neutral lipid (% of total lipid)	45.5 \pm 3.3 ^d	60.5 \pm 3.8 ^b	67.5 \pm 0.6 ^a	52.0 \pm 0.3 ^c

Table 13. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of neutral lipid fraction of whole body lake trout alevins from the Fish Oil Replacement Experiment before the start of the experiment and after being fed one of four diets for 8 weeks. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$) at the end of the experiment. nd = non-detectable. OA = Oleic acid, LO = Linseed oil, CLO = Cod liver oil, LE = Lecithin.

Fatty Acid	Dietary Treatment				
	Initial	OA	LO	CLO	LE
<i>n</i>	1	3	3	3	3
<i>Saturated</i>					
12:0	nd	nd	nd	nd	nd
14:0	1.5	1.7 \pm 0.2 ^b	0.6 \pm 0.1 ^d	2.9 \pm 0.1 ^a	1.2 \pm 0.2 ^c
15:0	0.2	0.2 \pm 0.0 ^c	0.1 \pm 0.0 ^d	0.3 \pm 0.0 ^b	0.4 \pm 0.0 ^a
16:0	9.0	9.4 \pm 1.1 ^{bc}	6.7 \pm 0.5 ^c	9.8 \pm 0.3 ^b	17.8 \pm 0.2 ^a
17:0	0.2	0.1 \pm 0.0 ^b	0.1 \pm 0.0 ^b	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a
18:0	3.1	3.3 \pm 0.5 ^{bc}	4.6 \pm 0.1 ^b	2.7 \pm 0.1 ^c	7.0 \pm 0.3 ^a
Σ Saturated	13.9	14.8 \pm 1.4 ^{bc}	12.0 \pm 0.7 ^c	15.9 \pm 0.5 ^b	26.6 \pm 0.3 ^a
<i>Monounsaturated</i>					
16:1 n -7	6.7	5.3 \pm 0.1 ^b	0.8 \pm 0.3 ^c	7.6 \pm 0.2 ^a	1.6 \pm 0.5 ^c
16:1 n -9	1.1	1.7 \pm 0.1 ^a	0.6 \pm 0.1 ^b	0.7 \pm 0.0 ^b	1.3 \pm 0.1 ^{ab}
17:1	0.3	0.4 \pm 0.0 ^a	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a	0.2 \pm 0.0 ^b
18:1 n -7	6.9	6.2 \pm 0.3 ^a	1.5 \pm 0.3 ^b	5.8 \pm 0.1 ^a	2.2 \pm 0.4 ^b
18:1 n -9	22.7	41.7 \pm 7.8 ^{ab}	27.0 \pm 0.6 ^a	22.9 \pm 0.5 ^b	21.2 \pm 1.6 ^{ab}
20:1 n -9	2.0	1.8 \pm 0.3 ^b	1.1 \pm 0.3 ^b	13.3 \pm 0.2 ^a	2.0 \pm 0.6 ^b
22:1 n -9	nd	nd	nd	1.1 \pm 0.0	nd
22:1 n -11	0.1	0.4 \pm 0.1 ^b	0.2 \pm 0.0 ^b	4.9 \pm 0.1 ^a	0.3 \pm 0.0 ^b
Σ Monounsaturated	39.7	57.4 \pm 8.3 ^{ab}	31.1 \pm 1.7 ^b	56.6 \pm 0.5 ^a	28.8 \pm 3.2 ^b
<i>Polyunsaturated</i>					
18:2 n -6	3.3	3.3 \pm 0.3 ^c	14.2 \pm 0.9 ^b	3.1 \pm 0.1 ^c	29.6 \pm 2.6 ^a
20:2 n -6	0.9	0.4 \pm 0.1 ^b	0.6 \pm 0.0 ^b	0.5 \pm 0.0 ^b	2.2 \pm 0.1 ^a
20:3 n -6	0.4	0.4 \pm 0.1 ^b	0.5 \pm 0.0 ^b	0.2 \pm 0.0 ^b	2.8 \pm 0.3 ^a
20:4 n -6	5.8	4.2 \pm 1.2 ^{ab}	0.6 \pm 0.2 ^b	0.5 \pm 0.0 ^b	3.4 \pm 0.1 ^a
22:4 n -6	0.9	0.6 \pm 0.1 ^{ab}	0.1 \pm 0.0 ^b	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a
22:5 n -6	1.4	0.8 \pm 0.3 ^{ab}	0.1 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.8 \pm 0.1 ^a
Σ n -6	12.8	9.6 \pm 1.8 ^{bc}	16.0 \pm 0.6 ^b	4.5 \pm 0.0 ^c	39.1 \pm 3.0 ^a
18:3 n -3	2.6	1.3 \pm 0.3 ^b	24.6 \pm 2.2 ^a	0.8 \pm 0.0 ^b	2.0 \pm 0.3 ^b
18:4 n -3	0.6	0.1 \pm 0.1 ^c	8.2 \pm 0.2 ^a	1.5 \pm 0.1 ^b	1.2 \pm 0.2 ^b
20:3 n -3	0.9	0.4 \pm 0.1 ^b	1.1 \pm 0.1 ^a	0.2 \pm 0.0 ^b	0.1 \pm 0.0 ^b
20:4 n -3	2.2	1.2 \pm 0.3 ^a	1.2 \pm 0.0 ^a	0.8 \pm 0.0 ^{ab}	0.2 \pm 0.0 ^b
20:5 n -3	6.4	3.1 \pm 1.0 ^{ab}	1.8 \pm 0.2 ^b	5.1 \pm 0.3 ^a	0.4 \pm 0.1 ^b

21:5 <i>n</i> -3	0.2	nd	nd	0.4 ± 0.0	nd
22:5 <i>n</i> -3	6.3	3.2 ± 0.5 ^{ab}	0.9 ± 0.2 ^b	1.9 ± 0.1 ^a	0.4 ± 0.1 ^b
22:6 <i>n</i> -3	14.3	8.8 ± 3.2 ^{ab}	3.2 ± 0.5 ^b	12.3 ± 0.6 ^a	1.3 ± 0.3 ^b
∑ <i>n</i> -3	33.6	18.1 ± 5.2 ^b	40.9 ± 1.7 ^a	23.0 ± 0.9 ^b	5.5 ± 0.2 ^c
∑ Polyunsaturated	46.4	27.8 ± 7.0 ^b	56.9 ± 2.3 ^a	27.5 ± 1.0 ^b	44.6 ± 2.9 ^a
∑ <i>n</i> -3/∑ <i>n</i> -6	2.6	1.9 ± 0.2 ^b	2.6 ± 0.0 ^b	5.1 ± 0.2 ^a	0.1 ± 0.0 ^c
DHA/EPA	2.2	2.8 ± 0.1 ^a	1.9 ± 0.1 ^c	2.4 ± 0.1 ^b	3.1 ± 0.1 ^a
ARA/EPA	0.9	1.3 ± 0.0 ^b	0.3 ± 0.1 ^c	0.1 ± 0.0 ^c	8.0 ± 1.4 ^a

Table 14. Fatty acid composition (expressed as % of total fatty acid detected, mean \pm standard deviation) of phospholipid fraction of whole body lake trout alevins from the Fish Oil Replacement Experiment before the start of the experiment and after being fed one of four diets for 8 weeks. Means with different superscript letters in a row indicate statistical significance ($p < 0.05$) at the end of the experiment. nd = non-detectable. OA = Oleic acid, LO = Linseed oil, CLO = Cod liver oil, LE = Lecithin.

Fatty Acid	Dietary Treatment				
	Initial	OA	LO	CLO	LE
<i>n</i>	1	3	3	3	3
<i>Saturated</i>					
12:0	nd	nd	nd	nd	nd
14:0	0.5	0.7 \pm 0.1 ^b	0.5 \pm 0.0 ^b	1.4 \pm 0.1 ^a	0.6 \pm 0.0 ^b
15:0	0.2	0.2 \pm 0.0 ^b	0.1 \pm 0.0 ^b	0.3 \pm 0.0 ^a	0.2 \pm 0.0 ^b
16:0	18.0	15.8 \pm 0.4 ^b	15.9 \pm 0.3 ^b	16.7 \pm 0.3 ^b	18.6 \pm 0.2 ^a
17:0	0.2	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b	0.3 \pm 0.0 ^a
18:0	5.9	6.3 \pm 0.2 ^a	7.0 \pm 0.1 ^a	4.5 \pm 0.1 ^b	6.1 \pm 0.3 ^a
Σ Saturated	25.0	23.1 \pm 0.4 ^b	23.7 \pm 0.4 ^b	23.1 \pm 0.4 ^b	25.8 \pm 0.1 ^a
<i>Monounsaturated</i>					
16:1 n -7	1.4	1.7 \pm 0.2 ^b	0.7 \pm 0.1 ^c	2.5 \pm 0.1 ^a	1.2 \pm 0.2 ^{bc}
16:1 n -9	0.8	2.3 \pm 0.3 ^a	1.8 \pm 0.2 ^a	1.4 \pm 0.1 ^a	1.8 \pm 0.1 ^a
17:1	0.2	0.3 \pm 0.0 ^a	0.1 \pm 0.0 ^c	0.2 \pm 0.0 ^b	0.1 \pm 0.0 ^c
18:1 n -7	4.3	3.5 \pm 0.2 ^a	1.7 \pm 0.2 ^b	4.0 \pm 0.1 ^a	2.0 \pm 0.1 ^b
18:1 n -9	10.1	23.5 \pm 2.2 ^a	17.1 \pm 0.3 ^b	14.2 \pm 0.3 ^b	14.5 \pm 0.7 ^b
20:1 n -9	1.2	1.2 \pm 0.2 ^b	0.7 \pm 0.2 ^b	3.3 \pm 0.1 ^a	0.9 \pm 0.2 ^b
22:1 n -9	nd	0.1 \pm 0.0 ^a	nd	0.1 \pm 0.0 ^a	nd
22:1 n -11	nd	nd	nd	0.4 \pm 0.0	nd
Σ Monounsaturated	18.0	32.5 \pm 3.1 ^a	22.0 \pm 1.0 ^b	26.1 \pm 0.6 ^{ab}	20.6 \pm 1.2 ^b
<i>Polyunsaturated</i>					
18:2 n -6	0.8	1.3 \pm 0.1 ^c	4.8 \pm 0.5 ^b	1.1 \pm 0.0 ^c	14.1 \pm 0.9 ^a
20:2 n -6	0.5	0.1 \pm 0.0 ^d	0.6 \pm 0.0 ^b	0.3 \pm 0.0 ^c	1.5 \pm 0.1 ^a
20:3 n -6	0.2	0.9 \pm 0.1 ^b	0.9 \pm 0.0 ^b	0.3 \pm 0.0 ^b	4.1 \pm 0.3 ^a
20:4 n -6	8.0	6.9 \pm 0.1 ^b	3.2 \pm 0.4 ^c	2.3 \pm 0.1 ^d	10.1 \pm 0.1 ^a
22:4 n -6	0.5	0.4 \pm 0.0 ^b	0.1 \pm 0.0 ^c	0.1 \pm 0.0 ^c	1.2 \pm 0.1 ^a
22:5 n -6	1.6	1.8 \pm 0.1 ^b	0.4 \pm 0.1 ^c	0.4 \pm 0.0 ^c	5.5 \pm 0.1 ^a
Σ n -6	11.5	11.5 \pm 0.4 ^b	10.0 \pm 0.2 ^b	4.4 \pm 0.1 ^c	36.5 \pm 1.3 ^a
18:3 n -3	0.5	0.1 \pm 0.0 ^b	6.9 \pm 1.1 ^a	0.2 \pm 0.0 ^b	0.9 \pm 0.1 ^b
18:4 n -3	0.1	nd	2.0 \pm 0.2 ^a	0.3 \pm 0.0 ^b	0.5 \pm 0.1 ^b
20:3 n -3	0.4	0.1 \pm 0.0 ^b	1.0 \pm 0.1 ^a	0.1 \pm 0.0 ^b	0.1 \pm 0.0 ^b
20:4 n -3	0.5	0.1 \pm 0.0 ^c	1.2 \pm 0.1 ^a	0.5 \pm 0.0 ^b	0.2 \pm 0.0 ^c
20:5 n -3	6.0	3.0 \pm 0.3 ^b	6.8 \pm 0.3 ^a	7.3 \pm 0.3 ^a	1.9 \pm 0.0 ^b

21:5 <i>n</i> -3	nd	nd	nd	0.2 ± 0.0	nd
22:5 <i>n</i> -3	4.5	2.0 ± 0.1 ^b	2.5 ± 0.1 ^a	2.1 ± 0.1 ^b	1.4 ± 0.1 ^c
22:6 <i>n</i> -3	33.6	27.7 ± 2.9 ^b	24.0 ± 0.4 ^b	35.8 ± 0.9 ^a	12.1 ± 0.4 ^c
∑ <i>n</i> -3	45.6	32.9 ± 3.1 ^b	44.3 ± 1.1 ^a	46.4 ± 1.1 ^a	17.1 ± 0.5 ^c
∑ Polyunsaturated	57.1	44.4 ± 2.7 ^b	54.4 ± 1.2 ^a	50.8 ± 1.0 ^{ab}	53.5 ± 1.2 ^a
∑ <i>n</i> -3/∑ <i>n</i> -6	4.0	2.9 ± 0.4 ^c	4.4 ± 0.1 ^b	10.5 ± 0.4 ^a	0.5 ± 0.0 ^d
DHA/EPA	5.6	9.3 ± 0.2 ^a	3.5 ± 0.2 ^d	4.9 ± 0.1 ^c	6.3 ± 0.1 ^b
ARA/EPA	1.3	2.4 ± 0.2 ^b	0.5 ± 0.1 ^c	0.3 ± 0.0 ^c	5.2 ± 0.1 ^a

Table 15. Correlation between dietary fatty acids and whole body fatty acids of lake trout from the Fish Oil Replacement Experiment.

Fatty Acids	Neutral Lipids			Phospholipids		
	Slope	r^2	P	Slope	r^2	P
18:1 n -9	0.34	0.99	<0.01	0.16	0.96	0.02
18:2 n -6	0.49	0.95	0.02	0.25	0.99	<0.01
18:3 n -3	0.52	0.99	<0.01	0.15	1.00	<0.01
20:4 n -6	-4.95	0.27	0.48	-11.17	0.38	0.38
20:5 n -3	0.35	0.70	0.17	0.35	0.39	0.38
22:6 n -3	0.62	0.61	0.22	1.15	0.55	0.26
Σ Saturated	0.84	0.83	0.09	0.16	0.73	0.15
Σ MUFA	0.80	0.84	0.08	0.20	0.46	0.32
Σ PUFA	0.49	0.78	0.12	0.17	0.90	0.05
Σn -3	0.71	0.86	0.07	0.55	0.60	0.22
Σn -6	0.60	0.99	<0.01	0.55	0.95	0.03

Figures:

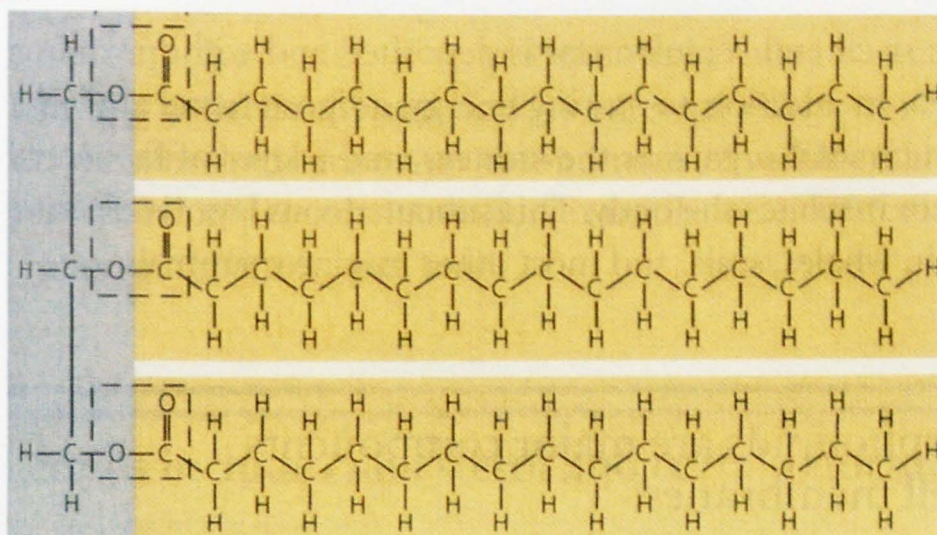


Figure 1. Schematic representation of triacylglycerol (TAG), used as energy or stored for later use, which contains a glycerol (gray) and three fatty acids (yellow) (Campbell and Reece 2002).

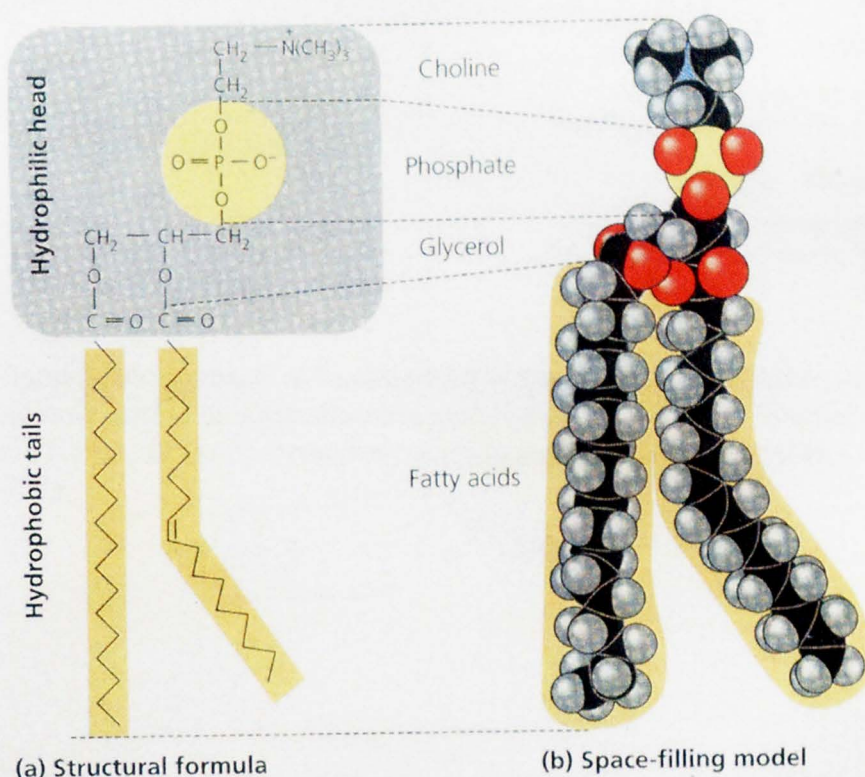


Figure 2. Schematic representation of a phospholipid containing a glycerol, phosphate, a polar group (choline, pictured here), and two fatty acids (Campbell and Reece 2002).

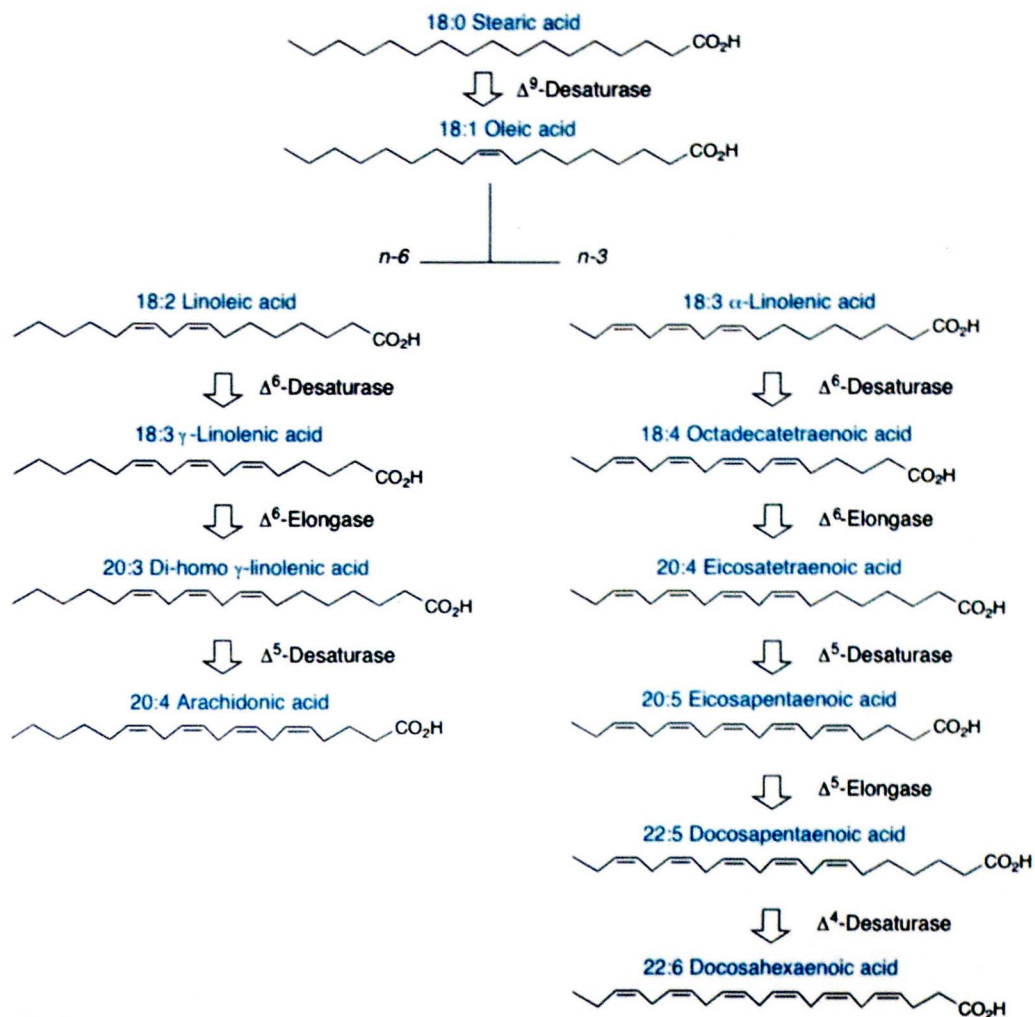


Figure 3. Biosynthesis of linoleic acid to arachidonic acid (ARA; 20:4n-6) and linolenic acid to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) through desaturase and elongation enzymes (Napier 2002).

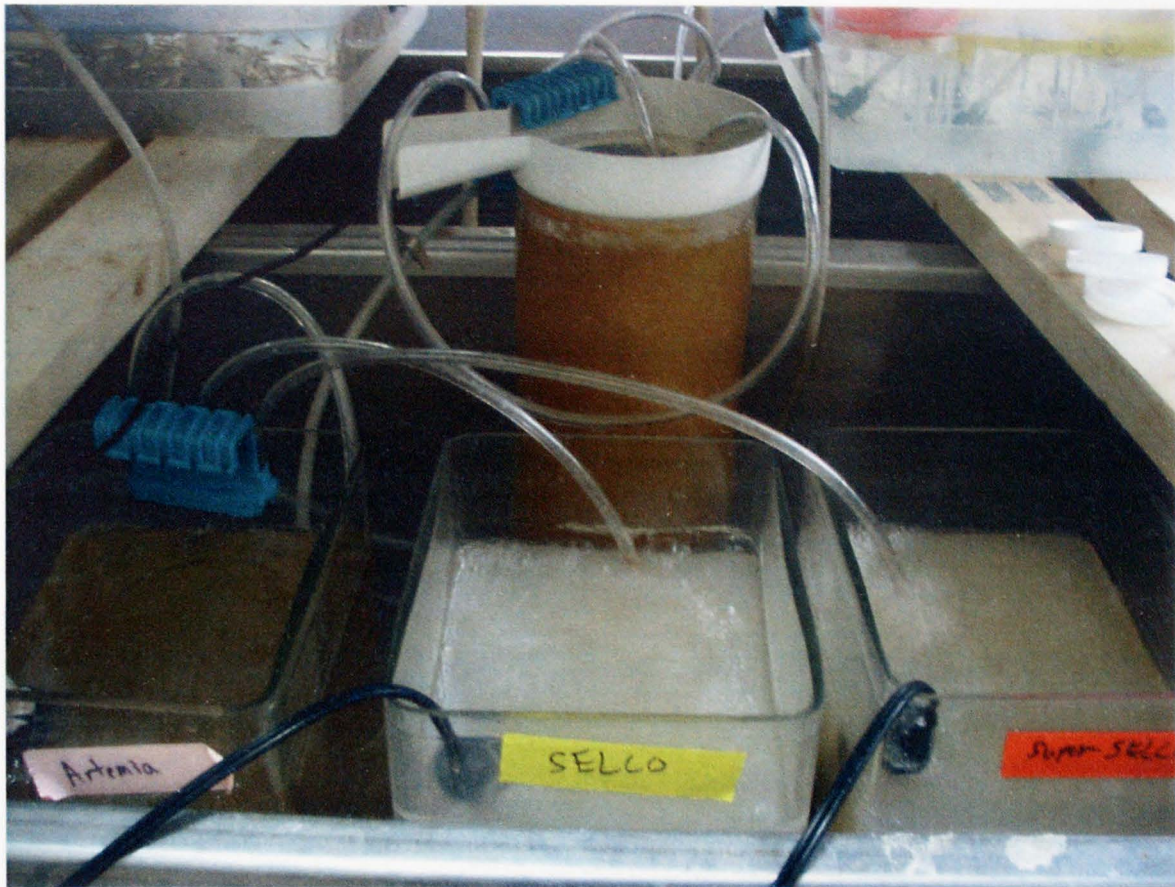


Figure 4. *Artemia* production. Stock *Artemia* were located in the McDonald hatching jar (back) for 24 h and were then placed in the respective 5-L tank (front).

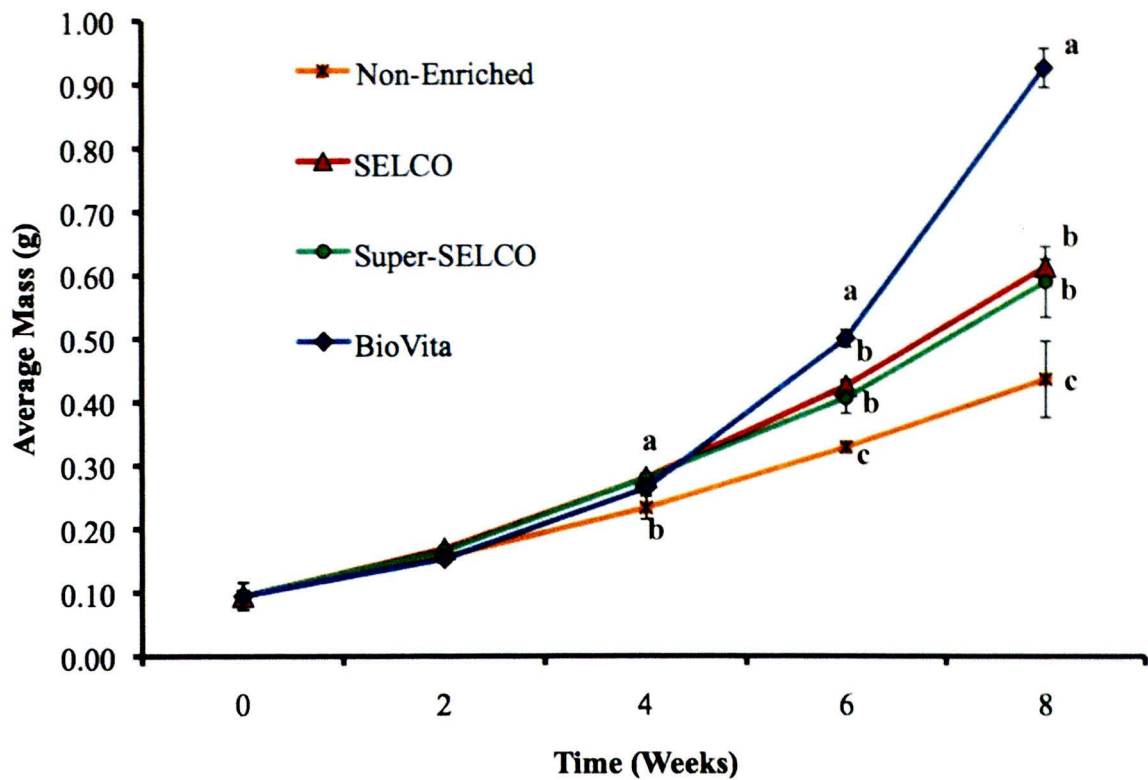


Figure 5. Change in lake trout mass throughout the duration of the *Artemia* Experiment. At each sampling date means with different superscript are statistically significant ($p < 0.05$). Non-enriched = Non-enriched *Artemia*, SELCO = SELCO-enriched *Artemia*, Super SELCO = Super SELCO-enriched *Artemia*.

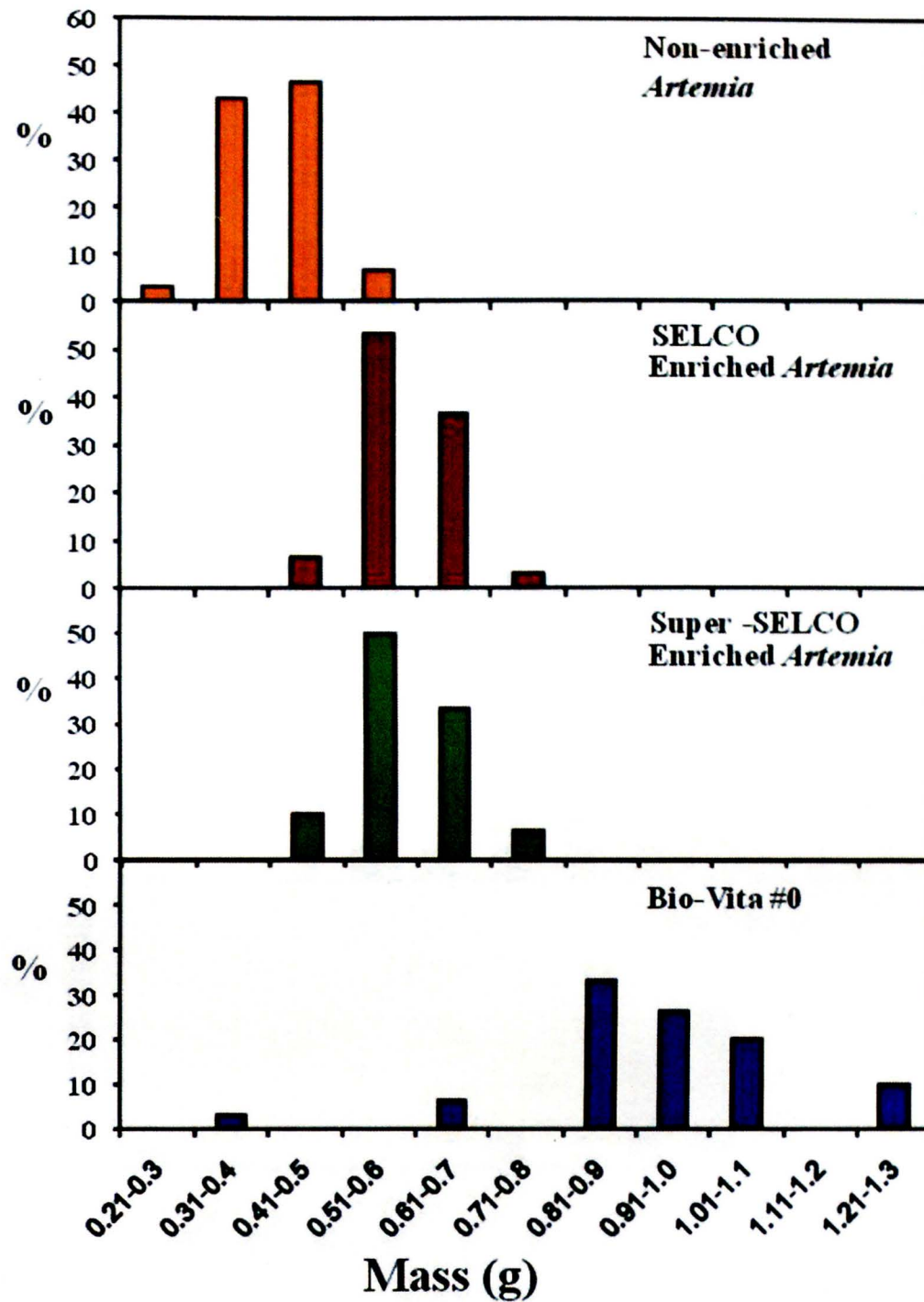


Figure 6. Final mass distribution after 8 weeks of feeding of lake trout fed one of the four diets used in the *Artemia* Experiment.

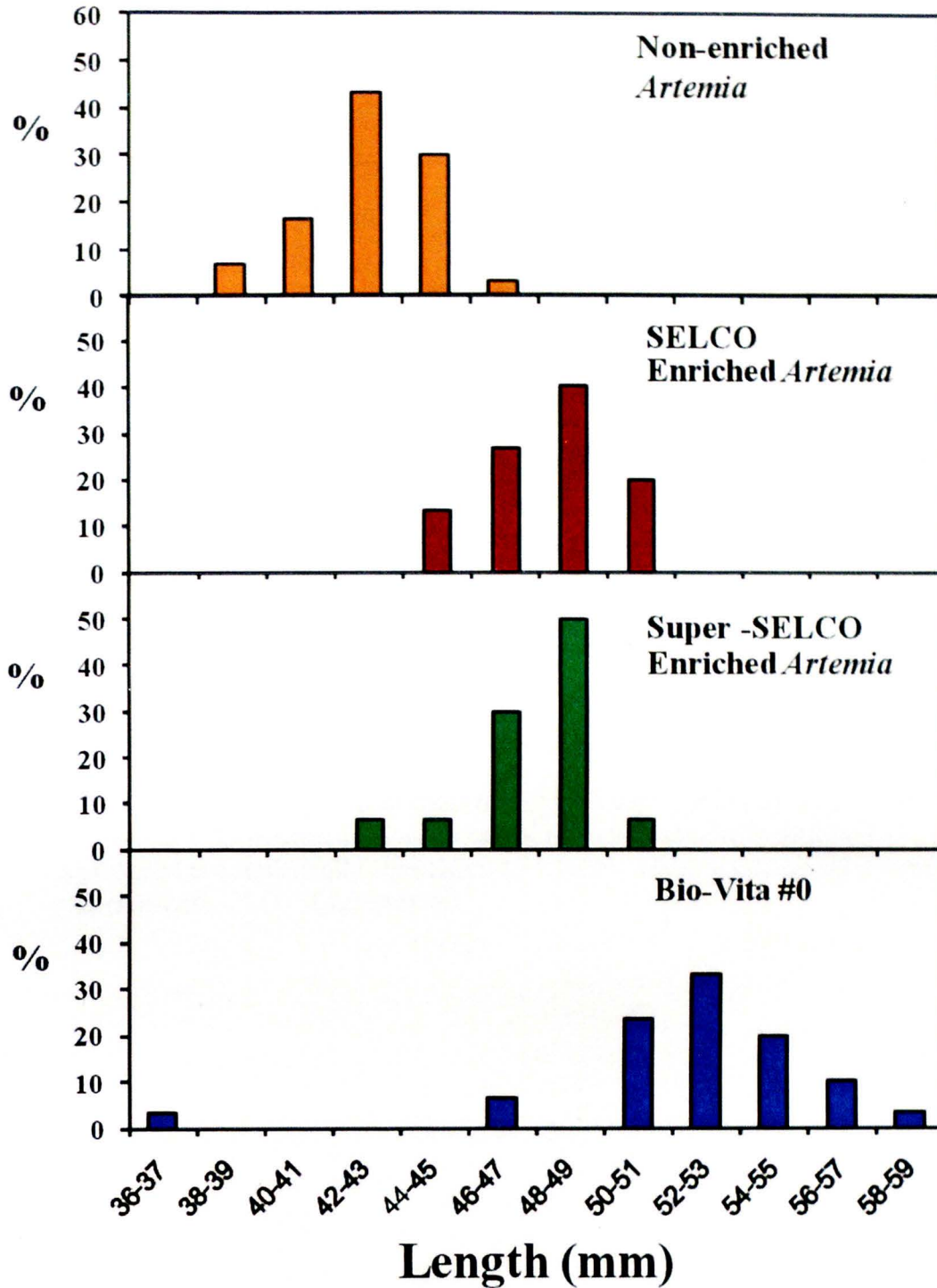


Figure 7. Final length distribution after 8 weeks of feeding of lake trout fed one of the four diets used in the *Artemia* Experiment.

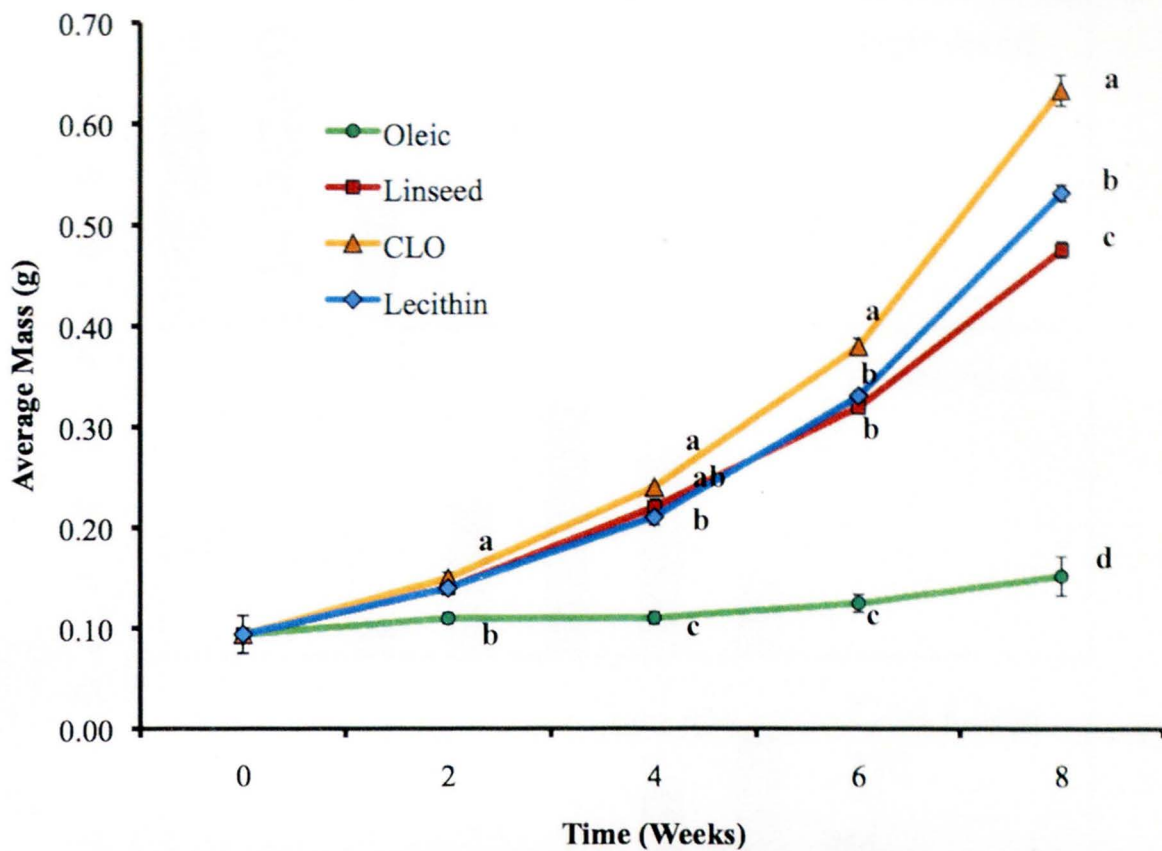


Figure 8. Change in lake trout mass throughout the duration of the Fish Oil Replacement Experiment. At each sampling date means with different superscript are statistically significant ($p < 0.05$). Oleic = Oleic acid, Linseed = Linseed oil, CLO = Cod liver oil.

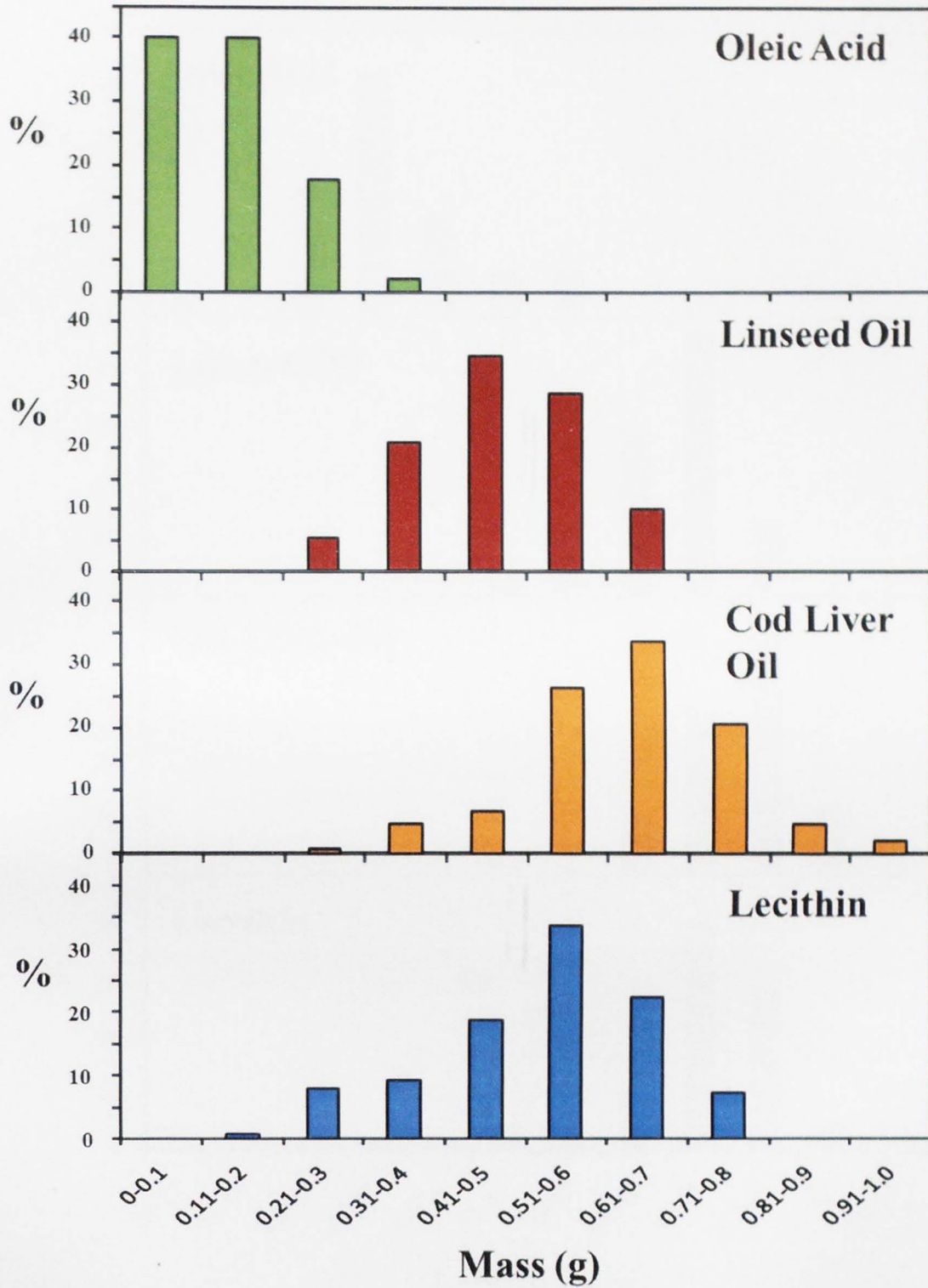


Figure 9. Final mass distribution after 8 weeks of feeding of lake trout fed one of the four diets used in the Fish Oil Replacement Experiment.

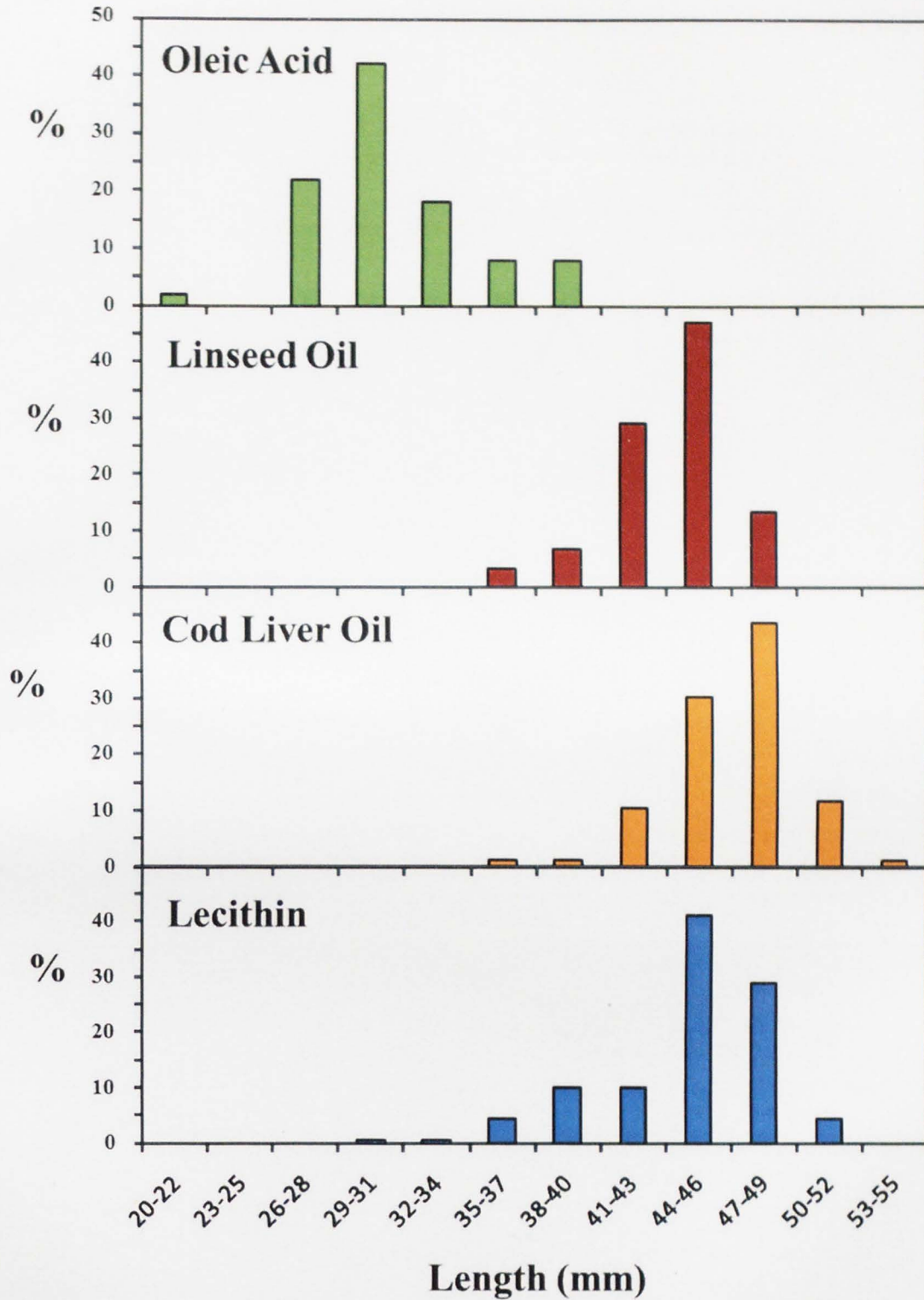


Figure 10. Final length distribution after 8 weeks of feeding of lake trout fed one of the four diets used in the Fish Oil Replacement Experiment.