DETERMINING THE NET GROWTH EFFICIENCY OF EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID IN ATLANTIC POLLOCK (*POLLACHIUS VIRENS*) USING A MASS BALANCE APPROACH

by

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ABTRACT

The essential fatty acids (EFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are required for the maintenance of good health in humans. Marine production of EPA and DHA is predicted to decrease as a result of warming seawater temperatures. With reduced production, it becomes critical to understand the efficiency with which these EFA are transferred through trophic systems. We employed a mass balance approach to determine the net growth efficiency (NGE) of EPA and DHA in Atlantic pollock (*Pollachius virens)* fed two low-lipid diets; one diet contained half the proportion of EPA and DHA as the other. NGEs for EPA and DHA were greater than 50% in fish fed diets that were rich in these EFA. However, in fish that received reduced dietary proportions of EPA and DHA, significantly lower NGEs were observed. This indicates that a limited ability to retain essential nutrients may exist when dietary supply is reduced.

LIST OF ABBREVIATIONS USED

AA	Arachidonic Acid
ADC	Apparent Digestibility Coefficient
AE	Assimilation Efficiency
ALA	α-Linolenic Acid
CCAC	Canadian Council on Animal Care
DHA	Docosahexaenoic Acid
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FID	Flame Ionization Detection
FAO	Food and Agriculture Organization
GC	Gas Chromatography
GGE	Gross Growth Efficiency
LC-PUFA	Long-chain Polyunsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate + Hydrogen
NGE	Net Growth Efficiency
NPP	Net Primary Production
NRCC	National Research Council of Canada
PL	Phospholipid
PUFA	Polyunsaturated Fatty Acid
SFA	Saturated Fatty Acid
SST	Sea Surface Temperature

TAG	Triacylglycerol
WHO	World Health Organization

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1.0 INTRODUCTION

1.1 MARINE FATTY ACIDS AND THEIR IMPORTANCE IN HUMAN HEALTH

Human health has been inextricably linked to dietary marine fatty acids (FAs) for millennia (Arts et al. 2001). Nearly 40% of the global population relies on finfish as their primary source of omega-3 long-chain polyunsaturated FA (LC-PUFA; Lloret et al. 2016). The structure of FAs consists of a carboxylic acid with a variable-length hydrocarbon chain. They are typically named using the "*a:bn-c*" notation, where *a* is the number of carbon atoms, *b* is the number of double bonds, and *c* is the position of the double bond relative to the terminal methyl group. The "*n-c*" nomenclature can be interchanged with the omega (ω) notation, which is fundamentally the same and is often encountered in nutrition literature. FAs with no double bonds in their structure are referred to as saturated fatty acids (SFAs), those with a single double bond are called monounsaturated fatty acids (MUFAs), and those with two or more are referred to as polyunsaturated fatty acids (PUFAs; Figure 1.1). LC-PUFAs are those with 20 carbons or more and are typically essential fatty acids (EFAs) in humans as they are not synthesized endogenously in significant amounts but are acquired through diet instead.

A broad range of health effects has been shown to be influenced by diets supplemented with significant amounts of omega-3 fatty acids, specifically eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). Briefly, such health effects include reduced occurrence of cardio-vascular disease (Mozaffarian & Wu 2011), reduced inflammation in patients with rheumatoid arthritis (Kremer 2000),

and lower risk of developing neurodegenerative disorders (Fotuhi et al. 2009). Omega-3 FA also appear to be important nutrients during fetal development in which they play a role in the development of the brain and retina (Swanson et al. 2012). Although suggested dietary intake varies by governing body, in the United States, the Academy of Nutrition and Dietetics recommends a daily intake of 500 mg of combined EPA and DHA for adults (Vannice & Rasmussen 2014). The World Health Organization (WHO) in collaboration with the Food and Agriculture Organization (FAO) recommends that n-3 LC-PUFA contribute 1-2% of total daily energy consumption (Nishida et al. 2004). The most readily available source of these important fatty acids is fish (Bézard et al. 1994, Ackman 2008).

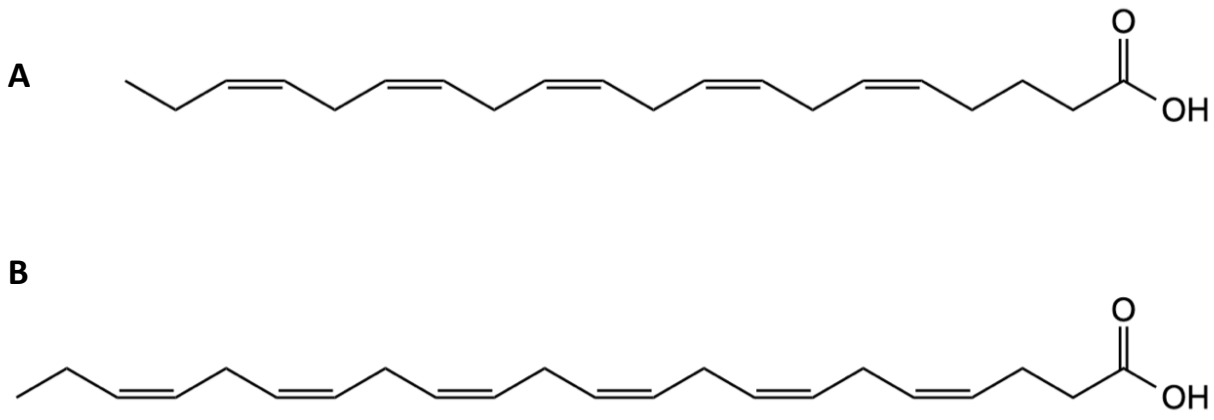


Figure 1.1 The structure of two physiologically essential n-3 LC-PUFA A) 20:5n-3; EPA and B) 22:6n-3.

The LC-PUFAs, EPA and DHA, are known to be abundant in marine ecosystems (Reitan et al. 1997, Budge et al. 2001, Dalsgaard & John 2004). The majority of EPA and DHA is first introduced into the marine food chain by primary producers, such as

bacteria, protists, and microalgae, through endogenous synthesis processes (Bell & Tocher 2009). On the contrary, terrestrial ecosystems, including freshwater aquatic ecosystems, are generally poor in *n-3* LC-PUFA and, instead, linoleic acid (18:2n-6) and α-linolenic acid (ALA; 18:3n-3) prevail (Ackman 1967). Primary producers in marine ecosystems are highly efficient at synthesizing *n-3* LC-PUFA and serve as an ample resource for primary consumers; however, in freshwater ecosystems, such a resource does not exist (Colombo et al. 2016). Within the marine realm, n-3 LC-PUFA production varies geospatially, with greater production being observed in polar and temperate waters compared to tropical environments (Colombo et al. 2016).

Table 1.1 The distribution of fatty acids in fish. Adapted from Ackman (2008).

Fatty Acid Common Name*	**Abbreviated Form**
Myristic Acid	14:0
Pentadecanoic Acid	15:0
Palmitic Acid	16:0
Palmitoleic Acid	16:1n-7
Stearic Acid	18:0
Vaccenic Acid	18:1n-7
Oleic Acid	18:1n-9
Linoleic Acid	18:2n-6
α-Linolenic Acid	18:3n-3
Gondoic Acid	20:1n-9
Arachidonic Acid	20:4n-6
Eicosapentaenoic Acid	20:5n-3
Erucic Acid	22:1n-9
Cetoleic Acid	22:1n-11
Adrenic Acid	22:4n-6
n-3 Docosapentaenoic Acid	22:5n-3
n-6 Docosapentaenoic Acid	22:5n-6
Docosahexaenoic Acid	22:6n-3

*All FA are in *cis* configuration

1.2 EFFECT OF CLIMATE CHANGE ON ESSENTIAL FATTY ACID PRODUCTION

As the world population continues to rise so does the global demand for EFA. Budge et al. (2014) estimated that the annual EPA production worldwide was scarcely enough to meet the nutritional needs of the present-day human population; henceforth, little disturbances in primary production would be required to elicit a major strain on the ability to supply global demands. Of the factors influencing EFA production

in marine ecosystems, warming ocean temperatures as a result of climate change pose one of the greatest threats. Estimates suggest mean sea surface temperatures (SSTs) could increase by as much as 2 °C globally by the year 2065, even under the most stringent mitigation scenarios (IPCC 2014). To some extent, the effects are already being observed as the past two decades have seen downward trends in net primary production (NPP) in the world's oceans (Signorini et al. 2015).

As ocean temperature increases, phytoplankton are predicted to modify their membrane composition through a process known as homeoviscous adaptation. This in turn has implications on the EPA and DHA composition of the organism. For example, an increase in water temperature by 2.5°C could result in a 28% decrease in global DHA production (Hixson & Arts, 2016). A decrease in the average cell size of phytoplankton communities has also been linked to increases in SST. For example, a 7% decrease in the proportion of large phytoplankton in the North Pacific subtropical biome has been predicted to occur over the course of the 21st century (Polovina et al. 2011). As such, shifts in phytoplankton community structure could result in a lower supply of EFA for higher trophic level consumers, including fish (Litzow et al. 2006).

Reduced dietary supply of EFA coupled with warming ocean temperatures is predicted to be detrimental for marine fish species (Vagner et al. 2019). Estimates of the influence of warming oceans on EFA production in primary producers can be used to extrapolate the effects on higher trophic level consumers. Pethybridge et al. (2015) predicted that an increase in SST by 1°C could elicit a decrease in EPA and DHA concentrations of 3% and 1.5% respectively in albacore tuna (Thunnus alalonga).

However, a major issue with such predictions is the lack of knowledge regarding the efficiency by which EFA are transferred from one trophic level to the next (Pethybridge et al. 2015).

1.3 ECOLOGICAL EFFICIENCIES

Understanding the efficiency by which EFA are transferred from primary producers to higher trophic level consumers, and eventually to humans, is crucial for predicting the effect of climate change on EFA availability in marine ecosystems. Trophic transfer efficiency, expressed as a percentage, is a ratio of the production of one trophic level to that of the previous trophic level (Lindeman 1942). However, in actuality, trophic transfer efficiency is nearly impossible to calculate because of complex consumer-resource interactions, such as widespread omnivory in aquatic ecosystems (Wetzel 2001). That said, understanding the efficiency by which an ingested nutrient is deposited into tissue can provide insight into the trophic dynamics of that nutrient.

While there is a general misunderstanding in regard to the appropriate nomenclature used to explain ecological efficiencies, most literature is in agreement when it comes to defining the three most important efficiencies: assimilation efficiency (AE), gross growth efficiency (GGE), and net growth efficiency (NGE). In an ecological context, the term ingestion describes the amount of food consumed by an organism and is the sum of both assimilated material and egested material. Assimilated material, or the fraction of ingested material that is incorporated into tissue, can be lost to metabolic pathways or it can be utilized for growth (Welch 1968). The term growth is often used synchronously with production; although, production traditionally

encompasses more than just somatic growth and includes such things as gamete production, among others (Schroeder 1981). Assimilation efficiency, the ratio of assimilated material to ingested material, describes a consumer's ability to extract energy and nutrients from its food. Gross growth efficiency, on the other hand, describes the overall efficiency with which ingested food is converted into tissue and does not discriminate among egested or respired material (Welch 1968). It is the ratio of growth, or production, to total ingested food. Net growth efficiency differs from GGE in that it considers only assimilated material, rather than total ingested material, and thus will always be smaller than GGE (Welch 1968). Net growth efficiency is defined in the present study as a ratio of the mass of a FA incorporated into biological tissue, and therefore used for growth, to the mass of that FA assimilated.

NGE is highly variable in poikilothermic organisms, like fish, but is known to range between 10% and 60% (Strayer 2012, and references therein). With respect to increasing seawater temperatures and the subsequent impact on EFA availability in marine ecosystems, it becomes necessary to identify whether higher trophic level consumers can adapt to decreases in primary production by becoming more efficient at incorporating these essential nutrients into tissue. There is some evidence that EPA and DHA are more efficiently retained when dietary supply is reduced in Atlantic salmon (Salmo salar; Torstensen et al. 2004); however, there is a general knowledge gap in the literature. In order to accurately quantify the NGE of a dietary nutrient in any organism, a firm knowledge of the digestive and metabolic processes that determine its fate is a prerequisite.

1.4 BIOSYNTHESIS AND β-OXIDATION OF FATTY ACIDS IN FISH

FAs deposited in fish tissue can originate from several processes. Firstly, they can be consumed in diet and deposited directly without modification. Alternatively, they can be consumed, modified by enzymatic activity, and then deposited. Further still, they can be synthesized *de novo* within the fish (Budge et al. 2006). Biosynthesis most often occurs in the liver and typically begins with the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) which are synthesized endogenously by the fatty acid synthetase enzyme (FAS) using acetyl-CoA as a carbon source (Tocher 2003). Fish can modify 16:0 and 18:0 FAs with limited success, as demonstrated in the synthesis of the monounsaturated oleic acid (18:1n-9) and palmitoleic acid (16:1n-7). This occurs through elongation and desaturation events carried by the endogenous desaturases Δ9, Δ6, and Δ5 found in all vertebrates (Henderson 1996; Iverson 2009). Desaturase enzymes operate by selectively removing two hydrogens from the existing carbon chain, creating a double bond (Lim et al. 2014). However, like all other animals, fish lack Δ12 and Δ15 desaturases and therefore are unable to insert a double bond between the terminal methyl group and the n-9 carbon (Bell & Tocher 2009).

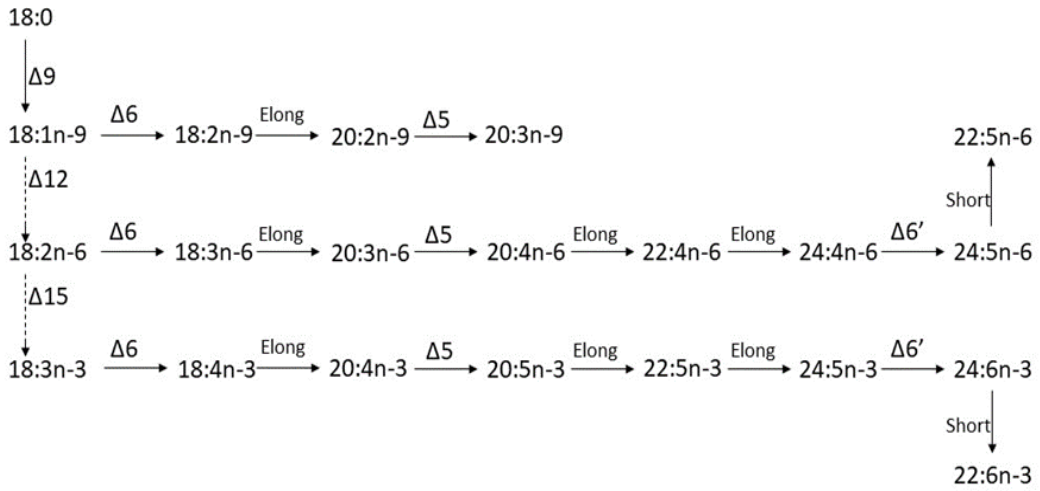


Figure 1.2 Pathway in which LC-PUFA (C_{20} and C_{22}) are synthesized from stearic acid (18:0) in rainbow trout (*Oncorhynchus mykiss*). Δ15, Δ12, Δ9, Δ6, Δ6', Δ5 represent fatty acyl desaturases, and Elong and Short represent fatty acyl elongases and chain shortening respectively. It is not known whether the Δ6 desaturase that acts on C_{18} and C_{24} is the same enzyme, hence the latter is given the notation Δ6'. Hashed arrows correspond to desaturase steps that are not possible in fish. Adapted from Tocher (2003).

Freshwater fish have demonstrated the ability to synthesize LC-PUFA, such as DHA, from shorter precursors supplied by diet, such as 18:3n-3, through an initial desaturation using Δ6 to yield 18:4n-3, followed by an elongation step to 20:4n-3, a Δ5 desaturation to 20:5n-3, and elongation to 22:5n-3 (Henderson 1996). The final product 22:6n-3 is the result of the elongation of 22:5n-3 to 24:5n-3, a subsequent Δ6 desaturation to 24:6n-3, and a final chain shortening step to yield 22:6n-3 (Tocher 2003). Elongation occurs in the endoplasmic reticulum in a sequence of steps, the first of which is the rate limiting step and is a condensation reaction between the fatty acyl chain and malonyl-CoA, forming a β-ketoacyl chain. Secondly, the β-ketoacyl chain is reduced, using NADPH, to produce 3-hydroxyacyl-CoA which, following a dehydration

reaction, is converted to trans-2, 3-enoyl-CoA. The final product, a two-carbon-extended FA, is derived from the reduction of enoyl-CoA (Castro et al. 2016).

In contrary to freshwater fish, marine fish appear to be incapable of synthesizing EPA and DHA from shorter chain precursors as they demonstrate little to no Δ5 desaturase activity (Tocher & Ghioni 1999). This trend has been observed in several marine fish species including cobia (*Rachycentron canadum*; Zheng et al. 2009a), Atlantic cod (*Gadus morhua*; Zheng et al. 2009b), and European seabass (*Dicentrarchus labrax*; Eroldoğan et al. 2013). The ability for FA biosynthesis in fish appears to be governed, to some extent, by the quality of the diet. The rate of lipogenesis has been shown to be inversely proportional to the availability of lipid in the diet, so if a high-fat content diet is available, little in the way of *de novo* synthesis is predicted to occur (Dalsgaard & John 2004). For example, tilapia (*Oreochromis sp*.), a freshwater fish, is capable of converting 18:2n-6 and 18:3n-3 to 20 and 22 carbon derivatives; however, when fed a commercial diet, rich in preformed LC-PUFA, this ability was suppressed (Olsen et al. 1990).

One metabolic fate of dietary PUFAs is the formation of eicosanoids. The term eicosanoid encompasses a family of biologically active derivatives of eicosapolyenoic acids and include the prostaglandins, thromboxanes, hydroperoxy- and hydroxyeicosatetraenoic acids, leukotrienes, and lipoxins (Mustafa & Srivastava 1989). They play a role in a number of physiological processes, including the immune response and reproduction (Tocher 2003). In fish, the major eicosanoid precursor is arachidonic acid (AA; 20:4n-6), with EPA making lesser contributions (Sargent et al. 1999). In fact,

eicosanoids formed from EPA precursors are known to inhibit the formation of eicosanoids from AA precursors (Sargent et al. 1999).

Although protein is a major source of energy in fish, FAs can also be exploited for this purpose. For instance, FAs contributed 20% and 10% to total oxidized substrate in fish at rest and while swimming, respectively (van den Thillart 1986). This process, known as β-oxidation, involves the breakdown of fatty acids into their acetyl-CoA constituents and it occurs in both the mitochondria and peroxisomes (Leaver et al. 2008). β-oxidation begins with the formation of a thiol ester between the thiol group of coenzyme A and the fatty acid which is catalyzed by acyl-CoA synthetases. A complete β-oxidation cycle yields a 2-carbon-shortened product as well as NADH which can be used to generate ATP (Tocher 2003). A number of factors, including chain length and degree of saturation, determine a FA's suitability as a substrate for oxidation, with saturated and monounsaturated short-chain FA having higher oxidation rates than LC-PUFA and, particularly, *n-3* LC-PUFA (Henderson 1996). The rate of β-oxidation can be influenced by the dietary FA concentrations (Turchini et al. 2003). In mice, elevated β-oxidation rates were associated with being fed diets rich in fish oils (Bargut et al. 2014). Existing methods for quantifying β-oxidation include the whole body fatty acid balance method (Turchini et al. 2007, Turchini & Francis 2009, Thanuthong et al. 2011) and the radiolabelling [1-^{14}C] approach (Ruyter & Thomassen 1999; Nanton et al. 2003, Torstensen & Stubhaug 2004).

1.5 MEASURING NET GROWTH EFFICIENCY: AN APPLICATION OF THE MASS BALANCE APPROACH

In the present study, we employed a mass balance approach to determine the net growth efficiency of EPA and DHA in Atlantic pollock (*Pollachius virens*). Existing methods for quantifying the efficiency at which a dietary nutrient is incorporated into tissue employ radiolabelled "tracer" FA, such as ^{3}H- and ^{14}C-labelled FA (Brown 2005); however, these methods require sophisticated instrumentation in order to achieve accurate results, as well as expensive specialized feeds that incorporate the labelled nutrient. Historically, reliability and consistency have been difficult to achieve using this method. The benefit of the fatty acid mass balance approach, originally described by Cunnane and Anderson (1997) in rats and more recently applied to finfish by Turchini et al. (2007), is its inherent simplicity and reliability. In terms of instrumentation, the mass balance approach requires little more than a gas chromatography (GC) unit to facilitate FA analysis. Virtually any diet can be used for the mass balance method given that its proximate composition is precisely known, and the FA composition is quantifiable. The whole body fatty acid balance method has been applied to a number of fish species including rainbow trout (*Oncorhynchus mykiss*; Turchini & Francis 2009), European seabass (Eroldoğan et al. 2013), Atlantic salmon (Norambuena et al. 2015), Murray cod (*Maccullochella peelii peelii*; Senadheera et al. 2011), and Atlantic cod (Hixson et al. 2014).

A fundamental requirement of the mass balance method is a controlled feeding study in which total digestion is quantified through collection of total faeces or by using

a digestibility marker. Initial and final measurements of body weight must be made, and likewise initial and final quantitative FA composition must be determined and expressed as mass per fish. Finally, the total feed consumption must be known to calculate the net intake of individual FA. Using this method, the fraction of dietary FA (as mass $fish^{-1}$) involved in each of three main pathways can be described. These pathways include: 1) accumulation – *FA from diet that are found in tissues*, 2) excretion – *total FA expelled in faeces*, and 3) appearance/disappearance – *FA involved in desaturation, elongation, or oxidation events*. The appearance/disappearance of a given FA is calculated by subtracting the intake and excretion values of that FA from the amount accumulated. Once these values are known, the method allows for computation of the SFA, MUFA, and PUFA (n-3 & n-6) balances, which requires the conversion of mass $fish^{-1}$ to mmol $fish^{-1}$. Next, a reverse calculation must be performed, i.e. backwards through the metabolic pathway of the individual FA, by which the mmol of longer/more unsaturated FA is subtracted from the number of mmol of the previous FA in the respective elongation/desaturation pathway. From here, it is possible to calculate the amount of a particular FA that has been elongated/desaturated or β-oxidized in mmol/day as well as derive the relative activities of desaturase and elongase enzymes.

In the present study, in which Atlantic pollock serve as a model organism to evaluate NGE in a marine fish, the mass balance calculation for EPA and DHA becomes considerably simplified because biosynthesis of these FA is assumed to be negligible in this species (Tocher 2010). Therefore, it can be expected that EPA and DHA in tissue is directly of dietary origin and the rate at which these EFA are incorporated into tissue

can be quantified accordingly. Pollock are a popular groundfish of the North Atlantic and are a member of the Gadidae family which includes commercially relevant species such as Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). In contrary to fatty marine fish, such as herring or mackerel, in which the depot fats are stored in muscle tissue, pollock are lean fish that store their primary energy reserves in the liver (Ackman 1967, Jensen 1979). In this regard, pollock presents a convenient test subject to estimate NGE of an essential nutrient in a secondary consumer.

1.6 OBJECTIVES

1. Determine the metabolic fate of EPA and DHA in the marine fish Atlantic pollock using the mass balance approach. More specifically, we will determine the efficiency with which dietary EPA and DHA are deposited in fish tissues. The rate at which dietary EPA and DHA is incorporated into biological tissue is not well characterized in many organisms; therefore, this work allows us to provide a basic framework for understanding how efficiently dietary EFAs are transferred through marine food webs.

2. Evaluate the influence of the dietary supply of EPA and DHA on NGE. Since climate change is predicted to reduce the supply of EPA and DHA in marine ecosystems by hampering primary production, this work will provide insight into how EPA and DHA net growth efficiency is influenced by changes in dietary supply. Tanks will receive either a fish oil – based diet (FO) which is high in EPA

and DHA, or a fish oil/canola oil – based diet (FOCO) in which the EPA and DHA content has been reduced.

2.0 METHODS

2.1 EXPERIMENTAL FISH

All aspects of the experiment were in full compliance with the guidelines set forth by the Canadian Council of Animal Care (CCAC 2005). Juvenile Atlantic pollock were caught on the 7th and 14th of August 2019 near Sambro, Nova Scotia using a commercial long line. Fish were transferred to Dalhousie University's Aquatron facility by truck in an oxygenated container filled with ambient seawater. Upon transfer to the Aquatron, fish were housed in oxygenated, 2000 L, circular, fiberglass tanks with a flow through system supplying ambient seawater from the Halifax harbour at a flowrate of ~17 L min^{-1}. Water temperature experienced seasonal variation so during the months of November and December, water was first heated before entering the tank. The temperature of the water remained between 11 and 13°C throughout the duration of the trial. The wet lab where the tanks were located was on a 12 h photoperiod such that the room was dark between 19:00 and 7:00 h. While acclimating to the captive environment, fish were fed a 5 mm commercial pellet intended for striped bass and barramundi (5 Aquasea; Corey Nutrition, Fredricton, NB) that was 48% protein and 18% fat and chopped haddock fillet for approximately two weeks. Tanks were regularly maintained throughout the duration of the trial; a syphon was used to remove waste material that had collected on the bottom of the tank and the scum line was regularly removed using a dampened cloth.

An initial sample of 12 fish were selected as controls and were euthanized by an overdose of anesthesia (MS-222; 100 mg L^{-1}). The carcasses were stored at -30°C until

they were processed (section 3.4). The remaining fish were anesthetized (MS-222; 50 mg L^{-1}) and tagged by placing a passive integrated transponder (PIT) tag anterior to the second dorsal fin. After the fish had recovered from the tagging procedure, they were divided into 6 tanks with 12 fish per tank. Fish were fed one of two diets; the first contained fish oil as the primary oil source and the other contained a 3:2 ratio of canola oil to fish oil. Fish were hand-fed once daily at approximately 10:00 h until apparent satiation was achieved. Care was taken during feeding to ensure that all food that entered the tank was consumed. To assess the extent to which weight gain was occurring, fish were re-weighed 2 months into the trial. Any fish that had lost weight were assumed to not have accepted the experimental diet, were eliminated from the trial, and were examined posthumously for any evidence of feeding. Pollock were maintained on their respective experimental diets for a period of 83 days.

Table 2.1 Formulated experimental diets.

	% of diet (as is basis)	
Ingredient	**Fish oil diet**	**Fish oil / Canola oil diet**
Fish Meal (Herring)†	10.00	10.00
Soy protein concentrate‡	21.00	21.00
Wheat gluten meal*	15.05	15.05
Corn protein concentrate (Empyreal 75)*	10.00	10.00
Krill meal¶	4.00	4.00
Wheat flour†	17.00	17.00
Fish oil (Menhaden; 21% EPA + DHA) *	8.00	3.20
Canola oil (0% EPA + DHA)	-	4.80
Corn starch	7.57	7.57
Calcium phosphate (monobasic)	3.90	3.90
Vitamin/Mineral premix†	0.40	0.40
Choline chloride (Vitamin B4) ‖	0.40	0.40
Vitamin C (Ascorbic acid; Stay-C) ‖	0.03	0.03
Vitamin E (α-tocopherol) ‖	0.03	0.03
L-Lysine††	1.28	1.28
L-Methionine‡‡	0.34	0.34
Taurine	0.50	0.50
Chromic oxide**	0.50	0.50
Total	**100.00**	**100.00**

† Corey Nutrition, Fredericton, NB

‡Archer Daniels Midland Ltd., Decatur, IL

* Northeast Nutrition, Truro, NS

¶ Lysaker, Norway

‖ DSM Nutritional Products Canada Inc., Ayr, ON

** Sigma-Aldrich, St. Louis, MO

†† JFFO Nutrition Inc., Saint-Hyacinthe, QC

‡‡ Evonik Industries, Germany

2.2 EXPERIMENTAL DIETS

Diets were prepared at the National Research Council of Canada (NRCC) Marine Research Station in Ketch Harbour, NS. To achieve the required formulations, ingredients were thoroughly mixed together in a stepwise fashion. Briefly, menhaden fish oil or a menhaden fish oil/canola oil blend was first added to a stainless-steel pitcher and gently warmed over a hotplate. The temperature of the oil was carefully adjusted so that it did not exceed 40 °C. Next, corn starch, vitamin/mineral premix, choline chloride (vitamin B4), ascorbic acid (vitamin C), α-tocopherol (vitamin E), L-lysine, L-methionine, taurine, and chromic oxide were combined together in a Globe® bench-top mixer (model SP-20, Globe Food Equipment Company, Dayton, OH) and mixed on low speed for 15 minutes. During this time, the herring fish meal, wheat gluten meal, corn protein concentrate (Empyreal® 75), soybean protein concentrate, krill meal (with shell), and wheat flour were combined in a Hobart® floor planetary mixer (model H600T, Rapids Machinery, Troy, OH) and mixed for 15 minutes at low speed. At this time, the contents of the first mixture were added to the Hobart mixer and both mixtures were blended for an additional 15 minutes at low speed. Finally, the oil component (either fish oil or a fish oil/canola oil blend) was poured slowly into the mixer and the final mixture was blended together for 15 minutes at medium speed to form a homogenous powder. The mixture was passed through a laboratory steam-

compression pelleting mill (California Pellet Mill, San Francisco, CA). The newly formed pellets, which measured 5 mm in diameter, were then dried in a forced-air drying oven at 70 – 80°C for 90 minutes before being packaged. Feed was stored in a freezer at -20°C until required.

Proximate composition analysis of the formulated diets was determined at the NRCC Marine Research Station in Ketch Harbor, NS. The moisture, ash, crude protein, and gross energy contents of the diets were determined following the procedure of Tibbetts et al. (2020). Briefly, moisture and ash contents were quantified gravimetrically by drying in an oven at 105°C until constant mass and by incineration in a muffle furnace at 550°C for 18 h respectively. The nitrogen (N) content was then determined by elemental analysis (950°C furnace) using a Leco N analyzer (model FP-528, Leco Corporation, St. Joseph, MI) using ultra-high purity oxygen as the combustion gas and ultra-high purity helium as the carrier gas. Crude protein was calculated as N x 6.25. Gross energy (MJ kg^{-1}) contents were quantified using an isoperibol oxygen bomb calorimeter (model 6400, Parr Instrument Company, Moline, IL). Crude lipid was extracted following the protocol of Tibbetts et al. (2015) using a Soxtec automated system (model 2050, FOSS North America, Eden Prairie, MN) in 33 x 80-mm cellulose extraction thimbles (CT33080, Rose Scientific Ltd., Edmonton, AB) employing petroleum ether at 150°C for 82 min. The final weight of the crude lipid extract was derived gravimetrically after oven-drying at 105°C for 90 min. Carbohydrate content was determined as 100% minus the sum of moisture, ash, crude protein, and crude lipid contents.

2.3 COLLECTION OF FECES AND CALCULATIONS OF APPARENT DIGESTIBILITY COEFFICIENTS

To ensure a constant flow of digesta through the intestinal tract prior to feces collection, pollock were fed to satiation twice daily, once at 10:00 h and again at 17:00 h, in the 2 weeks leading up to the conclusion of the trial. Feces was collected from euthanized pollock (n=18; 3 per tank) on the final day of the trial using the stripping technique (Windell et al. 1978). Briefly, fish were positioned over a plastic collection bag and pressure was applied to the lower portion of the intestine in a cephalic to caudal motion which facilitated the discharge of feces into the collection bag. Feces were kept frozen at -30°C prior to being freeze-dried (n = 18; 3 samples $tank^{-1}$). The chromic oxide content of feed and feces was determined using inductively coupled plasma – optical emission spectroscopy (ICP-OES). Samples were prepared for analysis by first subjecting them to a bromate-phosphoric acid digestion (Williams et al. 1962). To accomplish this, oven-dried and freeze-dried samples of feed and feces respectively were finely ground by mortar and pestle and were accurately weighed into 30 ml porcelain crucibles using an electronic balance. For samples of feed, aliquots of ~1 g were used; however, samples of feces varied in mass due to availability and ranged from 10.8 mg to 66.1 mg with mean mass of 42.0 mg (SD = 18.1 mg). All samples were then ashed at 600°C in a bench-top muffle furnace (Isotemp 550 series model 126, Thermo Fisher Scientific, Waltham, MA) for 1.5 h. Once cool, 3 ml of a solution containing 10% w/v manganese (II) sulfate tetrahydrate ($MnSO_4 \cdot 4H_2O$) in water and 85% phosphoric acid (H_3PO_4) in a 3:100 ratio was added to the contents of the crucible. Following this, 4 ml of a 4.5% potassium bromate ($KBrO_3$) solution in water was added and a watch glass was

positioned on top of the crucible before being placed on a previously heated hot plate. The solution was brought to a boil and digestion proceeded until a purple colour was observed. Finally, the sample was allowed to cool before the contents of the crucible were quantitatively transferred into a clean 200 ml volumetric flask and made up to the mark with distilled water. The resulting solution was then left to stand overnight before a 10 ml aliquot was extracted and analysed directly on a Thermo Scientific™ iCap7400 dual view ICP-OES unit (Thermo Fisher Scientific, Waltham, MA). Samples were read on an axial view at a wavelength of 267.716 nm. The chromium content was recorded in mg Kg^{-1}, and the corresponding mass of Cr_2O_3 was derived stoichiometrically. Subsequently, an apparent digestibility coefficient (ADC) could be derived for each FA of interest using the preestablished Cr_2O_3 values:

$$\mathrm{ADC} = 100 - 100 * \frac{\%\mathrm{Cr_2O_3 in\ feed}}{\%\mathrm{Cr_2O_3\ in\ feces}} * \frac{a}{b} \quad (1)$$

where "*a*" is the amount of a given FA in the feces and, similarly, "*b*" the amount of the same FA in the feed (Maynard & Loosli 1969).

2.4 SAMPLE PREPARATION

Whole fish were removed from the freezer and allowed to partially thaw. The entire liver was removed and weighed prior to analysis and a sample of muscle tissue (~1.5 g) was extracted from the dorsal area so that the muscle FA profile could be determined. The rest of the body, which included the remaining carcass without liver and muscle tissue sample, was homogenized in a bench top blender (Total Blender

Classic, Blendtec, Orem, UT). The liver and muscle tissue samples being too small for the blender, were finely minced using a scalpel to produce a fine homogenate. Once prepared, each sample was subject to a modified Folch extraction (Budge et al. 2006, Folch et al. 1957). Briefly, 1.5 g of homogenized fish tissues was immersed in a 2:1 chloroform ($CHCl_3$) to methanol (CH_3OH) solution, vigorously shaken, and refrigerated for a minimum of 1 h. Samples of feed and freeze-dried feces were prepared in a similar manner but were first ground by mortar and pestle. An internal standard of tricosanoic acid (23:0) with known mass was added to facilitate the quantification of FA in samples of tissue, feces, and diet.

Table 2.2 Amount of internal standard used, depending upon tissue type.

Sample Type	Mass of internal standard added (μg)[1]
Liver	1586.0
Muscle	572.0
Rest-of-body[2]	572.0
Feces	171.6
Diet	597.6

[1] internal standard used was tricosanoic acid (23:0; Nu-Chek Prep, Inc., Elysian, MN)

[2] Rest-of-body = Whole body – Liver – Muscle

Following this, samples were filtered and washed several times with 2:1 $CHCl_3$/ CH_3OH to bring total volume of 2:1 to 33 ml. Next, a 0.88% salt solution was added to each tube to bring total water content to 8.25 ml; tubes were then vortexed, followed by centrifugation. The resulting solution effectively contained two phases: an organic layer containing mostly $CHCl_3$ and an aqueous layer which consisted predominantly of

water and CH_3OH. The organic layer, containing the lipid, was carefully separated from the aqueous layer, filtered, and washed several times with $CHCl_3$. Finally, the solvent was evaporated under nitrogen and lipid mass was determined gravimetrically.

In preparation for gas chromatography (GC), fatty acids were transesterified to their equivalent fatty acid methyl esters (FAMEs) using the Hilditch method and employing an acidic catalyst (Budge et al. 2006). Briefly, a lipid sample of 100 mg or less was mixed with 1.5 ml dichloromethane (CH_2Cl_2) with 0.01% butylated hydroxytoluene (BHT) and 3.0 ml of Hilditch reagent (0.015% concentrated sulfuric acid (H_2SO_4) in CH_3OH). For samples containing greater than 100 mg of lipid, excess lipid was stored at -20°C in CH_2Cl_2 with 0.01% BHT under a nitrogen atmosphere. Tubes were flushed with nitrogen and vortexed before being heated for 1 h at 100°C. Tubes were then allowed to cool to room temperature and FAMEs were isolated following a hexane extraction. Samples were prepared for GC analysis at 10 mg ml^{-1} FAME in hexane and residual FAME was stored in hexane under a nitrogen atmosphere at -20°C.

2.5 GAS CHROMATOGRAPHY ANALYSIS

Fatty acid methyl esters were analysed using a Bruker 436 capillary gas chromatography (GC) unit (Bruker Corporation, Billerica, MA) equipped with a flame ionization detector (FID) and a polar column with a stationary phase of 50% cyanopropyl polysiloxane with dimensions 30 m in length with an inner diameter of 0.25 mm and 0.25 µm film thickness (J&W DB-23, Agilent Technologies, Santa Clara, CA). The carrier gas was helium with a column flow rate of 0.8 ml min^{-1}. A split injection mode was used with a split ratio of 1:100 and the injection volume was 1 µL. The initial oven

temperature was set to 150°C for 2 min and followed by a temperature ramp of 8°C min^{-1} until a final temperature of 220°C was achieved. The temperature was held at 220°C for 6.25 min which resulted in a total run time of 17 min. The temperature of the detector was 270°C and the flow of combustion gasses, hydrogen and air, was 30 and 300 ml min^{-1} respectively. The make-up gas was argon which had a flow rate of 50 ml min^{-1}. Compass CDS software (Bruker Corporation, Billerica, MA) was employed to determine peak identities. Samples were compared against a reference chromatogram of menhaden oil to confirm successful peak identification and peak area was manually integrated as required.

2.6 FATTY ACID MASS BALANCE

Final fatty acid body content - Initial fatty acid body content =		Fatty acid intake - Fatty acid excretion =	
Fatty acid accumulation	-	Fatty acid net intake	= Fatty acid appearance/ disappearance

Figure 2.1 Visual representation of the first step of the mass balance method, which involves the calculation of fatty acid accumulation and fatty acid net intake.

The FA proportions of tissues, feed, and feces determined by chromatography were normalized to 100% and FA with proportions less than 0.1% were eliminated from the data. The fatty acid mass balance method (Turchini et al. 2007) was employed to estimate the incorporation of EPA and DHA from diet to tissue (Figure 2.1).

Table 2.3 Values derived from each step of the mass balance method and EPA net growth efficiency in tank 1.

Tank #	$\gamma_{controlsEPA}$	$mass_{controls}$	$mass_{initial}$	$\gamma_{initialEPA}$	$\gamma_{finalEPA}$	α_{EPA}
	(g)	(g)	(g)	(g)	(g)	(g)
1	6.6	4167.4	3808.0	6.0	36.0	30.0
Tank #	i_{EPA}	ε_{EPA}	i_{NETEPA}	δ_{EPA}	NGE_{EPA}	
	(g)	(g)	(g)	(g)	(%)	
1	51.9	1.0	50.9	-20.9	58.9	

The final fatty acid concentration (mg g^{-1}) of the whole body of individual Atlantic pollock was elucidated by calculating the sum of EPA and DHA in all tissues (i.e. liver + muscle + rest of body; mg) and dividing by the respective mass of tissue extracted. For control fish (n=12), the sum of whole-body EPA and DHA of all individuals was divided by the sum of whole-body mass of all individuals. From this, the pre-trial EPA and DHA content of experimental fish was estimated in mg g^{-1} by assuming that the FA concentration of these fish was equivalent to that of the control fish and then multiplying the initial whole-body mass of the experimental fish by the mean proportion of that FA in the control fish. The final EPA and DHA content (mg) for each tank was resolved by simply calculating the EPA and DHA content of each individual in the tank and then summing for all fish in the tank (mg). For each experimental tank, accumulation of a given FA was derived by subtracting the initial mass of that FA from the final mass. In effect, accumulation (α) of a given FA within a tank was defined as:

$$\alpha_{FA} = \gamma_{final,\,FA} - \gamma_{initial,FA} \quad (2)$$

where γ_{final} is the final FA mass (g) in experimental fish per tank and $\gamma_{initial}$ is the initial FA mass per tank (g). Further, $\gamma_{initial}$ was defined as:

$$\gamma_{initial,FA} = \frac{\gamma_{controls,FA}}{mass_{controls}} * mass_{initial} \quad (3)$$

where $\gamma_{control}$ is the total mass of a given FA in all control fish (g), $mass_{controls}$ is the total mass of the same FA in all control fish (g), and $mass_{initial}$ is the total initial mass of experimental fish per tank (g). If we consider tank 1, $\gamma_{finalEPA}$ was 36 g, $\gamma_{controlEPA}$ was 6.6 g, $mass_{controls}$ was 4167.4 g, and $mass_{initial}$ was 3808.0 g. Hence,

$$\gamma_{initialEPA} = \frac{6.6\ g}{4167.4\ g} * 3808.0\ g = 6.0\ g \quad (3)$$

and:

$$\alpha_{EPA} = 36.0\ g - 6.0\ g = 30.0\ g \quad (2)$$

Net intake (i_{NET}; g) of a FA was established by subtracting excretion of that FA from intake. Intake was determined by multiplying the total feed intake of a tank (g) by the concentration of the FA of interest (mg g^{-1}) in the feed. Net intake was quantified using the following equation:

$$i_{NET,FA} = i_{FA} - \varepsilon_{FA} \quad (4)$$

where i is total intake (g) of a given FA per tank, and ε is the excretion of that FA (g). Fatty acid excretion was quantified using the following equation:

$$\varepsilon_{FA} = i_{FA} * \left(\frac{100 - ADC_{FA}}{100}\right) \quad (5)$$

where *ADC* is the apparent digestibility coefficient for a given FA such that 100 – *ADC* is equivalent to the "coefficient of indigestibility". Therefore, continuing with the above example:

$$\varepsilon_{EPA} = 51.9\ g * (\frac{100 - 98.14}{100}) = 1.0\ g \quad (5)$$

and:

$$i_{NET_{EPA}} = 51.9\ g - 1.0\ g = 50.9\ g \quad (4)$$

Ultimately, the appearance/disappearance of a FA was calculated as the result of the accumulation of that FA minus its net intake. The disappearance of FA typically corresponds to catabolic processes for energy production or conversion of FA into longer-chain products through elongation/desaturation events. Similarly, the appearance of FA can be attributed to the endogenous production of FA from shorter-chain reactants. Appearance or disappearance (δ) of a given FA could be quantified as such:

$$\delta = \alpha - i_{NET} \quad (6)$$

thus, with the example above we get:

$$\delta_{EPA} = 30.0 - 50.9 = -20.9 \quad (6)$$

Net growth efficiency (*NGE;* %) was calculated as the ratio of FA accumulation to FA net intake:

$$NGE = \left(\frac{\alpha}{i_{NET}}\right) * 100\% \quad (7)$$

and to conclude with the example for tank 1:

$$NGE_{EPA} = \left(\frac{30.0\ g}{50.9\ g}\right) * 100\% = 58.9\% \quad (7)$$

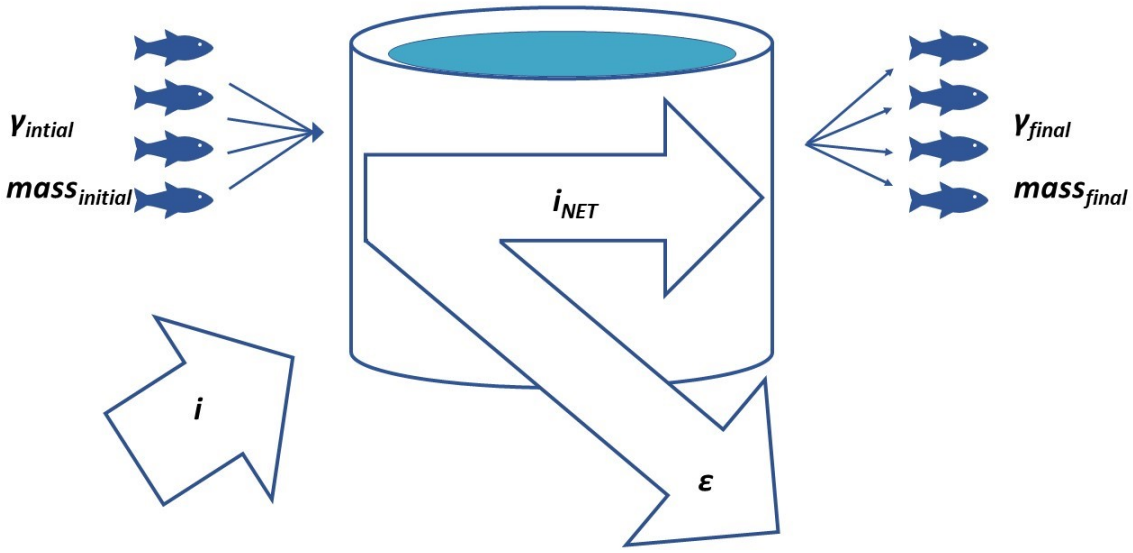


Figure 2.2 Visual representation of the variables involved in the calculation of net growth efficiency through the application of the mass balance method.

2.7 STATISTICAL ANALYSIS

Fatty acid proportions of tissue and diet were normalized to 100% and those with mean proportions less than 0.1% were eliminated. In total, 45 FA remained. Proportions were then renormalized, and FA were converted to mg g^{-1} using an internal standard of tricosanoic acid with known mass. All data were subject to a Shapiro-Wilks test for normality and a Levene's test for heteroskedasticity. One-way analysis of variance, (ANOVA; SPSS version 25, IBM Corporation, Armonk, NY) was used to determine differences in FA concentration and NGE between dietary treatments.

Individuals were grouped by tank such that growth characteristics (mass, length, feed conversion ratio, etc.), lipid proportions, and NGE were presented as mean per tank ± SD (n = 3 for each dietary treatment). Control fish were analyzed on an individual basis. All graphical representation was done using Microsoft Excel.

3.0 RESULTS

3.1 EXPERIMENAL DIETS

Proximate composition analysis (Table 3.1) revealed a high degree of consistency between diets. Crude protein, crude lipid, and carbohydrate contributed approximately 48%, 11%, and 26% of total diet respectively.

Table 3.1 Proximate composition of experimental diets. Values (mean [SD]) were calculated on an as-fed basis (n=3).

Proximate composition	**Fish oil diet**	**Fish oil / Canola oil diet**
Moisture (%)	6.5 (0.06)	6.9 (0.18)
Ash (%)	8.7 (0.10)	8.3 (0.30)
Crude protein (%)	48.0 (0.41)	47.9 (0.21)
Crude lipid (%)	10.9 (0.08)	10.8 (0.06)
Carbohydrate (%)	26.1 (0.38)	26.1 (0.42)
Gross energy (MJ kg^{-1})	20.0 (0.05)	20.0 (0.04)

[1] (100 – [Moisture + Ash + Crude protein + Crude lipid])

Mean EPA concentration was nearly twice as high in FO diets (mean = 9.9 mg g^{-1}, SD = 1.0 mg g^{-1}) compared to FOCO diets (mean = 5.3 mg g^{-1}, SD = 0.7 mg g^{-1}; Figure 3.1). Likewise, mean DHA proportion was 1.7x greater in FO diets (mean = 7.7 mg g^{-1}, SD = 0.7 mg g^{-1}) compared to FOCO diets (mean = 4.5 mg g^{-1}, SD = 0.5 mg g^{-1}). Other FA that showed notable differences between diets include oleic acid (18:1n-9) and linoleic acid (18:2n-6) which both had greater proportions in the FOCO diet. The quantity of 18:1n-9 was approximately fourfold greater in FOCO diets (mean = 37.7 mg g^{-1}, SD = 3.5 mg g^{-1}) relative to FO diets (mean = 9.3 mg g^{-1}, SD = 0.8 mg g^{-1}). Similarly, 18:2n-6 was approximately twice as abundant in FOCO diets (mean = 20.2 mg g^{-1}, SD = 2.0 mg g^{-1})

compared to FO diets (mean = 10.4 mg g^{-1}, SD = 0.9 mg g^{-1}). The most abundant FA in the FO diet was 16:0 (Figure 3.1; mean = 20.8 mg g^{-1}, SD = 2.0 mg g^{-1}) while 18:1n-9 was the most abundant FA in FOCO diets.

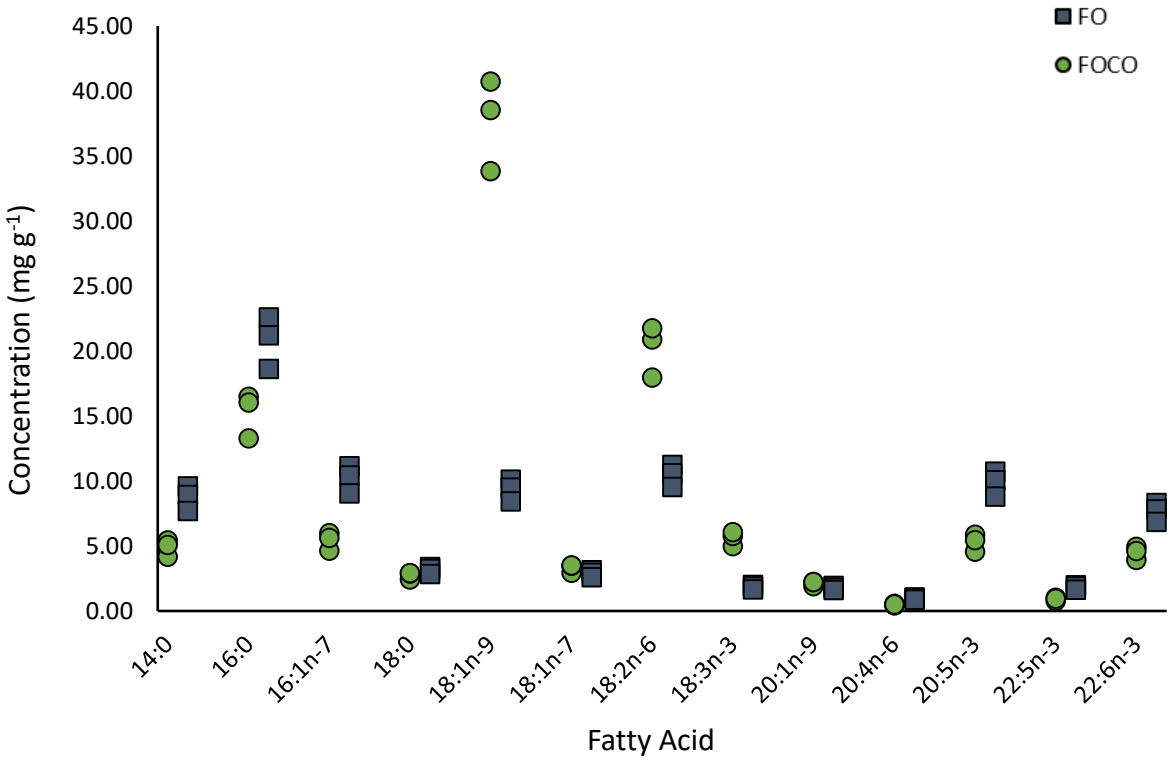


Figure 3.1 Concentration (mg g^{-1} dry mass) of select FA with greatest representation in diet (n = 3 for both diets).

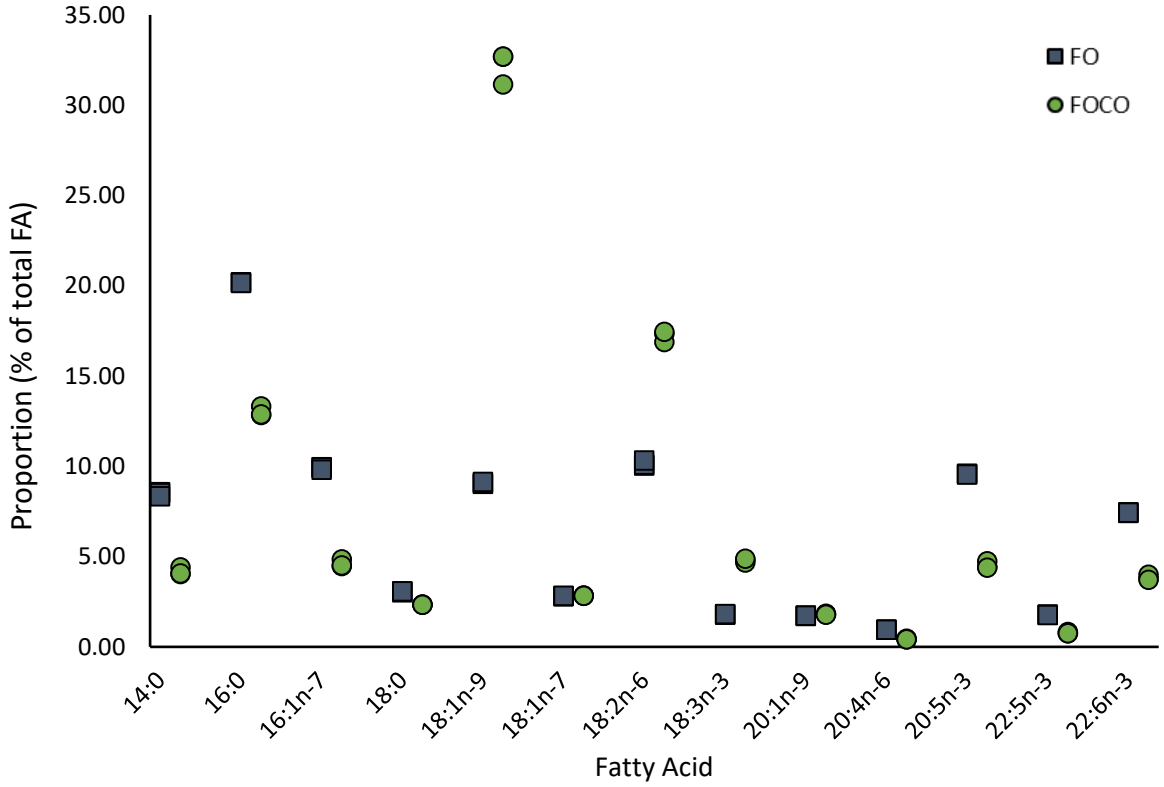


Figure 3.2 Proportions (% of total FA) of select FA with greatest representation in diet (n = 3 for both diets).

3.2 EXPERIMENTAL FISH

A higher degree of mortality was observed in FOCO tanks compared to FO tanks leading to a discrepancy in final sample sizes ($F_{1,4}$ = 16.200, p = 0.016; Table 3.2). Initial fish mass did not differ between treatment fish (FO and FOCO) and the controls ($F_{1, 16}$ = 0.940, p = 0.347; Table 3). All fish gained significant mass over the course of the 83-day feeding trial (t_5 = -11.7, p < 0.001) and mass gain was not significantly different across treatments ($F_{1,4}$ = 5.299, p = 0.083).

Table 3.2 Experimental tanks and their corresponding treatment and sample size at the end of the experiment. FO = fish oil diet, FOCO = fish oil/canola oil diet. A sample of 12 fish were removed at the start of the trial to serve as controls.

Tank #	Dietary Treatment	N
1	**FO**	**8**
2	**FOCO**	**4**
3	**FO**	**6**
4	**FOCO**	**4**
5	**FO**	**8**
6	**FOCO**	**5**

Fish in both dietary conditions showed similar initial lengths and no apparent difference in length existed among dietary conditions and controls ($F_{1,16} = 0.923$, $p = 0.351$; Table 3.3). Mean initial lengths ranged from 29.8 cm (SD = 5.7 cm; tank 2) to 36.1 cm (SD = 3.1 cm; tank 4). Increases in length were approximately 14% in FO tanks and 10% in FOCO tanks. Treatments were congruent in respect to change in length: $F_{1, 4} = 0.664$, $p = 0.461$.

Table 3.3 Growth characteristics of Atlantic pollock. Values for experimental fish correspond to mean (SE) unless stated otherwise; n = 3 for each treatment. Values for controls correspond to mean (SD); n = 12.

	FO	FOCO	Controls
Initial mass (g)	403.0 (39.4)	399.9 (54.7)	347.3 (125.2)
Final mass (g)	778.1 (50.5)	680.2 (71.8)	-
Mass gain (g)	375.2 (34.2)	280.3 (23.0)	-
Initial length (cm)	32.9 (1.3)	32.6 (1.8)	30.9 (4.3)
Final length (cm)	37.4 (0.7)	36.0 (1.5)	-
Change in length (cm)	4.5 (1.1)	3.4 (0.4)	-
$K_{initial}$ (g cm^{-3})[a]	1.14 (0.1)	1.14 (0.1)	1.14 (0.2)
K_{final} (g cm^{-3})[a]	1.45 (>0.01)	1.39 (0.1)	-
Apparent feed intake (g)[b]	651.29 (84.2)	604.98 (96.1)	-
FCR (g g^{-1})[c]	1.74 (0.1)*	2.16 (0.1)[†]	-
SGR (% day^{-1})[d]	0.80 (0.2)	0.65 (0.1)	-
Liver mass (g)	83.0 (9.8)*	57.1 (4.3)*	8.9 (4.7)[†]
HSI (g g^{-1})[e]	10.3 (0.4)*	8.0 (0.7)*	2.7 (1.6)[†]

[a] K = somatic condition factor = (body mass (g) / length^3 (cm^3))*100

[b] Apparent feed intake (mean [SD]) = total feed consumed per tank (g) / number of fish in tank

[c] FCR (mean [SD]) = feed conversion ratio = total feed consumed per tank (g) / total mass gain per tank (g)

[d] SGR (mean [SD]) = specific growth ratio = 100*[ln (final body weight)– ln (initial body weight)]/d

[e] HSI = hepatosomatic index = (liver mass (g) / (total fish mass (g))*100

Different superscripts within the same row indicate a significant difference

Initial somatic condition factors ($K_{initial}$) were highly consistent between experimental and control fish and increased in all tanks over the course of the feeding trial ($t_5 = -8.237$, $p < 0.001$). No significant differences in K_{final} were observed across treatments. Mean feed conversion ratios (FCRs) were consistently lower in FO tanks (1.74 g g^{-1}, SE = .05 g g^{-1}) compared to FOCO tanks (2.16 g g^{-1}, SE = .05 g g^{-1}): $F_{1, 4} = 33.138$, $p = 0.005$. On the contrary, mean SGR was the same across treatments ($F_{1,4} = 2.481$, $p = 0.190$). Apparent feed intake did not differ between treatments ($F_{1,4} = 0.394$, $p = 0.564$). Liver mass and hepatosomatic index HSI increased substantially over the duration of the feeding trial as evident when comparing treatment individuals to controls ($F_{1, 16} = 123.659$, $p < 0.001$ and $F_{1, 16} = 62.369$, $p < 0.001$ for liver mass and HSI respectively; Table 3.3). No difference was observed with respect to liver mass among treatments ($F_{1, 4} = 5.996$, $p = 0.072$). Hepatosomatic indices did not appear to vary with treatment ($F_{1,4} = 6.459$, $p = 0.064$).

3.3 LIPID CONTENT OF WHOLE FISH AND SELECT TISSUES

The total proportion of lipid in experimental Atlantic pollock increased greatly over the course of an 83-day feeding trial ($F_{1, 16} = 63.095$, $p < 0.001$; Table 3.4). Mean total lipid was four- and three-fold higher in FO and FOCO tanks respectively, compared to controls. Liver lipid content was the greatest contributor to total fish lipid, and, at approximately 58% lipid (SE = 0.6% and 1.7% for FO and FOCO groups respectively), was highly consistent among all tanks (Table 3.4). Lipid content did not vary with treatment in respect to muscle or rest-of-body samples. Muscle lipid was consistently less than 1% of total tissue mass and hence its contribution to total fish lipid was minimal. Likewise,

lipid contributed approximately 1% to rest-of-body mass further indicating that the majority of fish lipid originated from the liver. Similar to individual tissues, total lipid content of Atlantic pollock did not vary between treatments.

Table 3.4 Proportion of lipid (wet weight basis; mean [SE]) in select tissues from Atlantic pollock maintained on experimental diets.

	FO Tanks	FOCO Tanks	Controls[a]
Liver (%)	57.7 (0.6)*	57.4 (0.8)*	33.5 (14.8)†
Muscle (%)	0.6 (>0.01)*	0.8 (0.2)*	0.5 (0.1)†
Rest-of-body (%)[b]	1.0 (0.1)*	1.1 (0.1)*	0.7 (0.1)†
Whole fish (%)	6.9 (0.6)*	5.6 (0.4)*	1.8 (1.2)†

[a]values for controls are mean ± SD

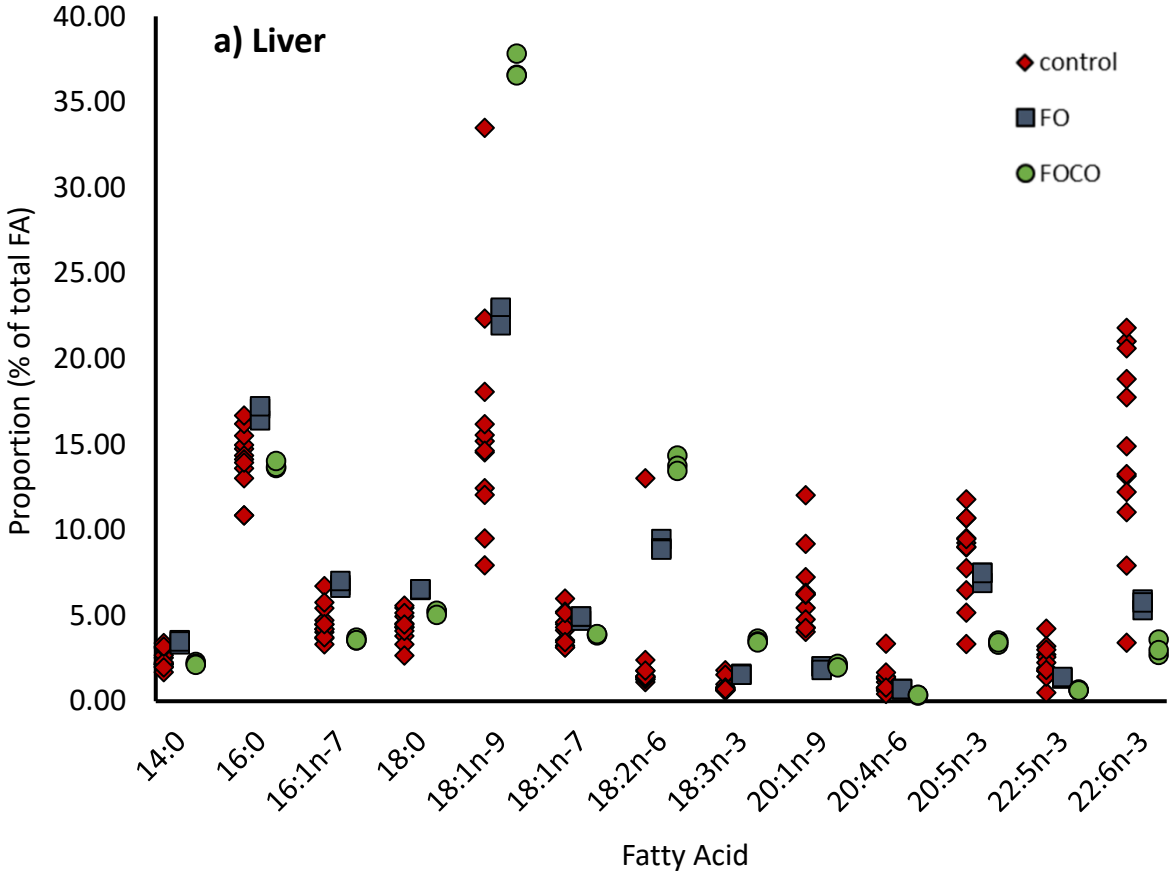
[b]rest of body = total fish mass – liver – muscle tissue sample

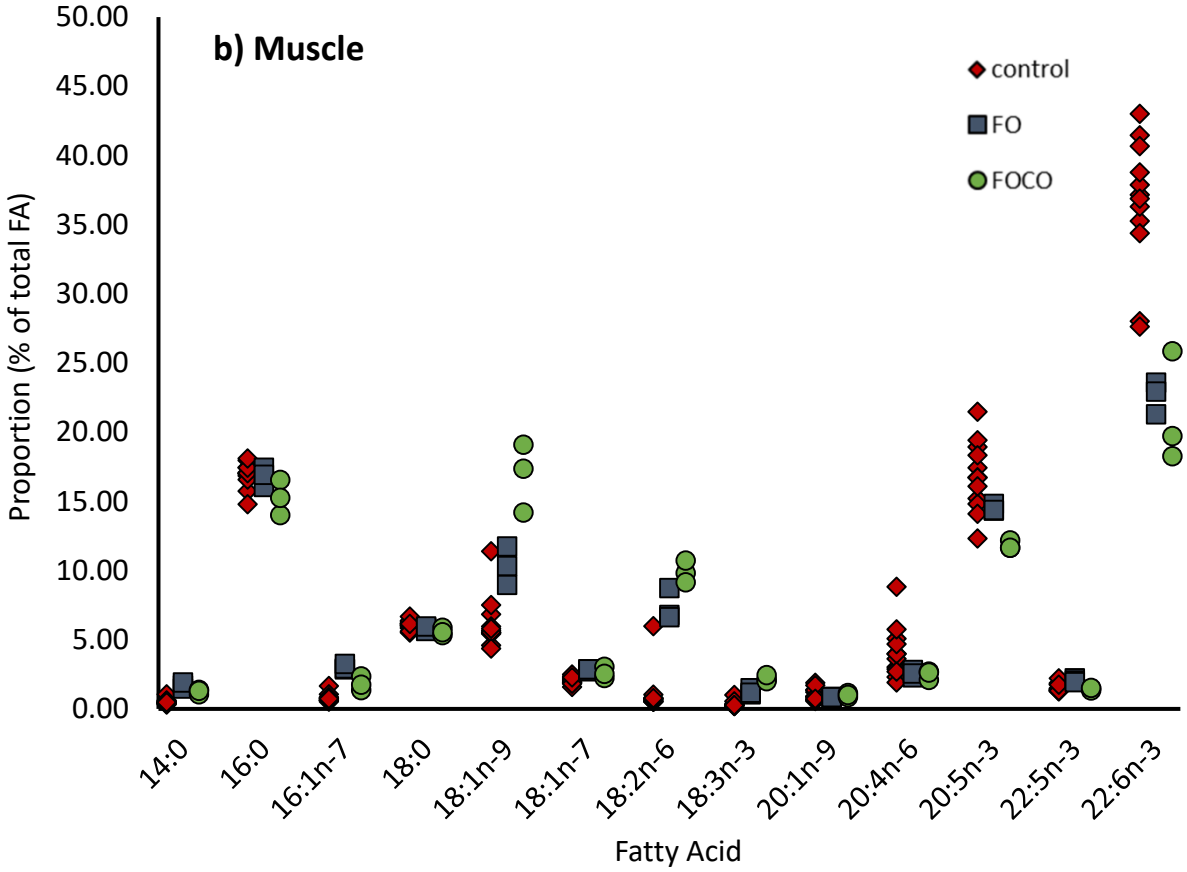
Different subscripts within the same row indicate a significant difference

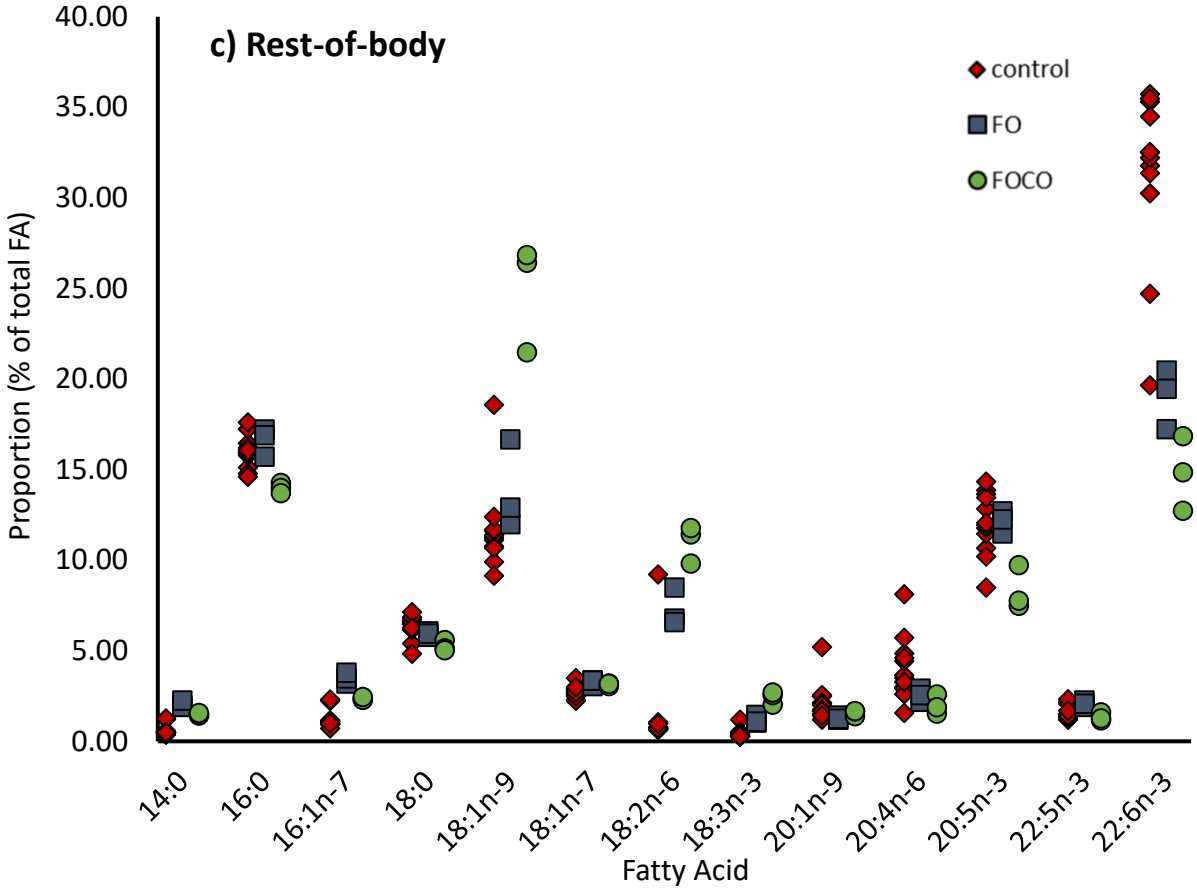
3.4 FATTY ACID COMPOSITION OF SELECT TISSUES

The general distribution of FAs across tissue types was consistent with approximately 13 FA contributing to over 87% of total FA (Figure 3.3). In all tissues, the majority of FA changed in proportion to reflect that of the diet. For instance, tanks fed the FOCO diet, which was characterized by a predominance of 18:1n-9, consistently showed greater proportions of that FA in tissue. This effect was most prominent in liver tissue where mean proportions of 18:1n-9 in FOCO tanks were approximately 14% greater than FO tanks ($F_{1, 4} = 731.355$, $p < .001$) and 21% greater than controls ($F_{1,13} = 27.961$, $p < 0.001$).

In muscle tissue, DHA was the most abundant FA and contributed to over 36% (SE = 1.37%) of total FA in controls, while more modest proportions were seen in FO and FOCO tanks ($F_{1,16} = 47.056$, $p < 0.001$). No difference was observed with respect to DHA content in muscle tissue between dietary groups despite receiving different concentrations in their respective diets ($F_{1,4} = 0.300$, $p = 0.613$). The proportion of DHA in rest-of-body followed a similar trend to that observed in muscle ($F_{1,4} = 7.726$, $p = 0.050$); however, significantly greater proportions were observed in the liver and total lipid of FOCO tanks compared to FO tanks with the concentration of DHA observed to be 1.8x and 1.4x greater in FOCO tanks for these tissue groups respectively ($F_{1,4} = 64.528$, $p = 0.001$, and $F_{1,4} = 12.893$, $p = 0.023$). The proportion of EPA in liver tissue of FO tanks was consistent with that of the controls ($F_{1,13} = 0.693$, $p = 0.420$) but was more than double that observed in FOCO tanks and thus similar to dietary proportions. Although not as prominent as in liver tissue, the mean proportion of EPA was significantly greater in FO tanks compared to FOCO tanks for all tissue groups ($F_{1,4} = 133.289$, $p < 0.001$, $F_{1,4} = 23.165$, $p = 0.009$, and $F_{1,4} = 243.072$, $p < 0.001$ for muscle, rest-of-body, and whole fish respectively).


a) Liver
control
FO
FOCO
Proportion (% of total FA)
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
14:0
16:0
16:1n-7
18:0
18:1n-9
18:1n-7
18:2n-6
18:3n-3
20:1n-9
20:4n-6
20:5n-3
22:5n-3
22:6n-3
Fatty Acid


b) Muscle
control
FO
FOCO
Proportion (% of total FA)
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
14:0
16:0
16:1n-7
18:0
18:1n-9
18:1n-7
18:2n-6
18:3n-3
20:1n-9
20:4n-6
20:5n-3
22:5n-3
22:6n-3
Fatty Acid


c) Rest-of-body
control
FO
FOCO
Proportion (% of total FA)
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
14:0
16:0
16:1n-7
18:0
18:1n-9
18:1n-7
18:2n-6
18:3n-3
20:1n-9
20:4n-6
20:5n-3
22:5n-3
22:6n-3
Fatty Acid

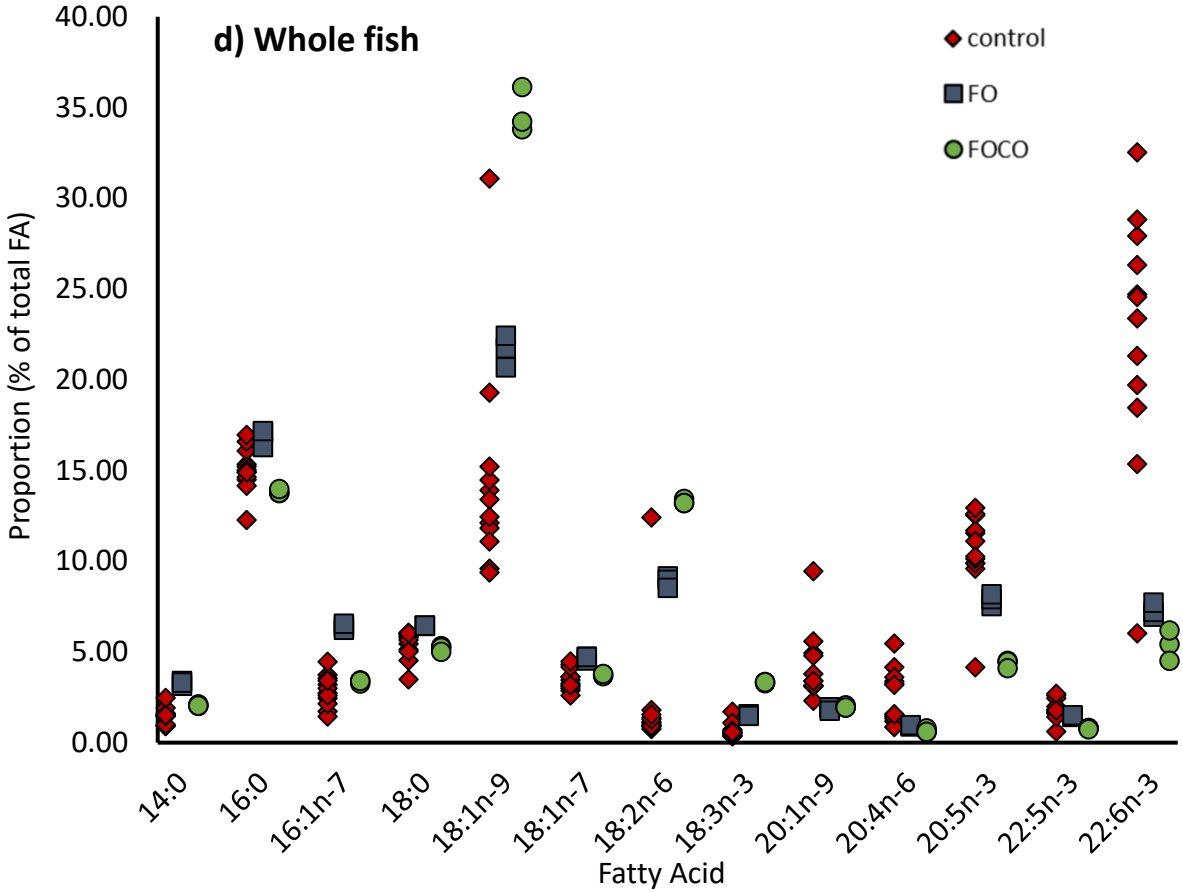


Figure 3.3 Proportions of select FA showing greatest abundance in a) liver, b) muscle, c) rest-of-body*, and d) whole fish. *rest-of-body = whole fish – liver – muscle tissue sample.

3.5 APPARENT DIGESTIBILITY COEFFICIENTS FOR EPA AND DHA

Mean ADCs for EPA and DHA were highly consistent across dietary treatments (Figure 3.4). Apparent digestibility coefficients calculated for EPA were approximately 97% in both treatment groups and 94% and 95% for DHA in FOCO and FO tanks respectively. No significant differences were seen in EPA and DHA ADCs between FO and FOCO treatments; however, EPA was observed to have, on average, a significantly greater ADC compared to DHA as indicated by the paired t-test: $t_5 = 7.836$, $p = 0.001$.

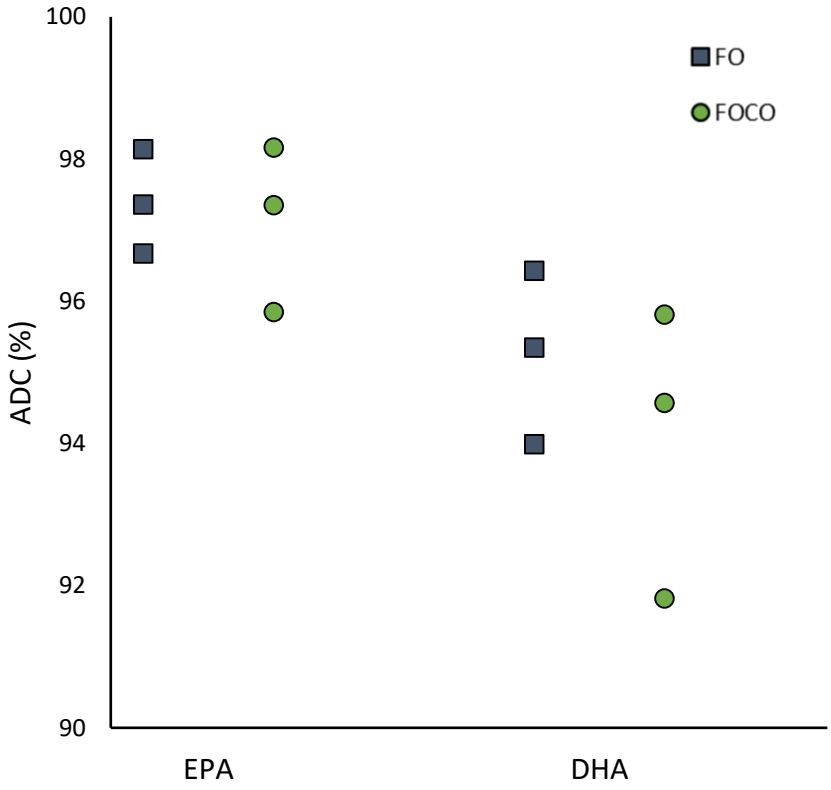


Figure 3.4 Apparent digestibility coefficients (ADCs; %) for EPA and DHA in experimental diets fed to Atlantic pollock (n = 3 for each dietary treatment).

3.6 NET GROWTH EFFICIENCY

Fatty acid accumulation, the first step of the mass balance method, was dramatically higher in FO tanks compared to FOCO tanks (Table 3.5). Mean EPA accumulation was approximately fivefold greater in FO tanks compared to FOCO tanks. Akin to the latter, mean DHA accumulation was more than 6x greater in FO tanks relative to FOCO tanks. Accumulation of both EFAs was greatest in tank 1 which was fed the FO diet (30.02 g and 20.13 g for EPA and DHA, respectively), while the lowest accumulation was found in tank 2 which was fed the FOCO diet (3.31 g and 1.66 g for

EPA and DHA, respectively), resulting in a nearly ten-fold difference in accumulation of these FA. Accumulation of EPA was significantly greater than DHA in FO tanks (t_2 = 7.466, p = 0.017) but not in FOCO tanks (t_2 = 3.780, p = 0.063). Net intake of EPA was always greater than that of DHA regardless of treatment (t_2 = 18.321, p = 0.003 and t_2 = 7.509, p = 0.017 for FO and FOCO tanks respectively) which remains consistent with the relative dietary proportions of these FA. Net intake of EPA was approximately 32% greater than that of DHA in FO tanks (t_2 = 18.321, p = 0.003) and 22% greater in FOCO tanks (t_2 = 7.509, p = 0.017). Net intake of both FA was greatest in tank 1, fed the FO diet, and, likewise, the intake of these FA was the lowest in tank 2 which was fed the FOCO diet. The disappearance of EPA was greater than that of DHA in FO tanks (t_2 = -6.680, p = 0.022) but no difference in disappearance was observed in FOCO tanks for these FA (t_2 = -1.175, p = 0.361). The disappearance of EPA and DHA were both greater in FO tanks compared to FOCO tanks ($F_{1,4}$ = 32.824, p = 0.005, and $F_{1,4}$ = 13.045, p = 0.023 respectively).

Table 3.5 Fatty acid mass balance data for EPA and DHA in Atlantic pollock fed experimental diets. Values for each treatment correspond to mean ± SE; n = 3.

		Tank 1 (FO)	Tank 2 (FOCO)	Tank 3 (FO)	Tank 4 (FOCO)	Tank 5 (FO)	Tank 6 (FOCO)	FO Tanks	FOCO Tanks
Accumulation[1]	EPA	30.02	3.31	25.95	4.75	22.60	6.15	26.19 (2.15)	4.74 (0.82)
	DHA	20.13	1.66	19.53	3.56	15.38	3.27	18.35 (1.49)	2.83 (0.59)
Net intake[2]	EPA	50.92	10.23	42.20	13.77	43.04	16.67	45.39 (2.78)	13.56 (1.86)
	DHA	38.86	8.43	32.10	11.18	32.51	13.79	34.49 (2.19)	11.13 (1.55)
Appearance / disappearance[3]	EPA	-20.90	-6.92	-16.25	-9.02	-20.45	-10.52	-19.20 (1.48)	-8.82 (1.04)
	DHA	-18.73	-6.76	-12.56	-7.62	-17.12	-10.52	-16.14 (1.85)	-8.30 (1.14)
Net growth efficiency[4]	EPA	58.96	32.34	61.50	34.49	52.49	36.90	57.65 (2.68)	34.58 (1.32)
	DHA	51.80	19.72	60.86	31.87	47.33	23.74	53.33 (3.98)	25.11 (3.57)

[1]FA accumulation (g) = sum of FA per $tank_{final}$ (g) – sum of FA per $tank_{initial}$ (g). The sum of a given FA per $tank_{initial}$ was calculated by multiplying the sum of initial fish mass in that tank by the ratio of the sum of the same FA mass in control fish divided by the sum of control fish mass.

[2]FA net intake (g) = FA intake (g) – FA excretion (g). FA intake is the total mass of a given FA consumed by a tank. FA excretion is FA intake multiplied by the "coefficient of indigestion" (i.e. 100 – ADC)

[3]FA appearance/disappearance (mg) = FA accumulation (g) – FA net intake (g)

[4]Net growth efficiency (%) = (FA accumulation (g) / FA net intake (g)) * 100

In general, EPA was incorporated into tissue more efficiently than DHA regardless of the dietary proportions of these FA ($t_5 = 3.280$, $p = 0.022$). The greatest differences in NGE for EPA and DHA were observed in tanks 2 and 6; the NGE of EPA was approximately 13% greater than DHA in these tanks. On average, FO tanks incorporated EPA 23% more efficiently compared to FOCO tanks: $F_{1, 4} = 59.625$, $p = 0.002$. This trend was more apparent for DHA in which FO tanks showed NGE values that were 28% greater than those observed in FOCO tanks: $F_{1, 4} = 59.625$, $p = 0.002$.

4.0 DISCUSSION

4.1 NET GROWTH EFFICIENCY

4.1.1 METHODOLOGICAL CONSIDERATION

The present study is the first of our knowledge to provide estimates for not only the NGE of EPA and DHA, but also for a nutrient in general, in a marine gadoid using a mass balance approach. Our findings revealed that EPA was accumulated more efficiently than DHA despite dietary treatment; however, NGE was always greatest in fish receiving the FO treatment compared to the FOCO treatment.

Few methods exist for determining NGE *in vivo* (Brown 2005). Traditional approaches for evaluating the metabolism and deposition of dietary material require the use of respiration chambers (Day et al. 1996, McKenzie et al. 2000, Moran & Manahan 2003) and radiotracers (Xu & Wang 2002, Pouil et al. 2017). Both techniques exhibit a reasonable degree of complexity, can be expensive, and are often impractical under conventional laboratory environments. The benefit of the mass balance approach, as demonstrated here, extends from its inherent simplicity as well as its adaptability to a variety of organisms and laboratory constraints.

The mass balance method has been carried out in a number of studies involving freshwater fish species, namely, Murray cod (Francis et al. 2009), rainbow trout (Senadheera et al. 2012), Atlantic salmon (Hixson et al. 2014), and barramundi (*Lates calcarifer*; Salini et al. 2017), to asses the metabolic fate of dietary FA and to quantify/qualify enzymatic activity. Compared to the aforementioned studies, the

application of the approach in the current study was not concerned with the metabolic pathways of EPA and DHA because Atlantic pollock, being a marine fish, was unlikely to synthesize these EFA endogenously and thus any accumulation within tissue was assumed to be directly of dietary origin (Dalsgaard *et al*. 2003). Hence, the use of the method in the present application resulted in a series of calculations that were considerably simplified.

4.1.2 IMPORTANCE OF NET GROWTH EFFICIENCY IN ECOSYSTEM PRODUCTIVITY

Since it was observed that a decrease in NGE was associated with decreased dietary supply of EPA and DHA, the results of the present study suggest that the transfer of EFA to higher trophic levels would become reduced as EFA production decreases. One effect of climate change is the warming of the oceans and, as seawater temperature increases, primary marine producers such as phytoplankton are predicted to modify their membrane lipid composition through a process known as homeoviscous adaptation. For example, an increase in water temperature by 2.5°C is predicted to translate to a 27.8% decrease in global DHA production (Hixson & Arts 2016). Barring an unprecedented reduction in global greenhouse gas emissions, DHA availability from marine and freshwater fish catches could decrease by more than 60% over the next century (Colombo et al. 2020). It is easy to envision how such a decrease in primary production would have huge cascading effects on higher trophic level consumers and ecosystem productivity as a whole. To add perspective, Budge et al. (2014) estimated that the global production of EPA in diatoms was 240 Mt $year^{-1}$. By performing a crude

calculation where the NGE for EPA derived from FO tanks is applied at each level of a 5-trophic-level food web (i.e. phytoplankton → zooplankton → planktivores → gadoids → humans), one would observe that the remaining primary production of EPA available to humans is approximately 27 Mt year^{-1}. Using the same example but implementing a 28% reduction in primary production (173 Mt year^{-1}) and applying the NGE for EPA derived from FOCO tanks, one would observe that only 2.6 Mt year^{-1} is available to humans. Studies such as the present are important for accurately modelling of the influence of climate change on ecosystem productivity.

Trophic transfer efficiency of nutrients requires ecosystem level measurements and is difficult, if not impossible, to quantify under experimental conditions (Twining et al. 2016 and references therein). A major contrast between net growth efficiency and trophic transfer efficiency is that the latter considers *all* production at a given trophic level and includes material that is unavailable to the next level, such as pupal skin, shed hair, and feathers that is lost from the predator-prey food chain to the saprophage chain (Schroeder 1981). That said, organism level calculations can provide insight into patterns of trophic transfer (Wetzel 2001). For instance, a higher NGE of a given EFA would correspond to a greater trophic transfer potential. Trophic transfer of bulk carbon is often cited as being roughly 10% (Lindeman 1942), which suggests losses of 90% from one trophic level to the next; however, evidence exists that carbon in the form of *n-3* LC-PUFA is likely an exception to this general rule and are transferred at much greater efficiencies (Gladyshev et al. 2010, Gladyshev et al. 2011, Koussoroplis et al. 2011, Sanders et al. 2016). Nutrient quality, specifically the presence of *n-3* LC-PUFA, is highly

indicative of total energy transfer at the autotroph-herbivore interface (De Mott & Müller-Navarra 1997). An increase in the ratio of EPA to carbon was correlated with increases in zooplankton growth and egg production (Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000), signifying that the quality of nutrients in the diet are much more important than the quantity of nutrients when it comes to the efficiency of carbon transfer to higher trophic level consumers. Therefore, the choice of the two experimental diets in the present study provided a suitable contrast to evaluate the influence of nutrient quality on NGE. We provide evidence that the quality of dietary nutrients is strongly related to the efficiency by which they are deposited in tissue.

4.1.3 THE FATE OF DIETARY EPA AND DHA IN ATLANTIC POLLOCK

As predicted for a marine fish, EPA and DHA both showed negative appearance, or rather "disappearance", values indicating dietary intake was greater than accumulation in tissue. Negative values correspond to either conversion to other FA or β-oxidation for energy production (Turchini et al. 2007). The disappearance of EPA could also be associated with its role as a precursor for eicosanoids; however, this is predicted to occur at minimal rates (Sargent *et al*. 1999).

A key assumption of the mass balance application in this study was that biosynthesis of EPA and DHA could not occur. The conversion of C_{18} FA to $C_{20\text{-}22}$ FA involves a series of steps in which 18:3n-3 is converted to 18:4n-3, through the action of a Δ6 desaturase, and is subsequently elongated to produce 20:4n-3. The conversion of 20:4n-3 to EPA and the conversion of EPA to DHA involves the action of a Δ5 and Δ6

desaturase respectively. While it is widely accepted that the Δ5 desaturase is not expressed in marine fish to any significant degree (Tocher & Ghioni, 1999, Zheng et al. 2009a) activity associated with the Δ6 enzyme cannot be completely disregarded. In Atlantic cod, evidence exists for the expression of a Δ6 desaturase gene, particularly in specialized tissues such as the nervous system (Tocher et al. 2006). Similar observations were made in cobia (*Rachycentron canadum*) which also demonstrated high activity in brain tissue (Zheng et al. 2009a). Expression of the Δ6 desaturase in cod was not influenced by changes in the dietary EPA and DHA content, which would suggest that dietary regulation of the gene did not exist in this species; however, contradictory evidence has been observed in other marine species (González-Rovira et al. 2009). In diadromous fish such as Atlantic salmon, conversion of dietary EPA to DHA and, to a lesser extent, retroconversion of DHA to EPA, has been demonstrated *in vivo* when fed diets devoid of one of the two (Thomassen et al. 2012, Bou et al. 2017). That said, an *in vitro* comparison of the Δ6 desaturase gene promoters from Atlantic salmon and Atlantic cod indicated that nutritional regulation of promoter activity occurred in salmon but not in cod (Zheng et al. 2009b).

While the potential capacity for the bioconversion of dietary EPA to DHA is supported by some literature, it is likely that this would only occur when major deficiencies in dietary EFA supply are present and/or during critical developmental periods (Mourente 2003, Morais et al. 2011). Further, bioconversion of EPA to DHA in hepatocytes, the primary storage location of lipid in pollock, is not known to occur in significant amounts (Bell et al. 2006, Almaida-Pagán et al. 2007, Mohd-Yusof et al.

2010). To this end, it is thought that the retention of a functional Δ6 desaturase gene in marine fish may be involved in minor "tuning" of the EPA:DHA ratio, particularly in specialized tissues such as the brain and heart, where there appears to be little tolerance for deviations from homeostatic equilibrium (Bell & Tocher 2009). However, it is important to highlight that these tissues would contribute very little to whole fish lipid in the present study.

More recently, an alternative synthesis route in which EPA is converted to 22:5n-3 and then directly to DHA by the action of a Δ4 desaturase has gained attention (Oboh et al. 2017). This pathway has been described in several marine fish such as rabbitfish (*Siganus canaliculatus*; Li et al. 2010) and Senegalese sole (Morais et al. 2012a). Nevertheless, these are likely exceptional cases as rabbitfish are herbivorous, feeding primarily on benthic algae and seagrasses, and Senegalese sole feeds heavily on polychaetes that contain very little DHA (Li et al. 2010, Morais et al. 2012a). It is doubtful that a similar phenomenon would be observed in Atlantic pollock whose natural diets are primarily comprised of copepods and other fish (Steele 1963, Rangeley & Kramer 1995) and, in the present study, were fed experimental diets with EFA concentrations that met nutritional demands.

Though not as widely characterized as desaturases, elongases, often abbreviated as *elovl*s (ELongation of Very Long chain fatty acids), are crucial in the LC-PUFA biosynthesis pathway (Tocher 2003). *Elovl*s are well described in a similar species, Atlantic cod, and at least 10 members of the *elovl* gene family have been identified (Xue

et al. 2014). The activity of *elovl*s toward substrate of varying chain length is inconsistent between species. For instance, *elovl* activity in Atlantic cod was very low and undetectable for C_{20-22} and C_{22-24} substrate respectively, but gilthead seabream, another marine fish, are very efficient at elongating C_{20-22} substrate, rivalling or surpassing the abilities of Atlantic salmon and catfish for both *n-3* and *n-6* substrates (Agaba et al. 2005). It is expected that Atlantic pollock would follow a typical gadoid trend with respect to *elovl* activity and hence, sufficient elongase activity to derive a quantifiable amount of DHA from dietary EPA is highly doubtful. When taken collectively, there is no evidence to support the bioconversion of dietary C_{18} PUFA to EPA in Atlantic pollock and conversion of EPA to DHA is almost certainly not occurring.

4.1.4 COMPARISON OF NET GROWTH EFFICIENCY TO OTHER FISH

Calow (1977) declared that the theoretical maximum at which poikilothermic organisms are able to convert ingested nutrients into biomass was between 70% and 80%; however, *in vivo* conversion efficiencies are likely significantly lower due to additional energy costs associated with maintaining homeostasis and the efficiency at which dietary nutrients are absorbed during digestion (Anderson et al. 2004). Mean, gross NGE in carnivorous fish was predicted to be 41% (Schroeder 1981 and references therein) suggesting that the NGE of EPA and DHA observed in FO tanks were within range of literature describing NGE of energy in general; however, NGE of EPA and DHA in FOCO tanks was almost twofold lower. Given the role of EPA and DHA as essential nutrients it would seem appropriate that NGE would be greater when dietary supply

was reduced (Stubhaug et al. 2007); albeit, the results observed in the present study would suggest the contrary. Torstensen et al. (2004) described a “retention efficiency” in the white muscle of Atlantic salmon fed diets containing fish oil with olive oil and rapeseed oil at various inclusion levels. Retention efficiency was derived from a ratio of the amount of the nutrient deposited in the fish to the amount of the same nutrient ingested by the fish. Because these values do not account for FA excretion, they are more accurately termed gross growth efficiencies (GGEs) and are likely an underestimation of true NGE (Welch 1968). In the present study, the proportion of consumed EPA and DHA that was lost to excretion ranged from 2% to 4% and from 4% to 8% for these FA respectively; therefore, GGE can serve as a rough approximation for NGE. GGE of EPA and DHA were generally low in Atlantic salmon fed diets incorporating 100% fish oil (5.5% and 12.4% for those FA respectively) but were approximately doubled when fish oil was substituted for 100% canola oil that was devoid of these EFA (12.6% and 22.6% for EPA and DHA respectively; Torstensen et al. 2004). Therefore, unlike the present study, Torstensen et al. observed an inverse relationship between the dietary availability of EPA and DHA and retention efficiency, likely because the salmon were able to synthesize EPA and DHA from shorter-chain precursors (Kjær et al. 2016). Also, retention efficiency of DHA was twice that of EPA, a trend not observed in the present study in which EPA was incorporated into tissue 4% and 9% more efficiently, on average, than DHA in FO and FOCO tanks respectively. Since evidence exists to suggest Atlantic salmon are capable of converting dietary 18:3n-3 to EPA and DHA (Tocher 2010) it is difficult to determine whether the deposition of EPA and DHA in tissue was directly

of dietary origin or rather the transformation product of shorter-chain precursors. Bendiksen & Jobling (2003) took this matter into consideration and evaluated *n-3* FA retention as a whole in salmon. Their observations suggested *n-3* FA retention efficiency was greater when fed diets with lower fat content (i.e. less mass of *n-3* FA) and/or when water temperature was colder; however, no discernable changes in retention efficiency existed when fish oil was replaced with vegetable oil substitutes, which is again contrary to the results observed in the current study. Overall, retention efficiencies in Atlantic salmon were high for *n-3* FA, ranging from 51% to 94% depending upon temperature and diet, and mean retention efficiencies were ca. 10% higher for *n-3* FA compared to *n-6* FA (Bendiksen & Jobling 2003).

Retention efficiencies of Atlantic cod, a closely related species, maintained on experimental diets with increasing dietary lipid levels were evaluated by Hansen et al. (2008). Retention of EPA and DHA increased with increasing dietary lipid levels; however, retention of DHA increased linearly while retention of EPA followed a second-degree polynomial regression and levelled out at high dietary lipid inclusion (Hansen et al. 2008). Retention values of approximately 30% and 60% were observed for EPA and DHA respectively in Atlantic cod fed diets containing 8% lipid and 6.2 g kg^{-1} of EPA and 12.9 g kg^{-1} of DHA. In the present study, pollock fed FO diets containing 11% lipid with 9.9 g kg^{-1} of EPA and 7.7 g kg^{-1} of DHA showed NGEs at 57.7% and 53.3% for EPA and DHA respectively. Hansen et al. (2008) cited the lower retention values for EPA relative to DHA as evidence for a higher oxidation rate of that FA. Our results do not support this claim as we have demonstrated that EPA was incorporated into tissue more efficiently

than DHA. A similar, but more apparent trend, was observed in FOCO diets in which NGEs of 34.6% and 25.1% were shown for EPA and DHA, respectively, and corresponded to dietary concentrations of 5.3 g kg^{-1} and 4.5 g kg^{-1} of EPA and DHA respectively in FOCO diets. Both studies are in agreement with respect to the influence of dietary FA concentrations on growth efficiency as increased dietary FA concentrations appeared to promote increased growth efficiency (Hansen et al. 2008).

4.1.5 COMPARISON OF NET GROWTH EFFICIENCY TO OTHER ORGANISMS AND IMPLICATIONS FOR TROPHIC TRANSFER EFFICIENCY

While estimates of NGE in high trophic level marine consumers are lacking in the literature, some have been described for more basal organisms. GGE was described for EPA and DHA in the calanoid copepod *Calanus finmarchicus* maintained on a cryptophyte diet (*Rhodomonas salina*) and mean values of 10% and 11% were observed for these FA respectively (Helenius et al. 2019). In comparison to net growth efficiencies described in the present study, which were more than 5-fold greater, these observations would suggest that an increase in NGE is associated with an increase in trophic level. This theory would be consistent with results from Kainz et al. (2006) in which accumulation factors for DHA, a ratio of the FA proportion of consumer lipid to the FA proportion of diet, in the diet, were much greater between macrozooplankton and fish than they were between seston and micro- and mesozooplankton. Similar observations have been made for EPA (Hessen & Leu 2006). This indicates a significant loss of EFA early in the trophic system due to inefficient transfer from primary producers to primary consumers. Recent NGE values calculated for EPA and DHA in *C. finmarchicus*

feeding on natural diets of dinoflagellates (*Heterocapsa triquetra*) and diatoms (*Thalassiosira weissflogii*) were more optimistic (Helenius et al. 2020). Helenius et al. (2020) cited GGE values in excess of 60% for DHA, which were much more consistent with the current study. While there is little supporting literature, this could indicate that the capacity for primary consumers to retain EFA is highly variable and influenced by a variety of elements, including prey availability and environmental factors.

4.1.6 EFFECT OF FATTY ACID DIGESTIBILITY ON NET GROWTH EFFICIENCY

Calculated digestibility values of EPA and DHA in the present study were in agreement with those observed for Atlantic cod fed diets containing similar lipid content (Hansen et al. 2008). It can be assumed that dietary FAs in the feeds, with fish oil and canola oil as the major contributors, exist predominantly in the form of TAG with small contributions of PL from fish- and krill meals. TAG and PL are highly digestible in teleost fish, particularly when compared with other lipid classes such as wax esters (Sigurgisladottir et al. 1992, Colombo-Hixson et al. 2011). In general, the digestibility of dietary lipid in fish is in excess of 90% and tends to be similar to that of dietary protein (Zhou et al. 2004, Tibbetts et al. 2004, Tibbetts et al. 2006). In the present study, digestibility of dietary lipid was consistent with literature with values of 91% and 94% observed in FO and FOCO tanks respectively. LC-PUFA, and particularly *n-3* LC-PUFA, are known to be highly digestible and are more efficiently digested than monounsaturates and saturates, with the possible exception of short-chain PUFA (Olsen & Ringo 1997, Hansen et al. 2008). In the present study, ADCs for EPA were approximately 97% in both dietary treatments and were significantly higher than ADCs for DHA, which were

approximately 95% and 94% for FO and FOCO diets respectively. This pattern is consistent with that of Tibbetts et al. (2020) in Atlantic salmon. Apparent digestibility of nutrients, including EPA and DHA, has been shown to be influenced by the presence of terrestrial oils in the diets of Murray cod (*Maccullochella peelii*), Atlantic halibut, and sharpsnout seabream (*Diplodus puntazzo*; Francis et al. 2007, Martins et al. 2007, and Piedecausa et al. 2007, respectively). That said, ADCs for both EPA and DHA did not differ significantly between dietary treatments in the current study suggesting digestibility was not impeded by the presence of canola oil.

The influence of terrestrial oils on digestive physiology in marine fish is not well known. Morais et al. (2012b) provided circumstantial evidence that terrestrial oil substitution in Atlantic cod may impact intestinal cell morphology. They noted discernable changes in the up-regulation of apoptotic-related transcripts as well as the downregulation of important structural proteins which, when taken collectively, suggest increased apoptosis and/or decreased cellular proliferation in intestinal cells (Morais et al. 2012b). This has the potential to impact the mechanical properties and regenerative capacity of the tissue which, in turn, would suggest reduced nutrient digestibility with latent impacts on the NGE of EFA. Bou et al. (2017) reported that some enterocytes were swollen and contained extensive supranuclear vacuolization, as well as a “foamy” cytoplasm, in Atlantic salmon fed EFA-deficient diets. No differences in ADC between dietary treatments were observed in the present study which would suggest that the function of intestinal cellular components was not compromised when fed diets containing canola oil as a partial substitute for fish oil.

4.2 LIPID DEPOSITION IN SELECT TISSUES AND IMPLICATIONS FOR NET GROWTH EFFICIENCY

4.2.1 LIPID DEPOSITION IN LIVER TISSUE

In fish, the extent of lipid storage varies with tissue, with the liver serving as the primary lipid depot in lean fish. As such, liver, muscle, and rest-of-body tissues were analyzed separately in the present study, to evaluate their function in lipid metabolism, particularly with respect to the incorporation of EFA into tissue. We found that livers of experimental pollock in both dietary treatments were high in lipid and represented the majority of whole fish lipid (78% - 89%). The mechanism responsible for this pattern of lipid deposition among tissues in gadoids appears to be a consequence of low plasma concentrations of very low-density lipoproteins (VLDL) which serve as the primary vector to facilitate lipid transport from liver to peripheral tissues (Sheridan 1988, Nanton et al. 2001, Kjaer et al. 2009). Therefore, marine gadoids deposit dietary lipid predominantly in the liver as opposed to the muscle or mesenteric fat, as in herring and mackerel (Ackman 1967; Jensen 1979). For example, high lipid diets fed to Atlantic cod have been shown to induce hypertrophic growth in liver tissue (Kjaer et al. 2009). While excess lipid in the liver can be pathological in species like salmonids (Dessen et al. 2020), gadoids appear to be rather robust towards large fluctuations in liver lipid content (Nanton et al. 2003, Zeng et al. 2010) and the present study was indicative of this. Cod fed diets of either prawn or herring, which were 3.5% and 12% lipid respectively, developed distinct deposition patterns depending upon the lipid content of the diet (dos Santos et al. 1993). Proximate composition of liver tissue revealed lipid proportions of 36% in cod fed prawn and 67% in those fed herring, and HSI values of 4% and 6% were

observed respectively (dos Santos et al. 1993). In contrast, the muscle tissue of fish in both dietary treatments were approximately 1% lipid, which is in agreement with the proportion of lipid in muscle tissue of pollock in our study.

The pattern of lipid deposition in gadoids may offer a particular advantage in determining NGE in these species compared to others. Standard methodology in traditional aquaculture studies usually requires the tripling of the initial mass of experimental organisms. For example, in studies evaluating the effect of alternative oil sources on fish growth performance, concluding the trial too early can result in committing a type II error (Turchini et al. 2013). In contrast, it has been shown that relatively little time is required to observe significant increases in lipid deposition (Budge et al. 2016). This is evident in the present study as the mean proportion of whole fish lipid in experimental Atlantic pollock increased threefold and fourfold for FOCO and FO treatments, respectively, compared to initial fish. In terms of FA mass, this corresponded to a 12-fold and fourfold increase in total EPA mass in the FO and FOCO tanks, respectively, compared to controls. This strongly indicates that a large proportion of the lipid content of pollock tissue would have originated from the experimental diets, while very little would be residual lipid remaining from the acclimation diet or from the wild diets. Hence, a lean fish being fed a “fatty” diet provides a convenient model for studying NGE.

The accretion of dietary lipid in fish is predicted to proceed either by dilution or turnover (Jobling 2003) and this is significant in the context of mass balance. In the case of dilution, existing fatty acid stores become diluted as the fish grows and deposits

increasing amounts of dietary FA while, in contrast, turnover indicates complete replacement of pre-existing FA deposits with those of dietary origin. Dilution is essential from a FA mass balance perspective because deriving estimates of FA accumulation using a mass balance approach requires that new material be deposited on top of pre-existing material. The dilution model has been supported in a number of species including Atlantic salmon (Budge et al. 2011), Murray cod (Turchini et al. 2006), gilthead sea bream (Benedito-Palos et al. 2009), and brown trout and turbot (Robin et al. 2003). Key assumptions of the dilution model are that dietary FA are deposited in tissues without influencing the metabolism of pre-existing FA deposits and the incorporation of dietary FA into tissue occurs independently of the FA composition of pre-existing FA stores (Robin et al. 2003). Traditionally, the application of the dilution model in lean fish, such as Atlantic pollock, which contain PL as the predominant lipid class of the fillet and do not deposit TAG as storage lipids in that tissue, is difficult due to PL being robust towards changes in FA composition following a dietary shift (Turchini et al. 2009). Maximizing nutritional value of the fillet is important in increasing the market value of the fish, therefore, studies in which the dilution model is tested are primarily concerned with fillet FA composition (Regost et al. 2003, Lane et al. 2006, Mørkøre et al. 2007). On the contrary, the present study was concerned with lipid deposition in the whole fish, to which fillet lipid stores, at approximately 1% lipid, contributed very little. We observed that the majority of dietary lipid was deposited in the liver, almost certainly in the form of TAG (Morais et al. 2001). The role of TAG lies primarily in providing a long-term energy source and, as a consequence, the FA structure is not highly conserved and

highly influenced by diet (Trushenski et al. 2008, Weil et al. 2013). Therefore, the validity of the dilution hypothesis is likely supported in the present study.

The FA profile of liver tissue was highly indicative of the dietary treatment and is consistent with similar observations in other gadoids following a change in dietary feed oils (Jobling et al. 2008, Budge et al. 2016). Mean concentrations of EPA and DHA in the liver tissue of FO fish were 2-fold greater than that of FOCO fish and indicative of their respective dietary proportions. The proportions of both EFA were lower in experimental fish liver relative to the controls due to the much higher proportion of C_{18} FA in livers of experimental fish. The similitude between dietary and tissue FA profile here would indicate that selective oxidation or retention of EPA and/or DHA had not occurred (Jobling 2004), thus providing further support for the dilution model and facilitation of accurate NGEs.

4.2.2 PROPORTIONS OF EPA AND DHA IN MUSCLE TISSUE

Muscle tissue lipid of experimental Atlantic pollock contributed less than 1% of tissue mass and was not significantly different than the muscle tissue of control fish. That said, fish were nearly double in mass so we would expect that the mass of lipid deposited in muscle tissue would have increase proportionally. Similar observations occurred in haddock fed increasingly fatty diets (Nanton et al. 2001; Tibbetts et al. 2005). Here, DHA was the most abundant FA in muscle tissue regardless of the dietary proportion. Further, DHA as a proportion of total FA was similar in muscle tissue despite dietary treatment, while, on the contrary, the proportion of EPA in this tissue reflected that of the diet. Likewise, the concentration of EPA and DHA (mg FA g tissue^{-1}; see

supplementary material) followed a similar trend as proportions. DHA, rather than EPA, has been cited in literature as being preferentially retained in the muscle tissue of species such as gilthead seabream (*Sparus aurata*; Menoyo et al. 2004) orange-spotted grouper (*Epinephelus coioides*; Lin et al. 2007), Atlantic cod (Hansen et al. 2008), and Senegalese sole (*Solea senegalensis*; Benítez-Dorta et al. 2013) when fed diets supplemented with plant oils.

DHA has been cited as a major component in neural and optic tissues (Mourente, 2003); however, its role in myocytes has not been well defined. Some evidence is provided in larval Atlantic cod, where the total cross-sectional area of white muscle fibers was 75% greater in fish fed a diet with a high DHA:EPA ratio compared to those that were fed a low DHA:EPA ratio (Galloway et al. 1999); albeit, overall growth performance of the fish was also reduced when fed the latter diet. In general, the role of DHA in biological membranes is complex and its presence is known to influence such physical properties as phase behaviour, fluidity, permeability, fusion, and elastic compressibility (Stillwell & Wassall, 2003, Arts & Kohler, 2009).

Compared to liver tissue in which TAG is the predominant lipid class, muscle tissue in gadoids is rich in PL. The PL content of muscle was shown to be 80 – 90% of total lipid in Atlantic cod (Hixson & Parrish 2014). Preferential incorporation of DHA into PL, especially phosphatidylcholine, the most abundant PL in fish tissue, is well known (Tocher 1995, van der Meeren et al. 2007). Comparison of the muscle tissue of farmed and wild turbot (*Scophthalmus maximus*) showed that DHA was the most abundant FA in muscle PL (ca. 30% of total FA) and was not significantly different between farmed

and wild individuals despite, by definition, receiving different diets (Sérot et al. 1998). In gilthead seabream fed diets in which DHA represented 1 – 7% of total FA, corresponding DHA concentrations in PL were 18 – 30% respectively (Kalogeropoulos et al. 1992). On the other hand, the same dietary concentrations of EPA resulted in only 7 – 11% of total FA in PL (Kalogeropoulos et al. 1992). Although DHA concentration in muscle tissue did not seem to conform to dietary proportions, all other FA appeared to do so. Evidence exists that substantial deposition of dietary FA occurred in this tissue despite being inherently resilient towards dietary alterations.

4.3 GROWTH PERFORMANCE

4.3.1 EFFECT OF DIET ON WEIGHT GAIN, SPECIFIC GROWTH RATES, AND SURVIVORSHIP: IMPLICATIONS FOR NET GROWTH EFFICIENCY

Fish gained 93% and 70% of their initial masses in FO and FOCO tanks respectively and there were no significant differences in mass gain between experimental treatments. However, there did appear to be a trend for lower mass gain when fed the FOCO diets and this is concomitant with trends for decreased liver mass and HSI, well-established condition indices in gadoids (Jensen 1979). Assimilation of LC-PUFA is linked to growth performance in zooplankton communities (De Mott & Müller-Navarra 1997); although, in fish, there is more support for dietary protein, rather than dietary lipid, as an indicator for growth success as long as minimum EFA requirements are met (Morais et al. 2001, Rosenlund et al. 2004, Albrektsen et al. 2006). For example, an increase in growth performance in haddock occurred independent of dietary lipid levels but was strongly associated with the levels of dietary protein (Tibbetts et al.

2005). The EFA requirements of juvenile gadoids are not well defined (Zeng et al. 2010); however, estimates from larval Pacific cod (*Gadus macrocephalus*) suggest DHA concentrations of approximately 1.0 g kg^{-1} (Zheng et al. 1996) and EPA:DHA ratios of 0.8:1 – 1.1:1 (Copeman & Laurel 2010) show good growth performance. This suggests that the nutritional requirements of EPA and DHA were met in our experimental diets and that they were likely richer in EFA than natural diets given that natural diets would be consumed moist while experimental diets were dry. Further, signs of EFA deficiency, such as fin erosion and shock syndrome, were not observed (Castell 1972). While mass gain was not statistically significant between treatments, its influence was apparent in NGE that were.

In addition to mass gain, SGR was also evaluated in experimental fish. In the present study, SGRs for experimental pollock ranged from 0.56% biomass day^{-1} to 0.97% biomass day^{-1} and were not significantly different between treatments. Our values were consistent with those reported for Atlantic cod (Grisdale-Helland et al. 2007, Karlsen et al. 2017) but much lower than those reported for haddock (2%; Perez-Casanova *et al.* 2009). This suggests that high variability of SGRs in gadoids exists and that, in general, SGRs are influenced by a host of environmental and physiological variables including temperature and size of the fish (Jobling 1988). Significant decreases in SGRs have been observed in literature when the nutritional requirements of EFA have been compromised (Peng et al. 2008, Turchini et al. 2013); however, the presence of terrestrial oils in diet was not known to influence SGR when minimum EFA requirements were met (Fountoulaki et al. 2009). SGRs observed in the present study inferred that

fish were in good condition; therefore, it would suggest that reduced NGE in FOCO tanks was not due to an underlying pathology, but rather a physiological inability to compensate for reduced dietary supply.

While evaluation of growth performance parameters yielded conflicting results, survivorship clearly favored FO tanks. Not all fish accepted the experimental diets, but this issue appeared to be exacerbated in FOCO tanks which led to a significant reduction in survivorship in this treatment group. This is contrary to a study in which a similar diet was used in the same species; however, in that study, pollock had a significant period of time to adjust to a FO based diet before receiving a diet with vegetable oil substitution (Budge et al. 2016). One caveat presented in the current study was that it was difficult to subject experimental fish to a long acclimation period to the new diet, for example using a commercial diet, without compromising the integrity of the experiment by inducing significant lipid deposition (Jobling 2008). Acclimation periods are typically required for sustaining wild fish in a captive setting which could be linked to poor survivorship in the present study (Dreyer et al. 2008). Given that a change in mass is a fundamental requirement of the mass balance approach, increasing the magnitude of mass gain results in increased sensitivity of the measurement (Turchini et al. 2007). Therefore, it was advantageous for initial measurements to be performed on a lean fish, which would not have been possible if they were first subject to a high-fat commercial diet for a long period of time. As with SGR, it is unlikely that poor survivorship is linked to illness since there were no obvious sign of pathology in fish that survived.

4.3.2 EFFECT OF DIET ON FEED CONVERSION RATIOS AND STRESS RESPONSE: IMPLICATIONS FOR NET GROWTH EFFICIENCY

FCR for Atlantic pollock in the present study (1.7 g g^{-1} and 2.2 g g^{-1} for FO and FOCO tanks respectively) were greater than those reported in literature (see below) and indicate poor feed utilization. Decreases in FCR were linked to decreases in muscle and liver *n-3* PUFA concentration in grass carp (Cai & Curtis 1990), which would suggest NGE and feed conversion are intrinsically related. The FCR is perhaps the single most important parameter in aquaculture due to its direct implications on production cost (Doupé & Lymbery 2003). Among farmed animals, fish are particularly efficient at utilizing feed to produce body mass because they are poikilothermic (Torrissen et al. 2011, Fry et al. 2018). For this reason, a body of literature is available describing FCR in a variety of fish species (Sales & Glencross 2011 and references therein). Compared to values observed here, FCR of 0.6 g g^{-1} to 1.0 g g^{-1} have been reported for related gadoids, as well as salmonids, under similar environmental conditions (Kim & Lall 2001, Nordgarden et al. 2003, Tibbetts et al. 2005, Hixson et al. 2014). FCR less than 1 are commonly reported in fish because feed consumption is measured in dry mass and fish flesh is moist. FCR reported here for FOCO fish resemble those observed in large freshwater species such as Nile tilapia (*Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), and migral carp (*cirrhinus mrigala*), where FCR can exceed 2.5 g g^{-1} (Robinson & Li 2010, Mengistu et al. 2020, and Jabeen et al. 2004 respectively).

As FCR was the only critical growth parameter that varied significantly among treatments, it's influence on NGE should be given special attention. A host of factors can

influence FCR in fish and may include the culture environment, body size, genetics, occurrence of disease, and the nutritional content of the diet; however, there is no evidence to attribute plant oil substitution as a cause for increased FCR when dietary nutrient requirements are satisfied, as they were in the present study (Lin et al. 2007, Al-Souti et al, 2012, Bou et al. 2017). Increased FCR has been associated with a stress coping strategy in farmed African catfish (*Clarias gariepinus*; Martins et al. 2005, van de Nieuwegiessen et al. 2010). Rainbow trout with low and high cortisol response to stress showed significantly different FCR (1.5 and 1.9 respectively; Øverli et al. 2006).

Although the higher than expected FCRs observed in FOCO tanks might not be directly responsible for decreases in NGE in these tanks, lower NGEs could be a consequence of stress response. There are metabolic costs associated with stress coping: for example, oxygen consumption in juvenile steelhead (*Salmo gairdneri*) was double in stressed fish compared to their unstressed counterparts (Barton & Schreck 1987). EPA and DHA digestibility were consistently high in both dietary conditions suggesting that absorption of these EFA from the diet was still efficient even if food utilization in general was not. Further, EPA and DHA disappearance as a proportion of net intake was significantly higher in FOCO tanks compared to FO tanks. This suggests that the lower NGE of EPA and DHA in FOCO tanks may be influenced by the metabolic costs associated with a stress response leading to greater mobilization of these EFA in FOCO tanks for energy. One potential cause of stress could be exposure to highly oxidized vegetable oils. Symptoms of oxidative stress include decreases in growth performance and increases in radical scavenging enzymes (Tocher et al. 2003, Gao et al.

2013, Wang et al. 2016). In the present study, peroxide values were obtained for fish oil but were not for canola oil as it was a popular commercial brand and assumed to be of high quality. While β-oxidation of EFA is not known to be influenced by the inclusion of dietary vegetable oils (Mourente et al. 2005), the impacts of highly oxidized oils on β-oxidation should be the subject of further investigation. Even if the difference in NGEs observed between the FO and FOCO treatments were not reliable, we can still be fairly confident in the integrity of NGE values for EPA and DHA derived from the FO treatment and this should serve as a benchmark for this fish species as well as a point of reference for investigations in other organisms. Further, despite some of the anomalous results that were observed in the present study, such as FCR that weren't typical of other gadoids, our NGE values were ultimately in agreement with those reported elsewhere.

5.0 CONCLUSIONS

The primary aim of our study was to address the knowledge gap that exists in the current understanding of trophic transfer efficiency by providing an estimate of the rate at which dietary EPA and DHA are incorporated into biological tissue. In doing so, we demonstrated that the mass balance method was an effective and inexpensive tool for quantifying the accumulation of EFAs in a lean, marine fish species. An alternative approach employing radiolabelled FAs could be used to achieve a similar result; however, this method is costly and requires more sophisticated instrumentation. We provided evidence that the efficiency at which an organism retained dietary EFAs, was, to some extent, governed by the nutritional composition of the diet. This is consistent with other literature that also showed that EFAs were more efficiently retained when they were provided in greater dietary proportions (Hansen et al. 2008). In the context of trophic ecology, the results of the present study indicate that decreases in marine primary production of EFA could elicit profound impacts on higher trophic level consumers.

The present study did not come without a substantial number of logistical challenges, chief among which was maintaining wild fish in a laboratory environment. The act of capture, as well as transport from dockside to laboratory, was highly stressful and, despite efforts to minimize their effects, the physiological toll of these events was apparent in many fish and resulted in mortalities. A farm-raised fish would have been favorable in many aspects of this study; however, an appropriate and readily available candidate was lacking.

While strategic feeding regimes were employed to minimize food waste, occasionally food pellets that were discarded by fish during feeding would be lost through the tank's central outflow. This could potentially lead to an overestimation of the mass of a given FA that was consumed by the fish since there was no way to discriminate between feed that was consumed and feed that was lost from the tank. This was a rare occurrence and its influence on the integrity of our results was minimal if at all; however, strategies to prevent this from happening in future trials should be implemented.

The collection of feces was necessary for determining the apparent digestibility of EPA and DHA but the method of collection in the present study was suboptimal. Due to the physiological structure of the intestine of pollock, fecal samples could only be stripped from the fish posthumously. Before fish were subject to FA analysis, the entire digestive tract had to be removed and rinsed thoroughly with distilled water to prevent remaining digesta from erroneously influencing the FA profile of the fish. This was an arduous process and could have introduced a source of contamination if not cleaned properly. Had experimental tanks been equipped with a fecal collection column, this could have been avoided and a greater number of fecal specimens could have been obtained at multiple stages of the feeding trial. That said, ADCs that were calculated for EPA and DHA in the present study agreed with those reported elsewhere in fish (Martins et al. 2007, Rahman et al. 2016, Tibbetts et al. 2020).

Finally, the desaturase and elongase enzymes involved in the synthesis pathways of EPA and DHA in pollock are lacking in literature; however, inferences can be made

from studies on closely related species such as cod. One of the major assumptions during our study was that endogenous production of EPA and DHA was not occurring and there is a body of literature to support this. That said, recent discoveries suggest that these pathways are more complex than traditionally thought (Oboh et al. 2017). Future investigations should attempt to delineate the synthesis pathways of *n-3* LC-PUFAs in gadoids as new evidence is presented.

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APPENDIX A Physical attributes of fish experimental tanks (mean [SD]). FCR, SGR, and apparent feed intake are presented as the sum of the tank.

	Tank 1 (FO)	Tank 2 (FOCO)	Tank 3 (FO)	Tank 4 (FOCO)
initial mass (g)	476.0 (110.8)	322.0 (128.7)	340.6 (88.3)	505.4 (154.6)
final mass (g)	872.8 (164.1)	556.5 (250.9)	761.0 (179.3)	805.1 (297.6)
change in mass (g)	396.8 (138.5)	234.5 (148.7)	420.5 (108.0)	299.8 (152.6)
initial length (cm)	34.8 (1.6)	29.8 (5.7)	30.3 (4.3)	36.1 (3.1)
final length (cm)	38.8 (1.9)	33.8 (4.2)	36.8 (2.8)	38.8 (4.9)
change in length (cm)	4.1 (2.3)	4.0 (3.5)	6.6 (2.1)	2.6 (1.8)
$K_{initial}$ (g cm^{-3})	1.13 (0.21)	1.23 (0.32)	1.28 (0.41)	1.04 (0.11)
K_{final} (g cm^{-3})	1.48 (0.16)	1.37 (0.16)	1.50 (0.11)	1.32 (0.09)
Apparent feed intake (g)	657.41	496.00	732.24	677.79
FCR (g g^{-1})	1.66	2.12	1.74	2.26
SGR (% day^{-1})	0.73	0.66	0.97	0.56
liver mass (g)	93.3 (24.6)	48.7 (36.7)	92.4 (31.0)	59.6 (35.0)
HSI (g g^{-1})	10.6 (1.2)	7.7 (3.2)	11.9 (1.8)	7.0 (2.2)
liver lipid (%)	57.5 (6.2)	55.9 (2.2)	58.8 (3.4)	57.7 (11.3)
muscle lipid (%)	0.6 (0.1)	1.2 (1.2)	0.7 (0.1)	0.5 (<0.1)
rest-of-body lipid (%)	0.8 (<0.1)	1.0 (0.1)	1.2 (0.5)	1.2 (0.3)
whole fish lipid (%)	7.2 (1.0)	5.6 (2.0)	8.3 (1.2)	5.2 (2.1)

	Tank 5 (FO)	Tank 6 (FOCO)
initial mass (g)	392.4 (130.2)	372.2 (60.7)
final mass (g)	700.5 (252.1)	678.9 (209.4)
change in mass (g)	308.2 (132.6)	306.7 (152.2)
initial length (cm)	33.6 (2.4)	32.0 (2.7)
final length (cm)	36.5 (3.9)	35.5 (3.4)
change in length (cm)	2.9 (1.9)	3.5 (2.1)
$K_{initial}$ (g cm^{-3})	1.01 (0.17)	1.16 (0.28)
K_{final} (g cm^{-3})	1.38 (0.11)	1.49 (0.20)
Apparent feed intake (g)	564.21	641.16
FCR (g g^{-1})	1.83	2.09
SGR (% day^{-1})	0.70	0.72
liver mass (g)	63.5 (27.5)	63.0 (26.2)
HSI (g g^{-1})	9.0 (1.1)	9.0 (1.6)
liver lipid (%)	56.8 (5.3)	58.6 (2.4)
muscle lipid (%)	0.6 (0.1)	0.6 (<0.1)
rest-of-body lipid (%)	0.9 (0.2)	1.2 (0.6)
whole fish lipid (%)	7.4 (1.1)	7.4 (1.8)

APPENDIX B Physical attributes of control fish.

	Control 1	Control 2	Control 3	Control 4
initial mass (g)	202.0	561.3	444.6	500.6
initial length (cm)	24.0	37.5	34.5	36.5
$K_{initial}$ (g cm^{-3})	1.4611	1.0644	1.0827	1.0294
liver mass (g)	13.8	17.7	13.1	5.3
HSI (g g^{-1})	7.4	3.3	3.0	1.1
liver lipid (%)	62.5	43.7	40.1	14.6
muscle lipid (%)	0.5	0.5	0.5	0.5
rest-of-body lipid (%)	0.9	0.6	0.7	0.6
whole fish lipid (%)	5.1	2.0	1.8	0.8
	Control 5	Control 6	Control 7	Control 8
initial mass (g)	391.0	242.1	319.7	426.4
initial length (cm)	32.0	29.0	32.0	31.5
$K_{initial}$ (g cm^{-3})	1.1931	.9928	.9756	1.3644
liver mass (g)	12.3	3.0	4.8	5.3
HSI (g g^{-1})	3.2	1.2	1.5	1.3
liver lipid (%)	36.5	9.0	19.7	22.9
muscle lipid (%)	0.7	0.5	0.5	0.5
rest-of-body lipid (%)	0.7	0.6	0.6	0.6
whole fish lipid (%)	1.8	0.7	0.9	0.9

	Control 9	Control 10	Control 11	Control 12
initial mass (g)	310.3	128.3	291.1	350.1
initial length (cm)	31.0	23.0	30.0	30.0
$K_{initial}$ (g cm^{-3})	1.0415	1.0547	1.0782	1.2965
liver mass (g)	8.5	3.1	10.0	10.1
HSI (g g^{-1})	2.8	2.4	3.6	3.0
liver lipid (%)	37.1	32.0	39.3	44.3
muscle lipid (%)	0.6	0.5	0.5	0.5
rest-of-body lipid (%)	0.6	0.6	0.9	0.6
whole fish lipid (%)	1.6	1.4	2.2	1.8

APPENDIX C Fatty acid proportions (% of total FA) for Atlantic pollock livers.

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Tank1	3.94	0.14	0.38	18.09	0.30	0.30	7.34	0.14
Tank1	3.72	0.14	0.36	18.54	0.28	0.32	7.45	0.14
Tank1	3.95	0.13	0.38	17.38	0.31	0.33	7.03	0.15
Tank1	3.37	0.12	0.34	17.13	0.29	0.36	6.63	0.13
Tank1	3.32	0.12	0.33	15.77	0.29	0.35	6.31	0.13
Tank1	3.33	0.11	0.32	16.35	0.30	0.36	6.41	0.12
Tank1	3.14	0.11	0.31	15.65	0.31	0.39	6.06	0.13
Tank1	3.71	0.12	0.36	17.83	0.29	0.32	6.95	0.13
Tank2	2.25	0.07	0.21	13.13	0.21	0.34	3.60	0.09
Tank2	2.01	0.07	0.20	13.73	0.19	0.28	3.55	0.09
Tank2	2.31	0.07	0.20	13.63	0.18	0.29	3.74	0.08
Tank2	2.58	0.09	0.25	13.96	0.20	0.30	3.94	0.09
Tank3	3.35	0.12	0.34	16.12	0.31	0.33	6.71	0.13
Tank3	3.61	0.12	0.36	17.46	0.29	0.32	7.06	0.16
Tank3	2.97	0.11	0.31	15.35	0.29	0.35	5.97	0.13
Tank3	3.32	0.13	0.34	16.07	0.30	0.32	6.66	0.13
Tank3	3.29	0.12	0.33	17.35	0.29	0.31	6.57	0.12
Tank3	3.31	0.12	0.34	16.03	0.29	0.32	6.83	0.13
Tank4	2.19	0.07	0.18	14.25	0.18	0.31	3.56	0.09
Tank4	2.27	0.09	0.24	14.24	0.21	0.30	3.68	0.09
Tank4	2.03	0.07	0.21	12.60	0.20	0.29	3.43	0.08
Tank4	2.21	0.07	0.23	13.69	0.22	0.30	3.48	0.08
Tank5	3.52	0.12	0.35	17.67	0.30	0.31	7.02	0.13
Tank5	3.53	0.12	0.35	18.08	0.29	0.31	6.79	0.13
Tank5	3.57	0.12	0.35	18.04	0.29	0.28	7.29	0.14
Tank5	3.73	0.13	0.39	16.89	0.31	0.29	7.52	0.15
Tank5	3.55	0.12	0.34	18.50	0.27	0.30	7.77	0.15
Tank5	3.12	0.11	0.32	15.25	0.30	0.32	6.25	0.13
Tank5	3.08	0.12	0.33	14.82	0.31	0.31	6.18	0.14
Tank5	3.76	0.12	0.37	18.47	0.29	0.28	7.31	0.15
Tank6	2.23	0.07	0.22	14.74	0.19	0.28	3.60	0.08
Tank6	2.19	0.06	0.20	14.55	0.17	0.32	3.43	0.08
Tank6	2.29	0.08	0.23	13.74	0.20	0.27	3.74	0.09
Tank6	1.86	0.06	0.18	13.58	0.15	0.28	3.34	0.08
Tank6	2.08	0.07	0.21	13.47	0.21	0.34	3.73	0.10
Control1	1.69	0.07	0.17	14.73	0.21	0.34	4.02	0.13
Control2	2.53	0.21	0.47	13.60	0.78	0.28	4.21	0.21
Control3	2.16	0.15	0.42	14.97	0.76	0.31	3.68	0.20
Control4	2.68	0.22	0.51	15.50	1.02	0.58	4.72	0.19
Control5	2.03	0.19	0.39	14.34	0.51	0.37	3.31	0.21
Control6	3.11	0.32	0.43	10.85	0.48	0.38	4.20	0.22
Control7	3.39	0.32	0.50	14.12	0.36	0.48	6.72	0.22

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Control8	2.16	0.20	0.40	13.01	0.46	0.35	4.47	0.18
Control9	2.18	0.21	0.42	16.19	0.57	0.42	3.73	0.20
Control10	2.27	0.40	0.59	13.93	0.38	0.55	5.43	0.22
Control11	3.19	0.28	0.44	10.85	0.44	0.27	5.77	0.27
Control12	1.97	0.13	0.30	16.68	0.38	0.42	4.50	0.17
	i-17:0	**17:1(b)**	**16:2n-4**	**17:0**	**16:3n-4**	**17:1**	**18:0**	**18:1n-11**
Tank1	0.21	0.16	0.68	0.42	0.80	0.34	6.80	0.27
Tank1	0.20	0.16	0.65	0.35	0.79	0.36	6.08	0.25
Tank1	0.21	0.16	0.66	0.45	0.77	0.31	7.01	0.34
Tank1	0.21	0.16	0.61	0.40	0.73	0.37	6.37	0.29
Tank1	0.19	0.14	0.56	0.41	0.63	0.29	6.66	0.49
Tank1	0.19	0.13	0.57	0.41	0.67	0.30	7.40	0.62
Tank1	0.15	0.13	0.44	0.32	0.46	0.26	5.42	0.43
Tank1	0.21	0.16	0.61	0.37	0.76	0.35	6.25	0.32
Tank2	0.11	0.08	0.27	0.26	0.29	0.23	5.42	0.44
Tank2	0.11	0.10	0.25	0.24	0.28	0.23	5.59	0.25
Tank2	0.10	0.08	0.30	0.22	0.35	0.25	4.89	0.23
Tank2	0.13	0.09	0.34	0.25	0.37	0.27	5.02	0.28
Tank3	0.20	0.14	0.58	0.41	0.69	0.31	6.88	0.43
Tank3	0.23	0.19	0.61	0.36	0.73	0.34	6.15	0.39
Tank3	0.20	0.17	0.49	0.35	0.55	0.29	6.63	0.40
Tank3	0.22	0.21	0.59	0.38	0.71	0.33	6.53	0.46
Tank3	0.21	0.16	0.56	0.34	0.69	0.33	6.41	0.27
Tank3	0.22	0.18	0.62	0.37	0.77	0.37	6.64	0.33
Tank4	0.10	0.10	0.25	0.20	0.27	0.23	5.01	0.15
Tank4	0.13	0.11	0.28	0.26	0.30	0.23	5.71	0.27
Tank4	0.11	0.09	0.28	0.23	0.31	0.24	5.20	0.23
Tank4	0.12	0.12	0.28	0.25	0.31	0.25	5.26	0.24
Tank5	0.21	0.18	0.62	0.34	0.78	0.34	6.38	0.25
Tank5	0.20	0.16	0.58	0.35	0.71	0.33	6.56	0.26
Tank5	0.21	0.17	0.64	0.36	0.78	0.34	6.45	0.29
Tank5	0.24	0.18	0.70	0.48	0.83	0.33	7.15	0.39
Tank5	0.20	0.18	0.66	0.31	0.79	0.34	5.39	0.25
Tank5	0.21	0.14	0.53	0.40	0.63	0.30	6.86	0.47
Tank5	0.22	0.18	0.53	0.40	0.61	0.29	7.14	0.65
Tank5	0.22	0.15	0.64	0.40	0.80	0.35	6.30	0.24
Tank6	0.12	0.08	0.27	0.21	0.30	0.24	5.05	0.27
Tank6	0.11	0.07	0.26	0.21	0.29	0.26	4.72	0.19
Tank6	0.11	0.09	0.29	0.25	0.32	0.24	5.25	0.29
Tank6	0.10	0.08	0.27	0.21	0.30	0.24	5.33	0.26
Tank6	0.11	0.10	0.24	0.22	0.24	0.23	4.78	0.48

	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
Control1	0.10	0.12	0.13	0.24	0.10	0.24	5.45	0.58
Control2	0.37	0.79	0.16	0.35	0.15	0.34	3.80	1.57
Control3	0.32	0.76	0.10	0.40	0.11	0.35	4.54	1.82
Control4	0.46	0.81	0.14	0.35	0.10	0.41	4.30	1.50
Control5	0.39	0.76	0.13	0.42	0.11	0.39	5.58	2.09
Control6	0.37	0.53	0.29	0.25	0.19	0.19	3.31	2.83
Control7	0.51	0.28	0.29	0.38	0.19	0.35	4.33	2.37
Control8	0.38	0.47	0.20	0.36	0.14	0.29	4.10	1.59
Control9	0.45	0.73	0.15	0.45	0.15	0.42	4.95	1.26
Control10	0.49	0.21	0.15	0.49	0.12	0.41	5.15	1.37
Control11	0.30	0.37	0.30	0.22	0.22	0.25	2.67	3.44
Control12	0.28	0.39	0.14	0.26	0.13	0.41	4.47	1.27
	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank1	20.94	4.78	0.20	8.37	0.41	0.23	0.35	1.50
Tank1	22.92	4.79	0.20	8.06	0.39	0.25	0.34	1.47
Tank1	20.45	4.49	0.20	10.12	0.36	0.23	0.31	1.71
Tank1	24.36	5.03	0.20	8.45	0.40	0.29	0.34	1.48
Tank1	23.31	4.79	0.22	10.25	0.36	0.25	0.28	1.66
Tank1	23.12	4.75	0.21	9.80	0.37	0.28	0.30	1.59
Tank1	25.21	4.23	0.23	11.89	0.26	0.21	0.21	1.79
Tank1	22.96	4.81	0.21	8.78	0.39	0.24	0.34	1.56
Tank2	35.87	3.90	0.20	14.70	0.18	0.20	0.15	3.51
Tank2	36.84	3.87	0.19	13.84	0.18	0.15	0.14	3.38
Tank2	37.80	3.91	0.13	14.09	0.20	0.18	0.18	3.88
Tank2	35.80	3.92	0.14	14.76	0.19	0.12	0.19	3.89
Tank3	21.86	5.02	0.22	9.86	0.39	0.23	0.31	1.66
Tank3	22.72	4.75	0.20	8.08	0.37	0.24	0.33	1.47
Tank3	23.88	4.77	0.00	10.75	0.34	0.24	0.27	1.68
Tank3	21.23	4.97	0.23	9.03	0.40	0.25	0.33	1.55
Tank3	24.40	4.90	0.21	7.92	0.38	0.23	0.32	1.40
Tank3	23.78	5.30	0.23	7.96	0.44	0.25	0.36	1.50
Tank4	37.67	3.79	0.00	13.49	0.16	0.20	0.13	3.22
Tank4	34.39	3.80	0.15	13.43	0.17	0.13	0.15	3.43
Tank4	37.80	3.97	0.14	14.36	0.20	0.20	0.16	3.69
Tank4	36.32	3.85	0.14	13.69	0.19	0.21	0.15	3.59
Tank5	22.64	5.05	0.20	8.11	0.41	0.25	0.35	1.47
Tank5	22.78	4.83	0.19	8.38	0.38	0.22	0.32	1.43
Tank5	21.96	5.00	0.18	8.37	0.41	0.21	0.34	1.51
Tank5	18.69	5.15	0.20	9.28	0.46	0.24	0.39	1.65
Tank5	22.78	4.90	0.18	8.35	0.38	0.24	0.34	1.50
Tank5	23.89	4.98	0.22	9.86	0.38	0.23	0.31	1.62

	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank5	20.71	4.82	0.25	10.00	0.36	0.23	0.29	1.66
Tank5	22.23	4.89	0.19	8.43	0.40	0.22	0.35	1.49
Tank6	37.49	3.84	0.13	13.08	0.17	0.17	0.12	3.40
Tank6	39.31	4.14	0.13	13.07	0.20	0.26	0.17	3.52
Tank6	36.40	3.77	0.00	14.04	0.18	0.14	0.14	3.61
Tank6	40.42	3.92	0.12	13.29	0.19	0.21	0.18	3.56
Tank6	35.45	3.87	0.21	13.74	0.15	0.19	0.12	3.03
Control1	33.48	3.24	0.27	13.02	0.08	0.14	0.06	1.80
Control2	15.18	3.15	0.50	1.26	0.08	0.07	0.06	0.67
Control3	14.53	3.57	0.44	1.11	0.09	0.05	0.07	0.64
Control4	15.54	4.49	0.29	1.29	0.08	0.07	0.08	0.64
Control5	12.44	4.14	0.46	1.38	0.12	0.07	0.07	0.76
Control6	7.94	3.13	0.25	1.38	0.08	0.08	0.07	0.72
Control7	14.62	5.99	0.32	1.46	0.13	0.10	0.12	0.61
Control8	12.04	4.60	0.36	1.41	0.12	0.11	0.10	0.81
Control9	16.18	4.31	0.42	1.76	0.09	0.13	0.07	0.98
Control10	18.07	5.22	0.37	2.40	0.08	0.17	0.11	1.55
Control11	9.51	3.45	0.51	1.45	0.13	0.10	0.07	0.80
Control12	22.35	5.15	0.46	1.79	0.13	0.06	0.07	0.71
	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank1	0.10	1.29	0.18	0.23	1.51	0.19	0.34	0.19
Tank1	0.10	1.25	0.18	0.22	1.44	0.22	0.33	0.19
Tank1	0.09	1.28	0.18	0.41	1.99	0.18	0.31	0.18
Tank1	0.10	1.22	0.20	0.30	1.72	0.18	0.31	0.18
Tank1	0.07	1.17	0.18	0.48	2.50	0.19	0.35	0.17
Tank1	0.09	1.18	0.18	0.41	2.13	0.17	0.33	0.17
Tank1	0.08	1.01	0.14	0.60	3.13	0.21	0.36	0.14
Tank1	0.09	1.26	0.20	0.31	1.73	0.17	0.32	0.18
Tank2	0.05	0.67	0.10	0.52	2.45	0.16	0.38	0.09
Tank2	0.04	0.65	0.11	0.37	2.32	0.15	0.34	0.09
Tank2	0.05	0.70	0.11	0.21	1.63	0.12	0.31	0.08
Tank2	0.06	0.64	0.11	0.25	1.85	0.11	0.34	0.09
Tank3	0.10	1.25	0.18	0.35	2.01	0.18	0.34	0.18
Tank3	0.09	1.29	0.18	0.30	1.96	0.24	0.30	0.18
Tank3	0.09	1.17	0.18	0.50	2.57	0.19	0.34	0.17
Tank3	0.11	1.39	0.20	0.38	2.19	0.21	0.35	0.18
Tank3	0.11	1.23	0.18	0.29	1.79	0.22	0.01	0.17
Tank3	0.10	1.31	0.21	0.26	1.58	0.22	0.31	0.18
Tank4	0.05	0.67	0.10	0.35	2.20	0.13	0.31	0.08
Tank4	0.05	0.73	0.10	0.40	2.58	0.17	0.33	0.08
Tank4	0.06	0.69	0.11	0.34	1.94	0.13	0.31	0.08

	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank4	0.06	0.66	0.10	0.29	1.98	0.17	0.32	0.08
Tank5	0.10	1.32	0.19	0.26	1.67	0.18	0.30	0.18
Tank5	0.10	1.24	0.17	0.30	1.81	0.20	0.32	0.18
Tank5	0.10	1.27	0.19	0.25	1.55	0.23	0.27	0.18
Tank5	0.11	1.39	0.21	0.26	1.60	0.19	0.33	0.20
Tank5	0.10	1.27	0.18	0.24	1.42	0.19	0.28	0.18
Tank5	0.09	1.20	0.19	0.47	2.39	0.23	0.36	0.18
Tank5	0.11	1.32	0.18	0.53	2.71	0.25	0.37	0.17
Tank5	0.11	1.26	0.20	0.23	1.50	0.17	0.31	0.19
Tank6	0.00	0.65	0.10	0.26	1.76	0.17	0.33	0.09
Tank6	0.04	0.63	0.10	0.24	1.56	0.14	0.31	0.08
Tank6	0.00	0.66	0.11	0.36	2.17	0.15	0.33	0.08
Tank6	0.04	0.63	0.10	0.22	1.51	0.14	0.28	0.08
Tank6	0.05	0.72	0.10	0.52	2.93	0.21	0.35	0.09
Control1	0.05	0.68	0.08	0.77	4.04	0.19	0.38	0.11
Control2	0.28	1.85	0.07	0.72	6.24	1.03	0.39	0.05
Control3	0.23	1.13	0.05	0.90	7.25	0.87	0.44	0.04
Control4	0.29	0.79	0.06	1.86	5.45	1.45	0.70	0.08
Control5	0.20	1.68	0.06	0.96	6.33	0.74	0.47	0.05
Control6	0.19	1.34	0.07	1.29	9.18	0.82	0.37	0.06
Control7	0.14	0.77	0.09	1.11	6.21	0.84	0.49	0.13
Control8	0.20	1.09	0.09	0.83	6.26	0.80	0.55	0.10
Control9	0.23	1.66	0.08	0.66	4.31	0.64	0.63	0.13
Control10	0.08	1.43	0.09	0.69	4.78	0.44	0.88	0.22
Control11	0.15	2.43	0.12	1.21	12.03	0.67	0.32	0.05
Control12	0.17	1.01	0.10	0.99	4.28	0.66	0.51	0.06
	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank1	0.74	0.18	0.81	7.57	1.09	0.18	0.22	0.37
Tank1	0.71	0.16	0.76	7.13	1.10	0.17	0.18	0.36
Tank1	0.72	0.13	0.69	7.10	1.47	0.17	0.07	0.35
Tank1	0.70	0.12	0.69	7.10	1.14	0.14	0.05	0.35
Tank1	0.67	0.12	0.64	6.70	1.98	0.23	0.04	0.33
Tank1	0.66	0.11	0.64	6.75	1.54	0.18	0.03	0.34
Tank1	0.60	0.11	0.53	5.77	2.46	0.25	0.05	0.29
Tank1	0.72	0.13	0.71	7.09	1.30	0.19	0.08	0.34
Tank2	0.36	0.10	0.31	3.24	1.60	0.15	0.16	0.15
Tank2	0.36	0.10	0.30	3.16	1.88	0.20	0.22	0.15
Tank2	0.34	0.10	0.33	3.49	0.96	0.12	0.12	0.17
Tank2	0.36	0.11	0.36	3.33	1.15	0.16	0.06	0.15
Tank3	0.79	0.14	0.71	7.60	1.38	0.17	0.04	0.36
Tank3	0.69	0.12	0.71	7.45	1.47	0.31	0.22	0.35

	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank3	0.70	0.12	0.62	7.00	2.03	0.20	0.04	0.33
Tank3	0.69	0.13	0.77	7.92	1.53	0.23	0.04	0.37
Tank3	0.68	0.13	0.68	7.40	1.25	0.21	0.05	0.35
Tank3	0.76	0.15	0.76	7.66	1.04	0.15	0.18	0.36
Tank4	0.31	0.09	0.31	3.32	1.64	0.20	0.05	0.15
Tank4	0.32	0.09	0.34	3.55	1.77	0.30	0.05	0.18
Tank4	0.33	0.09	0.33	3.58	1.14	0.14	0.03	0.18
Tank4	0.35	0.11	0.35	3.78	1.07	0.19	0.03	0.18
Tank5	0.69	0.13	0.75	7.49	1.17	0.20	0.05	0.36
Tank5	0.67	0.12	0.70	7.20	1.27	0.25	0.04	0.36
Tank5	0.74	0.12	0.73	7.53	1.11	0.17	0.33	0.36
Tank5	0.77	0.15	0.82	8.32	1.03	0.16	0.15	0.42
Tank5	0.72	0.12	0.73	7.46	1.07	0.19	0.36	0.36
Tank5	0.71	0.13	0.66	7.00	1.81	0.23	0.04	0.34
Tank5	0.66	0.13	0.70	7.61	1.86	0.33	0.31	0.36
Tank5	0.70	0.12	0.73	7.30	1.14	0.22	0.10	0.36
Tank6	0.43	0.10	0.33	3.65	1.06	0.20	0.34	0.17
Tank6	0.33	0.09	0.31	3.21	0.89	0.16	0.27	0.16
Tank6	0.32	0.10	0.33	3.45	1.46	0.24	0.05	0.18
Tank6	0.31	0.09	0.30	3.18	0.92	0.12	0.35	0.15
Tank6	0.38	0.10	0.32	3.63	2.16	0.23	0.28	0.17
Control1	0.72	0.13	0.21	3.33	4.17	0.27	0.05	0.12
Control2	0.73	0.18	0.72	9.53	4.31	1.83	0.11	0.32
Control3	0.80	0.28	0.53	9.25	3.49	1.13	0.20	0.25
Control4	1.43	0.31	0.55	6.48	3.12	1.43	0.48	0.18
Control5	0.60	0.18	0.62	8.98	3.05	0.75	0.41	0.29
Control6	1.12	0.17	0.49	5.17	9.49	2.16	0.47	0.18
Control7	1.32	0.22	0.46	7.76	4.91	1.24	0.43	0.34
Control8	1.40	0.32	0.55	10.69	3.15	0.94	0.11	0.30
Control9	1.68	0.29	0.77	10.71	2.54	0.47	0.09	0.26
Control10	3.35	0.51	0.75	11.78	2.17	0.45	0.09	0.22
Control11	0.43	0.18	0.64	9.02	9.82	1.53	0.19	0.41
Control12	0.80	0.24	0.44	9.46	2.83	0.54	0.08	0.23

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.17	0.26	1.48	5.57	0.30
Tank1	0.17	0.25	1.36	5.25	0.27
Tank1	0.19	0.25	1.38	5.28	0.13
Tank1	0.16	0.25	1.37	5.51	0.12
Tank1	0.14	0.22	1.28	5.29	0.12
Tank1	0.14	0.23	1.28	5.32	0.11
Tank1	0.13	0.18	1.10	4.98	0.14

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.16	0.24	1.35	5.29	0.11
Tank2	0.08	0.09	0.59	2.75	0.27
Tank2	0.09	0.09	0.58	2.75	0.27
Tank2	0.08	0.11	0.63	2.77	0.20
Tank2	0.11	0.12	0.74	2.57	0.13
Tank3	0.15	0.24	1.39	5.68	0.13
Tank3	0.16	0.24	1.36	5.67	0.17
Tank3	0.14	0.21	1.25	5.52	0.15
Tank3	0.16	0.24	1.44	6.61	0.16
Tank3	0.17	0.25	1.37	6.20	0.17
Tank3	0.17	0.26	1.44	5.92	0.29
Tank4	0.08	0.09	0.60	3.30	0.16
Tank4	0.08	0.10	0.75	3.75	0.20
Tank4	0.08	0.10	0.67	3.40	0.14
Tank4	0.10	0.11	0.75	3.91	0.14
Tank5	0.16	0.25	1.41	5.96	0.16
Tank5	0.16	0.24	1.37	5.86	0.17
Tank5	0.18	0.26	1.48	5.50	0.15
Tank5	0.19	0.27	1.59	5.95	0.17
Tank5	0.17	0.25	1.39	5.40	0.14
Tank5	0.16	0.22	1.30	5.31	0.14
Tank5	0.16	0.23	1.39	6.81	0.18
Tank5	0.17	0.25	1.39	5.34	0.15
Tank6	0.09	0.10	0.68	2.98	0.16
Tank6	0.07	0.09	0.61	2.69	0.09
Tank6	0.08	0.10	0.69	3.24	0.12
Tank6	0.07	0.09	0.57	2.50	0.12
Tank6	0.10	0.09	0.62	3.48	0.10
Control1	0.10	0.06	0.49	3.42	0.21
Control2	0.24	0.30	1.91	17.75	0.64
Control3	0.31	0.27	1.76	18.81	0.45
Control4	0.94	0.39	4.23	13.14	0.69
Control5	0.39	0.22	1.77	21.01	0.61
Control6	0.22	0.32	2.72	21.80	0.79
Control7	0.31	0.31	3.20	11.04	0.53
Control8	0.33	0.35	2.57	20.61	0.46
Control9	0.39	0.27	2.26	14.88	0.59
Control10	0.53	0.19	3.00	7.91	0.32
Control11	0.12	0.16	1.44	13.27	0.50
Control12	0.35	0.17	1.82	12.22	0.45

APPENDIX D Fatty acid proportions (% of total FA) of Atlantic pollock muscle tissue

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Tank1	2.06	0.08	0.34	16.96	0.30	0.30	3.14	0.19
Tank1	2.04	0.08	0.32	16.91	0.30	0.30	2.96	0.23
Tank1	1.95	0.06	0.36	19.03	0.29	0.31	3.30	0.19
Tank1	1.48	0.05	0.30	16.39	0.26	0.32	2.84	0.18
Tank1	1.54	0.05	0.30	17.01	0.27	0.35	2.60	0.19
Tank1	1.57	0.05	0.31	17.90	0.27	0.38	2.84	0.20
Tank1	1.02	0.03	0.25	18.09	0.23	0.26	1.98	0.16
Tank1	1.91	0.08	0.32	17.14	0.29	0.39	3.11	0.18
Tank2	1.82	0.06	0.29	15.63	0.30	0.36	3.24	0.21
Tank2	1.49	0.05	0.15	10.09	0.28	0.33	3.34	0.12
Tank2	1.40	0.05	0.21	14.49	0.24	0.56	1.83	0.15
Tank2	0.78	0.02	0.20	15.83	0.21	0.28	1.03	0.15
Tank3	1.55	0.06	0.30	16.48	0.28	0.31	3.20	0.19
Tank3	1.67	0.06	0.31	16.28	0.23	0.30	3.12	0.22
Tank3	1.31	0.05	0.28	16.04	0.28	0.35	2.98	0.19
Tank3	1.27	0.05	0.27	16.60	0.26	0.27	2.70	0.18
Tank3	1.94	0.06	0.31	16.76	0.31	0.37	3.40	0.19
Tank3	1.16	0.04	0.21	14.11	0.23	0.48	2.12	0.15
Tank4	0.86	0.03	0.17	16.48	0.19	0.41	1.22	0.13
Tank4	1.17	0.04	0.24	17.30	0.22	0.28	1.37	0.20
Tank4	1.13	0.03	0.23	16.35	0.23	0.40	1.49	0.16
Tank4	1.05	0.03	0.23	16.06	0.23	0.34	1.34	0.15
Tank5	1.91	0.07	0.32	17.02	0.25	0.27	3.30	0.22
Tank5	1.86	0.06	0.31	17.12	0.26	0.31	3.16	0.19
Tank5	2.20	0.08	0.33	18.01	0.26	0.27	3.75	0.21
Tank5	2.08	0.08	0.32	15.32	0.27	0.33	3.65	0.18
Tank5	1.81	0.06	0.26	16.40	0.24	0.27	3.05	0.17
Tank5	1.64	0.05	0.29	16.39	0.27	0.31	3.11	0.19
Tank5	1.62	0.06	0.31	18.00	0.26	0.29	2.62	0.20
Tank5	2.21	0.08	0.37	17.13	0.29	0.27	3.34	0.22
Tank6	1.44	0.04	0.24	15.26	0.25	0.50	1.92	0.16
Tank6	1.33	0.04	0.24	16.58	0.20	0.41	1.62	0.16
Tank6	1.28	0.04	0.24	15.77	0.20	0.30	1.61	0.17
Tank6	1.31	0.04	0.20	13.79	0.22	0.50	1.69	0.13
Tank6	1.14	0.04	0.21	14.92	0.22	0.45	1.93	0.16
Control1	1.05	0.03	0.25	17.08	0.27	0.25	1.66	0.18
Control2	0.47	0.02	0.26	17.46	0.38	0.16	0.64	0.19
Control3	0.41	0.02	0.24	17.02	0.36	0.16	0.61	0.17
Control4	0.41	0.02	0.24	16.99	0.43	0.24	0.64	0.17
Control5	0.61	0.05	0.23	15.75	0.36	0.22	1.06	0.19
Control6	0.52	0.03	0.25	14.80	0.28	0.19	0.76	0.22
Control7	0.48	0.03	0.25	16.86	0.22	0.21	0.82	0.23

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Control8	0.42	0.03	0.23	16.58	0.27	0.17	0.84	0.19
Control9	0.32	0.02	0.22	17.95	0.26	0.17	0.52	0.18
Control10	0.35	0.03	0.29	17.03	0.19	0.16	0.62	0.22
Control11	0.57	0.03	0.29	17.43	0.27	0.17	0.74	0.29
Control12	0.47	0.02	0.21	18.11	0.24	0.23	0.74	0.20
	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
Tank1	0.18	0.14	0.39	0.34	0.19	0.19	5.89	0.39
Tank1	0.19	0.15	0.37	0.37	0.18	0.16	6.54	0.45
Tank1	0.17	0.19	0.36	0.36	0.25	0.16	5.58	0.47
Tank1	0.17	0.17	0.30	0.34	0.14	0.19	5.95	0.45
Tank1	0.15	0.17	0.29	0.36	0.13	0.14	5.62	0.64
Tank1	0.16	0.18	0.32	0.36	0.16	0.16	5.58	0.60
Tank1	0.11	0.18	0.18	0.32	0.09	0.11	5.36	0.60
Tank1	0.18	0.20	0.39	0.33	0.21	0.18	6.41	0.55
Tank2	0.21	0.19	0.39	0.36	0.18	0.18	6.96	0.49
Tank2	0.10	0.10	0.25	0.17	0.26	0.24	4.14	0.23
Tank2	0.12	0.12	0.16	0.19	0.08	0.15	6.34	0.41
Tank2	0.11	0.14	0.09	0.26	0.01	0.12	5.98	0.29
Tank3	0.16	0.17	0.31	0.36	0.16	0.16	5.77	0.58
Tank3	0.18	0.13	0.35	0.33	0.18	0.16	5.50	0.59
Tank3	0.16	0.21	0.23	0.34	0.11	0.17	5.65	0.75
Tank3	0.16	0.24	0.24	0.32	0.11	0.16	6.00	0.50
Tank3	0.18	0.19	0.40	0.34	0.19	0.18	6.04	0.54
Tank3	0.11	0.14	0.16	0.23	0.01	0.17	4.81	0.55
Tank4	0.10	0.20	0.08	0.20	0.04	0.14	5.53	0.45
Tank4	0.12	0.16	0.15	0.24	0.09	0.12	5.23	0.50
Tank4	0.12	0.19	0.15	0.24	0.09	0.12	5.28	0.44
Tank4	0.10	0.23	0.13	0.25	0.06	0.12	5.32	0.38
Tank5	0.18	0.18	0.38	0.32	0.19	0.19	5.90	0.52
Tank5	0.17	0.18	0.34	0.34	0.19	0.18	5.84	0.49
Tank5	0.18	0.14	0.41	0.33	0.30	0.21	6.14	0.34
Tank5	0.20	0.18	0.42	0.37	0.23	0.24	6.83	0.33
Tank5	0.15	0.19	0.31	0.27	0.23	0.20	5.96	0.32
Tank5	0.15	0.16	0.30	0.33	0.15	0.16	5.22	0.61
Tank5	0.18	0.20	0.28	0.35	0.14	0.15	5.64	0.62
Tank5	0.20	0.20	0.39	0.40	0.20	0.20	6.08	0.41
Tank6	0.15	0.15	0.17	0.27	0.12	0.16	5.54	0.46
Tank6	0.15	0.13	0.15	0.27	0.10	0.15	5.68	0.36
Tank6	0.10	0.13	0.15	0.22	0.09	0.11	5.10	0.57
Tank6	0.14	0.13	0.15	0.25	0.10	0.17	6.57	0.37
Tank6	0.11	0.17	0.15	0.25	0.09	0.13	4.75	0.70

	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
Control1	0.15	0.24	0.06	0.35	0.03	0.14	5.94	0.77
Control2	0.13	0.60	0.02	0.24	0.00	0.11	5.51	0.43
Control3	0.15	0.53	0.02	0.30	0.00	0.12	6.06	0.54
Control4	0.20	0.48	0.01	0.26	0.00	0.15	6.17	0.39
Control5	0.22	0.61	0.03	0.31	0.02	0.19	6.27	0.87
Control6	0.17	0.28	0.01	0.30	0.00	0.12	5.84	0.71
Control7	0.24	0.24	0.04	0.31	0.00	0.13	6.37	0.50
Control8	0.19	0.33	0.04	0.29	0.01	0.13	6.03	0.40
Control9	0.16	0.39	0.02	0.28	0.00	0.12	6.15	0.34
Control10	0.18	0.15	0.02	0.36	0.00	0.11	6.67	0.30
Control11	0.15	0.32	0.04	0.24	0.00	0.09	5.61	0.92
Control12	0.16	0.37	0.02	0.28	0.01	0.14	6.15	0.43
	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank1	8.08	2.83	0.14	6.26	0.23	0.11	0.38	1.09
Tank1	7.69	2.84	0.16	5.48	0.22	0.10	0.34	0.98
Tank1	9.30	2.65	0.15	6.73	0.17	0.12	0.31	1.09
Tank1	10.12	2.96	0.16	7.16	0.23	0.13	0.39	1.13
Tank1	8.79	2.61	0.15	7.38	0.21	0.12	0.39	1.09
Tank1	8.92	2.64	0.15	7.40	0.21	0.13	0.40	1.09
Tank1	9.23	2.39	0.14	7.69	0.15	0.09	0.27	0.95
Tank1	9.59	2.81	0.15	6.20	0.20	0.10	0.33	0.99
Tank2	9.52	3.17	0.16	6.46	0.27	0.11	0.42	1.16
Tank2	36.30	4.21	0.19	14.55	0.17	0.15	0.15	3.30
Tank2	18.27	2.60	0.13	10.51	0.14	0.09	0.18	2.50
Tank2	12.32	2.21	0.11	7.70	0.11	0.06	0.15	1.64
Tank3	10.19	2.96	0.16	8.01	0.25	0.13	0.42	1.30
Tank3	8.94	2.94	0.15	6.89	0.22	0.11	0.38	1.20
Tank3	11.20	2.88	0.19	8.95	0.23	0.14	0.37	1.24
Tank3	8.67	2.72	0.16	6.67	0.22	0.12	0.41	1.05
Tank3	9.16	2.99	0.15	6.93	0.23	0.11	0.38	1.16
Tank3	22.30	2.69	0.16	14.97	0.13	0.12	0.19	3.03
Tank4	16.10	2.28	0.14	9.41	0.10	0.10	0.15	1.80
Tank4	11.58	2.11	0.11	7.82	0.08	0.05	0.11	1.77
Tank4	14.93	2.35	0.11	10.03	0.10	0.08	0.16	2.30
Tank4	14.14	2.20	0.11	9.38	0.11	0.08	0.17	2.14
Tank5	9.12	2.87	0.17	6.36	0.22	0.11	0.38	1.14
Tank5	9.66	2.97	0.15	6.57	0.23	0.12	0.35	1.07
Tank5	9.55	3.08	0.16	5.31	0.22	0.11	0.30	1.02
Tank5	9.43	3.04	0.18	6.86	0.28	0.13	0.44	1.23
Tank5	15.81	2.93	0.14	6.68	0.18	0.12	0.25	1.49
Tank5	12.38	2.74	0.16	8.75	0.19	0.12	0.35	1.42

	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank5	8.09	2.51	0.14	6.68	0.17	0.11	0.33	1.02
Tank5	8.59	2.70	0.14	5.81	0.20	0.11	0.32	1.07
Tank6	16.71	2.49	0.15	9.46	0.12	0.09	0.17	2.39
Tank6	16.19	2.42	0.13	9.17	0.11	0.11	0.16	2.23
Tank6	15.00	2.36	0.11	10.62	0.09	0.06	0.15	2.40
Tank6	20.30	2.77	0.14	10.71	0.13	0.10	0.18	2.46
Tank6	18.57	2.63	0.16	13.65	0.12	0.10	0.18	2.74
Control1	11.40	2.12	0.22	5.97	0.05	0.06	0.04	1.00
Control2	4.60	1.59	0.22	0.53	0.03	0.02	0.03	0.19
Control3	5.42	1.87	0.21	0.53	0.03	0.02	0.03	0.23
Control4	6.84	1.92	0.16	0.52	0.04	0.02	0.03	0.22
Control5	7.50	2.36	0.26	0.80	0.05	0.02	0.04	0.32
Control6	5.50	2.04	0.26	0.73	0.05	0.02	0.04	0.37
Control7	5.96	2.49	0.21	0.63	0.07	0.02	0.07	0.24
Control8	5.66	2.22	0.19	0.59	0.05	0.03	0.04	0.28
Control9	5.52	1.94	0.18	0.72	0.04	0.03	0.03	0.30
Control10	5.54	2.01	0.18	1.04	0.04	0.04	0.03	0.55
Control11	4.35	1.81	0.26	0.71	0.04	0.02	0.04	0.27
Control12	5.80	2.27	0.23	0.77	0.07	0.02	0.04	0.27
	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank1	0.14	0.63	0.17	0.09	0.76	0.06	0.65	0.26
Tank1	0.13	0.59	0.16	0.12	0.93	0.08	0.65	0.19
Tank1	0.14	0.69	0.13	0.16	0.80	0.06	0.46	0.20
Tank1	0.14	0.65	0.15	0.11	0.79	0.04	0.65	0.29
Tank1	0.15	0.59	0.15	0.10	0.77	0.04	0.63	0.28
Tank1	0.15	0.65	0.16	0.10	0.77	0.04	0.63	0.26
Tank1	0.12	0.42	0.10	0.14	0.78	0.03	0.44	0.25
Tank1	0.13	0.62	0.14	0.12	0.79	0.06	0.74	0.29
Tank2	0.14	0.70	0.18	0.09	0.75	0.05	0.62	0.25
Tank2	0.09	0.64	0.12	0.33	2.13	0.15	0.39	0.10
Tank2	0.09	0.40	0.12	0.08	1.03	0.03	0.82	0.16
Tank2	0.09	0.25	0.06	0.07	0.65	0.03	0.40	0.14
Tank3	0.14	0.72	0.18	0.13	0.79	0.05	0.48	0.26
Tank3	0.12	0.61	0.17	0.11	0.92	0.05	0.61	0.28
Tank3	0.15	0.65	0.17	0.14	0.81	0.04	0.51	0.26
Tank3	0.14	0.62	0.17	0.11	0.69	0.04	0.44	0.24
Tank3	0.14	0.69	0.17	0.10	0.76	0.05	0.60	0.24
Tank3	0.10	0.51	0.12	0.19	1.20	0.04	0.59	0.18
Tank4	0.11	0.33	0.08	0.11	0.88	0.03	0.48	0.15
Tank4	0.09	0.33	0.07	0.12	0.92	0.05	0.38	0.11
Tank4	0.10	0.37	0.08	0.08	0.82	0.06	0.52	0.14

	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank4	0.13	0.33	0.10	0.08	0.74	0.04	0.46	0.14
Tank5	0.13	0.66	0.17	0.12	0.87	0.05	0.52	0.22
Tank5	0.14	0.66	0.17	0.12	0.87	0.06	0.61	0.22
Tank5	0.12	0.70	0.17	0.11	0.84	0.08	0.42	0.20
Tank5	0.15	0.75	0.19	0.10	0.70	0.06	0.48	0.24
Tank5	0.12	0.60	0.15	0.13	0.96	0.06	0.40	0.15
Tank5	0.13	0.63	0.16	0.19	1.01	0.05	0.46	0.22
Tank5	0.15	0.58	0.15	0.12	0.73	0.03	0.47	0.21
Tank5	0.14	0.66	0.16	0.11	0.84	0.08	0.53	0.20
Tank6	0.11	0.45	0.12	0.10	0.97	0.03	0.64	0.14
Tank6	0.09	0.38	0.10	0.08	0.89	0.04	0.57	0.16
Tank6	0.07	0.33	0.09	0.15	1.04	0.05	0.45	0.14
Tank6	0.09	0.38	0.11	0.11	1.04	0.05	0.68	0.16
Tank6	0.11	0.44	0.12	0.17	1.12	0.04	0.55	0.18
Control1	0.11	0.36	0.04	0.27	1.38	0.05	0.49	0.15
Control2	0.23	0.29	0.02	0.11	0.88	0.08	0.18	0.05
Control3	0.19	0.21	0.10	0.19	1.22	0.10	0.23	0.10
Control4	0.21	0.15	0.02	0.13	0.68	0.10	0.24	0.08
Control5	0.23	0.50	0.02	0.33	1.89	0.12	0.29	0.11
Control6	0.13	0.28	0.03	0.16	1.35	0.09	0.25	0.10
Control7	0.11	0.14	0.10	0.13	0.86	0.07	0.23	0.12
Control8	0.14	0.22	0.06	0.14	0.92	0.10	0.26	0.08
Control9	0.15	0.19	0.01	0.07	0.57	0.05	0.26	0.11
Control10	0.06	0.22	0.03	0.10	0.64	0.03	0.36	0.19
Control11	0.13	0.33	0.03	0.24	1.70	0.05	0.20	0.05
Control12	0.15	0.22	0.03	0.14	0.72	0.05	0.29	0.07
	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank1	3.60	0.17	0.85	15.81	0.03	0.17	0.14	0.29
Tank1	2.18	0.15	0.81	14.90	0.02	0.17	0.15	0.26
Tank1	3.08	0.14	0.66	14.79	0.19	0.10	0.02	0.25
Tank1	3.08	0.18	0.88	15.12	0.14	0.09	0.02	0.33
Tank1	2.73	0.17	0.83	15.48	0.14	0.12	0.03	0.29
Tank1	2.38	0.16	0.83	14.76	0.11	0.11	0.02	0.30
Tank1	2.92	0.14	0.68	14.57	0.16	0.11	0.02	0.28
Tank1	2.43	0.21	0.77	13.01	0.13	0.13	0.03	0.24
Tank2	2.70	0.17	0.88	15.02	0.03	0.13	0.13	0.28
Tank2	0.69	0.11	0.34	4.82	0.01	1.53	0.18	0.16
Tank2	3.23	0.18	0.46	11.67	0.01	0.00	0.14	0.15
Tank2	4.16	0.14	0.44	15.04	0.02	0.13	0.14	0.16
Tank3	2.90	0.17	0.92	15.50	0.20	0.10	0.02	0.32
Tank3	2.71	0.17	0.89	15.70	0.20	0.10	0.03	0.37

	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank3	2.36	0.15	0.85	15.36	0.14	0.10	0.02	0.33
Tank3	1.92	0.14	0.90	15.92	0.14	0.10	0.02	0.32
Tank3	2.22	0.16	0.82	14.57	0.13	0.11	0.02	0.29
Tank3	1.53	0.14	0.49	8.97	0.02	0.32	0.12	0.20
Tank4	1.84	0.12	0.44	11.77	0.19	0.11	0.02	0.17
Tank4	2.27	0.09	0.38	12.97	0.15	0.16	0.02	0.14
Tank4	2.25	0.12	0.42	12.05	0.14	0.10	0.03	0.16
Tank4	2.04	0.13	0.43	11.88	0.12	0.12	0.02	0.16
Tank5	2.07	0.15	0.86	14.57	0.19	0.14	0.02	0.30
Tank5	2.03	0.15	0.83	13.92	0.22	0.14	0.02	0.29
Tank5	3.47	0.14	0.68	15.67	0.31	0.16	0.03	0.28
Tank5	2.86	0.16	0.90	15.96	0.20	0.10	0.03	0.33
Tank5	2.10	0.12	0.58	12.47	0.38	0.13	0.03	0.20
Tank5	2.45	0.14	0.75	13.74	0.38	0.12	0.02	0.29
Tank5	2.13	0.13	0.75	13.85	0.15	0.09	0.02	0.26
Tank5	3.32	0.16	0.74	14.76	0.19	0.11	0.02	0.26
Tank6	3.08	0.16	0.45	12.14	0.16	0.17	0.10	0.16
Tank6	4.19	0.17	0.46	12.42	0.15	0.11	0.03	0.16
Tank6	1.96	0.11	0.45	11.50	0.25	0.14	0.02	0.19
Tank6	2.41	0.17	0.45	11.94	0.17	0.12	0.03	0.17
Tank6	1.47	0.14	0.50	10.18	0.32	0.11	0.03	0.20
Control1	4.00	0.16	0.37	12.32	0.49	0.13	0.02	0.12
Control2	3.03	0.08	0.39	15.21	0.15	0.11	0.02	0.12
Control3	2.88	0.11	0.32	14.81	0.24	0.16	0.03	0.11
Control4	5.07	0.11	0.31	17.43	0.09	0.15	0.03	0.09
Control5	2.30	0.12	0.43	14.10	0.75	0.28	0.06	0.15
Control6	3.63	0.12	0.46	18.94	0.25	0.15	0.04	0.14
Control7	3.97	0.10	0.33	19.41	0.12	0.14	0.03	0.15
Control8	4.68	0.13	0.31	18.32	0.21	0.15	0.03	0.12
Control9	5.74	0.12	0.39	16.73	0.09	0.11	0.05	0.10
Control10	8.83	0.25	0.54	21.48	0.08	0.14	0.06	0.11
Control11	1.90	0.07	0.42	16.71	0.40	0.15	0.02	0.17
Control12	2.69	0.10	0.34	16.09	0.12	0.10	0.05	0.12

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.24	0.64	2.18	22.48	0.44
Tank1	0.17	0.56	1.97	26.03	0.37
Tank1	0.30	0.53	2.28	21.11	0.36
Tank1	0.23	0.68	2.37	21.96	0.30
Tank1	0.21	0.66	2.41	23.38	0.31
Tank1	0.19	0.64	2.20	23.27	0.27
Tank1	0.24	0.62	2.31	25.47	0.33

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.20	0.56	1.91	24.84	0.37
Tank2	0.23	0.62	2.14	22.51	0.22
Tank2	0.11	0.15	0.79	6.59	0.18
Tank2	0.15	0.42	1.43	18.17	0.35
Tank2	0.17	0.41	1.57	25.74	0.42
Tank3	0.23	0.57	2.36	20.27	0.23
Tank3	0.22	0.65	2.30	23.05	0.31
Tank3	0.21	0.56	2.16	20.46	0.23
Tank3	0.16	0.54	2.04	25.79	0.22
Tank3	0.21	0.57	1.99	23.39	0.26
Tank3	0.18	0.37	1.39	14.81	0.27
Tank4	0.12	0.42	1.33	24.68	0.32
Tank4	0.10	0.37	1.22	28.58	0.42
Tank4	0.12	0.40	1.29	23.75	0.31
Tank4	0.16	0.46	1.43	26.39	0.31
Tank5	0.18	0.57	1.96	24.31	0.31
Tank5	0.17	0.57	1.96	24.44	0.28
Tank5	0.20	0.53	1.90	20.72	0.36
Tank5	0.21	0.61	2.10	20.96	0.31
Tank5	0.13	0.39	1.35	21.67	0.36
Tank5	0.21	0.57	2.19	20.60	0.25
Tank5	0.19	0.56	1.99	27.12	0.35
Tank5	0.24	0.58	1.97	23.62	0.37
Tank6	0.17	0.40	1.55	20.07	0.41
Tank6	0.17	0.37	1.45	19.46	0.45
Tank6	0.11	0.42	1.44	23.88	0.35
Tank6	0.17	0.43	1.58	16.78	0.40
Tank6	0.19	0.36	1.51	18.42	0.27
Control1	0.18	0.32	1.35	28.03	0.35
Control2	0.14	0.42	1.27	43.00	0.39
Control3	0.24	0.42	1.30	41.47	0.50
Control4	0.29	0.39	1.86	35.25	0.75
Control5	0.35	0.34	1.38	37.16	0.74
Control6	0.14	0.37	1.44	37.88	0.56
Control7	0.14	0.50	1.80	34.39	0.56
Control8	0.16	0.38	1.38	36.29	0.68
Control9	0.21	0.41	1.49	36.87	0.43
Control10	0.30	0.30	2.20	27.62	0.33
Control11	0.09	0.38	1.30	40.67	0.30
Control12	0.28	0.33	1.75	38.78	0.33

APPENDIX E Fatty acid proportions (% of total FA) of Atlantic pollock rest-of-body

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Tank1	2.22	0.07	0.34	16.97	0.35	0.33	3.28	0.19
Tank1	2.21	0.08	0.34	16.45	0.27	0.30	3.40	0.19
Tank1	2.25	0.08	0.34	17.62	0.30	0.34	3.70	0.18
Tank1	2.05	0.07	0.33	17.30	0.28	0.35	3.58	0.17
Tank1	1.82	0.06	0.32	17.36	0.27	0.34	2.90	0.18
Tank1	1.68	0.06	0.31	17.49	0.28	0.35	2.78	0.17
Tank1	1.31	0.05	0.26	17.41	0.25	0.32	2.31	0.15
Tank1	2.24	0.08	0.33	17.10	0.29	0.37	3.58	0.17
Tank2	1.91	0.07	0.30	16.02	0.31	0.33	3.34	0.18
Tank2	1.36	0.04	0.20	13.04	0.24	0.37	2.34	0.13
Tank2	1.46	0.04	0.21	13.84	0.26	0.45	2.09	0.14
Tank2	0.97	0.03	0.19	14.19	0.31	0.37	1.60	0.12
Tank3	2.24	0.08	0.30	16.18	0.28	0.31	4.48	0.16
Tank3	2.14	0.08	0.33	16.81	0.27	0.31	3.48	0.18
Tank3	1.62	0.06	0.27	15.69	0.26	0.34	3.32	0.15
Tank3	1.69	0.06	0.27	16.08	0.25	0.28	3.34	0.15
Tank3	1.97	0.07	0.31	16.48	0.28	0.32	3.30	0.17
Tank3	1.74	0.06	0.20	13.02	0.21	0.38	2.92	0.11
Tank4	1.37	0.05	0.17	14.33	0.18	0.36	2.35	0.10
Tank4	1.54	0.05	0.23	14.44	0.23	0.35	2.23	0.14
Tank4	1.38	0.05	0.20	13.56	0.21	0.35	2.18	0.12
Tank4	1.52	0.05	0.21	13.58	0.21	0.31	2.39	0.10
Tank5	2.37	0.08	0.33	17.04	0.38	0.32	3.95	0.20
Tank5	1.97	0.07	0.33	17.55	0.30	0.32	3.23	0.19
Tank5	2.34	0.08	0.34	17.32	0.29	0.32	3.78	0.19
Tank5	2.34	0.08	0.35	16.28	0.30	0.35	3.93	0.17
Tank5	2.48	0.09	0.33	17.31	0.28	0.33	4.50	0.17
Tank5	2.26	0.08	0.30	15.47	0.27	0.31	4.36	0.15
Tank5	1.76	0.06	0.31	17.00	0.27	0.30	2.89	0.17
Tank5	2.47	0.08	0.36	17.22	0.31	0.31	3.68	0.18
Tank6	1.50	0.05	0.21	13.49	0.26	0.41	2.21	0.13
Tank6	1.49	0.05	0.21	14.54	0.21	0.42	2.12	0.12
Tank6	1.59	0.05	0.22	14.14	0.21	0.34	2.25	0.14
Tank6	1.51	0.05	0.19	13.58	0.20	0.38	2.43	0.09
Tank6	1.75	0.06	0.21	12.74	0.22	0.37	3.22	0.12
Control1	1.21	0.04	0.20	15.93	0.25	0.34	2.24	0.16
Control2	0.57	0.04	0.26	16.46	0.46	0.27	1.00	0.17
Control3	0.49	0.03	0.23	15.98	0.43	0.28	1.00	0.15
Control4	0.48	0.03	0.23	16.21	0.45	0.35	0.95	0.15
Control5	0.47	0.04	0.21	15.12	0.36	0.30	0.94	0.16
Control6	0.50	0.03	0.25	14.78	0.34	0.31	0.93	0.19
Control7	0.48	0.04	0.24	15.78	0.26	0.31	1.10	0.17

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
Control8	0.48	0.04	0.22	15.91	0.31	0.29	1.09	0.16
Control9	0.37	0.04	0.20	17.24	0.33	0.32	0.71	0.15
Control10	0.49	0.06	0.29	16.08	0.26	0.30	1.16	0.18
Control11	1.27	0.12	0.32	14.60	0.37	0.27	2.30	0.25
Control12	0.48	0.03	0.19	17.60	0.28	0.34	0.99	0.16
	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
Tank1	0.18	0.21	0.34	0.34	0.23	0.21	5.77	0.40
Tank1	0.16	0.18	0.36	0.34	0.23	0.18	5.73	0.45
Tank1	0.17	0.22	0.34	0.35	0.30	0.21	5.83	0.49
Tank1	0.18	0.24	0.33	0.35	0.27	0.22	6.31	0.45
Tank1	0.15	0.22	0.26	0.35	0.19	0.17	6.23	0.57
Tank1	0.16	0.21	0.24	0.34	0.17	0.16	6.24	0.58
Tank1	0.13	0.24	0.18	0.31	0.13	0.16	5.88	0.62
Tank1	0.16	0.21	0.35	0.36	0.29	0.20	6.47	0.54
Tank2	0.18	0.19	0.34	0.35	0.23	0.20	6.14	0.52
Tank2	0.12	0.20	0.18	0.22	0.17	0.18	4.97	0.48
Tank2	0.13	0.15	0.18	0.21	0.15	0.20	5.40	0.38
Tank2	0.12	0.22	0.12	0.22	0.10	0.20	5.88	0.42
Tank3	0.17	0.15	0.38	0.36	0.38	0.25	6.25	0.45
Tank3	0.17	0.17	0.36	0.34	0.27	0.21	5.53	0.56
Tank3	0.15	0.22	0.25	0.32	0.22	0.19	5.80	0.60
Tank3	0.16	0.23	0.29	0.33	0.25	0.21	6.13	0.46
Tank3	0.16	0.20	0.34	0.34	0.21	0.21	5.87	0.49
Tank3	0.10	0.10	0.22	0.23	0.22	0.21	4.99	0.44
Tank4	0.09	0.17	0.16	0.19	0.16	0.20	5.28	0.37
Tank4	0.12	0.22	0.19	0.23	0.16	0.18	5.24	0.51
Tank4	0.10	0.18	0.19	0.23	0.17	0.19	5.08	0.49
Tank4	0.10	0.19	0.20	0.23	0.19	0.18	4.93	0.25
Tank5	0.17	0.21	0.39	0.34	0.30	0.24	5.59	0.47
Tank5	0.17	0.26	0.32	0.35	0.22	0.22	6.04	0.45
Tank5	0.18	0.20	0.37	0.34	0.30	0.24	5.67	0.39
Tank5	0.19	0.23	0.39	0.38	0.31	0.24	6.64	0.37
Tank5	0.17	0.25	0.42	0.33	0.39	0.25	5.86	0.42
Tank5	0.16	0.16	0.39	0.36	0.38	0.24	5.91	0.52
Tank5	0.17	0.24	0.27	0.35	0.18	0.17	5.74	0.65
Tank5	0.18	0.27	0.36	0.37	0.28	0.22	6.06	0.55
Tank6	0.12	0.16	0.18	0.23	0.16	0.21	5.01	0.46
Tank6	0.12	0.14	0.17	0.23	0.16	0.21	5.24	0.48
Tank6	0.09	0.16	0.20	0.22	0.17	0.16	4.78	0.51
Tank6	0.11	0.13	0.19	0.22	0.19	0.22	5.65	0.39
Tank6	0.10	0.13	0.22	0.21	0.21	0.22	4.41	0.50

	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
Control1	0.13	0.25	0.07	0.25	0.04	0.17	5.39	0.64
Control2	0.18	0.86	0.03	0.27	0.00	0.18	6.16	0.73
Control3	0.18	0.90	0.03	0.32	0.00	0.20	6.18	0.96
Control4	0.22	0.66	0.00	0.29	0.00	0.23	6.52	0.53
Control5	0.20	0.75	0.03	0.36	0.01	0.21	6.73	0.91
Control6	0.19	0.58	0.03	0.30	0.01	0.16	6.45	0.93
Control7	0.24	0.33	0.04	0.32	0.01	0.20	6.61	0.68
Control8	0.20	0.50	0.02	0.32	0.02	0.20	6.54	0.56
Control9	0.22	0.63	0.02	0.31	0.03	0.19	6.84	0.46
Control10	0.22	0.27	0.03	0.39	0.02	0.20	7.13	0.57
Control11	0.20	0.45	0.09	0.25	0.07	0.19	4.83	1.93
Control12	0.18	0.58	0.02	0.26	0.00	0.21	6.27	0.57
	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank1	10.38	3.08	0.15	6.19	0.20	0.11	0.34	1.00
Tank1	10.53	3.05	0.14	6.19	0.20	0.11	0.33	1.07
Tank1	12.69	3.14	0.16	7.03	0.20	0.12	0.31	1.12
Tank1	13.75	3.35	0.16	7.02	0.23	0.14	0.34	1.11
Tank1	11.61	2.91	0.15	7.08	0.20	0.12	0.34	1.02
Tank1	11.90	2.95	0.14	6.90	0.19	0.12	0.33	0.96
Tank1	12.47	2.73	0.15	7.21	0.14	0.10	0.24	0.90
Tank1	12.47	3.10	0.15	6.63	0.20	0.12	0.32	1.03
Tank2	12.18	3.33	0.16	6.36	0.23	0.12	0.36	1.06
Tank2	26.51	3.15	0.14	12.34	0.13	0.10	0.15	2.61
Tank2	23.98	3.06	0.13	11.45	0.12	0.09	0.17	2.54
Tank2	23.23	3.20	0.18	9.09	0.13	0.07	0.14	1.88
Tank3	16.79	3.90	0.17	8.23	0.28	0.16	0.33	1.29
Tank3	11.19	3.05	0.15	6.48	0.20	0.11	0.33	1.08
Tank3	15.62	3.42	0.18	8.30	0.21	0.14	0.29	1.12
Tank3	13.23	3.30	0.16	6.58	0.23	0.13	0.33	1.02
Tank3	11.65	3.09	0.14	6.72	0.20	0.11	0.34	1.07
Tank3	31.56	3.40	0.15	14.60	0.14	0.15	0.15	3.14
Tank4	29.68	3.20	0.14	11.43	0.12	0.13	0.13	2.38
Tank4	22.78	2.85	0.13	10.60	0.09	0.08	0.14	2.38
Tank4	26.08	3.05	0.12	11.71	0.13	0.12	0.16	2.67
Tank4	27.14	3.10	0.12	11.92	0.12	0.14	0.16	2.79
Tank5	12.71	3.41	0.20	6.53	0.23	0.13	0.35	1.15
Tank5	11.85	3.15	0.15	6.32	0.21	0.12	0.32	0.98
Tank5	12.24	3.37	0.22	5.79	0.21	0.11	0.31	1.02
Tank5	12.29	3.35	0.16	6.77	0.26	0.13	0.37	1.16
Tank5	14.75	3.65	0.24	6.30	0.24	0.14	0.32	1.11
Tank5	16.76	3.76	0.18	8.41	0.27	0.16	0.32	1.29

	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
Tank5	11.07	2.99	0.21	6.61	0.18	0.10	0.31	0.99
Tank5	11.54	3.11	0.16	5.91	0.20	0.11	0.32	1.03
Tank6	24.15	3.11	0.16	10.76	0.11	0.10	0.16	2.59
Tank6	25.67	3.11	0.15	10.90	0.12	0.14	0.15	2.50
Tank6	22.86	2.76	0.12	11.57	0.11	0.08	0.15	2.62
Tank6	31.11	3.43	0.19	11.80	0.15	0.15	0.17	2.81
Tank6	30.48	3.44	0.17	13.89	0.13	0.15	0.13	2.93
Control1	18.57	2.29	0.20	9.21	0.04	0.07	0.04	1.19
Control2	11.10	2.23	0.29	0.66	0.03	0.02	0.03	0.25
Control3	11.36	2.68	0.42	0.65	0.04	0.02	0.04	0.27
Control4	11.23	2.61	0.21	0.62	0.04	0.02	0.03	0.26
Control5	10.77	2.76	0.43	0.75	0.05	0.02	0.03	0.30
Control6	9.91	2.52	0.42	0.77	0.04	0.02	0.04	0.33
Control7	11.37	3.49	0.24	0.72	0.06	0.02	0.05	0.27
Control8	10.68	2.93	0.24	0.69	0.05	0.03	0.04	0.31
Control9	11.62	2.49	0.21	0.72	0.04	0.03	0.03	0.29
Control10	11.68	2.87	0.22	1.05	0.04	0.05	0.03	0.52
Control11	9.15	2.69	0.38	0.98	0.07	0.05	0.04	0.43
Control12	12.39	2.99	0.26	0.96	0.05	0.02	0.04	0.29
	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank1	0.16	0.57	0.15	0.11	1.14	0.11	0.72	0.28
Tank1	0.14	0.60	0.16	0.17	1.27	0.11	0.72	0.26
Tank1	0.14	0.67	0.13	0.21	1.28	0.10	0.54	0.24
Tank1	0.13	0.69	0.13	0.17	1.19	0.09	0.59	0.26
Tank1	0.16	0.55	0.14	0.15	1.14	0.07	0.62	0.29
Tank1	0.15	0.53	0.12	0.15	1.15	0.07	0.62	0.30
Tank1	0.14	0.40	0.07	0.18	1.26	0.07	0.52	0.25
Tank1	0.15	0.65	0.14	0.15	1.20	0.10	0.68	0.29
Tank2	0.16	0.63	0.16	0.13	1.09	0.08	0.63	0.26
Tank2	0.10	0.46	0.09	0.23	1.70	0.10	0.53	0.16
Tank2	0.10	0.39	0.08	0.13	1.39	0.07	0.71	0.17
Tank2	0.13	0.26	0.00	0.16	1.32	0.09	0.46	0.14
Tank3	0.12	0.84	0.17	0.24	1.42	0.11	0.46	0.22
Tank3	0.14	0.64	0.17	0.15	1.19	0.08	0.59	0.27
Tank3	0.13	0.64	0.15	0.24	1.44	0.12	0.52	0.24
Tank3	0.15	0.68	0.16	0.19	1.29	0.10	0.48	0.23
Tank3	0.15	0.61	0.14	0.11	1.12	0.10	0.60	0.26
Tank3	0.06	0.55	0.11	0.38	2.06	0.10	0.49	0.14
Tank4	0.08	0.46	0.10	0.26	1.74	0.09	0.41	0.12
Tank4	0.11	0.41	0.14	0.24	1.84	0.11	0.58	0.14
Tank4	0.10	0.44	0.08	0.20	1.49	0.08	0.47	0.15

	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
Tank4	0.10	0.47	0.08	0.18	1.57	0.10	0.43	0.12
Tank5	0.16	0.70	0.17	0.16	1.22	0.13	0.56	0.24
Tank5	0.15	0.58	0.15	0.16	1.17	0.10	0.65	0.26
Tank5	0.14	0.65	0.17	0.15	1.15	0.10	0.54	0.23
Tank5	0.15	0.72	0.18	0.15	1.13	0.09	0.54	0.25
Tank5	0.16	0.76	0.18	0.17	1.26	0.11	0.56	0.22
Tank5	0.13	0.82	0.18	0.29	1.68	0.14	0.47	0.23
Tank5	0.16	0.55	0.15	0.19	1.18	0.08	0.58	0.24
Tank5	0.17	0.65	0.18	0.16	1.21	0.11	0.63	0.24
Tank6	0.11	0.43	0.12	0.16	1.48	0.08	0.66	0.18
Tank6	0.08	0.40	0.12	0.13	1.42	0.07	0.63	0.18
Tank6	0.09	0.41	0.08	0.19	1.59	0.08	0.55	0.16
Tank6	0.07	0.46	0.12	0.18	1.42	0.08	0.50	0.15
Tank6	0.06	0.64	0.10	0.43	2.46	0.12	0.45	0.13
Control1	0.11	0.34	0.05	0.38	2.49	0.09	0.64	0.19
Control2	0.36	0.31	0.02	0.26	2.07	0.22	0.23	0.06
Control3	0.28	0.22	0.14	0.35	2.56	0.23	0.29	0.05
Control4	0.26	0.15	0.02	0.27	1.49	0.24	0.31	0.08
Control5	0.28	0.32	0.13	0.35	2.12	0.17	0.30	0.07
Control6	0.18	0.23	0.12	0.26	2.01	0.13	0.29	0.12
Control7	0.16	0.12	0.02	0.20	1.51	0.13	0.27	0.11
Control8	0.21	0.20	0.01	0.22	1.74	0.18	0.30	0.07
Control9	0.29	0.17	0.02	0.13	1.20	0.12	0.27	0.10
Control10	0.12	0.28	0.02	0.25	1.67	0.11	0.42	0.17
Control11	0.19	0.90	0.05	0.64	5.21	0.27	0.25	0.05
Control12	0.23	0.19	0.02	0.24	1.42	0.14	0.32	0.08
	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank1	3.41	0.18	0.81	13.86	0.03	0.34	0.18	0.29
Tank1	2.35	0.17	0.82	13.39	0.02	0.38	0.16	0.33
Tank1	2.99	0.15	0.67	12.32	0.49	0.17	0.03	0.29
Tank1	2.86	0.16	0.77	12.21	0.42	0.15	0.03	0.32
Tank1	2.93	0.15	0.74	13.07	0.36	0.18	0.03	0.30
Tank1	2.90	0.15	0.73	12.94	0.31	0.14	0.03	0.31
Tank1	3.21	0.14	0.61	12.25	0.39	0.18	0.03	0.26
Tank1	2.56	0.22	0.76	11.51	0.46	0.19	0.07	0.26
Tank2	2.83	0.17	0.78	13.31	0.02	0.33	0.16	0.31
Tank2	1.75	0.14	0.38	7.60	0.02	0.82	0.18	0.18
Tank2	2.66	0.16	0.42	8.89	0.01	0.41	0.15	0.17
Tank2	3.09	0.14	0.36	9.10	0.02	0.42	0.21	0.17
Tank3	2.21	0.15	0.75	11.27	0.75	0.18	0.04	0.33
Tank3	2.73	0.15	0.77	13.69	0.39	0.18	0.04	0.32

	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
Tank3	2.52	0.14	0.69	12.27	0.68	0.19	0.07	0.30
Tank3	2.16	0.14	0.77	12.72	0.54	0.19	0.03	0.33
Tank3	2.52	0.15	0.75	13.39	0.32	0.18	0.03	0.31
Tank3	1.00	0.12	0.36	5.49	0.01	1.15	0.15	0.17
Tank4	1.24	0.10	0.34	6.65	0.95	0.17	0.03	0.17
Tank4	1.73	0.13	0.38	7.95	0.72	0.25	0.04	0.16
Tank4	1.70	0.12	0.38	7.89	0.60	0.16	0.03	0.18
Tank4	1.39	0.12	0.38	7.42	0.68	0.18	0.03	0.17
Tank5	2.11	0.16	0.79	12.52	0.48	0.18	0.04	0.30
Tank5	2.44	0.16	0.77	12.47	0.36	0.18	0.03	0.27
Tank5	3.29	0.16	0.69	13.10	0.42	0.18	0.05	0.29
Tank5	2.74	0.16	0.80	13.00	0.38	0.15	0.11	0.33
Tank5	2.14	0.14	0.70	11.23	0.54	0.17	0.04	0.29
Tank5	2.09	0.15	0.72	11.06	0.94	0.18	0.04	0.32
Tank5	2.54	0.14	0.71	12.34	0.39	0.15	0.03	0.29
Tank5	3.02	0.16	0.72	12.39	0.41	0.19	0.04	0.29
Tank6	2.47	0.14	0.42	9.01	0.50	0.17	0.05	0.17
Tank6	2.72	0.15	0.39	8.22	0.44	0.19	0.04	0.16
Tank6	1.76	0.12	0.40	8.69	0.62	0.21	0.03	0.18
Tank6	1.50	0.13	0.37	6.94	0.58	0.15	0.03	0.17
Tank6	0.94	0.12	0.38	5.95	1.63	0.20	0.04	0.19
Control1	3.50	0.16	0.29	8.48	1.78	0.23	0.04	0.12
Control2	3.00	0.11	0.38	10.67	0.53	0.37	0.05	0.15
Control3	2.91	0.14	0.31	10.20	0.54	0.33	0.14	0.13
Control4	4.85	0.13	0.29	12.84	0.24	0.30	0.04	0.10
Control5	2.60	0.11	0.36	11.45	0.48	0.30	0.23	0.13
Control6	3.64	0.13	0.41	13.86	0.41	0.31	0.11	0.13
Control7	4.42	0.10	0.29	13.65	0.24	0.24	0.04	0.16
Control8	4.61	0.16	0.29	13.46	0.35	0.32	0.05	0.12
Control9	5.71	0.10	0.32	11.77	0.18	0.20	0.05	0.09
Control10	8.11	0.27	0.43	14.33	0.47	0.27	0.06	0.11
Control11	1.57	0.08	0.46	11.93	3.17	0.64	0.07	0.25
Control12	3.28	0.10	0.28	12.05	0.30	0.23	0.06	0.12

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.26	0.65	2.33	20.65	0.89
Tank1	0.21	0.60	2.17	22.71	0.78
Tank1	0.30	0.57	2.31	18.31	0.66
Tank1	0.23	0.60	2.19	17.76	0.47
Tank1	0.23	0.67	2.37	20.47	0.58
Tank1	0.22	0.68	2.31	20.92	0.58
Tank1	0.24	0.63	2.26	22.55	0.69

	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9
Tank1	0.22	0.57	2.03	20.36	0.68
Tank2	0.24	0.65	2.25	20.81	0.63
Tank2	0.19	0.33	1.24	13.83	0.61
Tank2	0.17	0.39	1.41	14.69	0.64
Tank2	0.18	0.35	1.47	18.07	0.91
Tank3	0.21	0.47	1.99	14.13	0.38
Tank3	0.21	0.60	2.19	21.09	0.62
Tank3	0.21	0.56	2.08	17.56	0.53
Tank3	0.19	0.57	2.06	21.32	0.55
Tank3	0.23	0.60	2.08	21.67	0.66
Tank3	0.13	0.23	0.97	7.60	0.27
Tank4	0.10	0.26	0.98	12.59	0.42
Tank4	0.13	0.32	1.26	17.39	0.88
Tank4	0.13	0.32	1.17	15.01	0.63
Tank4	0.15	0.32	1.18	14.36	0.44
Tank5	0.20	0.53	1.99	19.64	0.65
Tank5	0.20	0.59	2.06	21.58	0.56
Tank5	0.23	0.58	2.18	19.17	0.92
Tank5	0.23	0.61	2.22	18.44	0.60
Tank5	0.18	0.47	1.82	17.87	0.66
Tank5	0.21	0.49	2.01	14.97	0.44
Tank5	0.21	0.60	2.13	23.65	0.71
Tank5	0.27	0.60	2.15	20.36	0.80
Tank6	0.18	0.38	1.52	15.11	0.74
Tank6	0.17	0.34	1.33	13.42	0.77
Tank6	0.13	0.36	1.33	16.86	0.66
Tank6	0.14	0.29	1.18	9.73	0.49
Tank6	0.13	0.21	1.00	8.47	0.32
Control1	0.22	0.25	1.19	19.65	0.85
Control2	0.19	0.43	1.44	35.73	1.16
Control3	0.26	0.41	1.41	35.31	0.96
Control4	0.40	0.41	2.19	31.77	1.29
Control5	0.27	0.32	1.32	35.49	1.29
Control6	0.16	0.33	1.47	34.50	1.16
Control7	0.18	0.49	2.07	31.37	1.20
Control8	0.20	0.35	1.47	32.51	1.30
Control9	0.27	0.36	1.51	32.21	1.40
Control10	0.39	0.31	2.33	24.70	1.06
Control11	0.11	0.32	1.40	30.26	0.83
Control12	0.31	0.33	1.69	32.53	0.91

APPENDIX F Fatty acid proportions (% of total FA) for experimental diets

	14:0	i-15:0	15:0	16:0	16:1n-11	16:1n-9	16:1n-7	16:1n-5
FOCO1	4.03	0.12	0.30	12.84	0.17	0.15	4.48	0.12
FOCO2	4.40	0.12	0.32	13.32	0.19	0.16	4.85	0.13
FOCO3	4.07	0.12	0.31	12.87	0.17	0.14	4.51	0.12
FO1	8.56	0.26	0.64	20.17	0.32	0.23	9.95	0.22
FO2	8.51	0.26	0.63	20.20	0.33	0.23	9.93	0.22
FO3	8.34	0.25	0.63	20.17	0.32	0.23	9.81	0.22
	i-17:0	17:1(b)	16:2n-4	17:0	16:3n-4	17:1	18:0	18:1n-11
FOCO1	0.09	0.05	0.49	0.22	0.53	0.09	2.33	0.06
FOCO2	0.09	0.06	0.53	0.23	0.58	0.10	2.35	0.04
FOCO3	0.09	0.05	0.49	0.22	0.53	0.10	2.33	0.05
FO1	0.19	0.10	1.14	0.43	1.28	0.14	3.02	0.07
FO2	0.18	0.10	1.14	0.43	1.29	0.14	3.07	0.07
FO3	0.19	0.14	1.10	0.43	1.26	0.14	3.07	0.06
	18:1n-9	18:1n-7	18:1n-5	18:2n-6	18:2n-4	18:3n-6	18:3n-4	18:3n-3
FOCO1	32.69	2.84	0.07	17.35	0.10	0.10	0.11	4.82
FOCO2	31.15	2.82	0.08	16.88	0.11	0.11	0.14	4.66
FOCO3	32.69	2.82	0.07	17.45	0.09	0.10	0.13	4.87
FO1	9.02	2.81	0.13	10.04	0.25	0.24	0.33	1.80
FO2	9.04	2.81	0.12	10.09	0.24	0.24	0.32	1.79
FO3	9.14	2.82	0.13	10.32	0.24	0.24	0.32	1.81
	18:3n-1	18:4n-3	18:4n-1	20:1n-11	20:1n-9	20:1n-7	20:2n-6	20:3n-6
FOCO1	0.01	0.85	0.10	0.15	1.83	0.09	0.10	0.08
FOCO2	0.02	0.88	0.10	0.13	1.80	0.09	0.10	0.08
FOCO3	0.02	0.82	0.09	0.13	1.79	0.09	0.10	0.07
FO1	0.06	1.71	0.20	0.21	1.72	0.18	0.16	0.20
FO2	0.06	1.70	0.20	0.17	1.70	0.17	0.16	0.20
FO3	0.06	1.69	0.20	0.18	1.74	0.17	0.17	0.20
	20:4n-6	20:3n-3	20:4n-3	20:5n-3	22:1n-11	22:1n-9	22:1n-7	21:5n-3
FOCO1	0.41	0.05	0.36	4.41	1.89	0.22	0.07	0.20
FOCO2	0.46	0.05	0.39	4.75	1.89	0.22	0.07	0.22
FOCO3	0.41	0.05	0.36	4.38	1.86	0.22	0.07	0.20
FO1	0.94	0.11	0.87	9.59	1.89	0.23	0.08	0.46
FO2	0.95	0.12	0.87	9.59	1.86	0.24	0.09	0.45
FO3	0.95	0.12	0.86	9.54	1.95	0.25	0.08	0.45
	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:1n-9			
FOCO1	0.09	0.15	0.76	3.78	0.22			
FOCO2	0.09	0.17	0.82	3.99	0.22			
FOCO3	0.09	0.14	0.75	3.71	0.22			
FO1	0.20	0.36	1.78	7.43	0.29			
FO2	0.20	0.36	1.79	7.45	0.29			
FO3	0.19	0.36	1.77	7.43	0.29			