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Changes in Tissue-Specific Fatty Acid Composition of the Freshwater Alewife (Alosa pseudoharengus) in Response to Temperature

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Abstract of Thesis

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A balance between fluidity and rigidity of cell membranes is essential for proper cell membrane function. Ectotherms are known to alter the composition of the cell membrane to counter the increased order (when challenged with decreasing temperatures) or disorder (when challenged with increasing temperatures) that results from changes in environmental temperature. One mechanism by which this can be accomplished is the alteration of the fatty acid composition of affected cellular membranes. In this study, tissue-specific fatty acid composition in the alewife (*Alosa pseudoharengus*) was examined in response to both warm and cold temperature challenges administered over an approximate one month period. Gill, muscle, and liver tissues were analyzed prior to the start of the temperature challenge (initial), following the temperature challenges (survivors), and on those fish that did not survive the temperature challenges (mortalities). Significant differences were found between fatty acid composition of initial fish and survivors for membrane-incorporated fatty acids (polar) and for stored fatty acids (neutral). In the cold challenge, gill tissues exhibited significant remodeling in membrane fatty acids (polar), including decreases in palmitic acid and saturated fatty acids and increases in highly unsaturated fatty acids. In the warm challenge, significant increases in saturated fatty acids were observed in polar lipids of muscle and liver tissue. Notably, a large increase in palmitic acid (C16:0) was observed in response to increased temperatures. Fatty acid profiles of fish that died during the cold challenge exhibited significantly higher levels of C16:0 in muscle tissues when compared to survivors. The observed changes in membrane (polar) fatty acids would be expected to promote appropriate membrane fluidity in response to temperature. Results of this study suggest that freshwater alewives respond to temperature challenges in accordance with what would be predicted by homeoviscous adaptation, although the pattern and extent of changes in response to temperature differed greatly among the tested tissues.

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Of particular importance to me, I greatly value the time spent with Dr. Snyder in the laboratory validating the analytical procedures and ensuring I was fully comfortable with all aspects of the lab work prior to collecting any data. The extra time that was spent demonstrating accuracy and reproducibility of the procedures provided a confidence that allowed us to complete a considerable amount of analytical work in a relatively short period of time. What could have been a frustrating and time consuming endeavor was instead rewarding throughout. The benefit of this approach is something I will never forget.

I would also like to thank my committee members and the professors with whom I studied during my experience at Buffalo State. My professional career has and will continue to benefit from the education and confidence I gained as a student at Buffalo State.

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Abbreviations and Shorthand Notation	
ARA	Arachidonic acid (C20:4n6)[1]
DHA	Docosahexanenoic acid (C22:6n3)
EPA	Eicosapentaenoic acid (C20:5n3)
HUFA	Highly unsaturated fatty acids
MUFA	Monounsaturated fatty acids
n3	Omega-3 series fatty acids
n6	Omega-6 series fatty acids
PUFA	Polyunsaturated fatty acids
SAFA	Saturated fatty acids

[1] The shorthand notation used in the text, tables, and figures below indicates the following; Carbon chain length (C20 in this example), the number of double bonds in the carbon chain (4 in this example), and the position of the first double bond counting from the methyl end of the fatty acid (n6 in this example).

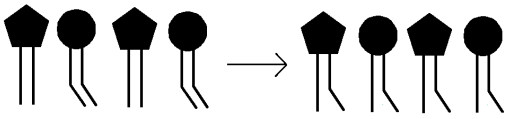
INTRODUCTION

Many organisms are known to address thermodynamic changes in membrane fluidity by altering the molecular composition of the biomembrane, a physiological response known as homeoviscous adaptation. Homeoviscous adaptation is common in ectotherms, and we know of several mechanisms by which organisms can modify the cell membrane in response to changing temperatures.

One approach is to alter membrane cholesterol incorporation. Cholesterol interacts with the hydrophobic fatty acid tails of phospholipids and thereby increases the rigidity of the cell membrane (Alberts et al. 2002). Increased incorporation of cholesterol is known to occur in response to increased environmental temperatures, whereas reduced incorporation occurs as temperatures decrease (Robertson and Hazel 1995). It is important to note that the thickening effect of cholesterol is actually reversed at temperatures near zero Celsius. As temperatures approach zero, cholesterol molecules contained in the phospholipid bilayer, instead of promoting thickening, disrupt bonding and can inhibit phase transitions (Alberts et al. 2002). However, with regard to changes

in cholesterol incorporation in response to ambient temperature, cholesterol incorporation increases in response to increasing temperature within the range of temperatures normally experienced by most ectotherms.

Another mechanism by which homeoviscous adaptation can occur is molecular species remodeling, which involves an adjustment of fatty acid position/pairing in existing membrane phospholipids (see image below). In this response, total fatty acid composition (or signature) remains unchanged; modification of membrane fluidity is achieved by altering the pairing or assortment of fatty acids, and may even be further modified by the chemical character of the polar head group to which they are bound. The reason for the change in membrane fluidity despite the identical molecular makeup of the membrane is that certain fatty acids, when associated with particular polar head groups, are more fluid than others. In response to a cold challenge, the fatty acid/polar head group combinations may undergo modification to favor a greater number of those combinations that contribute to fluidity. Molecular species remodeling has been observed in rainbow trout (Hazel and Landrey 1988) and carp (Fodor et al. 1995) in response to decreased temperatures.



Molecular Species Remodeling

A third means of adjusting biomembrane fluidity is to alter incorporation of the various common phospholipid polar head groups. Increases in phosphatidylinositol and phosphatidlyethanolamine (both have a destabilizing effect on the biomembrane) and decreases in phosphatidylcholine (stabilizing effect) have been observed in response to decreasing temperatures (Greene and Selivonchick 1987, Henderson and Tocher 1987).

One of the more commonly employed mechanisms of membrane fluidity adaptation is a change in the degree of saturation of phospholipid-bound fatty acids. Specifically, during cold acclimation the relative abundance of polyunsaturated (carbon chain containing more than one double bond) and highly unsaturated fatty acids (carbon chain containing four or more double bonds) increases as the abundance of saturated (carbon chain containing no double bonds) fatty acids decreases (Henderson and Tocher 1987, Snyder and Hennessy 2003). This response is not only common among fish species (Hazel and Williams, 1990), but is observed in microorganisms, fungi, plants, and invertebrates (Hazel 1995).

My research is principally aimed towards changes in fatty acid composition as they pertain to predicted effects on membrane fluidity. With respect to the organism of interest in this research effort, the alewife, a possible failure to effectively modify membranes in response to temperature is of interest for several reasons. First, of the several mechanisms by which cell membranes can be modified to respond to changes in environmental temperatures, changes in the fatty acid composition in response to temperature is not only a common approach but has been demonstrated in alewives with analysis of whole body fatty acid profiles (Snyder and Hennessy, 2003).

Secondly, the increase in the degree of unsaturation of acyl chains in cell membranes in response to decreased temperatures is in part accomplished by increased incorporation of long chain, n3 polyunsaturated fatty acids. The ability to incorporate higher amounts of these fatty acids in membranes is largely dependent on diet. Several physiologically important long chain, n3 polyunsaturated fatty acids (DHA, EPA, ARA) are considered essential fatty acids for fish due to inability of any known fish species to

synthesize them from shorter chain (18:3n3) fatty acids (Sargent et al. 2002). [As described in the terminology table above, the shorthand notation for fatty acids indicates the length of the fatty acid carbon chain (18 in this example), the number of double bonds contained in the fatty acid (3 in this example), and the position of the first double bond counting from the methyl end of the fatty acid (n3 in this example).] Should these EFA's not be readily available in diet, the deficiency, which has been demonstrated to adversely affect numerous metabolic processes in fish (Roberts 2002, Sargent et al. 2002), would not be addressable by modification of shorter chain unsaturated fatty acids.

Lastly, and strongly associated with the point above, is that alewives are primarily anadromous and as such have evolved with a diet rich in PUFA's. However, freshwater populations of alewives that do not have the dietary availability of n3 polyunsaturated fatty acids of their anadromous relatives may be more susceptible to temperature change due to the decreased dietary availability of EFA's.

Storage lipids

In addition to the fatty acid composition of membranes, fatty acids primarily stored as triacylglycerols are also of interest. Triacylglycerols are stored in a variety of tissues including muscle, liver, and mesenteries. Triacylglycerols serve as a major energy reservoir in a variety of animals, and provide approximately five times the energy provided by an equal weight of glycogen (Berg et al. 2002). As a primary energy storage molecule, triacylglycerol levels increase when food is abundant and decrease when food is scarce. These lipid stores are of critical importance to fish health as they are necessary

for overwinter survival, successful reproductive performance and development, and adequate response to environmental stress (Adams 1999).

Though fatty acids stored as triacylglycerols do not directly impact membrane function, availability of fatty acids as triacylglycerols is important during periods of food shortage or starvation. As triacylglycerol stores are depleted, mobilization of membrane associated fatty acids occurs (Tocher and Sargent 2003). The use of structural lipids (phospholipids) for energy production when food is scarce may cause changes in membrane composition away from the optimal state. The use of membrane fatty acids in response to low stored fatty acid reserves appears to be a contributor to the compromised osmoregulation observed in lipid deprived fish species (Adams 1999). Consequently, membrane and stored fatty acids are intimately tied.

Stored fatty acids may also respond to temperature change to support and promote membrane change should modification in membranes be occurring. Stored fatty acids are a reservoir of energy, but may also serve to store physiologically important fatty acids for use as structural components when needed (as phospholipids). This would be particularly critical for an organism for which the necessary fatty acids are not readily available through the diet or cannot be synthesized endogenously.

Addressing changes in the fatty acid profiles of storage lipids when analyzing lipid response to temperature change is becoming increasingly important. Though homeoviscous adaption theory was originally based on the response of membrane lipids, there is evidence that suggests homeoviscous adaptation applies to stored lipids as well. Stored lipids undergo physical property changes in response to low temperatures just as phospholipids do (by increased acyl chain bonding/association). This effect can cause

triacylglycerols to solidify to a point that they are not suitable for lipase activity. As such, they may become unsuitable as an energy source (Kostal and Simek 1998). Similar to the response that is observed in membranes challenged with decreasing temperatures, increased unsaturation of the involved fatty acids helps alleviate the temperature induced cellular stress. Therefore, maintenance of fluidity may be just as critical to stored lipids as to those lipids contained in the cell membranes (Van Dooremalen et al. 2010).

Temperature Acclimation

The importance of fatty acid compositional changes in response to decreased temperatures has been extensively studied. Salmon from different latitudes show a pattern of elevated highly unsaturated fatty acid DHA (C22:6n3) levels with decreasing temperatures, suggesting an important role for DHA in temperature acclimation (Olsen 1999). Similarly, DHA levels increased in plasma phospholipids of cold acclimated trout (Wallert and Babin 1994). In addition to studies that examined fatty acid compositional changes in cold challenged fish, other studies in which dietary levels of PUFA were modified also demonstrate the importance of fatty acid modification to survivability. Fish fed higher dietary levels of PUFA demonstrated higher cold tolerance (Craig et al. 1995, Kelly and Kohler 1999). Furthermore, a study performed with alewives (*Alosa pseudoharengus*) indicated higher levels of membrane saturated fatty acids and lower levels of DHA in whole body extracts of cold challenged fish that were not able to survive a cold challenge (Snyder and Hennessey 2003).

Relative to cold acclimation, our understanding of warm acclimation as it pertains to changes in lipid dynamics is not as extensive. The effects of high temperature on

nutritional requirements and fatty acid signatures are poorly documented (Person Le-Ruyet et al. 2004). In a study using sea bass, warm acclimated fish held at either of two temperatures (22° and 29° C) showed a greater influence of temperature on membrane lipid than storage lipid. Furthermore, the group maintained at the higher temperature (29°C) demonstrated lower levels of HUFA. Both observations are in accordance with predictions based on homeoviscous adaptation theory, i.e., increased saturation in response to decreasing temperatures and decreased saturation in response to increasing temperatures (Person-Le Ruyet 2004).

Fish can adapt relatively quickly to higher temperatures, often in less than 24 hours; conversely, adaptation to lower temperatures is a slower process, and requires up to 20 days in some species (Doudoroff 1942, Brett 1944). The rate at which acclimation to warmer temperatures occurs suggests that this response may be of less importance to an organism's survival than cold acclimation. However, study of the response to warmer temperatures is of value as it pertains to investigation of homeoviscous adaptation: response in fatty acid composition, particularly in the membranes, would be expected to behave in opposition to changes observed for cold challenged fish (Hazel and Williams 1990).

Tissue-Specific Fatty Acid Composition

Different species of fish employ different strategies for fat deposition and mobilization, and the location and availability of these stores have been shown to affect survival in response to temperature change (Kelly and Kohler 1999). Similarly, tissue-specific differences in fatty acid composition are common in fishes due to the many

physiological processes in which fatty acids are involved. Lower levels of DHA (C22:6n3), n3 fatty acids, low n3/n6 ratios and elevated levels of ARA (C20:4n6) have been observed in gill membrane lipids when compared to other tissues (Fountoulaki et al. 2003, Skalli et al. 2006). Lower levels of C18:2n6 and EPA (C20:5n3) were observed in liver storage lipids when compared to gill and muscle tissues of European Sea bass (Skalli et al. 2006). Fatty acid signatures of tissues, and any responses exhibited in tissue fatty acid composition due to temperature change, have the potential to vary for different tissues due to the many functions fatty acids serve. Where a decrease in DHA might be predicted for both gill and liver tissue in response to increasing temperatures, such a change may not occur for reasons other than the predicted need to modify membrane fluidity (e.g. DHA requirement as a precursor for docasenoids in gill tissue, or increased preservation of a scarce essential fatty acid in liver neutral lipids).

Alewives (*Alosa pseudoharengus*) in the Great Lakes

The species of fish used in this research is the alewife, which inhabits many northeastern U.S. lakes (including all of the Laurentian Great Lakes and the New York State Finger Lakes). Alewives are an important source of food for salmon and trout (Stewart and Ibarra 1991), and as such changes in alewife populations are predicted to affect the population size and health of these predatory game species. Population dynamics of freshwater alewives remain poorly understood, and of particular concern, this species is subject to periodic mass mortalities. One of the most notable of these events occurred in 1967, when an alewife fish kill of an estimated 300 million pounds occurred in Lake Michigan (Brown 1972). The unpredictability and size of alewife mass

mortalities are consequently of great interest due to their potential impact on the yield of game fish stocking efforts.

Several possible causes for these mortalities have been explored. Included in these are shortages in food supply, failure to adequately osmoregulate, failure to extract iodine, and combinations of these potential causes (Colby 1973). However, studies of these mechanisms as contributors to alewife die-offs have in many cases been inconclusive and sometimes contradictory. One example of such a contradiction was reported by O'Gorman and Schneider (O'Gorman and Schneider 1986), where alewives in poor condition prior to the winter season did not suffer unexpectedly large mortalities. Another example involves a study of the Lake Michigan mortality in 1967 in which it was noted that many of the dead alewives collected were in robust condition (Brown 1968). Despite efforts to date, the underlying physiological reasons for the mass-mortalities remain poorly understood and the predictability of die-offs is inadequate for modeling freshwater alewife populations.

Another physiological mechanism which has been considered as a factor in temperature acclimation in alewives is maintenance of appropriate biomembrane fluidity. As discussed above, with changes in temperature the interactions among membrane-bound phospholipids change (with respect to fatty acids as well as polar head groups). Changes in membrane fluidity are known to affect several cellular functions, including the activity of membrane bound enzymes, cell division, and channel function; changes in channel function in particular may lead to osmoregulatory difficulties, which have been reported for alewives subjected to low temperatures (Stanley and Colby 1971). As mass mortalities often coincide with colder than average winters (Colby 1973, O'Gorman and

Schneider 1986) and low water temperatures are believed to adversely alewife growth and survival (Brown 1972, Colby 1973), a connection between changes in biomembrane fluidity and the health of freshwater alewife populations is evident. A temperature-induced loss of biomembrane fluidity might be significant enough to have a deleterious effect on an organism's health, and a failure to compensate for these changes may result in decreased survival and the occurrence of large mortality events.

The purpose of this study is to identify temperature-induced changes that occur in fatty acid signatures in each of three tissues (gill, muscle, liver) of freshwater alewives. Assessment of fatty acid signature changes, by comparing difference in both individual fatty acids and in fatty acid classes, may provide additional evidence of homeoviscous adaptation in this species as well as a more complete view of the role of fatty acids in the physiology of fishes in general in response to temperature change.

MATERIALS AND METHODS

Overview of the experimental design

In this laboratory-based experiment, alewives were maintained at a constant temperature (15 °C) and fed a lipid-rich diet of frozen *Mysis* (a small freshwater shrimp) for 9 weeks. Immediately following the acclimation period, two groups (N=25 for each group) of fish were subjected to a 4-5 week increasing temperature challenge, and two groups (N=25) were subjected to an approximately 30-day decreasing temperature challenge. Temperature challenges were executed by changing temperature 0.5° C per day (increasing or decreasing), intended to be reflective of what might be expected in seasonal temperature shifts. This rate of temperature change also afforded the fish the opportunity to undergo membrane modification in response to the stress.

Fatty acid composition of gill, liver, and muscle tissues were determined for fish samples taken prior to the temperature challenge (*initial*), fish that died during the trial (*mortalities*), and fish that survived the trial (*survivors*). Polar (membrane-incorporated) and neutral (triaclyglyceride) fatty acid fractions were analyzed to allow for independent observation of changes that occur within biomembranes and stored fatty acids, respectively. Comparison of *initial* and *survivor* samples allowed for determination of temperature (warm vs. cold) and tissue-specific modification of fatty acid composition. Analysis of warm and cold temperature *mortalities* were used to determine if these fish failed to modify fatty acid composition in the same manner observed in their respective *survivor* group.

Laboratory Temperature Challenge

Approximately 400 adult alewives were procured from Waneta Lake in the Finger Lakes Region of central New York, USA and were transported to the Buffalo State College Aquatic Research Laboratory for this study. Individuals selected for use in the temperature challenge were similarly sized and weighed approximately 20 grams each. Selected individuals were distributed among four 760-liter circular tanks to provide a final density of 50 fish per tank. Experimental tanks initially shared a common recirculating water supply and biofiltration system equipped with a mixed media filter and carbon filter. Tank temperature was regulated using two in-line 1.5 horsepower chilling units. Water contained 1% sodium chloride to minimize osmotic stress.

During the conditioning stage of the study, tank temperature was maintained at 15.0 ± 0.5°C for 9 weeks. During this conditioning phase, fish received feedings of frozen *Mysis* twice daily. *Mysis* was used as it is a natural high quality food source with a high lipid content, and it is rich in essential fatty acids EPA (C20:5n3) and DHA (C22:6n3). Thawed *Mysis* were provided in a daily ration constituting approximately 2-3% of total alewife mass (number of fish x approximate wet body fish weight). *Mysis* were procured from Piscine Energetics Inc, British Columbia, Canada.

An Aquanode monitoring system (Aquadyne, San Diego, CA) was used to continuously monitor water temperature, pH, and dissolved oxygen concentrations. A 12 L:12 D photoperiod was used for the duration of the experiment. At the culmination of the nine week acclimation period, five fish were removed from each of the tanks and were euthanized with Ethyl 3-aminobenzoate methanesulfonate (MS-222) at 275 mg/L.

These fish were placed in plastic bags, fully immersed in water, and sealed prior to storage. Samples were frozen and stored at -80°C for analysis at a later date. Length (cm) and weight (g) of these *Initial Fish* were recorded.

The alewives were then subdivided into four groups, each in its own separate tank and consisting of 25 fish. Two tanks were subjected to an identical warm (increasing) temperature challenge, and the remaining two tanks were subjected to a cold (decreasing) temperature challenge. In-line electric heaters were used to gradually increase the water temperature (0.5°C / day) of the warm challenge tanks from the initial temperature (15°C) to approximately 34°C over 39 days. The cold challenge tanks were set to gradually decrease in temperature (0.5°C / day) from the initial 15°C to approximately 1°C over 31 days. The duration of the temperature challenges was based on the occurrence of mortality: challenges were continued until individual mortalities numbers were adequate for analysis. Following completion of the temperature challenges, all surviving fish were euthanized with MS-222 at 275 mg/L. Fish were placed in plastic bags, immersed in water, and were frozen and stored at -80°C as described above for *Initial Fish.* Photoperiod and feeding schedule during the temperature challenges were consistent with those used in the conditioning period. Alewives were fed daily during the temperature challenges; however, the feeding schedule was modified towards the end of the temperature challenges to account for declining feeding rates.

Sampling

At each daily feeding, tanks were examined for *mortalities* (deceased fish). *Mortalities* were removed from the tanks upon observation, measured (cm length) and weighed (g). Carcasses were sealed in plastic bags, labeled (treatment, tank, and date) and stored frozen in water as describe above (-80°C) for subsequent analysis of fatty acid signatures.

At the culmination of the temperature treatments (~30 days from start of the temperature challenge), all surviving fish were sacrificed with a lethal dose of MS-222. All fish were measured, weighed, stored and processed as described above for the *initial* and *mortality* samples. A summary of the design of the temperature challenges and overall survivorship during the experiment is provided in Table 1.

Determination of Fatty Acid Signatures

Determination of fatty acid signatures is a three step process, and each of these is described in greater detail in the sections below. For detailed descriptions of analytical protocols, reference Appendixes A, B, and C. In short, tissues were removed from collected fish, after which fatty acids bound to phospholipids and triglycerides were extracted. Extracted fatty acids were separated into polar (phospholipids) and neutral (triglycerides) fractions to allow independent analysis. These products were then derivitized, a process which essentially frees the fatty acids from the glycerol backbone and facilitates detection. Fatty acid derivatives were then analyzed using gas chromatography.

General Laboratory Procedures and Sample Handling

Tissue samples were kept under nitrogen and sealed with Teflon-lined caps to minimize oxidation of fatty acids. Solvents used in volumes in excess of 1 ml were supplemented with the addition of 2,6-Di-*tert*-butyl-4-methylphenol (BHT, Sigma CAS 128-37-0) added at 10mg/L to inhibit oxidation of samples. Samples were spun in a centrifuge for 10 minutes to separate tissue from solvent, or aqueous phase from organic phase. All sample/solvent transfers were performed in glass. Reusable glassware was washed thoroughly, rinsed in methanol, and dried completely before re-use to prevent sample-to-sample contamination.

Sample Selection and Preparation

A minimum of three samples were prepared for each of five sampling points. Sampling points included *initial fish*, *warm survivors*, *cold survivors*, *warm mortalities*, and *cold mortalities*. Individual samples consisted of two fish (composite sampling) from which each of three tissues were removed; gills, liver, and muscle. Composite samples were used to ensure that enough tissue was available for reproducible and accurate fatty acid analysis. Prior to the start of the experiment, *mortality* samples were to be selected such that the sampling dates for both the warm and cold *mortalities* were similar for comparative purposes. The majority of the mortalities occurred during the end phase of both warm and cold temperature challenges and made selection of temporally comparable *mortality* samples possible. Furthermore, *mortalities* occurred for both the warm and cold temperature challenge in numbers large enough to allow for the preparation of composite samples.

Tissue collection

Ice was removed from frozen fish samples by immersion in lukewarm water. Following removal of ice, fish were set aside to thaw at room temperature (~ 30 minutes), after which tissue samples were excised and weighed. All sample weights were recorded as wet weight (mg).

Gill Samples: Following removal of the operculum, gill structures were excised from the fish and set on a dissection tray to isolate the gill tissues from the surrounding structure (see Appendix A). Scissors were used to collect as much of the gill tissue (avoiding gill rakers and supporting structure) as the sample affords. Excised gill tissue was weighed (wet weight) on a Mettler digital analytical balance and recorded. Weights of gill tissue were recorded separately for each of the two composite fish and summed to provide a maximum sample weight. Tissue collection from the composite was adjusted by removing sample from the greater of the two tissue yields so that contribution of each tissue to the total sample would be approximately 50%.

Liver Samples: A cut was made along the ventral side of the fish from the anal fin to the operculum. Another cut was then made from the anal fin to the lateral line, after which the cut moved laterally to the dorsal-most portion of the operculum. Tissue (muscle/ribs) was pulled back with a pair of forceps to expose the liver. This method causes minimal liver tissue disruption, facilitating a clean collection of the tissue even when the integrity of the liver is compromised. From each of the two composite fish, approximately 150 mg of tissue were removed for a total of approximately 300 mg. As

with the gill samples, tissue collections were adjusted so that the collection from each fish contributed approximately 50% of the total sample weight.

Muscle Samples: Skin was removed from the fish, starting at the lateral line and moving upwards towards the dorsal fin. Using a paper towel, fat and other subcutaneous material were gently abraded from the exposed muscle. Using a scalpel, a small wedge of muscle tissue was excised from the dorsal portion of the fish. Approximately 150 mg of tissue was collected from each of the composites to provide a 300 mg sample.

As mentioned above, collections of the three tissues were intended to obtain approximately 300 mg samples. However, in cases where sample availability was below 300 mg due to a low body weight or poor tissue integrity of one or both fish used for the composite, smaller samples in the 150 mg - 200 mg range were used and are more than adequate as the method employed has been successfully validated for samples as small as 20 mg (Herbes and Allen 1983). If a fish had degraded or was of questionable quality, the questionable sample was discarded and replaced with another from its group. For all three tissue types, poor quality samples were identified by low tissue integrity/firmness.

Fatty Acid Extraction

The extraction procedure used was similar to a lipid microquantitation procedure reported by Herbes and Allen (1983), which produces consistent lipid recoveries with relatively small biological tissue samples. The extraction process was performed twice for each sample, as a second extraction improved fatty acid recovery in our lab and facilitates a cleaner transfer of the extract. The ratio of tissue to organic solvent used (1:20 assuming a 300 mg tissue sample) is sufficient for extraction of tissues with lipid

content in excess of those found in this study (Iverson et al. 2001), and eliminates concerns with tissue-specific differences in total lipid content, or variations in the extraction efficiency of specific fatty acid types due to affinity/solubility in the organic solvent.

Tissue samples were transferred to a 20 mL glass vial containing 6 mL of a 1:1 chloroform/methanol mix (10mg/L BHT). Samples were homogenized for 1 minute using a homogenizer (Brinkman polytron) and visually inspected to ensure that homogenization was thorough. Samples were transferred to 15 mL glass tubes, sealed under nitrogen, and spun down (Clinical Centrifuge) to pellet the tissue residue. The supernatant was transferred to a glass conical tube containing 3 ml of distilled water to form a biphasic separation (Folch et al. 1957). This sample was vortexed for 1 minute, then centrifuged as before, after which the organic (bottom) layer was transferred to a second 15 mL glass tube (collection tube). A second extraction was performed on the remaining aqueous phase using an additional 3 mL of chloroform, a volume which maintains the organic/aqueous solvent ratio reported by Iverson (Iverson et al. 2001). The extraction process was repeated (vortex/spin), and the second organic aliquot was added to the original collection tube. This extraction was then placed in a warming block (~40°C) and dried under a stream of nitrogen. After the solvent completely evaporated, the sample was reconstituted in 500 uL of hexane.

Polar/Neutral Fraction Separation

Separation of polar (membrane) and neutral (storage) fractions was performed using solid phase extraction columns by a method previously described (Juaneda and

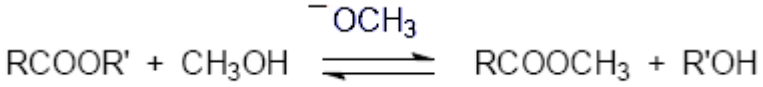
Rocquelin 1985). The choice of eluent alters the affinity of the analytes (polar vs. neutral) to the silica stationary phase. The solubility of the neutral fraction in chloroform is greater than that of the polar fraction, allowing for a fast elution of the neutral fraction and retention of the polar fraction. The retained and theoretically isolated polar fraction was eluted using methanol (decreases affinity of polar fraction to silica). Efficient separation of the two fractions was validated prior to running test samples by measuring the recovery of known amounts of applied lipid fraction. Fish oil (triaclyglycerides) and phosphotidylcholine (Sigma) were used for this purpose.

Prior to sample loading, solid phase extraction cartridges (Sep-pack "classic" SPE cartridges, 690 mg Waters) were charged with 5-10 mL of chloroform. Lipid extracts in hexane (~500 uL) were transferred to the column, and elution of the neutral fraction was achieved by passing 20 mL of chloroform in 60 seconds using a glass syringe. The polar fraction was subsequently eluted by passing 30 mL of methanol through the cartridge, again for 60 seconds. Fractions were collected in glass 250 mL round flasks, and solvent was removed using a rotovap (~45-50°C, vacuum~18 inches HG). After solvent removal, two chloroform rinses of 2 mL each were used to recover lipid fractions from the flasks. Recovered fractions were transferred to 15 mL round bottom glass tubes and dried down under nitrogen. Fractions were reconstituted in 1 mL toluene for storage and subsequent methanolysis.

SPE cartridges were rinsed with 20 mL methanol and 20 mL chloroform prior to the next use. A single SPE cartridge was used for the separation of 6 fractions (Juaneda and Rocquelin 1985), after which it was discarded.

Fatty Acid Methyl Ester (FAME) synthesis:

Fatty acids were derivitized to facilitate detection/separation during gas chromatography. Underivitized fatty acids are non-volatile and present adsorption issues during analysis. Fatty acids methyl esters (FAME) are more easily vaporized and therefore move through the GC column more efficiently. Additionally, FAME are more stable than their fatty acid counterparts, which is beneficial both for analysis and extended periods of storage. Base catalyzed methanolysis (reaction below) provides an inexpensive, fast, and efficient (95%+ yield) means of derivitization and was employed in this research effort.



The process is a transesterification which dissociates the fatty acid from the glycerol molecule and replaces the newly available bonding site on the fatty acid with a methyl group (CH_3). This derivitization is sufficient for both phospholipid and triacylglyceride-bound fatty acids and was therefore usable for both the polar and neutral fractions. Widespread use of these fatty acid derivatives (fatty acid methyl esters, or FAME) provides an extensive understanding of their analysis and multiple options for established and validated GC separations. Additionally, GC stationary phases have been specifically designed (for example, "FAME-WAX" columns by Restek, Inc.) to optimize retention/separation of these analytes, resulting in greater peak symmetry, baseline resolution, and retention consistency. For these reasons, validation of the GC method employed was not required. However, minor modifications to established methods were used to optimize the separation for the purposes in this research. Reference Appendix C

and the *Gas Chromatography* section below for details regarding the chromatographic method used.

For methanolysis, fatty acid fractions in 1 mL toluene were transferred to 15 mL conical glass tubes containing 2 mL of methanolic base (metallic sodium in anhydrous methanol, 0.6N). The presence of water decreases the yield of the derivitization, so a drop of methyl acetate was added to adsorb any residual water prior to initiating the reaction and provide consistency of derivitization. Tubes were sealed under nitrogen to minimize oxygen exposure and were vortexed for 10 seconds to ensure adequate mixing. The mixture was heated at 50 °C in a warm water bath for 10 minutes, after which the tubes were removed from the water bath and were allowed to cool for 1 minute. Two drops of glacial acetic acid were added to stop the reaction, after which 1 mL of hexane and 1 mL of double distilled water were added to the tubes. Samples were vortexed briefly (10 seconds) to ensure adequate mixing, during which impurities with higher water solubility were separated from the FAME. The sample was then spun for 1 minute to separate layers, after which the lower water/methanol layer was transferred to a second 15 mL glass conical tube. To this, 1 mL of hexane was added, vortexed, and spun as before to provide a second recovery. The uppermost layer (hexane + FAME) of this separation was then added to the initial recovery sample.

This sample was vortexed briefly and spun to concentrate any remaining water into the bottom of the tube. Any residual water visible in the bottom of the tube was carefully removed with a Pasteur pipette, after which approximately 30 mg of sodium sulfate (hygroscopic) was added to the tube to adsorb any water not collected with the pipette. The sample was then placed in a warming block and dried down under a stream

of nitrogen until the volume of the sample was 100 uL or less, at which point the sample was transferred to a pre-weighed glass vial and dried down (completely) under a stream of nitrogen. After drying, the vial was reweighed to determine the absolute amount of FAME present in that sample. These data were useful in determining an appropriate amount of hexane with which to reconstitute the sample to provide consistent sample concentrations for chromatographic analysis. Reference Appendix C for additional information regarding the utility of this sample weight.

For purposes of GC detection and extended storage, hexane (including an internal standard – see below) was added to FAME samples in an amount to provide a final FAME concentration adequate for detection on the GC. Based on previous validation work in lab, the ratio of hexane to recovered FAME used was approximately 35 uL of hexane for 1 mg of FAME, but this was adjusted as necessary depending on the response of the chromatograms. Due to the high evaporative rate of hexane, the ratio of hexane to sample could not be relied upon following reconstitution. Consequently, chromatograms that provided peak responses too low to detect were in many cases concentrated (by allowing hexane evaporation) and re-injected. Similarly, chromatograms that exhibited peak responses (areas) in excess of levels determined to be within the determined linear response were diluted and re-injected.

The chosen hexane/FAME ratio provided a volume adequate for GC analysis (17-35 uL) even for samples with low FAME yields (0.5-1.0 mg). Furthermore, FAME yields using the methods described above were adequate and provided an excess of material for GC analysis even for samples that exhibited the lowest FAME quantities (the

quantity of reconstituted fame required for injection is only 1 uL). Therefore, deviation from the above procedures was not required for any samples.

Gas Chromatography

FAME were analyzed by gas chromatography. A Hewlett-Packard 5890 series II Gas Chromatograph equipped with a microcapillary column using a stationary phase designed for the analysis of FAME (Restek FAMEWAX Crossbond PEG, 30 meter, 0.25 mm ID) was used for chromatographic analysis. Detection was achieved using a flame ionization detector (FID), a standard means of detection for these fatty acid derivatives. Data output was performed by a Hewlett-Packard 3396 Series III integrator. The chromatographic method employed was as follows:

Injector temperature: 130°C
Detector temperature: 230°C
Initial column temperature : 130°C
Temperature ramp : 6°C/minute for 15 minutes, followed by a 15 minute hold
Split ratio : 10:1
Injection volume: 1 uL
Carrier Gas: Helium

Methods similar to this (e.g. Restek #108-01[001]) have been shown to be effective in the separation and quantification of a large number of physiologically common fatty acids, 33 of which were considered for this study (Appendix E).

Fatty acid methyl ester (FAME) standards were procured from Nu-Chek Prep, Inc., and were analyzed using the above method to determine and assign retention times for the various analytes of interest. Known concentrations of the standards allowed for the determination of signal response factors for individual FAME (signal strength/amount), which are expected to differ based on differences in molecular weight

(larger molecules typically respond with higher response due to increased conductance per molecule). Calculated response factors specific to each FAME were uploaded to the integrator, thus allowing the integrator to report data for identified peaks as both a measure of *signal strength* and a corrected *amount*.

Additionally, the integrator identified and measured the signal from a single internal standard in each sample run. This allowed the integrator to adjust the FID signal for the entire chromatogram to a preset internal standard 'expected response'. This adjustment accounts for any inconsistency in injection volume or variation that can occur from sample delivery to the column through the split injector. As an added precaution, chromatograms were manually reviewed to ensure that peak responses did not exceed established upper limits of detection.

Resulting chromatograms provided data which were adjusted in two ways; normalization of data in response to the internal standard signal, and a recalculation of the signal strength for each analyte using the appropriate response factor. Data generated was therefore indicative not of percent weight contribution to total fatty acid content of the sample, but is a measure of the relative number of fatty acid types, e.g. a 24 carbon fatty acid would be expected to elicit a larger signal than a 10 carbon fatty acid. This difference is accounted for by the use of measured response factors for all reported fatty acids.

The internal standard was prepared by dissolving a synthetic FAME (C23:0) in BHT-treated hexane. Naturally occurring fatty acids are typically synthesized/modified 2 carbons at a time and consequently contain an even number of carbons. The use of an internal standard with an odd number (23) of carbons greatly reduces the likelihood of

co-elution with FAME contained in the test samples. Furthermore, the internal standard is structurally similar to the analytes of interest, and therefore is expected to be responsive to any variable/unwanted interference that might affect the elution of analytes in the test sample (e.g. purity of carrier gas, decomposition of stationary phase, failure to maintain temperature, etc).

C23:0 was dissolved in hexane at a concentration of 0.25 ug/uL for use as a stock solution, from which "working aliquots" were transferred to 2 mL vials to reduce contamination or concentration changes due to gradual solvent evaporation. Dried FAME samples from the various tissues examined in this study were resuspended in hexane to which a known amount of internal standard (23:0) had been added.

Reproducibility of the GC analysis was confirmed by comparison of multiple injections of both standards and samples. To determine the upper limit of detection, standards were run at increasing concentrations (serial dilutions) and plotted to examine linearity of signal response. The concentration at which the sample concentration/signal ratio loses linearity due to detector saturation was determined. The upper limit of detection was set at 75% of this response (as determined by peak area). Any test samples that contained peaks that exhibited areas above the upper limit of detection were further diluted in hexane and re-analyzed to ensure all peak areas were within the validated response limits.

Lower limits of detection were determined using the calibration curve. Peak signals determined to be too small for reproducible detection were excluded from the data at the time of integration (minimum detectable peak signal). When considering the high sensitivity of flame ionization detection (<1.0 ng) and the low contribution of these peaks

to the overall abundance of FAME in the sample, the fatty acid signature of any particular sample is essentially unaffected by the exclusion of trace FAME. To prevent inadvertent exclusion of significant peaks when samples contain an unusually low absolute FAME concentration, chromatograms were checked to verify that a minimum of 20 FAME were detected by the integrator during each run. Any samples that contained fewer than 20 FAME were concentrated (i.e., partially dried under nitrogen) and re-injected until the resulting sample concentration was sufficient for a reliable report of the fatty acid signature.

Data Reporting and Statistical Analysis

Individual fatty acid values reported in tables, figures, and appendices are representative of the number of fatty acids (percent contribution of fatty acid units) in a given sample (percent contribution of fatty acid units). As described on page 31 above, known response factors for the individual fatty acids were used to account for differences in detection strength.

Fatty acid index values reported are the sum total of all applicable individual fatty acid values as described below and in Appendix E, pg 86. Saturated Fatty Acids (saturates) represent the sum total of individual fatty acids 14:0, 16:0, 18:0, 20:0, 22:0, 24:0. Monounsaturated Fatty Acids (monoenes) represent the sum total of individual fatty acids: 14:1, 16:1, 18:1, 20:1, 22:1, 24:1. Polyunsaturated Fatty Acids (PUFA) represent the sum total of individual fatty acids 16:2n4, 16:3n4, 16:4n1, 18:3, 18:4, 18:4n1, 20:2, 20:3n6, 20:4, 20:3, 20:4n3, 20:5, 21:5n3, 22:4, 22:5n6, 22:5, 22:6.

Fatty acid indices are also reported for fatty acids of the n6, n3, n3 HUFA classes. Omega-6 (n6) fatty acids include 18:2, 18:3 (γ-Linolenic), 20:2, 20:3n6, 20:4, 22:4, 22:5n6. Omega-3 (n3) fatty acids include 18:3 (α-Linolenic), 18:4, 20:4n3, 20:5, 21:5n3, 22:5, 22:6. Omega-3 Highly unsaturated fatty acids (n3 HUFA) include 20:4n3, 20:5, 21:5n3, 22:5, 22:6. Finally, the n3/n6 ratio is provided for all test groups.

One-way ANOVA's and post-hoc Tukey tests were used for comparison of initial fish and survivor test groups following the temperature challenge. Comparison of fatty acid profiles of survivors and mortalities (both cold and warm) were made using t-tests. Data were arcsine transformed where appropriate, and significance for one-way ANOVA's and t-tests was assumed at the $p < 0.05$ level. SPSS v.14.0 statistical software (SPSS Inc, Chicago, IL, USA) was used for all statistical comparisons.

RESULTS

For the purposes of reporting results, individual fatty acids to be reported and discussed below were selected based on overall contribution to the fatty acid signature and responsiveness to temperature challenges. For clarity of presentation, fatty acids C16:0, C18:0, C18:1n9, C18:1n7, C20:4, C20:5, and C22:6 are the only individual fatty acids included in Figures 1-6. All tested fatty acids were grouped and analyzed in appropriate fatty acid indices (reference Appendix E for detail) to capture overall trends of saturation/unsaturation in response to the temperature challenges. These indices are commonly used in the field and can allow a basis for comparison across studies. Furthermore, reporting fatty acid composition change in fatty acid groups or classes can be more effective in identifying predicted changes in membrane order than by looking at responses of a large number of individual fatty acids (see below).

Changes in PUFA are correlated with temperature acclimation (Olsen 1999) and are expected to increase in response to colder temperatures where modification of membrane composition is occurring in response to temperature. Changes in SAFA can be expected to occur in opposition to PUFA as the predicted effect on membrane order is in opposition to that of PUFA. Changes in SAFA can therefore be used to indicate change in membrane order/fluidity independently of PUFA results. HUFA in the n3 class are in large part essential fatty acids in fishes and are therefore a class of interest when attempting to identify fatty acid dietary deficiencies. n3/n6 ratios can be indicators of dietary sources and are of growing interest due to the importance of these ratios to eicosanoid/docosenoid production. All fatty acid data generated in this study are

available in Appendix E and includes data for individual fatty acids and the calculated indices.

Graphical representation of all the analyzed fatty acids would be of limited value as the majority of the individual fatty acids either did not exhibit statistically significant changes or were present in such low composition that the overall impact of the individual fatty acid on homeoviscous adaptation would be expected to be minimal. For example, fatty acid C20:2 (eicosadienoic acid) in liver neutral samples responded to both cold and warm temperature treatments. Differences between initial fish (1.3 ± 0.02%), cold survivors (1.5 ± 0.02%), and warm survivors (0.7 ± 0.02%) are statistically significant and are therefore potentially of interest. However, as this fatty acid constitutes not more than 1.5% of the total fatty acid signature in any of the three samples, the implications of these results as they pertain to changes in membrane fluidity are not expected to be large due to the low abundance of the *individual* fatty acid. These data are of greater interest when looked at as a contributor to overall polyunsaturated fatty acid change. The change in levels of C20:2 in response to temperature is therefore captured in the appropriate fatty acid index (PUFA). For these reasons, only select individual fatty acids which in most cases were those occurring in the greatest amounts are graphically presented in Figures 1-6. However, all statistically significant differences observed are reported in Tables 2-4.

Initial "Control" samples vs Survivors of Temperature Challenges

Gill Tissue

Gill storage lipids were generally not responsive to either the cold or warm temperature challenges (Figure 1). When considering all individual fatty acids and the

calculated fatty acid indices, the only significant change was observed in the individual fatty acid DPA (C22:5n3): warm survivor DPA levels (2.5 ± 0.05%) were significantly higher than DPA levels in initial fish (2.2 ± 0.09%).

Gill membrane lipids were very responsive to the temperature challenges when compared to the neutral lipids (Figure 1). In response to the cold challenge, gill membrane lipids of survivors had significantly lower levels of C16:0 and SAFA. In both cases, the change was an approximate 20% decrease from initial levels. The cold challenge also resulted in increases in three individual fatty acids, C18:1n7 (vaccenic), ARA (C20:4n6) , and DHA (C22:6n3) as shown in Figure 1. Furthermore, PUFA, total n6, total n3, and n3 HUFA levels were all significantly higher than levels found in initial fish (Figure 1). The change observed in PUFA in the cold challenge gill polar samples represents a 25% increase over initial levels.

In response to the warm challenge, gill membrane lipids showed little change. The only significant change in individual fatty acids was a decrease in the level of C18:1n-7. No significant changes were observed in the fatty acid indices, and as illustrated in Figure 1, gill membrane lipids were generally more responsive to the cold challenge than the warm challenge.

Liver Tissue

Liver storage lipids exhibited a number of changes in response to both warm and cold temperature challenges. With regard to the cold temperature challenge, significantly lower levels of C16:0 and SAFA were observed in cold challenge survivors when compared to initial fish. Of interest, the decrease in SAFA in the cold challenged liver storage fatty acids is almost entirely attributable to the decrease in the individual fatty

acid C16:0. Conversely, significantly higher levels of both C16:0 and SAFA were observed in the neutral lipids of warm challenged fish (Figure 2).

Liver storage lipids in warm temperature challenged fish also exhibited a statistically significant increase in DHA (C22:6n3) along with increases in several of the fatty acid indices. Increases in total PUFA, total n3, n3 HUFA, and n3/n6 ratios were also observed, and C18:1n9, C18:1n7, and MUFA all decreased significantly in these tissues (Figure 2).

Liver membrane lipids were also responsive to both temperature challenges. Levels of the individual fatty acid C18:1n7 changed significantly in response to both challenges, exhibiting higher levels in response to the cold challenge and lower levels in response to the warm temperature challenge. In addition to the reduced levels of C18:1n7 observed in the warm temperature challenge, survivors showed reduced levels of two other unsaturated fatty acids, C18:1n9 and EPA (C20:5n3), and reductions in the amounts of MUFA, PUFA and total n6 (Figure 2). Warm survivors also exhibited significantly higher C16:0, C18:0, SAFA and n3/n6 ratios. Generally, more changes in liver lipids resulted from the warm temperature challenge than the cold temperature challenge.

Muscle Tissue

Muscle storage lipids were generally unresponsive to both the cold and warm temperature challenges. The only statistically significant difference observed was a decrease in eicosenoic acid (C20:1) in warm challenged fish when compared to initial fish (Table 4).

Membrane lipids in muscle tissue did not exhibit significant changes in response to the cold challenge. However, membrane lipids in muscle tissue were responsive to the warm temperature challenge as evidenced by changes in individual fatty acids and fatty acid indices. With regard to individual fatty acids, an increase C16:0 and a decrease in C18:1n7 were observed. Fatty acid indices for muscle polar lipids exhibited increases in SAFA and decreases in PUFA and total n6 in response to the warm temperature challenge (Figure 3). In this study, results indicate that the warm temperature challenge promoted greater fatty acid change than did the cold temperature challenge.

Survivor and Mortality Comparisons

Gill Tissue

Gill neutral fatty acids signatures were essentially identical for both the warm and cold temperature challenge survivor and mortality samples. Similarly, gill membrane lipids were essentially unchanged in response to the cold challenge when comparing survivors to mortalities (Figure 4). However, a number of significant differences existed between survivors and mortalities for fish subjected to the warm temperature treatment. Warm mortalities had higher levels of SAFA when compared to warm survivors. Furthermore, warm mortalities showed significantly lower levels of DHA (C22:6n3) (approximately 33% less DHA than levels present in warm survivors). Several fatty acid indices were significantly lower in warm mortalities (vs survivors), including PUFA, total n3, n3 HUFA, and n3/n6 ratios (Figure 4).

Liver Tissue

Fatty acids in liver storage lipids in survivor and mortality groups were essentially identical in cold challenged samples, but demonstrated a number of differences in the warm temperature challenges (Figure 5). Similar to what was observed for gill polar lipids in warm mortality samples, levels of DHA (C22:6n3) in liver storage samples of mortalities were significantly lower than those of survivors (levels of DHA were approximately 30% lower than found in the comparable survivor tissue). There were also several significant differences in the fatty acid indices for these test groups, as warm mortalities showed lower levels of PUFA, total n6, total n3, n3 HUFA, and lower n3/n6 ratios when compared to survivors (Figure 5).

Liver polar lipids exhibited differences between survivors and mortalities as well, but again only in response to the warm temperature challenge. Levels of individual fatty acids C18:1n7 and ARA (C20:4n6) were significantly higher in warm mortalities, and levels of DHA (C22:6n3) were significantly lower in these samples when compared to warm survivors (Figure 5). The fatty acid indices demonstrated a number of significant differences, including higher levels of MUFA and total n6 (opposite of what was observed in the liver neutral lipids mortalities), and decreases in PUFA, n3, n3 HUFA, and the n3/n6 ratio in mortalities when compared to surviving fish (Figure 5).

Muscle Tissue

Muscle neutral lipids did not demonstrate any significant differences in either the warm or cold temperature challenge when comparing survivors to mortalities (Figure 6). There were, however, two statistically significant differences in muscle polar lipids.

Cold mortalities exhibited higher levels of C16:0 and lower levels of C18:1n7 when compared to cold survivors (Figure 6). There were no significant differences in polar lipids of the warm temperature challenged fish when comparing survivors to mortalities.

Survivorship in Response to Temperature Challenges

As discussed on page 19 above, *mortality* samples were to be removed and recorded throughout the duration of the temperature challenges. *Mortality* specimens would be selected for lipid analysis with a preference for mortalities that occurred toward the end of the study. The intent was to provide samples that would most closely match the surviving counterpart with regard to time and temperature exposure. This approach was expected to provide more valuable comparisons than, for example, a comparison of 15 day warm temperature mortality fish to 32 day warm temperature survivors.

As seen in Table 1, the majority of mortality events occurred in the last few days of the study and therefore occurred almost concurrently with survivor fish sampling. Consequently, selection of *mortality* samples did not require inclusion of mortality specimens that died several days or weeks prior to the sampled survivor fish. The warm challenge was extended beyond the originally intended 30 days to ensure mortalities occurred.

DISCUSSION

This study explored changes in stored fatty acid reserves and biomembrane fatty acid composition in response to changing temperatures (homeoviscous adaptation), and ultimately provides data to help predict the implications of these adaptations on freshwater alewife survivability in response to temperature change. Previous work with this species demonstrated that changes in fatty acid composition in response to cold temperature stress were in accordance with predictions made by homeoviscous adaptation, in that increased HUFA and PUFA (and decreases in SAFA) were observed in whole body analysis of alewives (Snyder and Hennessey 2003). The tissue-specific data generated in my study indicates that fatty acid composition in membrane and storage lipids changes in response to both cold and warm temperature challenges, and these results provide further evidence of homeoviscous adaptation in alewives. In addition, comparisons among tissues demonstrate tissue specific lipid modification in response to temperature, as the changes that occurred in individual fatty acids and fatty acid classes were not shared among the tissues I examined.

Polar lipids from alewives subjected to the cold temperature challenge would be expected to demonstrate decreased saturation to counter the decrease in membrane fluidity that results from thermodynamic interaction between fatty acids. Gill polar lipids responded accordingly, demonstrating significant decreases in the saturated fatty acid C16:0 (palmitic acid) and in SAFA as a whole. Increases in the unsaturated fatty acids DHA (C22:6n3) and ARA (C20:4n6) were also observed in this tissue, which would be expected to maintain membrane fluidity in response to the low temperatures. Furthermore, HUFA and n-3 fatty acids, as well as n-6 fatty acids, showed significant

increases. Each of the above changes are indicative of homeoviscous adaptation (Hazel 1993), and support the likelihood that gill tissue membranes are responsive to cold temperatures by remodeling membrane fatty acid signatures.

Polar lipids (membrane fatty acids) of both liver and muscle tissue were essentially unaffected by the cold temperature challenge. There was an increase in the level of C18:1n7 observed in the liver tissue of cold challenged fish, but overall the data indicate that membrane fatty acid composition of liver and muscle tissue of alewives are unresponsive to cold temperature challenge as performed in this study. Though these results were not anticipated, they demonstrate the tissue-specific nature of the homeoviscous adaptation response and support the importance of examining these changes at the tissue versus whole body level (discussed further below).

Polar lipids in warm challenged survivor samples were expected to undergo changes in opposition to those of the cold temperature challenge. Increases in the relative amount of saturated phospholipids, and decreases in membrane HUFA and PUFA would be predicted to occur during warm acclimation due to the resulting increase in membrane order that would be needed to offset the temperature-induced increase in membrane fluidity (Hazel and Williams 1990). Under the premise that membrane function is optimal at a specific fluidity/order, the increased inclusion of saturated fatty acids could provide the additional viscosity/inter-membrane order needed to effectively return membrane order to the desired state.

There are multiple means by which increased membrane saturation could take place. One possibility would be for cells to undergo small to moderate changes in the quantities of a number individual saturated fatty acids (e.g. C14:0, C16:0, C18:0, C20:0).

Another possibility would be to reduce HUFA levels and increase MUFA (or possibly PUFA), as the overall decrease in the number of double bonds would generally be expected to decrease membrane interaction and therefore "thicken" the membrane. Per unit double bond, monoenes (MUFA) have a greater impact on modifying physiological properties of membranes than more highly unsaturated fatty acids (Hazel 1995) and for this reason could serve as a medium for increased membrane fluidity. However, a modification of membrane composition from low to high MUFA could increase the order of a cell membrane if the increase in MUFA occurred concurrently with a decrease in HUFA, PUFA, or both. Another approach to increase membrane order or viscosity would be for cell membranes to undergo a large increase in the amount of a single saturated fatty acid.

In liver and muscle tissues, increases in SAFA indices were significant, but in both cases this increase was nearly entirely attributable to an increase in a single fatty acid, palmitic acid (C16:0; see Figures 2 and 3). As described in the results section above, levels of palmitic acid doubled in both liver and muscle membranes of warm challenge survivors. In liver membranes, C18:0 also increased dramatically (2x), but this fatty acid accounted for less than 5% of membrane composition in initial samples and less than 10% in the warm survivors. In contrast, palmitic acid accounted for over 30% of membrane fatty acid composition in warm survivors versus approximately 15% in initial fish. Of the several mechanisms by which a cell might increase saturation in response to increasing temperatures, in this study the increased saturation was largely accounted for by the increased inclusion of palmitic acid in both liver and muscle tissues.

One expectation of this study was that of the three tissues, phospholipids from the gill would be the most responsive to the temperature challenges. This was predicted based on the importance of the gill in osmoregulatory function, and the association of compromised osmoregulatory function as a suspected cause of winter mortality in this species in the Laurentian Great Lakes (Stanley and Colby 1971). As discussed above, gill phospholipids were highly responsive to the cold challenge. However, they were essentially unresponsive to the warm challenge with regard to modification of fatty acid signatures. In light of the significant fatty acid signature modification of both liver and muscle phospholipids in response to warm temperatures, the possibility remains that gill membranes were responsive to warm temperatures as tested in this study, but that the acclimation/response resulted from a mechanism other than changes in fatty acid composition (e.g. molecular species remodeling, changes in cholesterol incorporation, polar head group modification etc.). A growing body of evidence supports tissue-specific responses to temperature stress and further study is required to better understand the complexities of these responses (Crockett and Londraville 2006). However, based on the results of this study, it appears gill phospholipids do not undergo significant changes in fatty acid composition in response to warm temperatures as tested in this study.

It was anticipated that changes in stored lipids (neutral fraction) would reflect the lipid modification that occurs in the membrane (polar). The rationale for this hypothesis was twofold. First, increases or decreases in those lipids required to maintain membrane fluidity might undergo a similar change in lipid stores to ensure appropriate amounts of the required fatty acids are available for use in membranes. Secondly, though homeoviscous adaptation is primarily thought of as a membrane response, there is

increasing evidence that changes in fatty acid composition are applicable to stored lipids for similar thermodynamic reasons. For example, as temperatures decrease, storage lipids become less fluid and their availability as substrates for lipases can be compromised (Kostal and Simek 1998). Maintaining lipid fluidity in triaclyglycerols is consequently of great importance to ensure bioavailability of these lipids for both energy and for transport and use as membrane components.

Data from this study demonstrate that, in some cases, the responses of stored lipid to changes in temperature show patterns that are similar to those observed in membrane lipids. For example, in warm-challenged alewives, membrane and storage lipids of liver tissue exhibited significant increases in C16:0 and SAFA, and significant decreases in C18:1n9, C18:1n7, and MUFA. However, as discussed further below, there are numerous examples of lipid change in the neutral fractions which do not mirror the changes in the polar fraction. Furthermore, there are examples in which the observed changes in the polar and neutral lipids of a given tissue actually respond in opposition to each other.

In liver tissue of warm-challenged fish, PUFA decreased significantly in membrane lipids when compared to initial fish. This decrease in unsaturated fatty acids is what would be predicted by homeoviscous adaptation theory, as the removal of double bonds (in the form of PUFA in this case) would lead to increased membrane order to counter the disordering effect of increased temperatures. However, the opposite response occurred in PUFA in stored lipids of warm challenged alewives, and the levels of this fatty acid class underwent a significant increase. Another example can be found in the response of DHA (C22:6n3) in liver tissue. Membrane lipids in liver tissue of warm-

challenged fish remained unchanged with regard to levels of DHA. DHA levels would therefore be expected to remain unchanged if neutral fractions generally mirrored the responses of the polar fraction. However, DHA levels increased significantly (nearly doubled) in the neutral fraction of warm-challenged liver tissue. Not only was this response dissimilar to that seen in the polar lipids found in the membranes of this tissue, the response is in opposition to what is predicted by homeoviscous adaptation. In response to warmer temperatures, increased saturation of the fatty acids would be expected as this would counter the decreased membrane order that results from increasing temperatures. A doubling of DHA represents a large decrease in saturation (DHA contains 6 double bonds). This response, however, may be to preserve stores/availability of this essential fatty acid as it is not readily available in freshwater alewife diets (Snyder and Hennessey 2003) and is needed as a precursor to hormone-like compounds (e.g. eicosanoids) essential for proper cellular and organismal function in fishes (Henderson and Tocher 1987).

One hypothesis of this study was that changes in membrane fatty acid signature would be reflected by the stored lipid counterpart (pg. 45). Analysis of the liver tissue in warm survivors discussed above indicates that this premise is not correct and that the modifications to fatty acid composition are complex and cannot be assumed to correlate to changes observed in cell membranes. Not only are changes that occur in membrane lipids not necessarily mirrored by their neutral counterparts, but responses of individual fatty acids or fatty acid indices may undergo a change counter to what was observed in the polar fractions. For example, as observed in Figure 2, PUFA levels in storage lipids

increased in response to the warm temperature challenge yet showed a statistically significant decrease in the membrane fraction of warm survivors.

I compared fatty acid composition of survivors and mortalities in each treatment to identify where significant differences in fatty acid signatures existed. Where evidence of fatty acid signature modification is evident in survivors, a comparison against mortalities might provide an opportunity to identify a failure to maintain appropriate membrane fluidity. By examining the predicted impact of these differences on membrane fluidity, we might gain a better understanding of which lipid modifications are most critical for alewife survival in response to changes in ambient temperature.

Comparison of survivors and mortalities in cold challenged fish indicate very few differences in the tested tissues. In both liver and gill tissues, no differences were observed in either the membrane or storage lipids of survivors and mortalities in response to the cold challenge. However, in muscle membrane lipids, there were significant differences between survivors and mortalities in two fatty acids. Cold survivors exhibited higher levels of C18:n7 in membrane lipids of muscle tissue, but this change would be expected to have little impact as the fatty acid accounts for less than 5% of the total membrane composition. However, palmitic acid (C16:0) was present in significantly lower amounts in cold challenge survivors. This observation is in accordance with previous research, where higher levels of SAFA were associated with cold challenge mortality in alewives (Snyder and Hennessey 2003). Furthermore, the abundance of palmitic acid has been identified as a key difference in membrane composition of cold and warm acclimated fish species, where considerably higher levels

of palmitic acid have been found in cell membranes of warm acclimated fish (Palmerini 2009).

When comparing palmitic acid levels in muscle membranes of initial fish to cold survivors, it is apparent that the amount of palmitic acid did not decrease in response to the cold challenge. However, the increased inclusion of palmitic acid in the cold temperature mortalities may have been the critical factor in the mortality of cold-challenged fish. Addition of palmitic acid would contribute to membrane order in system where membrane order is already being increased (by a decrease in temperature) and away from optimal fluidity.

As mentioned above, survivor and mortality comparisons of warm-challenged fish indicate a number of significant changes in gill and liver tissue, but none in muscle tissue. In gill tissue, differences in fatty acid signatures were limited to membrane lipids, where decreases in DHA (C22:6n3), PUFA, n3, and n3 HUFA were observed. All of these differences in membrane composition, as well as the observed increase in SAFA, would be expected to provide increased membrane order when compared to the survivors. Implications of these observations to membrane fluidity, speaking strictly to predicted changes in response to membrane lipid composition (not accounting for changes in polar head groups, molecular remodeling, cholesterol incorporation, etc.) are that mortalities were better suited to address the decreased order that resulted from the increasing temperatures.

Similarly, liver tissues of warm temperature survivors and mortalities exhibited a number of fatty acid differences in storage and membrane fractions. Differences shared in both membrane and storage lipids were decreases in DHA (C22:6n3), PUFA, n3, and

n3 HUFA. Again, assuming changes in membrane fluidity based solely on fatty acid composition, each of these differences would be predicted to increase membrane order, and effectively counter the decreased order that occurs at higher temperatures. As concluded for the gill tissues, the differences in survivors and mortalities actually suggest that mortalities were better suited to address the warm temperature challenge than the survivors.

Membrane physiology and fluid dynamics are extremely complex. Modification of lipid composition of biomembranes has been demonstrated in response to temperature changes using a variety of approaches in fish species, including changes in cholesterol incorporation, molecular species remodeling (Hazel and Landrey 1988) and modification of polar head groups of phospholipids (Greene and Selivonchick 1987). The analysis of fatty acid signatures as performed in this study does not account for changes in polar head groups or membrane remodeling. The use of fatty acid signatures and changes in lipid classes (degree of saturation) was selected due to its use in a variety of organisms to address thermal stress, and this response has been shown to be prevalent in a variety of fishes (Hazel and Williams 1990). Moreover, previous work has indicated that alewives alter fatty acid composition in response to temperature changes (Snyder and Hennessey 2003).

The differences demonstrated in fatty acid composition of survivors and mortalities in this study are potentially a reflection of a number of factors, including increased use of n3 and n6 fatty acids as eicosanoid/docosenoid precursors in response to stress, changes in energy demands, the inability to feed effectively towards the end of the challenges, or other physiological disruptions. However, as many of the observed

differences are in opposition of what would be expected from our current understanding of homeoviscous adaptation, for this species future studies of response to warm acclimation should consider additional factors (e.g. polar head composition and molecular remodeling) in addition to the extent of lipid saturation. Furthermore, this species may not prove to be an effective model to identify warm acclimation response as it is generally considered a cold-adapted organism and is likely to avoid warm temperatures in nature (Colby 1973).

Conclusions

Evidence of changes in fatty acid composition in response to temperature were found in all tissues tested, and this study provides further evidence of homeoviscous adaptation response in alewives. With regard to comparisons of initial fish to survivors of the temperature challenges, results were generally in accordance with expectations of homeoviscous adaptation theory. In cold-challenged alewives, polar lipids in gill tissues exhibited decreases in SAFA and increases in n3 and n6 HUFA, and polar lipids in liver and muscle tissue from warm-challenged alewives displayed increases in SAFA and decreases in PUFA. The changes in lipid saturation that were observed in survivors strongly correspond to anticipated requirements to maintain appropriate membrane fluidity.

In polar tissues of cold-challenged alewives, the increases in HUFA (n3 and n6) observed in the gill may be of ecological importance to populations of this species in the Laurentian Great Lakes, as abundance of these fatty acids in fresh water systems is low relative to those found in marine environments (Arts and Kohler 2009). These results

suggest that in fresh water habitats, the scarcity of these essential fatty acids in the alewife diet may leave populations susceptible to cold temperature stress and mortality due to an inability to effectively increase membrane composition of n3 and n6 fatty acids, the acclimation response observed in the cold water challenge in this study. Lastly, results of this study indicate that changes in lipid composition are highly tissue-specific, and differ greatly for both cold-challenged and warm-challenged alewives.

Future Research

The differences observed in survivors and mortalities in the warm temperature challenge were unexpected, as the majority of lipid differences were in opposition of what would be predicted by homeoviscous adaptation theory. If homeoviscous adaptation is occurring, these data suggest that other factors, potentially molecular species remodeling or polar head group changes, may be involved and require further study in this species.

Regarding execution of the temperature challenges, the rate of temperature change, as well as the start and finish temperatures used in this study, proved very suitable as mortality events occurred towards the end of both temperature challenges with little modification to the proposed temperature regime. Moreover, the consistency of lipid analysis (i.e. little variation in triplicate data for survivors) indicates that sufficient time was provided for individuals to acclimate. A more rapid change in temperature during the experiments may have had the consequence of challenging the populations so quickly that temperature acclimation may not have been given sufficient time to occur.

It should be noted that the lengthy conditioning phase performed prior to the temperature challenges was critical to the experiment. At the onset of the conditioning phase, the change in diet to frozen *Mysis* from a previous diet of dried prepared fish food precipitated a number of changes in fatty acid composition (Wei 2007). These changes occurred in the first few weeks of the conditioning period and were confirmed by GC analysis over the course of the nine week conditioning phase. By verifying stability of fatty acid signatures prior to the execution of the temperature challenge, the initial fish could confidently be used as a control for temperature-challenged fish. This is common practice in the field, but of the factors known to modify fatty acid signature (other than temperature), changes in diet are one of the more pronounced. Should a study of this type be repeated it is recommended that a lengthy conditioning phase be performed and that fatty acid signatures demonstrate stability following the change in diet prior to execution of the temperature challenges. A separate group of unchallenged fish would be useful as an additional control. However, establishing a baseline lipid signature is more critical to observing temperature effects than comparison to a population maintained at initial temperatures that has not fully adjusted to the change in dietary lipid.

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Table 1. Temperature challenge design and survivorship.

Cold Temperature Challenge Design			
	Initial Temperature	Temperature at 25 Days	Temperature at 31 days (Final)
Tank C1	15°C	1.1°C	1.1°C
Tank C2	15°C	1.1°C	1.1°C
Cold Temperature Challenge Survivorship			
	Initial Number of Fish	Final Number of Fish	Percent Survival (%)
Tank C1	20[a]	13	65
Tank C2	25	6	24
All mortality occurred during the last five (5) days of the cold challenge (days 27-31)			
Warm Temperature Challenge Design			
	Initial Temperature	Temperature at 39 Days (Final)	
Tank W1	15°C	34.0°C	
Tank W2	15°C	34.0°C	
Warm Temperature Challenge Survivorship			
	Initial Number of Fish	Final Number of Fish	Percent Survival (%)
Tank W1	25	17	68
Tank W2	25	17	68
All mortality occurred during the last three (3) days of the warm temperature challenge (days 37-39)			

[a] All tanks originally stocked with 25 fish per tank. Mortality in tank C1 occurred several weeks before the temperature challenge resulted in an initial N of 20.

Table 2. Statistically significant changes in fatty acid composition (% of total fatty acids) of gill tissue in initial, cold-challenged and warm-challenged survivors. Data are $\overline{X}$ ± SE. Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

	Initial	Cold Challenge Survivors	Warm Challenge Survivors
Neutral FA			
DPA (22:5)	2.2 ± 0.09 [a]	2.2 ± 0.02 [a]	2.5 ± 0.05 [b]
Polar FA			
Palmitic (16:0)	27.9 ± 0.84 [a]	22.1 ± 0.79 [b]	29.9 ± 0.53 [a]
(16:3n4)	0.5 ± 0.04 [ab]	0.6 ± 0.03 [a]	0.4 ± 0.01 [b]
Oleic (18:1)	12.1 ± 1.14 [ab]	10.5 ± 0.09 [a]	14.5 ± 0.40 [b]
Vaccenic (18:1)	5.1 ± 0.29 [a]	6.2 ± 0.03 [b]	3.8 ± 0.08 [c]
Eicosen (20:1)	0.9 ± 0.11 [ab]	1.0 ± 0.11 [a]	0.5 ± 0.05 [b]
Eicosad(20:2)	0.7 ± 0.3 [ab]	0.7 ± 0.002 [a]	0.5 ± 0.03 [b]
Arachidonic (20:4)	5.0 ± 0.32 [a]	6.2 ± 0.15 [b]	4.2 ± 0.13 [a]
EPA (20:5)	3.5 ± 0.24 [ab]	4.4 ± 0.26 [a]	2.8 ± 0.20 [b]
(22:5n6)	2.1 ± 0.08 [a]	3.0 ± 0.05 [b]	2.0 ± 0.14 [a]
DPA (22:5)	1.2 ± 0.10 [ab]	1.5 ± 0.03 [a]	1.1 ± 0.04 [b]
DHA (22:6)	17.5 ± 0.71 [a]	22.5 ± 0.30 [b]	18.0 ± 0.83 [a]

Table 3. Statistically significant changes in fatty acid composition (% of total fatty acids) of liver tissue in initial, cold-challenged and warm-challenged survivors. Data are $\overline{X}$ ± SE. Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

	Initial	Cold Challenge Survivors	Warm Challenge Survivors
Neutral FA			
Myristic (14:0)	5.5 ± 0.32 [a]	5.4 ± 0.02 [a]	4.3 ± 0.12 [b]
Palmitic (16:0)	15.9 ± 0.39 [a]	12.5 ± 0.27 [b]	20.4 ± 0.57 [c]
Stearic (18:0)	2.2 ± 0.13 [ab]	1.71 ± 0.07 [a]	2.4 ± 0.22 [b]
Oleic (18:1)	22.8 ± 2.46 [a]	23.1 ± 2.38 [a]	13.8 ± 0.37 [b]
Vaccenic (18:1)	5.4 ± 0.30 [a]	5.5 ± 0.22 [a]	4.2 ± 0.08 [ab]
gLinolenic (18:3)	0.3 ± 0.01 [a]	0.3 ± 0.01 [a]	0.9 ± 0.03 [b]
aLinoleic (18:3)	4.2 ± 0.43 [a]	4.1 ± 0.24 [a]	2.7 ± 0.08 [b]
Arachidic (20:0)	0.2 ± 0.03 [a]	0.2 ± 0.02 [ab]	0.1 ± 0.00 [b]
Eicosen (20:1)	2.3 ± 0.96 [ab]	3.3 ± 0.55 [a]	0.5 ± 0.25 [b]
Eicosad (20:2)	1.3 ± 0.02 [a]	1.5 ± 0.02 [b]	0.7 ± 0.03 [c]
(20:3n6)	0.4 ± 0.04 [a]	0.3 ± 0.00 [ab]	0.2 ± 0.03 [b]
Erucic (22:1)	0.8 ± 0.07 [a]	0.9 ± 0.12 [a]	0.2 ± 0.01 [b]
(21:5n3)	0.33 ± 0.03 [a]	0.35 ± 0.01 [a]	0.43 ± 0.02 [b]
DPA (22:5)	1.5 ± 0.10 [ab]	2.0 ± 0.09 [b]	3.3 ± 0.02 [c]
DHA (22:6)	9.2 ± 1.02 [a]	10.5 ± 0.80 [a]	19.4 ± 0.63 [b]
Nervonic (24:1)	1.4 ± 0.12 [a]	1.6 ± 0.14 [a]	0.3 ± 0.03 [b]
Polar FA			
Palmitic (16:0)	15.0 ± 0.27 [a]	12.1 ± 1.83 [a]	32.5 ± 1.05 [b]
Stearic(18:0)	4.3 ± 0.42 [a]	3.5 ± 0.40 [a]	9.0 ± 1.35 [b]
Oleic(18:1)	8.9 ± 0.85 [a]	10.5 ± 0.83 [a]	6.0 ± 0.25 [b]
Vaccenic(18:1)	5.5 ± 0.05 [a]	6.5 ± 0.19 [b]	2.0 ± 0.09 [c]
Linoleic(18:2)	1.7 ± 0.13 [a]	1.4 ± 0.08 [a]	0.7 ± 0.04 [b]
aLinoleic(18:3)	1.4 ± 0.14 [a]	1.2 ± 0.07 [a]	0.3 ± 0.16 [b]
Steridonic(18:4)	0.7 ± 0.10 [a]	0.6 ± 0.07 [ab]	0.3 ± 0.04 [b]
Eicosen(20:1)	2.4 ± 0.33 [a]	2.9 ± 0.54 [a]	0.3 ± 0.04 [b]
Eicosad(20:2)	2.3 ± 0.18 [a]	2.4 ± 0.05 [a]	0.6 ± 0.04 [b]
(20:3n6)	0.8 ± 0.06 [a]	0.5 ± 0.05 [ab]	0.2 ± 0.08 [b]
(20:4n3)	1.8 ± 0.05 [a]	1.3 ± 0.07 [b]	0.4 ± 0.02 [c]
EPA (20:5)	3.5 ± 0.11 [a]	3.2 ± 0.05 [ab]	2.3 ± 0.38 [b]
Docosatet (22:4)	1.8 ± 0.29 [a]	1.3 ± 0.17 [ab]	0.8 ± 0.05 [b]
(22:5n6)	2.8 ± 0.04 [a]	3.2 ± 0.19 [a]	1.2 ± 0.07 [b]
DPA (22:5)	1.9 ± 0.02 [a]	2.6 ± 0.22 [b]	1.3 ± 0.07 [a]

Table 4. Statistically significant changes in fatty acid composition (% of total fatty acids) of muscle tissue in initial, cold-challenged and warm-challenged survivors. Data are $\overline{X}$ ± SE. Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

	Initial	Cold Challenge Survivor	Warm Challenge Survivor
Neutral FA			
Eicosen (20:1)	1.9 ± 0.05 [a]	2.2 ± 0.15 [a]	0.8 ± 0.41 [b]
Polar FA			
Palmitic (16:0)	15.8 ± 1.62 [a]	16.1 ± 0.40 [a]	29.0 ± 0.43 [b]
Vaccenic(18:1)	4.7 ± 0.47 [a]	4.3 ± 0.04 [a]	2.2 ± 0.06 [b]
Linoleic(18:2)	2.1 ± 0.27 [a]	1.8 ± 0.11 [a]	1.1 ± 0.04 [b]
aLinoleic(18:3)	2.0 ± 0.34 [a]	1.7 ± 0.17 [ab]	0.7 ± 0.05 [b]
Eicosen (20:1)	0.8 ± 0.11 [ab]	0.8 ± 0.13 [a]	0.3 ± 0.11 [b]
Eicosad (20:2)	0.9 ± 0.06 [a]	0.9 ± 0.03 [a]	0.6 ± 0.02 [b]
(20:4n3)	1.2 ± 0.04 [a]	1.2 ± 0.04 [a]	0.4 ± 0.01 [b]
(22:5n6)	3.3 ± 0.12 [a]	3.5 ± 0.17 [a]	2.5 ± 0.08 [b]
DPA (22:5)	2.8 ± 0.05 [a]	2.6 ± 0.06 [a]	1.6 ± 0.05 [b]

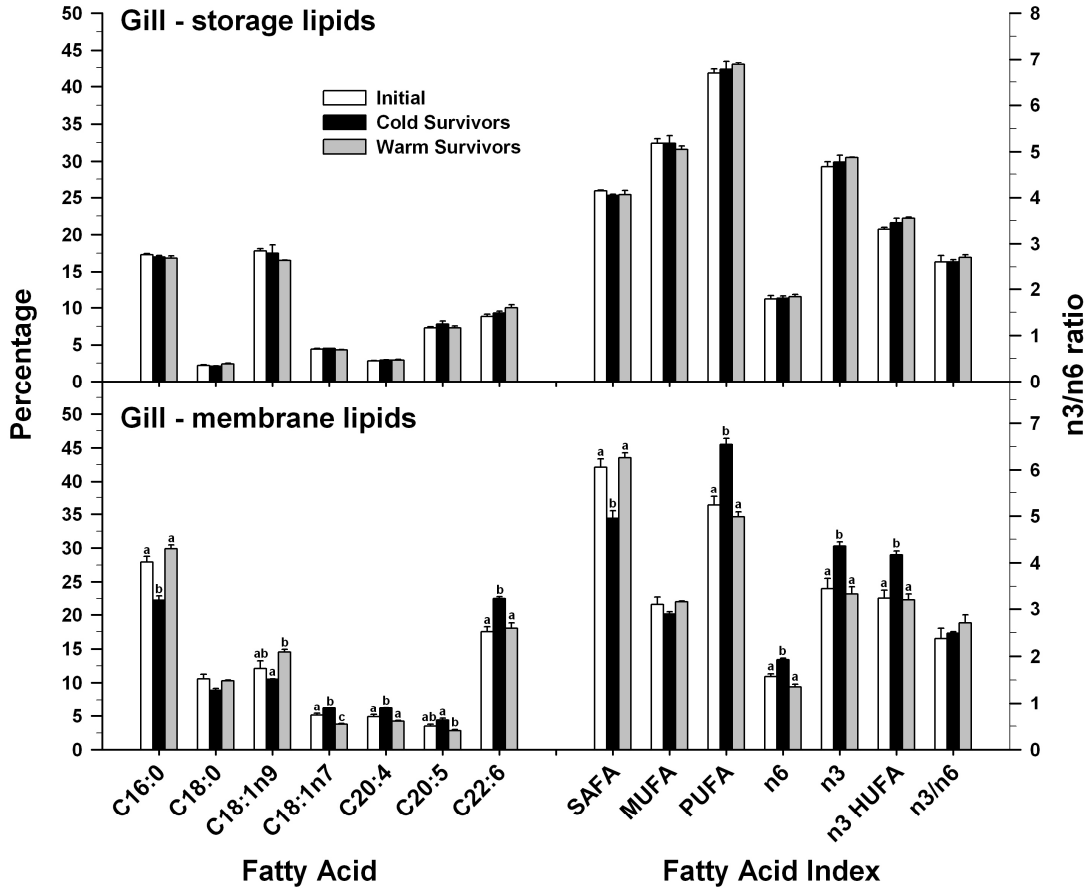


Figure 1 – Storage and membrane lipids in alewife gill tissue. Select individual fatty acids and fatty acid indices of initial, warm-challenged, and cold-challenged fish (survivors only). Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

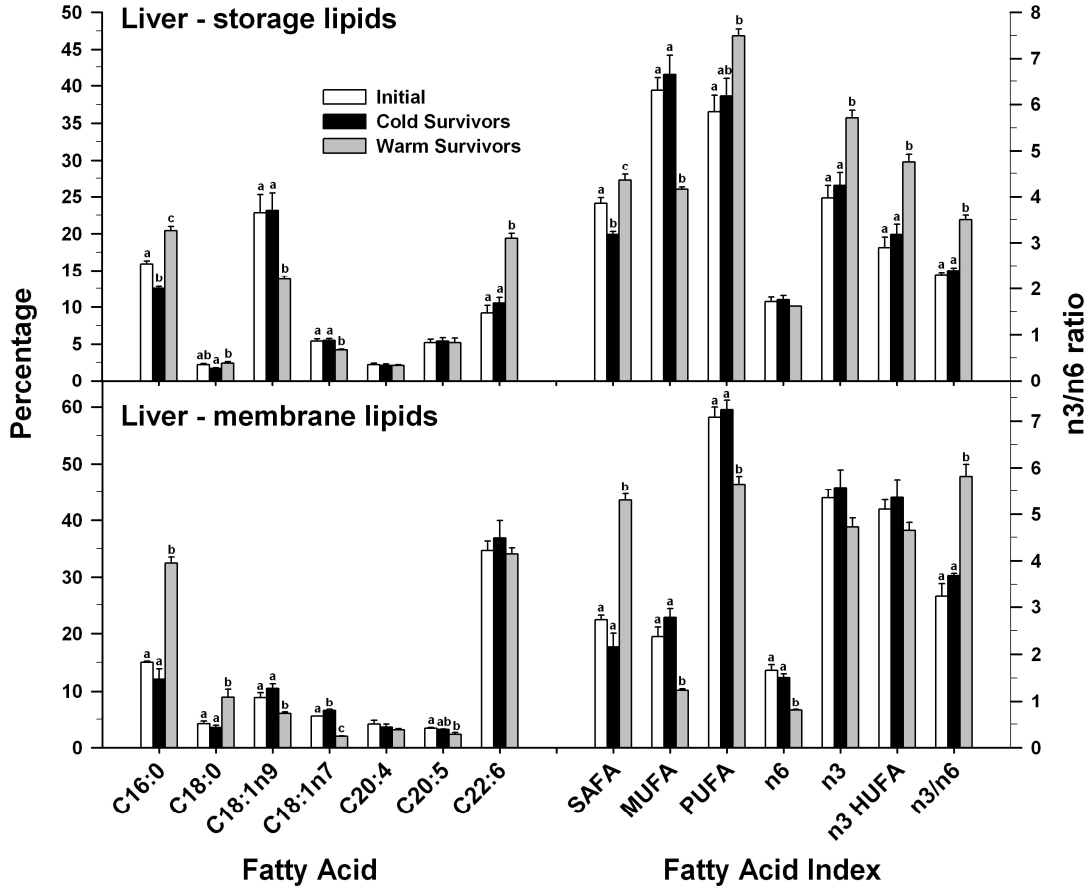


Figure 2 - Storage and membrane lipids in alewife liver tissue. Select individual fatty acids and fatty acid indices of initial, warm-challenged, and cold-challenged fish (survivors only). Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

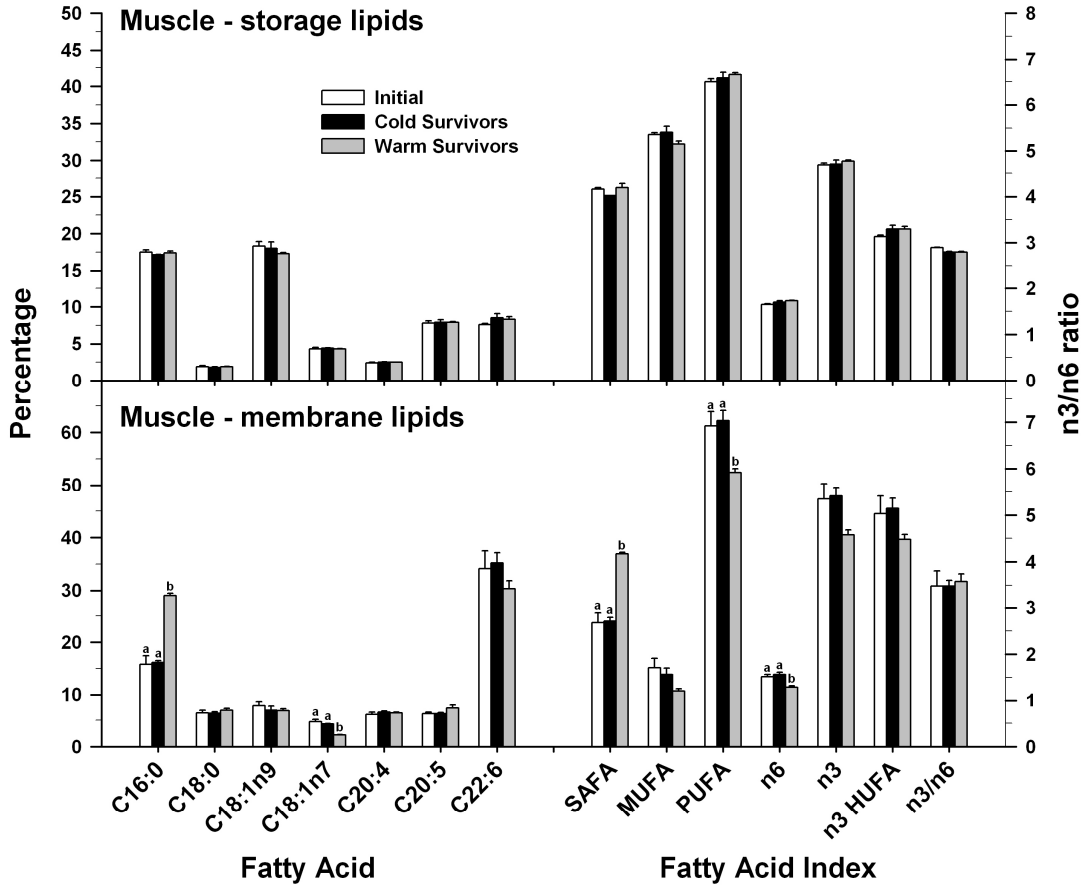


Figure 3 - Storage and membrane lipids in alewife muscle tissue. Select individual fatty acids and fatty acid indices of initial, warm-challenged, and cold-challenged fish (survivors only). Mean values marked with different letters indicate statistically significant differences at $p < 0.05$.

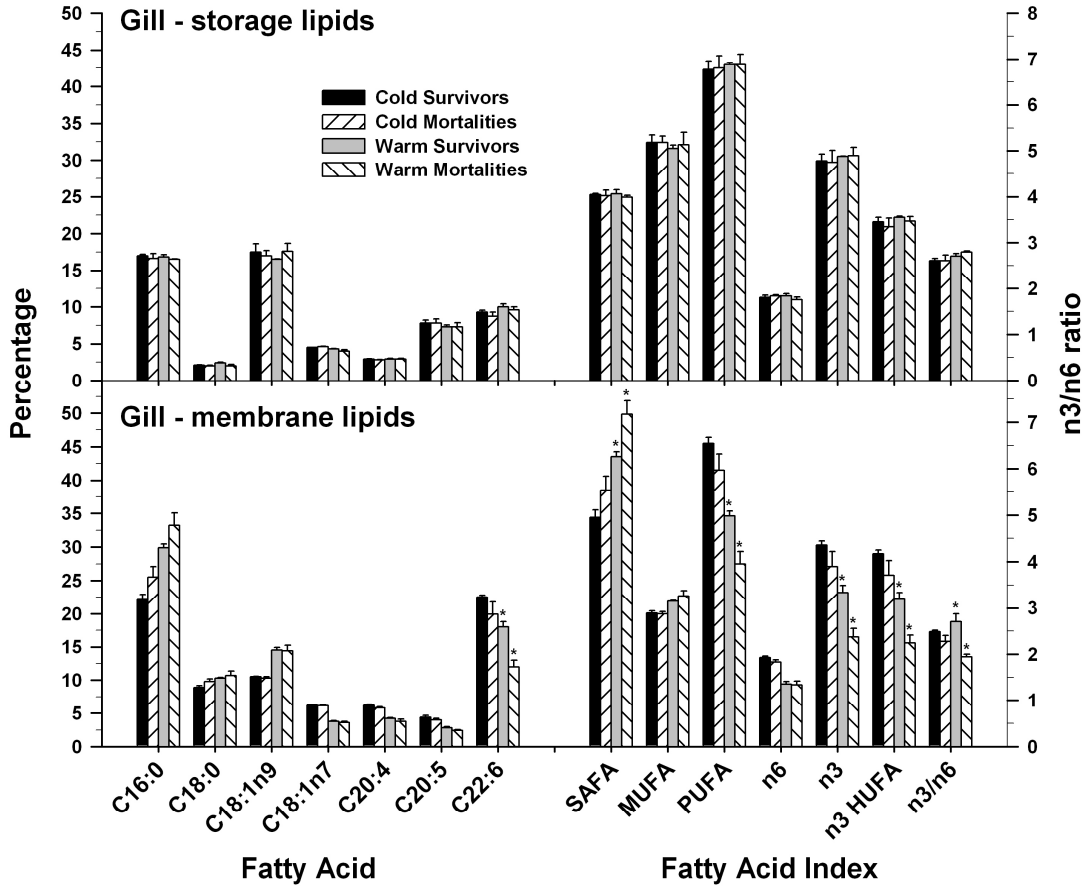


Figure 4 - Storage and membrane lipids in alewife gill tissue from survivors and mortalities. Select individual fatty acids and fatty acid indices of warm- and cold-challenged survivors and mortalities. Asterisks (*) indicate statistically significant differences ($p < 0.05$) as determined using t-tests.

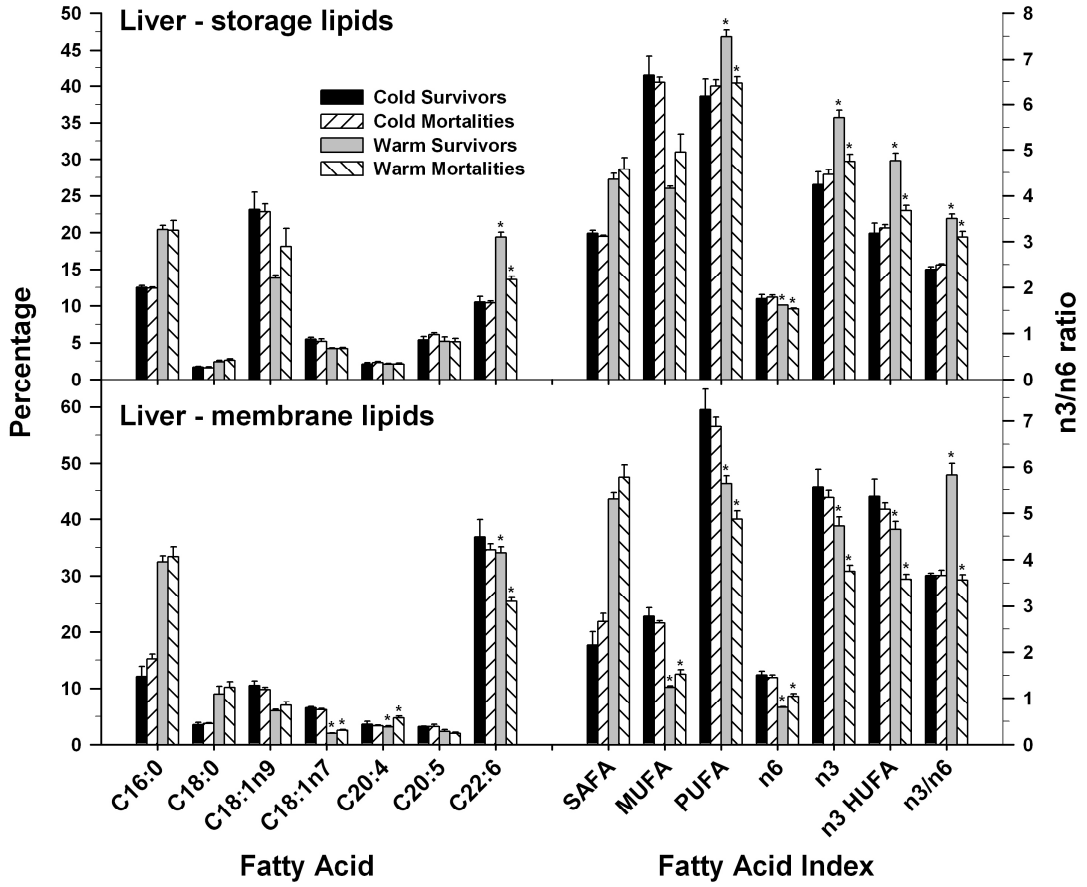


Figure 5 – Storage and membrane lipids in alewife liver tissue from survivors and mortalities. Select individual fatty acids and fatty acid indices of warm- and cold-challenged survivors and mortalities. Asterisks (*) indicate statistically significant differences ($p < 0.05$) as determined using t-tests.

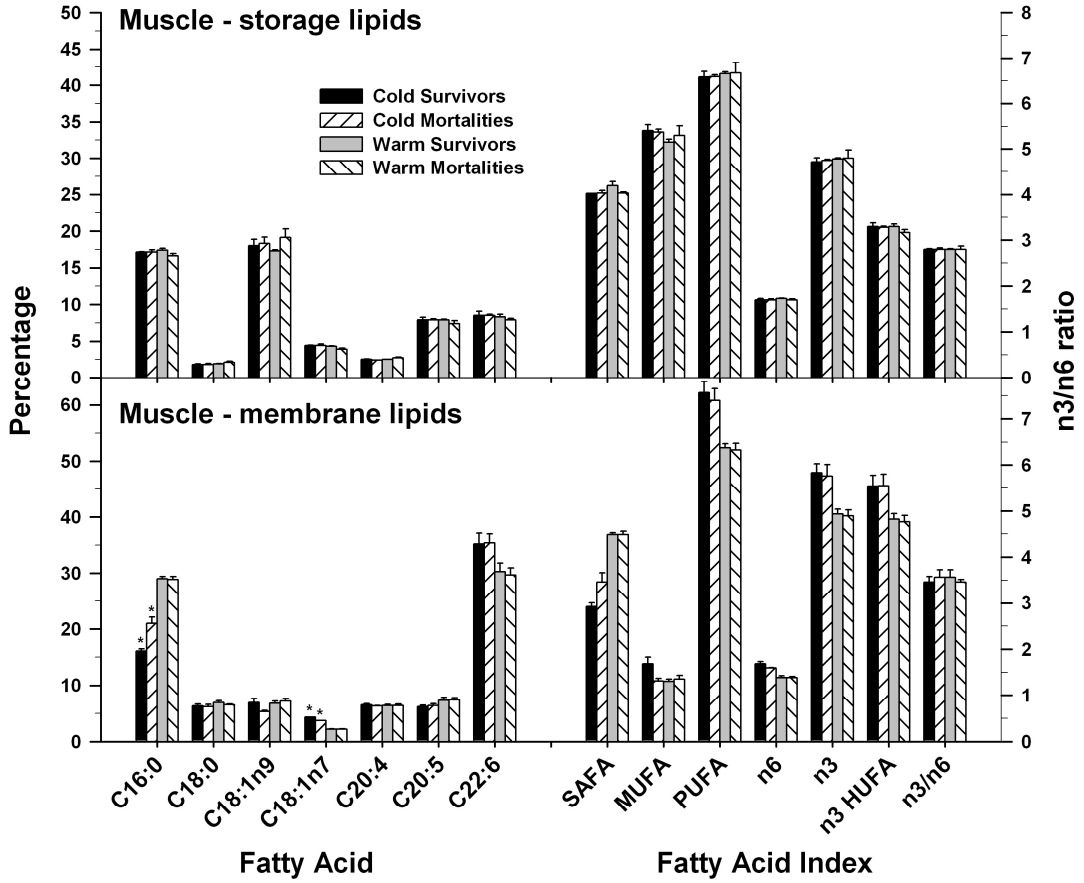


Figure 6 – Storage and membrane lipids in alewife muscle tissue from survivors and mortalities. Select individual fatty acids and fatty acid indices of warm- and cold-challenged survivors and mortalities. Asterisks (*) indicate statistically significant differences ($p < 0.05$) as determined using t-tests.

Appendix A - Sample Preparation and Extraction of Fatty Acids.

Procedure 1: Extraction of Lipids from Tissues

Equipment:
Brinkman Polytron Homogenizer (~½ inch O.D.)
Glass Flat Bottom Screw Top Vials (~20 ml capacity) with Teflon lined caps
15 ml Conical Test tubes with Teflon lined caps

Consumables:
Chloroform
Methanol
Butylated Hydroxytoluene (BHT)

Description: Below is a procedure which allows for reproducible extraction of lipids (polar and neutral) from small amounts of tissue. The procedure is qualitative, but can be modified for quantitative recovery. Briefly, the tissues are homogenized in a solvent, after which the lipids are recovered by two applications of chloroform. The chloroform washes are then cleaned further with a low volume application/removal of water. Solvents are evaporated, and the recovered lipids are reconstituted and stored in hexane.

1) Fill a screw-top homogenizer vial with 6 ml of chloroform:methanol (50:50 by volume). Cap vial to prevent loss of solvent to evaporation.
2) Carefully extract and weigh tissue sample on tared plastic weigh boat (small size). 150-200 mg is recommended – larger amounts may saturate solvent, and lesser amounts may not provide enough fatty acid for analysis. Should the sample not afford 150 mg, Gill samples of less than 100 mg have been successfully extracted and analyzed.
3) Record the weight of the sample and add the tissue to the homogenizer vial.
4) Homogenize the tissue sample for 1 min at speed 5 in the fume hood. After 30 seconds of homogenization, visually inspect the sample, as the sample may need

to be capped and shaken to ensure that tissues are suspended in the solvents. Between samples, rinse the homogenizer first in chloroform:methanol (50:50) and then in methanol. To prevent sample carry over, wipe the homogenizer with a kimwipe and visually inspect for tissue. Use tweezers to remove any tissue that remains on the homogenizer. Rinse the homogenizer in chloroform:methanol (50:50) and then in methanol and wipe dry before storage.

5) Transfer entire homogenate to a labeled 15 ml round-bottom screw-top test tube with a Pasteur pipette. Flush test tube with nitrogen and seal with Teflon-lined screw cap. Place in centrifuge, balance centrifuge as needed, and spin for 10 min at maximum speed (#7). Visually inspect tube. Solvents should be free of solids, and the tissue should have formed a pellet.
6) Supernatant Tube - Transfer supernatants to a 15 ml conical test tube, leaving tissue residue behind.
7) Add 3 ml of distilled water to the supernatant tube. Vortex tubes for 1 min and centrifuge again for 10 min at maximum speed.
8) Organic Wash #1 – With a Pasteur pipette, carefully transfer the lower layer (containing the extracted lipids) to a labeled 15ml conical test tube. To this, add 10 drops of distilled water with a Pasteur pipette and seal under nitrogen.
9) Add 3 mls chloroform to the supernatant tube (which still contains MeOH and H2O), vortex for 1 min, and centrifuge for 10 min at maximum speed.
10) Organic Wash #2 - Carefully transfer the lower layer containing the extracted lipids to organic wash #1.

11) Vortex the organic wash tube for 1 minute as a final purification step (10 drops of H2O added at step 8 intended to collect any remaining water soluble components). Centrifuge for 10 min at maximum speed. Transfer bottom lipid containing layer to a 15 ml round bottomed tube, being sure not to carry any of the aqueous layer into the round bottomed tube.
12) Place lipid containing sample tube in warming block, and evaporate the solvent with a stream on nitrogen to dryness. Resuspend the lipids in 0.5 ml hexane. Flush the tube with nitrogen and seal with Teflon-lined screw cap.
13) Store extracted lipids in the freezer or proceed directly to *Procedure 2: Separation of Neutral and Polar Fractions*.

Technical Notes:

1) For Qualitative analysis, tissue free transfer of the supernatant (step 6) is more important than recovering the majority of the solvents. If pellet formation is poor, spin down for a greater duration or leave a small quantity of solvent on the pellet to ensure that no tissue is inadvertently transferred. Small amounts of tissue transferred with the solvent can result in poorly defined organic and aqueous phases (large, cloudy interface) and may require discard of the sample. Should a sample not be replaceable, spinning the sample down in centrifuge tube to re-pellet the transferred tissue is preferable to performing multiple extractions as solvent ratios used for extraction are critical. Should a large interface appear immediately following step 7, tissue transfer is a likely cause.
2) Organic phase transfers in steps 6 and 8 are performed with a Pasteur pipette. For cleaner samples, it is recommended that the pipette be pushed though the interface

quickly to prevent mixing of the two phases. Once through, a *gentle* press of the bulb performed just prior to drawing up the organic (lower) phase will discharge any of the upper phase trapped in the pipette. This technique minimizes any phase mixing that may occur during transfer and is of greater importance if multiple draws are required to remove the bottom layer.

3) Scale-up: If using larger volumes of solvent for larger sample sizes, volume ratios throughout the procedure cannot be changed without additional validation procedures. Extractions/separations using these methods are robust using the organic/aqueous ratios described, but may not remain consistent should ratios be modified in the process of scale-up.
4) Separatory Funnels – For larger sample sizes, separation of organic/aqueous phases may be improved with the use of separatory funnels. The separation of larger volumes in centrifuge tubes may require additional equipment (centrifuge and higher capacity tubes), and and additional equipment to that listed above. Separatory funnels allow for easy mixing of solvents and provide a precise removal of the organic phase with very little solvent loss. The use of separatory funnels is recommended should a true quantitation of fatty acid content in tissues be desired as the organic phase can be almost entirely removed without collecting interface or aqueous phase. However, allot 24 hours to allow a very clean phase separation .

Appendix B - Separation of Polar and Neutral Fractions

Equipment:
Rotovap
Ringstand
Stainless Steel SPE valve
250 ml boiling flasks (2 for each sample)

Consumables:
SPE Column
Chloroform
Methanol
Pasteur Pipettes

Description: Fatty acids are bound to a glycerol molecule in both the phospholipid (polar) and triacylglyceride (neutral) form. The procedure below allows for the separation of polar and neutral lipid fractions using a Solid Phase Extraction Cartridge. Briefly, a sample containing both polar and neutral lipids is applied to an SPE cartridge. The neutral fraction elutes with the application of chloroform. The polar fraction, collected separately, elutes with the application of methanol. Solvents are then evaporated and the fractions are collected into tubes.

1) Fill the water bath with deionized water and turn it on (should be done first as it takes some time to come to temperature).
2) Secure a SPE-pack extraction cartridge on a ring stand with the large stem pointed upwards. Attach the stainless steel on-off valve to the bottom of the cartridge. Adjust the height of the cartridge to allow a 250 ml boiling flask to fit underneath.
3) Place a labeled boiling flask under the cartridge, close the valve, and fill the cartridge to the base of the stem with chloroform using a Pasteur pipette. A properly “charged” cartridge will appear translucent. Any air bubbles present in the cartridge (white areas) can be removed by eluting approximately 5ml

chloroform through the cartridge using the glass syringe designated for chloroform (must be done with valve open).

4) Close valve and add the lipid extract (0.5ml) to the neck of the cartridge using a Pasteur pipette, being careful not to overfill. If sample volume is too large to fit into the neck, open the on-off valve briefly to allow the sample to drain into the cartridge, and then close the valve again. Continue filling neck with sample until the entire sample is loaded. At this time, the polar fraction will bind to the stationary phase in the column.
5) Fill a 30 ml glass syringe with 20ml chloroform. Use a few drops of chloroform from the syringe to completely fill the stem of the SPE cartridge to ensure no air is remaining in either the syringe luer lock or the SPE cartridge neck. This is to prevent the formation of an air pocket in the cartridge during elution. Attach the 30 ml syringe upside-down to the top of the cartridge.
6) Push the 20ml chloroform evenly through the cartridge over a 1 min period until the syringe is empty. Close the on-off valve at the base of the cartridge and remove the syringe.
7) The flask contains the neutral fraction (storage lipids). Cap and set aside.
8) Place a second clean flask underneath the SPE cartridge.
9) Fill a 30 ml glass syringe with 30ml Methanol. Use a few drops of methanol from the syringe to completely fill the stem of the SPE cartridge to ensure no air is remaining in either the syringe luer lock or the SPE cartridge neck. Attach the 30 ml syringe upside-down to the top of the cartridge.

10) Push the 30ml of methanol evenly through the cartridge over a 1 min period until the syringe is empty. Close the valve at the base of the cartridge and remove the syringe.
11) The flask contains the polar fraction (phospholipids). Cap and set aside.

Rotovap Drydown

12) To concentrate the sample, use a Rotovap until the majority of the solvent has been completely removed. Recover the lipid fraction from the flask by using three 2 ml rinses of chloroform. These recoveries should be pooled in a small round bottom tube and dried down completely with nitrogen.
13) Reconstitute the sample in 1 ml Toluene – Store in freezer if derivitization cannot be performed immediately.

Technical Notes:

1) Overfill of the SPE neck is not a critical issue if doing qualitative fatty acid analysis. Should overfill occur, be sure to adequately rinse the outside of the cartridge with chloroform and methanol to prevent transfer of overfill (both polar and neutral fractions) to flasks during elution.
2) As the cartridge is charged with chloroform, the neutral fraction is not bound to the stationary phase during sample loading. If the valve is opened to facilitate sample loading, chloroform runoff may contain neutral lipids. For quantitative

analysis, the neutral collection flask should be placed beneath the valve during sample loading to collect any neutral lipids eluting prior to the wash sequence.

3) At step 5, care should be taken not to introduce an air bubble to the cartridge after sample loading. However, if air is present between the cartridge and the syringe prior to elution, the sample can be eluted without introducing the air bubble to the stationary phase. Constant pressure on the syringe (vs. erratic) will prevent the air bubble from transferring to the SPE column and will ensure appropriate elution of the analytes.
4) BHT content of solvents should be kept low (<0.01%). The final steps of the separation procedure involve a drydown/concentration of the sample. High BHT solvent concentrations, in addition to concentration of BHT in extracts (30ml to 0.5ml) can lead to very large BHT peaks during GC analysis and will add a significant weight contribution (0.5mg or more) should a quantitative recovery be performed. The boiling point of BHT is 265°C. Therefore, any BHT added during separation, extraction, or derivitization is carried through the procedure.
5) A supply of the solvent used to elute each fraction should be available to rinse the neck of the flask after isolating the fractions (prior to removing the flask from the base of the SPE column). Sample may splash on to the flask neck during elution. Due to the volatility of the solvents, the sample can adhere to the neck and will not be recovered for the next steps should it not be washed down to the base of the flask.
6) SPE cartridges can be used for 6 samples. Following each sample, the cartridge should be rinsed with methanol and chloroform.

7) A complete dry down is necessary as water can interfere with the derivitization in the next procedure.

Appendix C - Derivitization to Fatty Acid Methyl Esters (FAME) and Chromatographic Analysis

Equipment:
Hot water bath or
Heating plate and glass beaker
15 ml glass conical tubes (with teflon lined cap)
Gas Chromatograph with Split Injector (Hewlett-Packard 5890 series II Gas Chromatograph)
Microcapillary column - Restek FAMEWAX Crossbond PEG, 30 meter, 0.25 mm ID
Flame Ionization Detector
Data Integrator – Hewlett-Packard 3396 Series III
Hamilton Syringe capable of 1uL injection

Consumables:
Pasteur Pipettes
Methanolic base (0.6N metallic sodium in anhydrous methanol)
Methyl Acetate
Glacial Acetic Acid
Hexane (spike with 23:0 Internal Standard – see Appendix D)
Double distilled water
Sodium Sulfate
Nitrogen
Chromatographic Grade Helium – Carrier Gas

Derivitization of Fatty Acid Fractions to FAME

1. Prepare a warm water bath (50°C) using a hot water bath or hot plate.
2. Transfer fatty acid fraction from the round bottom glass tube to a 15 mL glass conical tube containing 2 mL of methanolic base (if the sample was frozen for storage, see technical notes #1 and #2 below).
3. Add 1 drop of methyl acetate to the tube, seal under nitrogen, and vortex for 10 seconds to ensure adequate mixing.
4. Place the conical tube in the water batch (50°C) for 10 minutes, remove and allow to cool for 1 minute.

5. Using a Pasteur pipette, add two drops of glacial acetic acid to stop the reaction. After vortexing briefly, add 1 mL of hexane and 1 mL of double distilled water. Vortex again for 10 seconds to ensure adequate mixing.
6. Place tube in centrifuge and spin until layers form (can be achieved in less than 1 minute). Using a pasteur pipette, transfer the bottom (aqueous) layer to a second, properly labeled 15 mL glass conical tube.
7. To the second tube containing the bottom layer of the sample, add 1 mL of hexane. Vortex briefly and centrifuge to form layers. Using a Pasteur pipette, remove the top layer and add it to the initial sample (top layer only – see Technical Note #3).
8. Briefly spin the 15 mL conical sample tube which now contains the top layer of the initial sample prep, and the top layer of the sample 'wash'. Should any water be visible in the bottom of the tube, remove with a pasteur pipette.
9. Add approximately 30 mg of sodium sulfate to the sample tube.
10. Place tube in a warming block (low heat) and dry down under a stream of nitrogen until the sample volume is approximately 100 uL or less.
11. Transfer sample to a pre-weighed, sealable glass vial, and continue to dry down under a stream of nitrogen for approximately 10 minutes to remove any remaining volatile solvent. See Technical Note #4.
12. After final drydown, record sample weight. To the sample vial, add approximately 35 uL of hexane for each mg of FAME recovered (see Technical Note #5). Hexane used for sample reconstitution should be spiked with Internal Standard (synthetic FAME 23:0) present at a concentration of 0.25 ug/ul.

Chromatographic Analysis

13. If the sample was stored in the freezer, allow sample to come to room temperature prior to injection (keep sample capped during thaw to prevent formation of condensate in the sample).

14. Remove sample cap and draw approximately 2uL of sample into an appropriate glass Hamilton syringe. Draw sample and replace vial cap immediately to minimize hexane evaporation from sample (evaporation causes sample concentration and can result in FID signals outside the limits of detection).

15. Holding the syringe with the plunger down, tap gently on the barrel of the syringe until all air bubbles have travelled to the base of the needle. In a fume hood with gloved hands, dispense sample as necessary to remove air trapped in the syringe.

16. Inject 1 uL of sample in the GC for analysis using the following chromatographic parameters.

    Injector temperature: 130° C
    Detector temperature: 230° C
    Initial column temperature : 130° C
    Temperature ramp : 6 deg/min for 15 minutes, followed by a 15 minute hold
    Split ratio : 10:1
    Carrier Gas: Helium

17. Following analysis, ensure that all peaks of interest are within detection limits using the areas generated by the integrator (peaks which have been cut off by the integrator may still be within detection limits.)

18. If peaks of interest are not within detection limits, concentrate or dilute the sample with Hexane as necessary to provide an acceptable analysis.

19. Samples can be frozen for storage.

Technical Notes:

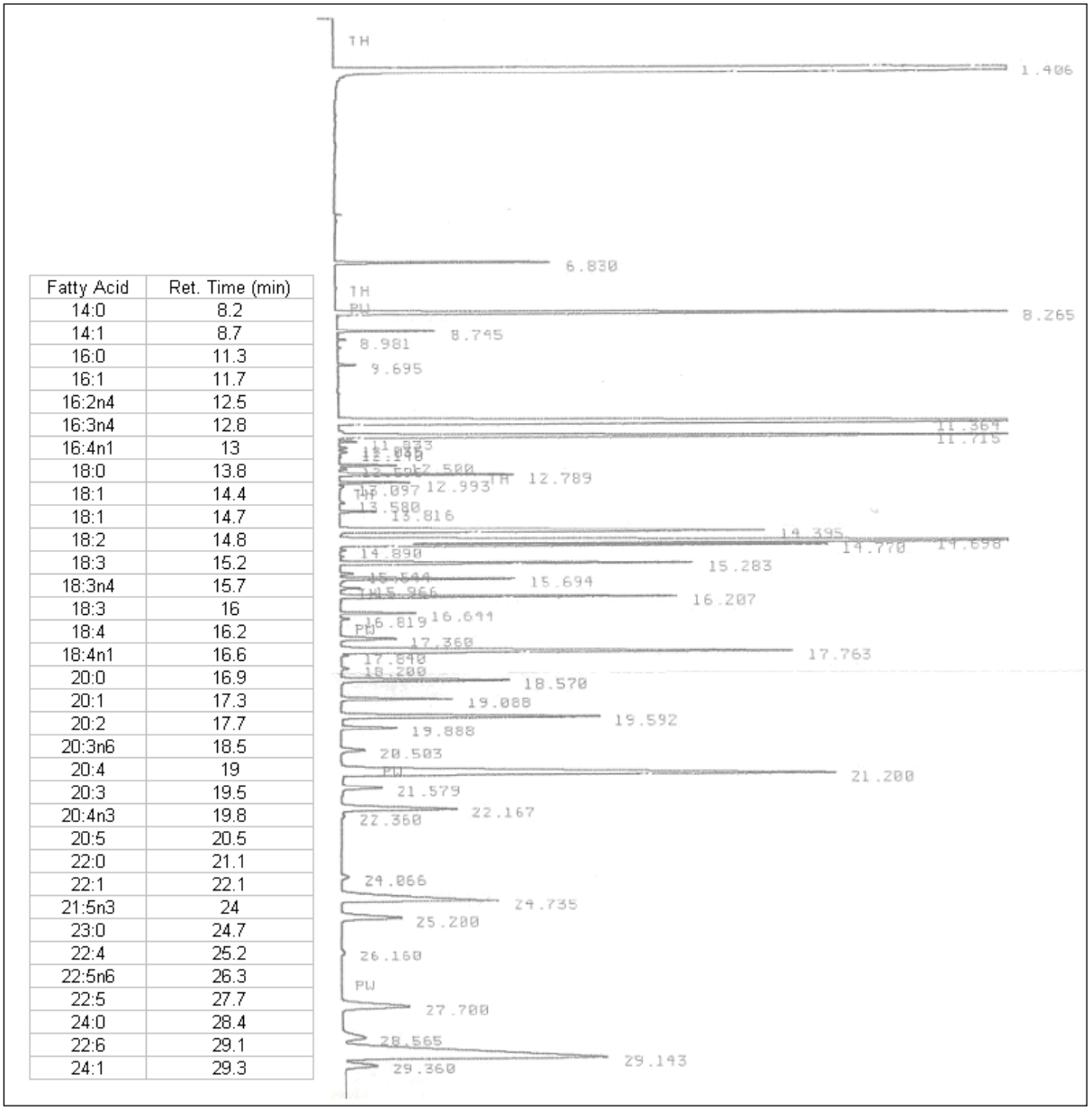
1. Wherever possible, the derivitization of the fatty acid fractions to FAME should be performed as soon as possible following fraction collections. FAME are more stable than their non-derivitized fatty acid precursors and therefore can be stored with greater confidence for extended periods.
2. Should fatty acid fractions suspended in toluene be frozen prior to derivitization, allow the round bottom tube to come to room temperature while still capped. This minimizes/prevents the formation of condensate which can interfere with methanolysis.
3. During separation of layers following methanolysis, water is added as a solvent to help separate and remove impurities from the FAME. When the initial separation is performed, the removal of the bottom (aqueous) layer procedure should be performed such that none of the bottom layer is left in the conical sample tube. This may require some incorporation of the upper layer in the aliquot. The second extraction performed on the 'wash' (steps 6 and 7 above) will recover any FAME lost during this initial transfer. Following this procedure will provide cleaner samples with minimal loss of FAME.
4. Weighing the final FAME yield is useful for determining the appropriate amount of solvent needed for sample reconstitution. An overly concentrated sample may require multiple injections/dilution before an acceptable analysis is achieved. This is a time consuming process but be avoided by taking the time to determine the amount of FAME recovered following derivitization. Furthermore, glassware

contamination, solvent contamination, SEP cartridge failure, fraction misidentification, poor derivitization, etc. can result in faulty sample preparations which may not be recognized even after GC analysis. By weighing the absolute recovery of FAME, which adds little time to the procedure, faulty extractions have a better chance to be identified.

5. The ratio of FAME to Hexane used to reconstitute samples for GC analysis provided chromatography within detection limits for the majority of samples prepared for this study. However, due to the volatility of hexane and the differences in sample weights, dilution or concentration of preparations was occasionally required. Though the amount of FAME that is delivered to the column is inherently inconsistent due to the use of the split injector, FAME recoveries from similar sample sizes should remain fairly consistent in all steps upstream of the chromatographic injection. The 35 uL to 1 mg ratio is a recommendation but may need to be modified due to differences in reproducibility of sample delivery.

APPENDIX D – Chromatogram and Approximate Retention Time of Individual Fatty Acids

Fatty Acid	Ret. Time (min)
14:0	8.2
14:1	8.7
16:0	11.3
16:1	11.7
16:2n4	12.5
16:3n4	12.8
16:4n1	13
18:0	13.8
18:1	14.4
18:1	14.7
18:2	14.8
18:3	15.2
18:3n4	15.7
18:3	16
18:4	16.2
18:4n1	16.6
20:0	16.9
20:1	17.3
20:2	17.7
20:3n6	18.5
20:4	19
20:3	19.5
20:4n3	19.8
20:5	20.5
22:0	21.1
22:1	22.1
21:5n3	24
23:0	24.7
22:4	25.2
22:5n6	26.3
22:5	27.7
24:0	28.4
22:6	29.1
24:1	29.3



Calibration standard (33 commonly occurring fatty acids) analyzed with Restek FAMEWAX Crossbond microcapillary column using the separation method described above. Fatty Acid 23:0 (Retention Time ~24.7 min) is the Internal Standard.

APPENDIX E – Raw Data for All GC Runs

The following tables include raw data from the chromatographic runs of all tissues used in the above described research effort. For each test group, tissue, and fraction below, data from 33 fatty acids are reported (where detected) as a percentage of total sample fatty acid content. These fatty acid signatures have been adjusted using measured response factors and normalized within each analysis against an internal standard as described in the Methods section (*Gas Chromatography*) above. Data presented include the triplicate runs used for each sample point, means, standard errors, and a summary of Fatty Acid Indices for each particular data set, where:

Saturated Fatty Acids (Saturates) : 14:0, 16:0, 18:0, 20:0, 22:0, 24:0
Monounsaturated Fatty Acids (Monoenes): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1
Polyunsaturated Fatty Acids (PUFA): 16:2n4, 16:3n4, 16:4n1, 18:3, 18:4, 18:4n1, 20:2, 20:3n6, 20:4, 20:3, 20:4n3, 20:5, 21:5n3, 22:4, 22:5n6, 22:5, 22:6
n6 (all n6 fatty acids): 18:2, 18:3 (γ-Linolenic), 20:2, 20:3n6, 20:4, 22:4, 22:5n6
n3 (all n3 fatty acids): 18:3 (α-Linolenic), 18:4, 20:4n3, 20:5, 21:5n3, 22:5, 22:6
n3 HUFA (Highly unsaturated n3 fatty acids): 20:4n3, 20:5, 21:5n3, 22:5, 22:6
n3/n6 ratio : % n3 ÷ % n6

Treatment : Initial
Tissue: Gill
Fraction: Neutral

Name	1=Run 148	2=Run 180	3=Run 236		Mean	SE
Myristic(14:0)	6.00	5.69	5.75		5.8	0.09
Myristolic(14:1)	0.00	0.00	0.11		0.0	0.04
Palmitic(16:0)	17.11	17.41	17.46		17.3	0.11
Palmitol(16:1)	7.60	7.40	7.62		7.5	0.07
(16:2n4)	0.46	0.50	0.56		0.5	0.03
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.74	0.40	0.43		0.5	0.11
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.37	2.01	2.15		2.2	0.11
Oleic(18:1)	17.27	18.37	17.87		17.8	0.32
Vaccenic(18:1)	4.45	4.53	4.20		4.4	0.10
Linoleic(18:2)	4.44	4.18	4.42		4.3	0.08
gLinolen(18:3)	0.36	0.37	0.09		0.3	0.09
(18:3n4)	0.00	0.00	0.10		0.0	0.03
aLinolen(18:3)	5.28	5.18	5.85		5.4	0.21
Stearidon(18:4)	2.85	3.10	3.59		3.2	0.22
(18:4n1)	0.00	0.22	0.20		0.1	0.07
Arachidic(20:0)	0.47	0.37	0.46		0.4	0.03
Eicosen(20:1)	2.03	2.03	0.37		1.5	0.55
Eicosad(20:2)	1.12	1.04	0.98		1.0	0.04
(20:3n6)	0.48	0.31	0.34		0.4	0.05
Arachidon(20:4)	3.02	2.70	2.81		2.8	0.09
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.86	2.07	2.18		2.0	0.09
EPA(20:5)	7.48	7.03	7.37		7.3	0.14
Behenic(22:0)	0.00	0.15	0.18		0.1	0.06
Erucic(22:1)	0.47	0.38	0.38		0.4	0.03
(21:5n3)	0.36	0.34	0.39		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.95	0.77	0.92		0.9	0.06
(22:5n6)	1.75	1.26	1.40		1.5	0.15
DPA(22:5)	2.19	2.00	2.32		2.2	0.09
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	8.26	9.45	8.80		8.8	0.34
Nervonic(24:1)	0.63	0.72	0.69		0.7	0.03

Fatty Acid Indices

Saturates	25.9	25.6	26.0		25.9	0.11
Monoenes	32.4	33.4	31.2		32.4	0.63
PUFA	41.6	40.9	42.8		41.8	0.53
n6	12.1	10.6	11.0		11.2	0.46
n3	28.3	29.2	30.5		29.3	0.64
n3 HUFA	20.2	20.9	21.1		20.7	0.28
n3/n6	2.3	2.7	2.8		2.6	0.14

Treatment : Initial
Tissue: Gill
Fraction: Polar

Name	1=Run 168	2=Run 182	3=Run 238		Mean	SE
Myristic(14:0)	1.99	2.88	3.61		2.8	0.47
Myristolic(14:1)	1.67	0.00	0.00		0.6	0.56
Palmitic(16:0)	28.02	29.26	26.35		27.9	0.84
Palmitol(16:1)	2.03	2.41	3.27		2.6	0.37
(16:2n4)	0.96	1.11	0.78		1.0	0.10
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.56	0.44	0.56		0.5	0.04
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	10.64	11.62	9.39		10.6	0.64
Oleic(18:1)	14.41	10.76	11.26		12.1	1.14
Vaccenic(18:1)	4.70	5.63	4.89		5.1	0.29
Linoleic(18:2)	1.45	1.25	1.62		1.4	0.11
gLinolen(18:3)	0.00	0.00	0.29		0.1	0.10
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.83	0.84	1.36		1.0	0.18
Stearidon(18:4)	0.00	0.40	0.67		0.4	0.19
(18:4n1)	0.00	0.00	0.41		0.1	0.14
Arachidic(20:0)	1.14	0.64	0.70		0.8	0.16
Eicosen(20:1)	0.99	0.73	1.08		0.9	0.11
Eicosad(20:2)	0.68	0.63	0.75		0.7	0.03
(20:3n6)	0.00	0.00	0.21		0.1	0.07
Arachidon(20:4)	5.52	4.90	4.43		4.9	0.32
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.00	0.45	0.60		0.4	0.18
EPA(20:5)	3.01	3.60	3.83		3.5	0.24
Behenic(22:0)	0.00	0.00	0.00		0.0	0.00
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	2.06	1.21	1.33		1.5	0.27
(22:5n6)	1.95	2.23	2.10		2.1	0.08
DPA(22:5)	1.24	1.04	1.38		1.2	0.10
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	16.15	17.96	18.49		17.5	0.71
Nervonic(24:1)	0.00	0.00	0.62		0.2	0.21

Fatty Acid Indices

Saturates	41.8	44.4	40.1		42.1	1.26
Monoenes	23.8	19.5	21.1		21.5	1.24
PUFA	34.4	36.1	38.8		36.4	1.28
n6	11.7	10.2	10.7		10.9	0.42
n3	21.2	24.3	26.3		24.0	1.48
n3 HUFA	20.4	23.0	24.3		22.6	1.15
n3/n6	1.8	2.4	2.5		2.2	0.20

Treatment : Initial
Tissue: Liver
Fraction: Neutral

Name	Run 151	Run 226	Run 243		Mean	SE
Myristic(14:0)	4.82	5.87	5.69		5.5	0.32
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	15.16	16.23	16.41		15.9	0.39
Palmitol(16:1)	6.84	6.13	7.07		6.7	0.28
(16:2n4)	0.40	0.43	0.47		0.4	0.02
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.46	0.50	0.35		0.4	0.04
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.37	1.96	2.34		2.2	0.13
Oleic(18:1)	19.79	27.67	20.93		22.8	2.46
Vaccenic(18:1)	5.58	5.87	4.87		5.4	0.30
Linoleic(18:2)	3.58	3.19	3.83		3.5	0.19
gLinolen(18:3)	0.33	0.30	0.33		0.3	0.01
(18:3n4)	0.00	0.17	0.00		0.1	0.06
aLinolen(18:3)	4.15	3.47	4.97		4.2	0.43
Stearidon(18:4)	2.49	2.18	2.97		2.5	0.23
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.25	0.18	0.28		0.2	0.03
Eicosen(20:1)	2.93	0.36	3.49		2.3	0.96
Eicosad(20:2)	1.29	1.28	1.22		1.3	0.02
(20:3n6)	0.40	0.38	0.28		0.4	0.04
Arachidon(20:4)	2.57	1.86	2.14		2.2	0.21
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	2.09	1.62	1.74		1.8	0.14
EPA(20:5)	5.59	4.31	5.65		5.2	0.44
Behenic(22:0)	0.00	0.55	0.17		0.2	0.16
Erucic(22:1)	0.72	0.94	0.81		0.8	0.07
(21:5n3)	0.38	0.28	0.35		0.3	0.03
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.91	1.26	1.22		1.5	0.22
(22:5n6)	1.81	1.49	1.52		1.6	0.10
DPA(22:5)	1.56	1.34	1.69		1.5	0.10
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	11.24	8.49	7.95		9.2	1.02
Nervonic(24:1)	1.32	1.66	1.28		1.4	0.12

Fatty Acid Indices

Saturates	22.6	24.8	24.9		24.1	0.75
Monoenes	37.2	42.6	38.4		39.4	1.65
PUFA	40.2	32.6	36.7		36.5	2.21
n6	11.9	9.8	10.5		10.7	0.62
n3	27.5	21.7	25.3		24.8	1.69
n3 HUFA	20.9	16.1	17.4		18.1	1.43
n3/n6	2.3	2.2	2.4		2.3	0.05

Treatment: Initial
Tissue: Liver
Fraction: Polar

Name	1=Run 152	2=Run 189	3=Run 249		Mean	SE
Myristic(14:0)	1.69	2.58	2.89		2.4	0.36
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	14.90	14.53	15.47		15.0	0.27
Palmitol(16:1)	1.72	2.34	2.76		2.3	0.30
(16:2n4)	0.00	0.69	0.61		0.4	0.22
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.00	0.28	0.23		0.2	0.09
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	4.80	3.42	4.51		4.2	0.42
Oleic(18:1)	7.21	9.54	9.93		8.9	0.85
Vaccenic(18:1)	5.43	5.39	5.57		5.5	0.05
Linoleic(18:2)	1.46	1.59	1.90		1.7	0.13
gLinolen(18:3)	0.00	0.10	0.00		0.0	0.03
(18:3n4)	0.00	0.21	0.00		0.1	0.07
aLinolen(18:3)	1.18	1.34	1.64		1.4	0.14
Stearidon(18:4)	0.48	0.67	0.81		0.7	0.10
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.00	0.17	0.28		0.1	0.08
Eicosen(20:1)	2.01	2.03	3.02		2.4	0.33
Eicosad(20:2)	2.52	1.99	2.52		2.3	0.18
(20:3n6)	0.87	0.68	0.80		0.8	0.06
Arachidon(20:4)	5.56	3.57	3.30		4.1	0.71
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.88	1.71	1.76		1.8	0.05
EPA(20:5)	3.30	3.66	3.38		3.4	0.11
Behenic(22:0)	1.83	0.00	0.00		0.6	0.61
Erucic(22:1)	0.00	0.18	0.23		0.1	0.07
(21:5n3)	0.00	0.15	0.00		0.1	0.05
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	2.39	1.45	1.58		1.8	0.29
(22:5n6)	2.78	2.78	2.91		2.8	0.04
DPA(22:5)	1.84	1.84	1.88		1.9	0.02
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	36.16	36.64	31.44		34.7	1.66
Nervonic(24:1)	0.00	0.48	0.58		0.4	0.18

Fatty Acid Indices

Saturates	23.2	20.7	23.2		22.4	0.83
Monoenes	16.4	20.0	22.1		19.5	1.67
PUFA	60.4	59.3	54.8		58.2	1.73
n6	15.6	12.2	13.0		13.6	1.03
n3	44.8	46.0	40.9		43.9	1.54
n3 HUFA	43.2	44.0	38.5		41.9	1.72
n3/n6	2.9	3.8	3.1		3.3	0.27

Treatment: Initial
Tissue: Muscle
Fraction: Neutral

Name	1=Run 153	2=Run 183	3=Run 241		Mean	SE
Myristic(14:0)	6.20	6.12	5.91		6.1	0.09
Myristolic(14:1)	0.09	0.00	0.11		0.1	0.03
Palmitic(16:0)	17.35	18.09	17.21		17.5	0.27
Palmitol(16:1)	8.53	8.05	7.57		8.0	0.28
(16:2n4)	0.46	0.45	0.46		0.5	0.00
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.40	0.37	0.39		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.76	1.84	2.19		1.9	0.13
Oleic(18:1)	17.10	18.56	19.18		18.3	0.62
Vaccenic(18:1)	4.57	4.43	3.84		4.3	0.22
Linoleic(18:2)	4.56	4.32	4.60		4.5	0.09
gLinolen(18:3)	0.07	0.38	0.07		0.2	0.10
(18:3n4)	0.11	0.00	0.10		0.1	0.03
aLinolen(18:3)	6.10	5.69	6.21		6.0	0.16
Stearidon(18:4)	3.72	3.63	3.90		3.7	0.08
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.26	0.25	0.30		0.3	0.02
Eicosen(20:1)	1.91	2.02	1.85		1.9	0.05
Eicosad(20:2)	1.25	1.10	0.94		1.1	0.09
(20:3n6)	0.27	0.30	0.31		0.3	0.01
Arachidon(20:4)	2.43	2.26	2.64		2.4	0.11
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.93	2.04	2.08		2.0	0.04
EPA(20:5)	8.32	7.54	7.41		7.8	0.28
Behenic(22:0)	0.20	0.00	0.17		0.1	0.06
Erucic(22:1)	0.41	0.38	0.39		0.4	0.01
(21:5n3)	0.41	0.37	0.39		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.49	0.41	0.52		0.5	0.03
(22:5n6)	1.28	1.28	1.27		1.3	0.00
DPA(22:5)	1.75	1.70	2.04		1.8	0.11
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	7.59	7.95	7.39		7.6	0.16
Nervonic(24:1)	0.46	0.50	0.56		0.5	0.03

Fatty Acid Indices

Saturates	25.8	26.3	25.8		26.0	0.18
Monoenes	33.1	33.9	33.5		33.5	0.25
PUFA	41.2	39.8	40.7		40.6	0.41
n6	10.4	10.0	10.4		10.3	0.11
n3	29.8	28.9	29.4		29.4	0.26
n3 HUFA	20.0	19.6	19.3		19.6	0.20
n3/n6	2.9	2.9	2.8		2.9	0.01

Treatment: Initial
Tissue: Muscle
Fraction: Polar

Name	1=Run 157	2=Run 185	3=Run 242		Mean	SE
Myristic(14:0)	1.27	0.98	1.70		1.3	0.21
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	18.92	14.83	13.56		15.8	1.62
Palmitol(16:1)	1.90	1.22	2.56		1.9	0.39
(16:2n4)	0.00	0.28	0.47		0.3	0.14
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.00	0.21	0.29		0.2	0.09
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	7.33	5.79	6.13		6.4	0.47
Oleic(18:1)	8.20	5.92	9.02		7.7	0.93
Vaccenic(18:1)	4.23	4.18	5.60		4.7	0.47
Linoleic(18:2)	2.00	1.62	2.56		2.1	0.27
gLinolen(18:3)	0.00	0.00	0.00		0.0	0.00
(18:3n4)	0.00	0.11	0.00		0.0	0.04
aLinolen(18:3)	1.86	1.48	2.63		2.0	0.34
Stearidon(18:4)	0.82	0.49	1.18		0.8	0.20
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.00	0.00	0.00		0.0	0.00
Eicosen(20:1)	0.76	0.60	0.98		0.8	0.11
Eicosad(20:2)	0.88	0.84	1.05		0.9	0.06
(20:3n6)	0.00	0.33	0.52		0.3	0.15
Arachidon(20:4)	6.98	5.79	5.69		6.1	0.41
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.17	1.27	1.28		1.2	0.04
EPA(20:5)	6.81	5.98	5.73		6.2	0.33
Behenic(22:0)	0.00	0.00	0.71		0.2	0.24
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.33	0.26		0.2	0.10
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.74	0.60	0.94		0.8	0.10
(22:5n6)	3.11	3.51	3.20		3.3	0.12
DPA(22:5)	2.70	2.86	2.74		2.8	0.05
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	30.31	40.79	31.22		34.1	3.35
Nervonic(24:1)	0.00	0.00	0.00		0.0	0.00

Fatty Acid Indices

Saturates	27.5	21.6	22.1		23.7	1.90
Monoenes	15.1	11.9	18.2		15.1	1.80
PUFA	57.4	66.5	59.7		61.2	2.72
n6	13.7	12.7	13.9		13.4	0.39
n3	43.7	53.2	45.0		47.3	2.97
n3 HUFA	41.0	51.2	41.2		44.5	3.37
n3/n6	3.2	4.2	3.2		3.5	0.33

Treatment: Cold Survivors
Tissue: Gill
Fraction: Neutral

Name	1=Run 159	2=Run 193	3=Run 247		Mean	SE
Myristic(14:0)	5.52	5.69	5.65		5.6	0.05
Myristolic(14:1)	0.00	0.08	0.09		0.1	0.03
Palmitic(16:0)	17.18	16.62	17.12		17.0	0.18
Palmitol(16:1)	7.39	7.58	7.82		7.6	0.13
(16:2n4)	0.49	0.58	0.52		0.5	0.03
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.42	0.40	0.43		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.12	2.18	1.99		2.1	0.05
Oleic(18:1)	18.32	15.31	18.99		17.5	1.13
Vaccenic(18:1)	4.52	4.48	4.41		4.5	0.03
Linoleic(18:2)	4.17	4.55	4.36		4.4	0.11
gLinolen(18:3)	0.36	0.37	0.09		0.3	0.09
(18:3n4)	0.00	0.09	0.11		0.1	0.03
aLinolen(18:3)	4.89	5.53	5.14		5.2	0.19
Stearidon(18:4)	2.86	3.27	3.03		3.1	0.12
(18:4n1)	0.00	0.09	0.24		0.1	0.07
Arachidic(20:0)	0.46	0.43	0.44		0.4	0.01
Eicosen(20:1)	2.32	1.94	0.38		1.5	0.59
Eicosad(20:2)	1.12	1.22	1.16		1.2	0.03
(20:3n6)	0.43	0.28	0.33		0.3	0.04
Arachidon(20:4)	2.88	3.09	2.78		2.9	0.09
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.93	1.92	1.98		1.9	0.02
EPA(20:5)	7.38	8.67	7.40		7.8	0.43
Behenic(22:0)	0.17	0.14	0.15		0.2	0.01
Erucic(22:1)	0.45	0.39	0.46		0.4	0.02
(21:5n3)	0.38	0.40	0.37		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.84	0.78	0.68		0.8	0.05
(22:5n6)	1.41	1.48	1.49		1.5	0.03
DPA(22:5)	2.19	2.19	2.15		2.2	0.02
Lignocer(24:0)	0.00	0.00	0.08		0.0	0.03
DHA(22:6)	8.99	9.71	9.29		9.3	0.21
Nervonic(24:1)	0.80	0.55	0.87		0.7	0.10
Fatty Acid Indices						
Saturates	25.5	25.1	25.4		25.3	0.13
Monoenes	33.8	30.3	33.0		32.4	1.05
PUFA	40.7	44.6	41.5		42.3	1.18
n6	11.2	11.8	10.9		11.3	0.26
n3	28.6	31.7	29.3		29.9	0.92
n3 HUFA	20.9	22.9	21.2		21.6	0.63
n3/n6	2.6	2.7	2.7		2.7	0.05

Treatment: Cold Survivors
Tissue: Gill
Fraction: Polar

Name	1=Run 169	2=Run 196	3=Run 248		Mean	SE
Myristic(14:0)	2.48	2.09	2.32		2.3	0.12
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	23.57	20.85	21.93		22.1	0.79
Palmitol(16:1)	1.84	2.23	2.11		2.1	0.11
(16:2n4)	1.05	1.26	1.04		1.1	0.07
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.51	0.62	0.61		0.6	0.03
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	9.25	8.37	9.15		8.9	0.28
Oleic(18:1)	10.43	10.63	10.31		10.5	0.09
Vaccenic(18:1)	6.20	6.21	6.11		6.2	0.03
Linoleic(18:2)	1.23	1.53	1.24		1.3	0.10
gLinolen(18:3)	0.00	0.28	0.27		0.2	0.09
(18:3n4)	0.00	0.12	0.00		0.0	0.04
aLinolen(18:3)	0.76	1.09	0.96		0.9	0.10
Stearidon(18:4)	0.00	0.53	0.48		0.3	0.17
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.63	0.66	0.75		0.7	0.04
Eicosen(20:1)	0.93	0.84	1.20		1.0	0.11
Eicosad(20:2)	0.72	0.73	0.73		0.7	0.00
(20:3n6)	0.00	0.16	0.30		0.2	0.09
Arachidon(20:4)	6.46	6.32	5.95		6.2	0.15
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.60	0.63	0.55		0.6	0.02
EPA(20:5)	4.67	4.73	3.93		4.4	0.26
Behenic(22:0)	0.00	0.00	0.92		0.3	0.31
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.11		0.0	0.04
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.71	1.90	1.88		1.8	0.06
(22:5n6)	2.95	2.90	3.07		3.0	0.05
DPA(22:5)	1.52	1.54	1.43		1.5	0.03
Lignocer(24:0)	0.00	0.30	0.00		0.1	0.10
DHA(22:6)	22.48	23.00	21.96		22.5	0.30
Nervonic(24:1)	0.00	0.49	0.70		0.4	0.21

Fatty Acid Indices

Saturates	35.9	32.3	35.1		34.4	1.10
Monoenes	19.4	20.4	20.4		20.1	0.34
PUFA	44.7	47.3	44.5		45.5	0.92
n6	13.1	13.8	13.4		13.4	0.22
n3	30.0	31.5	29.4		30.3	0.62
n3 HUFA	29.3	29.9	28.0		29.0	0.57
n3/n6	2.3	2.3	2.2		2.3	0.03

Treatment: Cold Survivors
Tissue: Liver
Fraction: Neutral

Name	1=Run 163	2=Run 198	3=Run 252		Mean	SE
Myristic(14:0)	5.46	5.41	5.37		5.4	0.02
Myristolic(14:1)	0.00	0.00	0.08		0.0	0.03
Palmitic(16:0)	12.81	12.77	11.98		12.5	0.27
Palmitol(16:1)	6.95	7.62	6.61		7.1	0.30
(16:2n4)	0.46	0.52	0.43		0.5	0.03
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.47	0.48	0.48		0.5	0.00
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.78	1.78	1.58		1.7	0.07
Oleic(18:1)	21.46	20.05	27.78		23.1	2.38
Vaccenic(18:1)	5.73	5.69	5.06		5.5	0.22
Linoleic(18:2)	3.48	3.88	3.21		3.5	0.20
gLinolen(18:3)	0.30	0.31	0.28		0.3	0.01
(18:3n4)	0.15	0.12	0.11		0.1	0.01
aLinolen(18:3)	4.01	4.50	3.66		4.1	0.24
Stearidon(18:4)	2.55	2.88	2.21		2.5	0.20
(18:4n1)	0.00	0.11	0.00		0.0	0.04
Arachidic(20:0)	0.19	0.19	0.14		0.2	0.02
Eicosen(20:1)	3.17	2.48	4.35		3.3	0.55
Eicosad(20:2)	1.49	1.46	1.43		1.5	0.02
(20:3n6)	0.27	0.27	0.26		0.3	0.00
Arachidon(20:4)	2.23	2.39	1.77		2.1	0.18
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.68	1.76	1.47		1.6	0.09
EPA(20:5)	5.36	6.15	4.66		5.4	0.43
Behenic(22:0)	0.25	0.00	0.07		0.1	0.07
Erucic(22:1)	0.85	0.70	1.12		0.9	0.12
(21:5n3)	0.33	0.37	0.34		0.3	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.26	1.43	1.32		1.3	0.05
(22:5n6)	2.10	2.05	1.67		1.9	0.14
DPA(22:5)	2.19	2.03	1.87		2.0	0.09
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	11.38	11.31	8.94		10.5	0.80
Nervonic(24:1)	1.65	1.28	1.75		1.6	0.14

Fatty Acid Indices

Saturates	20.5	20.1	19.1		19.9	0.40
Monoenes	39.8	37.8	46.8		41.5	2.71
PUFA	39.7	42.0	34.1		38.6	2.35
n6	11.1	11.8	9.9		11.0	0.54
n3	27.5	29.0	23.1		26.6	1.76
n3 HUFA	20.9	21.6	17.3		19.9	1.35
n3/n6	2.5	2.5	2.3		2.4	0.05

Treatment: Cold Survivors
Tissue: Liver
Fraction: Polar

Name	1=Run 164	2=Run 201	3=Run 253		Mean	SE
Myristic(14:0)	1.12	1.67	2.03		1.6	0.26
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	8.48	13.35	14.42		12.1	1.83
Palmitol(16:1)	1.68	2.10	2.39		2.1	0.21
(16:2n4)	0.64	0.69	0.56		0.6	0.04
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.22	0.24	0.29		0.2	0.02
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.67	3.89	3.87		3.5	0.40
Oleic(18:1)	9.96	9.43	12.13		10.5	0.83
Vaccenic(18:1)	6.33	6.87	6.30		6.5	0.19
Linoleic(18:2)	1.25	1.52	1.42		1.4	0.08
gLinolen(18:3)	0.00	0.14	0.16		0.1	0.05
(18:3n4)	0.27	0.29	0.25		0.3	0.01
aLinolen(18:3)	1.09	1.34	1.25		1.2	0.07
Stearidon(18:4)	0.45	0.64	0.68		0.6	0.07
(18:4n1)	0.00	0.00	0.41		0.1	0.14
Arachidic(20:0)	0.15	0.21	0.42		0.3	0.08
Eicosen(20:1)	2.58	2.23	3.99		2.9	0.54
Eicosad(20:2)	2.30	2.48	2.38		2.4	0.05
(20:3n6)	0.50	0.35	0.53		0.5	0.05
Arachidon(20:4)	4.64	3.31	2.83		3.6	0.54
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.37	1.38	1.16		1.3	0.07
EPA(20:5)	3.20	3.06	3.22		3.2	0.05
Behenic(22:0)	0.56	0.00	0.25		0.3	0.16
Erucic(22:1)	0.18	0.22	0.32		0.2	0.04
(21:5n3)	0.00	0.00	0.13		0.0	0.04
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.34	1.58	1.00		1.3	0.17
(22:5n6)	3.55	3.07	2.93		3.2	0.19
DPA(22:5)	3.08	2.38	2.43		2.6	0.22
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	42.02	37.05	31.57		36.9	3.02
Nervonic(24:1)	0.37	0.51	0.68		0.5	0.09

Fatty Acid Indices

Saturates	13.0	19.1	21.0		17.7	2.42
Monoenes	21.1	21.4	25.8		22.8	1.53
PUFA	65.9	59.5	53.2		59.5	3.67
n6	13.6	12.5	11.2		12.4	0.67
n3	51.2	45.8	40.4		45.8	3.11
n3 HUFA	49.7	43.9	38.5		44.0	3.22
n3/n6	3.8	3.7	3.6		3.7	0.05

Treatment: Cold Survivors
Tissue: Muscle
Fraction: Neutral

Name	1=Run 161	2=Run 195	3=Run 250		Mean	SE
Myristic(14:0)	6.04	6.13	5.61		5.9	0.16
Myristolic(14:1)	0.10	0.00	0.00		0.0	0.03
Palmitic(16:0)	16.92	17.04	17.24		17.1	0.09
Palmitol(16:1)	8.22	8.64	7.45		8.1	0.35
(16:2n4)	0.44	0.51	0.42		0.5	0.03
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.41	0.37	0.40		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.76	1.71	1.95		1.8	0.07
Oleic(18:1)	18.82	16.23	18.86		18.0	0.87
Vaccenic(18:1)	4.46	4.55	4.31		4.4	0.07
Linoleic(18:2)	4.34	4.58	4.05		4.3	0.15
gLinolen(18:3)	0.41	0.39	0.37		0.4	0.01
(18:3n4)	0.10	0.19	0.18		0.2	0.03
aLinolen(18:3)	5.51	5.68	5.04		5.4	0.19
Stearidon(18:4)	3.69	3.75	3.21		3.6	0.17
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.28	0.26	0.28		0.3	0.01
Eicosen(20:1)	2.22	1.95	2.49		2.2	0.15
Eicosad(20:2)	1.20	1.28	1.13		1.2	0.04
(20:3n6)	0.31	0.26	0.29		0.3	0.01
Arachidon(20:4)	2.33	2.55	2.55		2.5	0.07
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.91	1.84	1.91		1.9	0.02
EPA(20:5)	8.07	8.47	7.27		7.9	0.35
Behenic(22:0)	0.16	0.00	0.00		0.1	0.05
Erucic(22:1)	0.47	0.38	0.46		0.4	0.03
(21:5n3)	0.40	0.39	0.38		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.45	0.47	0.59		0.5	0.04
(22:5n6)	1.29	1.47	1.46		1.4	0.06
DPA(22:5)	1.73	1.77	1.96		1.8	0.07
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	7.44	8.71	9.39		8.5	0.57
Nervonic(24:1)	0.50	0.41	0.75		0.6	0.10

Fatty Acid Indices

Saturates	25.2	25.1	25.1		25.1	0.03
Monoenes	34.8	32.2	34.3		33.8	0.81
PUFA	40.0	42.7	40.6		41.1	0.81
n6	10.3	11.0	10.4		10.6	0.21
n3	28.8	30.6	29.2		29.5	0.57
n3 HUFA	19.6	21.2	20.9		20.6	0.50
n3/n6	2.8	2.8	2.8		2.8	0.00

Treatment: Cold Survivors
Tissue: Muscle
Fraction: Polar

Name	1=Run 162	2=Run 199	3=Run 251		Mean	SE
Myristic(14:0)	1.89	0.95	1.01		1.3	0.30
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	16.86	15.56	15.78		16.1	0.40
Palmitol(16:1)	2.40	1.41	1.37		1.7	0.34
(16:2n4)	0.31	0.41	0.29		0.3	0.04
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.00	0.20	0.19		0.1	0.07
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	5.77	6.43	6.84		6.3	0.31
Oleic(18:1)	8.49	6.08	6.24		6.9	0.78
Vaccenic(18:1)	4.37	4.22	4.27		4.3	0.04
Linoleic(18:2)	2.02	1.84	1.63		1.8	0.11
gLinolen(18:3)	0.00	0.17	0.00		0.1	0.06
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	2.00	1.58	1.45		1.7	0.17
Stearidon(18:4)	1.03	0.65	0.57		0.7	0.14
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.00	0.11	0.00		0.0	0.04
Eicosen(20:1)	1.03	0.58	0.90		0.8	0.13
Eicosad(20:2)	0.93	0.87	0.81		0.9	0.03
(20:3n6)	0.32	0.35	0.34		0.3	0.01
Arachidon(20:4)	6.23	6.91	6.33		6.5	0.21
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.21	1.10	1.22		1.2	0.04
EPA(20:5)	6.14	6.62	5.88		6.2	0.22
Behenic(22:0)	0.85	0.00	0.00		0.3	0.28
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.30	0.34	0.24		0.3	0.03
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.68	0.80	0.69		0.7	0.04
(22:5n6)	3.20	3.80	3.59		3.5	0.17
DPA(22:5)	2.55	2.74	2.61		2.6	0.06
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	31.43	36.30	37.74		35.2	1.91
Nervonic(24:1)	0.00	0.00	0.00		0.0	0.00
Fatty Acid Indices						
Saturates	25.4	23.0	23.6		24.0	0.70
Monoenes	16.3	12.3	12.8		13.8	1.26
PUFA	58.3	64.7	63.6		62.2	1.95
n6	13.4	14.7	13.4		13.8	0.45
n3	44.7	49.3	49.7		47.9	1.62
n3 HUFA	41.6	47.1	47.7		45.5	1.93
n3/n6	3.3	3.3	3.7		3.5	0.12

Treatment: Warm Survivors
Tissue: Gill
Fraction: Neutral

Name	1=Run 171	2=Run 213	3=Run 267		Mean	SE
Myristic(14:0)	5.59	5.37	5.62		5.5	0.08
Myristolic(14:1)	0.08	0.09	0.09		0.1	0.00
Palmitic(16:0)	16.73	16.21	17.42		16.8	0.35
Palmitol(16:1)	7.34	8.06	7.46		7.6	0.22
(16:2n4)	0.66	0.50	0.61		0.6	0.05
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.35	0.36	0.40		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.40	2.21	2.58		2.4	0.11
Oleic(18:1)	16.45	16.71	16.49		16.5	0.08
Vaccenic(18:1)	4.22	4.43	4.26		4.3	0.06
Linoleic(18:2)	4.67	4.58	4.41		4.6	0.08
gLinolen(18:3)	0.39	0.09	0.10		0.2	0.10
(18:3n4)	0.00	0.12	0.07		0.1	0.04
aLinolen(18:3)	5.49	5.32	5.12		5.3	0.11
Stearidon(18:4)	3.09	2.96	2.80		2.9	0.09
(18:4n1)	0.00	0.13	0.08		0.1	0.04
Arachidic(20:0)	0.52	0.46	0.48		0.5	0.02
Eicosen(20:1)	1.67	2.09	1.95		1.9	0.12
Eicosad(20:2)	1.10	1.23	1.08		1.1	0.05
(20:3n6)	0.29	0.28	0.29		0.3	0.00
Arachidon(20:4)	3.14	2.81	2.81		2.9	0.11
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.96	2.00	1.92		2.0	0.02
EPA(20:5)	7.73	7.41	6.89		7.3	0.25
Behenic(22:0)	0.31	0.17	0.13		0.2	0.06
Erucic(22:1)	0.37	0.40	0.33		0.4	0.02
(21:5n3)	0.35	0.38	0.34		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.76	0.79	0.73		0.8	0.02
(22:5n6)	1.63	1.65	1.61		1.6	0.01
DPA(22:5)	2.58	2.42	2.54		2.5	0.05
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	9.28	10.08	10.70		10.0	0.41
Nervonic(24:1)	0.82	0.70	0.71		0.7	0.04
Fatty Acid Indices						
Saturates	25.6	24.4	26.2		25.4	0.53
Monoenes	31.0	32.5	31.3		31.6	0.46
PUFA	43.5	43.1	42.5		43.0	0.29
n6	12.0	11.4	11.0		11.5	0.28
n3	30.5	30.6	30.3		30.5	0.08
n3 HUFA	21.9	22.3	22.4		22.2	0.15
n3/n6	2.5	2.7	2.7		2.7	0.06

Treatment: Warm Survivors
Tissue: Gill
Fraction: Polar

Name	1=Run 172	2=Run 215	3=Run 257		Mean	SE
Myristic(14:0)	3.11	2.33	2.58		2.7	0.23
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	30.21	28.88	30.67		29.9	0.53
Palmitol(16:1)	2.84	2.61	2.63		2.7	0.08
(16:2n4)	1.23	1.16	1.03		1.1	0.06
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.45	0.41	0.41		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	10.35	10.05	10.44		10.3	0.12
Oleic(18:1)	14.99	14.89	13.74		14.5	0.40
Vaccenic(18:1)	3.69	3.68	3.93		3.8	0.08
Linoleic(18:2)	1.06	1.17	1.08		1.1	0.03
gLinolen(18:3)	0.38	0.36	0.21		0.3	0.05
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.61	0.79	0.73		0.7	0.05
Stearidon(18:4)	0.22	0.26	0.31		0.3	0.02
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.63	0.71	0.42		0.6	0.09
Eicosen(20:1)	0.59	0.44	0.59		0.5	0.05
Eicosad(20:2)	0.43	0.54	0.45		0.5	0.03
(20:3n6)	0.00	0.18	0.17		0.1	0.06
Arachidon(20:4)	4.23	4.34	3.91		4.2	0.13
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.27	0.34	0.33		0.3	0.02
EPA(20:5)	2.61	3.24	2.64		2.8	0.20
Behenic(22:0)	0.00	0.00	0.00		0.0	0.00
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	1.43	0.00	0.00		0.5	0.48
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.14	1.34	1.13		1.2	0.07
(22:5n6)	2.15	2.19	1.74		2.0	0.14
DPA(22:5)	1.04	1.19	1.12		1.1	0.04
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	16.33	18.58	18.99		18.0	0.83
Nervonic(24:1)	0.00	0.31	0.76		0.4	0.22

Fatty Acid Indices

Saturates	44.3	42.0	44.1		43.5	0.74
Monoenes	22.1	21.9	21.6		21.9	0.14
PUFA	33.6	36.1	34.3		34.6	0.75
n6	9.4	10.1	8.7		9.4	0.41
n3	22.5	24.4	24.1		23.7	0.58
n3 HUFA	21.7	23.3	23.1		22.7	0.51
n3/n6	2.4	2.4	2.8		2.57	0.12

Treatment: Warm Survivors
Tissue: Liver
Fraction: Neutral

Name	1=Run 177	2=Run 228	3=Run 261		Mean	SE
Myristic(14:0)	4.11	4.24	4.51		4.3	0.12
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	20.37	19.38	21.34		20.4	0.57
Palmitol(16:1)	6.89	7.21	7.10		7.1	0.09
(16:2n4)	0.49	0.53	0.53		0.5	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.39	0.39	0.48		0.4	0.03
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.10	2.30	2.84		2.4	0.22
Oleic(18:1)	13.08	14.15	14.21		13.8	0.37
Vaccenic(18:1)	4.03	4.18	4.31		4.2	0.08
Linoleic(18:2)	3.15	3.13	3.05		3.1	0.03
gLinolen(18:3)	0.88	0.81	0.93		0.9	0.03
(18:3n4)	0.00	0.14	0.08		0.1	0.04
aLinolen(18:3)	2.83	2.56	2.69		2.7	0.08
Stearidon(18:4)	3.28	2.93	3.22		3.1	0.11
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.11	0.11	0.11		0.1	0.00
Eicosen(20:1)	0.96	0.23	0.19		0.5	0.25
Eicosad(20:2)	0.76	0.78	0.69		0.7	0.03
(20:3n6)	0.20	0.24	0.28		0.2	0.03
Arachidon(20:4)	2.26	2.03	2.12		2.1	0.07
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.38	1.56	1.71		1.5	0.09
EPA(20:5)	6.09	5.28	4.11		5.2	0.57
Behenic(22:0)	0.00	0.00	0.00		0.0	0.00
Erucic(22:1)	0.18	0.23	0.22		0.2	0.01
(21:5n3)	0.42	0.42	0.47		0.4	0.02
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.10	1.30	1.40		1.3	0.09
(22:5n6)	1.79	1.87	1.66		1.8	0.06
DPA(22:5)	3.30	3.31	3.25		3.3	0.02
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	19.61	20.34	18.20		19.4	0.63
Nervonic(24:1)	0.26	0.36	0.29		0.3	0.03

Fatty Acid Indices

Saturates	26.7	26.0	28.8		27.2	0.83
Monoenes	25.4	26.4	26.3		26.0	0.32
PUFA	47.9	47.6	44.9		46.8	0.96
n6	10.1	10.1	10.1		10.1	0.01
n3	36.9	36.4	33.7		35.7	1.01
n3 HUFA	30.8	30.9	27.7		29.8	1.04
n3/n6	3.6	3.6	3.3		3.5	0.10

Treatment: Warm Survivors
Tissue: Liver
Fraction: Polar

Name	1=Run 192	2=Run 230	3=Run 262		Mean	SE
Myristic(14:0)	1.76	2.31	1.65		1.9	0.20
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	31.83	34.53	31.05		32.5	1.05
Palmitol(16:1)	1.70	1.71	1.31		1.6	0.13
(16:2n4)	0.37	0.36	0.34		0.4	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.14	0.17	0.16		0.2	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	7.53	7.79	11.69		9.0	1.35
Oleic(18:1)	5.72	6.47	5.70		6.0	0.25
Vaccenic(18:1)	2.11	2.13	1.85		2.0	0.09
Linoleic(18:2)	0.69	0.70	0.56		0.6	0.04
gLinolen(18:3)	0.00	0.11	0.13		0.1	0.04
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.49	0.44	0.00		0.3	0.16
Stearidon(18:4)	0.41	0.34	0.26		0.3	0.04
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.00	0.11	0.09		0.1	0.04
Eicosen(20:1)	0.30	0.40	0.27		0.3	0.04
Eicosad(20:2)	0.63	0.71	0.57		0.6	0.04
(20:3n6)	0.00	0.18	0.26		0.1	0.08
Arachidon(20:4)	3.12	2.62	3.44		3.1	0.24
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.36	0.40	0.43		0.4	0.02
EPA(20:5)	2.98	2.22	1.68		2.3	0.38
Behenic(22:0)	0.00	0.00	1.26		0.4	0.42
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.90	0.82	0.74		0.8	0.05
(22:5n6)	1.27	1.28	1.07		1.2	0.07
DPA(22:5)	1.45	1.37	1.21		1.3	0.07
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	36.25	32.82	33.36		34.1	1.06
Nervonic(24:1)	0.00	0.00	0.90		0.3	0.30

Fatty Acid Indices

Saturates	41.1	44.7	45.8		43.9	1.41
Monoenes	9.8	10.7	10.0		10.2	0.27
PUFA	49.1	44.6	44.2		45.9	1.56
n6	6.6	6.4	6.8		6.6	0.10
n3	41.9	37.6	36.9		38.8	1.57
n3 HUFA	41.0	36.8	36.7		38.2	1.43
n3/n6	6.3	5.8	5.5		5.9	0.26

Treatment: Warm Survivors
Tissue: Muscle
Fraction: Neutral

Name	1=Run 173	2=Run 224	3=Run 258		Mean	SE
Myristic(14:0)	6.56	5.90	6.76		6.4	0.26
Myristolic(14:1)	0.00	0.10	0.11		0.1	0.03
Palmitic(16:0)	17.43	16.95	17.76		17.4	0.24
Palmitol(16:1)	8.77	9.18	8.56		8.8	0.18
(16:2n4)	0.39	0.42	0.42		0.4	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.52	0.36	0.36		0.4	0.05
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.82	1.95	2.04		1.9	0.06
Oleic(18:1)	17.13	17.61	17.24		17.3	0.15
Vaccenic(18:1)	4.30	4.41	4.16		4.3	0.07
Linoleic(18:2)	4.66	4.68	4.71		4.7	0.01
gLinolen(18:3)	0.38	0.07	0.08		0.2	0.10
(18:3n4)	0.00	0.12	0.11		0.1	0.04
aLinolen(18:3)	6.17	5.77	5.95		6.0	0.12
Stearidon(18:4)	3.52	3.29	3.32		3.4	0.07
(18:4n1)	0.00	0.06	0.00		0.0	0.02
Arachidic(20:0)	0.28	0.30	0.32		0.3	0.01
Eicosen(20:1)	1.64	0.41	0.39		0.8	0.41
Eicosad(20:2)	1.13	1.27	1.16		1.2	0.04
(20:3n6)	0.27	0.33	0.31		0.3	0.02
Arachidon(20:4)	2.53	2.55	2.54		2.5	0.01
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.87	1.93	1.98		1.9	0.03
EPA(20:5)	8.11	7.78	7.78		7.9	0.11
Behenic(22:0)	0.14	0.15	0.17		0.2	0.01
Erucic(22:1)	0.30	0.40	0.38		0.4	0.03
(21:5n3)	0.35	0.40	0.39		0.4	0.02
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.45	0.59	0.48		0.5	0.04
(22:5n6)	1.27	1.41	1.42		1.4	0.05
DPA(22:5)	2.05	2.17	2.12		2.1	0.04
Lignocer(24:0)	0.00	0.00	0.09		0.0	0.03
DHA(22:6)	7.61	8.92	8.33		8.3	0.38
Nervonic(24:1)	0.37	0.53	0.54		0.5	0.05
Fatty Acid Indices						
Saturates	26.2	25.2	27.1		26.2	0.55
Monoenes	32.5	32.7	31.4		32.2	0.40
PUFA	41.3	42.1	41.5		41.6	0.25
n6	10.7	10.9	10.7		10.8	0.07
n3	29.7	30.3	29.9		29.9	0.17
n3 HUFA	20.0	21.2	20.6		20.6	0.35
n3/n6	2.8	2.8	2.8		2.8	0.01

Treatment: Warm Survivors
Tissue: Muscle
Fraction: Polar

Name	1=Run 176	2=Run 227	3=Run 264		Mean	SE
Myristic(14:0)	1.11	1.08	0.07		0.8	0.34
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	29.71	29.03	28.23		29.0	0.43
Palmitol(16:1)	1.40	1.42	1.09		1.3	0.11
(16:2n4)	0.25	0.26	0.25		0.3	0.00
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.16	0.18	0.15		0.2	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	6.34	6.85	7.56		6.9	0.35
Oleic(18:1)	6.92	7.30	6.12		6.8	0.35
Vaccenic(18:1)	2.15	2.22	2.36		2.2	0.06
Linoleic(18:2)	1.04	1.12	0.99		1.0	0.04
gLinolen(18:3)	0.00	0.00	0.14		0.0	0.05
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.82	0.76	0.65		0.7	0.05
Stearidon(18:4)	0.28	0.24	0.18		0.2	0.03
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.12	0.14	0.09		0.1	0.01
Eicosen(20:1)	0.38	0.45	0.10		0.3	0.11
Eicosad(20:2)	0.56	0.62	0.57		0.6	0.02
(20:3n6)	0.14	0.16	0.15		0.2	0.01
Arachidon(20:4)	6.35	6.70	6.21		6.4	0.15
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.42	0.43	0.42		0.4	0.01
EPA(20:5)	7.87	7.81	6.15		7.3	0.56
Behenic(22:0)	0.00	0.00	0.39		0.1	0.13
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.60	0.70	0.58		0.6	0.04
(22:5n6)	2.47	2.70	2.46		2.5	0.08
DPA(22:5)	1.55	1.58	1.71		1.6	0.05
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	29.36	28.25	33.18		30.3	1.49
Nervonic(24:1)	0.00	0.00	0.22		0.1	0.07

Fatty Acid Indices

Saturates	37.3	37.1	36.3		36.9	0.29
Monoenes	10.8	11.4	9.9		10.7	0.44
PUFA	51.9	51.5	53.8		52.4	0.70
n6	11.2	12.0	11.1		11.4	0.29
n3	40.3	39.1	42.3		40.6	0.93
n3 HUFA	39.2	38.1	41.4		39.6	0.99
n3/n6	3.6	3.3	3.8		3.6	0.16

Treatment: Cold Morts
Tissue: Gill
Fraction: Neutral

Name	1=Run 281	2=Run 327	3=Run 349		Mean	SE
Myristic(14:0)	5.85	5.98	6.01		5.9	0.05
Myristolic(14:1)	0.10	0.10	0.08		0.1	0.01
Palmitic(16:0)	15.70	17.95	16.29		16.6	0.67
Palmitol(16:1)	8.18	7.95	9.13		8.4	0.36
(16:2n4)	0.55	0.56	0.53		0.5	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.43	0.48	0.40		0.4	0.02
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.99	2.12	1.82		2.0	0.09
Oleic(18:1)	16.08	18.28	16.57		17.0	0.67
Vaccenic(18:1)	4.41	4.69	4.62		4.6	0.08
Linoleic(18:2)	4.65	4.73	4.89		4.8	0.07
gLinolen(18:3)	0.42	0.09	0.08		0.2	0.11
(18:3n4)	0.00	0.10	0.14		0.1	0.04
aLinolen(18:3)	5.68	5.18	5.83		5.6	0.20
Stearidon(18:4)	3.33	2.66	3.79		3.3	0.33
(18:4n1)	0.18	0.21	0.15		0.2	0.02
Arachidic(20:0)	0.35	0.48	0.35		0.4	0.04
Eicosen(20:1)	1.83	2.13	0.45		1.5	0.52
Eicosad(20:2)	1.18	1.18	1.34		1.2	0.05
(20:3n6)	0.30	0.32	0.36		0.3	0.02
Arachidon(20:4)	2.90	2.78	2.73		2.8	0.05
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	2.00	1.86	1.87		1.9	0.04
EPA(20:5)	8.20	6.71	8.51		7.8	0.56
Behenic(22:0)	0.11	0.16	0.13		0.1	0.01
Erucic(22:1)	0.34	0.38	0.35		0.4	0.01
(21:5n3)	0.39	0.32	0.43		0.4	0.03
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.68	0.71	0.65		0.7	0.02
(22:5n6)	1.56	1.47	1.52		1.5	0.03
DPA(22:5)	2.26	1.96	1.96		2.1	0.10
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	9.82	7.79	8.57		8.7	0.59
Nervonic(24:1)	0.52	0.69	0.47		0.6	0.07
Fatty Acid Indices						
Saturates	24.0	26.7	24.6		25.1	0.81
Monoenes	31.5	34.2	31.7		32.5	0.89
PUFA	44.5	39.1	43.7		42.5	1.70
n6	11.7	11.3	11.6		11.5	0.12
n3	31.7	26.5	31.0		29.7	1.63
n3 HUFA	22.7	18.6	21.3		20.9	1.19
n3/n6	2.7	2.3	2.7		2.6	0.12

Treatment: Cold Morts
Tissue: Gill
Fraction: Polar

Name	1=Run 286	2=Run 319	3=Run 352		Mean	SE
Myristic(14:0)	3.13	2.12	2.34		2.5	0.31
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	27.80	22.48	26.19		25.5	1.58
Palmitol(16:1)	2.63	1.91	2.46		2.3	0.22
(16:2n4)	1.46	1.14	1.17		1.3	0.10
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.62	0.42	0.54		0.5	0.06
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	10.56	9.76	9.20		9.8	0.39
Oleic(18:1)	10.72	10.20	10.04		10.3	0.20
Vaccenic(18:1)	6.22	5.79	6.30		6.1	0.16
Linoleic(18:2)	1.43	1.25	1.35		1.3	0.05
gLinolen(18:3)	0.31	0.27	0.29		0.3	0.01
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.85	0.91	0.83		0.9	0.02
Stearidon(18:4)	0.41	0.41	0.35		0.4	0.02
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.71	0.66	0.67		0.7	0.02
Eicosen(20:1)	0.89	0.91	0.79		0.9	0.04
Eicosad(20:2)	0.75	0.72	0.71		0.7	0.01
(20:3n6)	0.00	0.24	0.24		0.2	0.08
Arachidon(20:4)	5.41	5.96	5.94		5.8	0.18
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.50	0.59	0.45		0.5	0.04
EPA(20:5)	3.57	4.19	4.20		4.0	0.21
Behenic(22:0)	0.00	0.00	0.00		0.0	0.00
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.65	1.67	1.59		1.6	0.03
(22:5n6)	2.51	3.16	2.52		2.7	0.21
DPA(22:5)	1.30	1.61	1.30		1.4	0.10
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	16.58	23.18	19.99		19.9	1.90
Nervonic(24:1)	0.00	0.48	0.54		0.3	0.17

Fatty Acid Indices

Saturates	42.2	35.0	38.4		38.5	2.08
Monoenes	20.5	19.3	20.1		20.0	0.35
PUFA	37.3	45.7	41.5		41.5	2.41
n6	12.1	13.3	12.6		12.7	0.35
n3	23.2	30.9	27.1		27.1	2.21
n3 HUFA	22.0	29.6	25.9		25.8	2.20
n3/n6	1.9	2.3	2.1		2.1	0.12

Treatment: Cold Morts
Tissue: Liver
Fraction: Neutral

Name	1=Run 301	2=Run 332	3=Run 359		Mean	SE
Myristic(14:0)	5.44	5.45	4.96		5.3	0.16
Myristolic(14:1)	0.09	0.10	0.00		0.1	0.03
Palmitic(16:0)	12.12	12.53	12.69		12.4	0.17
Palmitol(16:1)	7.60	7.42	8.34		7.8	0.28
(16:2n4)	0.45	0.45	0.41		0.4	0.02
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.48	0.53	0.52		0.5	0.02
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.88	1.51	1.55		1.6	0.12
Oleic(18:1)	22.78	24.64	20.86		22.8	1.09
Vaccenic(18:1)	4.76	4.86	5.87		5.2	0.35
Linoleic(18:2)	3.93	3.72	3.88		3.8	0.06
gLinolen(18:3)	0.33	0.29	0.33		0.3	0.01
(18:3n4)	0.00	0.07	0.00		0.0	0.02
aLinolen(18:3)	4.64	4.32	4.43		4.5	0.09
Stearidon(18:4)	2.70	2.53	3.05		2.8	0.16
(18:4n1)	0.00	0.08	0.00		0.0	0.03
Arachidic(20:0)	0.16	0.15	0.00		0.1	0.05
Eicosen(20:1)	3.26	3.11	2.59		3.0	0.20
Eicosad(20:2)	1.31	1.39	1.54		1.4	0.07
(20:3n6)	0.27	0.30	0.24		0.3	0.02
Arachidon(20:4)	2.38	2.02	2.50		2.3	0.15
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.62	1.67	1.87		1.7	0.08
EPA(20:5)	6.08	5.61	6.51		6.1	0.26
Behenic(22:0)	0.00	0.08	0.00		0.0	0.03
Erucic(22:1)	0.69	0.74	0.65		0.7	0.03
(21:5n3)	0.33	0.37	0.35		0.3	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.09	1.26	1.46		1.3	0.11
(22:5n6)	1.80	1.76	1.74		1.8	0.02
DPA(22:5)	2.20	2.19	1.86		2.1	0.11
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	10.66	9.88	10.74		10.4	0.28
Nervonic(24:1)	0.95	1.01	1.06		1.0	0.03
Fatty Acid Indices						
Saturates	19.6	19.7	19.2		19.5	0.16
Monoenes	40.1	41.9	39.4		40.5	0.74
PUFA	40.3	38.4	41.4		40.0	0.88
n6	11.1	10.7	11.7		11.2	0.28
n3	28.2	26.6	28.8		27.9	0.68
n3 HUFA	20.9	19.7	21.3		20.6	0.48
n3/n6	2.5	2.5	2.5		2.5	0.02

Treatment: Cold Morts
Tissue: Liver
Fraction: Polar

Name	1=Run 302	2=Run 334	3=Run 358		Mean	SE
Myristic(14:0)	2.84	2.68	2.01		2.5	0.25
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	16.86	15.08	13.77		15.2	0.90
Palmitol(16:1)	2.73	2.16	2.88		2.6	0.22
(16:2n4)	0.60	0.56	0.68		0.6	0.04
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.26	0.20	0.28		0.2	0.02
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	3.95	3.69	3.51		3.7	0.13
Oleic(18:1)	9.94	10.29	9.07		9.8	0.36
Vaccenic(18:1)	5.88	6.42	6.29		6.2	0.16
Linoleic(18:2)	1.57	1.26	1.73		1.5	0.14
gLinolen(18:3)	0.00	0.10	0.00		0.0	0.03
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	1.39	1.10	1.65		1.4	0.16
Stearidon(18:4)	0.69	0.50	0.84		0.7	0.10
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.20	0.19	0.22		0.2	0.01
Eicosen(20:1)	2.51	2.85	2.05		2.5	0.23
Eicosad(20:2)	2.37	2.31	2.59		2.4	0.09
(20:3n6)	0.43	0.38	0.47		0.4	0.02
Arachidon(20:4)	3.18	3.15	3.54		3.3	0.12
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.06	1.22	1.41		1.2	0.10
EPA(20:5)	3.24	2.54	3.82		3.2	0.37
Behenic(22:0)	0.74	0.00	0.00		0.2	0.25
Erucic(22:1)	0.00	0.20	0.00		0.1	0.07
(21:5n3)	0.00	0.13	0.00		0.0	0.04
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	1.20	1.11	1.93		1.4	0.26
(22:5n6)	2.86	2.96	2.58		2.8	0.11
DPA(22:5)	2.64	2.85	2.47		2.7	0.11
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	32.46	35.55	35.67		34.6	1.05
Nervonic(24:1)	0.40	0.48	0.55		0.5	0.04

Fatty Acid Indices

Saturates	24.6	21.6	19.5		21.9	1.48
Monoenes	21.4	22.4	20.8		21.6	0.46
PUFA	54.0	56.0	59.7		56.5	1.68
n6	11.6	11.3	12.8		11.9	0.47
n3	41.5	43.9	45.9		43.8	1.27
n3 HUFA	39.4	42.3	43.4		41.7	1.19
n3/n6	3.6	3.9	3.6		3.7	0.11

Treatment: Cold Morts
Tissue: Muscle
Fraction: Neutral

Name	1=Run 303	2=Run 322	3=Run 353		Mean	SE
Myristic(14:0)	6.03	5.83	6.08		6.0	0.08
Myristolic(14:1)	0.09	0.10	0.00		0.1	0.03
Palmitic(16:0)	16.53	17.67	17.24		17.1	0.33
Palmitol(16:1)	8.23	7.86	9.12		8.4	0.37
(16:2n4)	0.40	0.44	0.40		0.4	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.35	0.38	0.40		0.4	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.85	1.94	1.50		1.8	0.13
Oleic(18:1)	18.70	19.59	16.66		18.3	0.87
Vaccenic(18:1)	4.14	4.23	4.76		4.4	0.20
Linoleic(18:2)	4.44	4.42	4.51		4.5	0.03
gLinolen(18:3)	0.38	0.07	0.41		0.3	0.11
(18:3n4)	0.09	0.11	0.00		0.1	0.03
aLinolen(18:3)	5.76	5.56	5.35		5.6	0.12
Stearidon(18:4)	3.52	3.50	3.92		3.6	0.14
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.26	0.29	0.23		0.3	0.02
Eicosen(20:1)	2.39	0.36	1.94		1.6	0.62
Eicosad(20:2)	1.18	1.19	1.37		1.2	0.06
(20:3n6)	0.26	0.30	0.29		0.3	0.01
Arachidon(20:4)	2.38	2.48	2.37		2.4	0.03
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.96	2.00	1.84		1.9	0.05
EPA(20:5)	7.63	8.02	8.07		7.9	0.14
Behenic(22:0)	0.11	0.14	0.00		0.1	0.04
Erucic(22:1)	0.40	0.45	0.37		0.4	0.03
(21:5n3)	0.38	0.41	0.41		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.45	0.47	0.44		0.5	0.01
(22:5n6)	1.38	1.45	1.44		1.4	0.02
DPA(22:5)	1.92	1.93	1.66		1.8	0.09
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	8.33	8.30	8.79		8.5	0.16
Nervonic(24:1)	0.45	0.52	0.43		0.5	0.03
Fatty Acid Indices						
Saturates	24.8	25.9	25.0		25.2	0.33
Monoenes	34.4	33.1	33.3		33.6	0.41
PUFA	40.8	41.0	41.7		41.2	0.26
n6	10.5	10.4	10.8		10.6	0.14
n3	29.5	29.7	30.0		29.8	0.16
n3 HUFA	20.2	20.7	20.8		20.6	0.17
n3/n6	2.8	2.9	2.8		2.8	0.03

Treatment: Cold Morts
Tissue: Muscle
Fraction: Polar

Name	1=Run 300	2=Run 326	3=Run 392		Mean	SE
Myristic(14:0)	0.89	1.31	0.96		1.1	0.13
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	19.31	23.22	20.41		21.0	1.17
Palmitol(16:1)	1.13	1.46	1.24		1.3	0.10
(16:2n4)	0.29	0.24	0.30		0.3	0.02
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.17	0.00	0.18		0.1	0.06
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	6.04	6.95	5.74		6.2	0.36
Oleic(18:1)	5.07	5.70	5.17		5.3	0.20
Vaccenic(18:1)	3.56	3.75	3.66		3.7	0.05
Linoleic(18:2)	1.43	1.50	1.69		1.5	0.08
gLinolen(18:3)	0.10	0.19	0.00		0.1	0.05
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	1.23	1.18	1.24		1.2	0.02
Stearidon(18:4)	0.49	0.49	0.52		0.5	0.01
(18:4n1)	0.00	0.16	0.00		0.1	0.05
Arachidic(20:0)	0.08	0.21	0.00		0.1	0.06
Eicosen(20:1)	0.50	0.77	0.42		0.6	0.10
Eicosad(20:2)	0.72	0.81	0.72		0.7	0.03
(20:3n6)	0.26	0.37	0.30		0.3	0.03
Arachidon(20:4)	6.45	6.41	6.13		6.3	0.10
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.93	0.91	0.95		0.9	0.01
EPA(20:5)	6.97	5.99	6.38		6.4	0.29
Behenic(22:0)	0.00	0.00	0.00		0.0	0.00
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.29	0.26	0.69		0.4	0.14
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.56	0.52	0.74		0.6	0.07
(22:5n6)	3.45	3.21	3.63		3.4	0.12
DPA(22:5)	2.47	2.19	2.47		2.4	0.09
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	37.60	32.21	36.46		35.4	1.64
Nervonic(24:1)	0.00	0.00	0.00		0.0	0.00

Fatty Acid Indices

Saturates	26.3	31.7	27.1		28.4	1.67
Monoenes	10.3	11.7	10.5		10.8	0.44
PUFA	63.4	56.6	62.4		60.8	2.11
n6	13.0	13.0	13.2		13.1	0.08
n3	50.0	43.2	48.7		47.3	2.07
n3 HUFA	48.3	41.6	46.9		45.6	2.05
n3/n6	3.9	3.3	3.7		3.6	0.16

Treatment: Warm Morts
Tissue: Gill
Fraction: Neutral

Name	1=Run 305	2=Run 338	3=Run 361		Mean	SE
Myristic(14:0)	5.73	5.89	5.46		5.7	0.13
Myristolic(14:1)	0.10	0.12	0.12		0.1	0.01
Palmitic(16:0)	16.60	16.44	16.53		16.5	0.05
Palmitol(16:1)	7.90	8.19	7.58		7.9	0.18
(16:2n4)	0.52	0.40	0.59		0.5	0.06
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.53	0.66	0.57		0.6	0.04
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.81	2.38	1.92		2.0	0.18
Oleic(18:1)	17.86	15.57	19.32		17.6	1.09
Vaccenic(18:1)	4.36	3.71	4.05		4.0	0.19
Linoleic(18:2)	4.39	4.87	4.40		4.6	0.16
gLinolen(18:3)	0.08	0.10	0.08		0.1	0.00
(18:3n4)	0.11	0.11	0.09		0.1	0.01
aLinolen(18:3)	5.35	6.56	5.28		5.7	0.42
Stearidon(18:4)	2.95	3.48	2.95		3.1	0.18
(18:4n1)	0.06	0.35	0.39		0.3	0.10
Arachidic(20:0)	0.42	0.44	0.48		0.4	0.02
Eicosen(20:1)	1.84	0.36	2.21		1.5	0.56
Eicosad(20:2)	1.02	1.02	1.00		1.0	0.01
(20:3n6)	0.31	0.28	0.30		0.3	0.01
Arachidon(20:4)	2.82	3.13	2.71		2.9	0.13
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	2.03	1.79	2.08		2.0	0.09
EPA(20:5)	6.75	8.42	6.76		7.3	0.55
Behenic(22:0)	0.13	0.16	0.17		0.2	0.01
Erucic(22:1)	0.34	0.27	0.38		0.3	0.03
(21:5n3)	0.35	0.37	0.35		0.4	0.01
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.64	0.69	0.64		0.7	0.02
(22:5n6)	1.56	1.53	1.42		1.5	0.04
DPA(22:5)	2.44	2.43	2.38		2.4	0.02
Lignocer(24:0)	0.00	0.10	0.00		0.0	0.03
DHA(22:6)	10.31	9.58	9.03		9.6	0.37
Nervonic(24:1)	0.66	0.60	0.75		0.7	0.04

Fatty Acid Indices

Saturates	24.7	25.4	24.6		24.9	0.26
Monoenes	33.1	28.8	34.4		32.1	1.68
PUFA	42.2	45.8	41.0		43.0	1.42
n6	10.8	11.6	10.6		11.0	0.32
n3	30.2	32.6	28.8		30.6	1.12
n3 HUFA	21.9	22.6	20.6		21.7	0.59
n3/n6	2.8	2.8	2.7		2.8	0.02

Treatment: Warm Morts
Tissue: Gill
Fraction: Polar

Name	1=Run 307	2=Run 342	3=Run 362		Mean	SE
Myristic(14:0)	3.83	3.51	3.83		3.7	0.11
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	32.33	30.40	36.82		33.2	1.90
Palmitol(16:1)	3.09	3.09	2.71		3.0	0.13
(16:2n4)	0.97	1.23	0.82		1.0	0.12
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.67	0.51	0.94		0.7	0.12
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	12.00	10.42	9.80		10.7	0.66
Oleic(18:1)	12.74	15.59	15.01		14.4	0.87
Vaccenic(18:1)	3.29	3.79	3.82		3.6	0.17
Linoleic(18:2)	1.11	1.16	0.85		1.0	0.10
gLinolen(18:3)	0.42	0.41	0.37		0.4	0.02
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.79	0.72	0.55		0.7	0.07
Stearidon(18:4)	0.30	0.23	0.00		0.2	0.09
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	1.11	0.88	0.96		1.0	0.07
Eicosen(20:1)	0.98	0.81	0.82		0.9	0.05
Eicosad(20:2)	0.49	0.53	0.43		0.5	0.03
(20:3n6)	0.00	0.16	0.79		0.3	0.24
Arachidon(20:4)	3.82	4.33	3.35		3.8	0.28
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.40	0.28	0.25		0.3	0.04
EPA(20:5)	2.45	2.53	2.15		2.4	0.12
Behenic(22:0)	0.33	0.15	0.67		0.4	0.15
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	2.05	1.22	1.00		1.4	0.32
(22:5n6)	1.80	2.20	1.52		1.8	0.19
DPA(22:5)	0.86	1.07	0.74		0.9	0.10
Lignocer(24:0)	1.06	0.55	0.69		0.8	0.15
DHA(22:6)	11.98	13.78	10.39		12.0	0.98
Nervonic(24:1)	1.14	0.45	0.72		0.8	0.20

Fatty Acid Indices

Saturates	50.7	45.9	52.8		49.8	2.03
Monoenes	21.2	23.7	23.1		22.7	0.75
PUFA	28.1	30.4	24.2		27.5	1.81
n6	9.7	10.0	8.3		9.3	0.52
n3	16.8	18.6	14.1		16.5	1.32
n3 HUFA	15.7	17.7	13.5		15.6	1.19
n3/n6	1.7	1.9	1.7		1.8	0.05

Treatment: Warm Morts
Tissue: Liver
Fraction: Neutral

Name	1=Run 311	2=Run 348	3=Run 375		Mean	SE
Myristic(14:0)	5.50	5.53	5.36		5.5	0.05
Myristolic(14:1)	0.07	0.07	0.07		0.1	0.00
Palmitic(16:0)	19.82	22.76	18.27		20.3	1.32
Palmitol(16:1)	7.84	7.35	7.10		7.4	0.22
(16:2n4)	0.47	0.45	0.51		0.5	0.02
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.44	0.35	0.56		0.5	0.06
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	2.31	3.02	2.44		2.6	0.22
Oleic(18:1)	18.19	13.89	22.36		18.1	2.45
Vaccenic(18:1)	4.43	4.18	3.96		4.2	0.14
Linoleic(18:2)	3.61	3.68	3.39		3.6	0.09
gLinolen(18:3)	0.05	0.08	0.60		0.2	0.18
(18:3n4)	0.10	0.05	0.08		0.1	0.01
aLinolen(18:3)	3.93	4.14	3.45		3.8	0.21
Stearidon(18:4)	2.99	3.00	2.68		2.9	0.11
(18:4n1)	0.08	0.06	0.00		0.0	0.02
Arachidic(20:0)	0.18	0.27	0.16		0.2	0.03
Eicosen(20:1)	0.27	0.28	0.24		0.3	0.01
Eicosad(20:2)	0.94	0.88	0.79		0.9	0.04
(20:3n6)	0.25	0.19	0.30		0.2	0.03
Arachidon(20:4)	1.98	2.39	1.86		2.1	0.16
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.57	1.26	1.76		1.5	0.15
EPA(20:5)	5.15	6.01	4.24		5.1	0.51
Behenic(22:0)	0.08	0.09	0.09		0.1	0.01
Erucic(22:1)	0.31	0.25	0.43		0.3	0.05
(21:5n3)	0.37	0.32	0.40		0.4	0.02
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.92	0.83	1.29		1.0	0.14
(22:5n6)	1.65	1.58	1.52		1.6	0.04
DPA(22:5)	2.31	0.00	2.62		1.6	0.83
Lignocer(24:0)	0.00	2.29	0.00		0.8	0.76
DHA(22:6)	13.75	14.25	12.76		13.6	0.44
Nervonic(24:1)	0.46	0.51	0.69		0.6	0.07

Fatty Acid Indices

Saturates	27.9	34.0	26.3		29.4	2.33
Monoenes	31.6	26.5	34.9		31.0	2.42
PUFA	40.5	39.5	38.8		39.6	0.50
n6	9.4	9.6	9.8		9.6	0.10
n3	30.1	29.0	27.9		29.0	0.62
n3 HUFA	23.1	21.8	21.8		22.3	0.44
n3/n6	3.2	3.0	2.9		3.0	0.10

Treatment: Warm Morts
Tissue: Liver
Fraction: Polar

Name	1=Run 316	2=Run 347	3=Run 377		Mean	SE
Myristic(14:0)	4.21	2.87	4.16		3.7	0.44
Myristolic(14:1)	0.00	0.00	0.06		0.0	0.02
Palmitic(16:0)	30.02	35.86	34.39		33.4	1.75
Palmitol(16:1)	2.72	2.07	2.09		2.3	0.21
(16:2n4)	0.40	0.36	0.39		0.4	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.23	0.19	0.20		0.2	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	9.14	12.09	9.29		10.2	0.96
Oleic(18:1)	7.63	5.85	7.66		7.0	0.60
Vaccenic(18:1)	2.71	2.61	2.23		2.5	0.15
Linoleic(18:2)	1.11	0.84	0.86		0.9	0.09
gLinolen(18:3)	0.00	0.22	0.11		0.1	0.06
(18:3n4)	0.00	0.00	0.05		0.0	0.02
aLinolen(18:3)	1.05	0.65	0.76		0.8	0.12
Stearidon(18:4)	0.74	0.42	0.48		0.5	0.10
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.00	0.24	0.16		0.1	0.07
Eicosen(20:1)	0.76	0.53	0.10		0.5	0.19
Eicosad(20:2)	0.73	0.82	0.66		0.7	0.05
(20:3n6)	0.23	0.00	0.29		0.2	0.09
Arachidon(20:4)	5.35	4.36	4.36		4.7	0.33
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.57	0.31	0.49		0.5	0.07
EPA(20:5)	2.42	1.86	1.76		2.0	0.21
Behenic(22:0)	0.00	0.00	0.10		0.0	0.03
Erucic(22:1)	0.00	0.00	0.13		0.0	0.04
(21:5n3)	0.00	0.00	0.06		0.0	0.02
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.67	0.61	0.56		0.6	0.03
(22:5n6)	1.35	1.46	1.22		1.3	0.07
DPA(22:5)	1.37	1.33	1.52		1.4	0.06
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	26.59	24.46	25.56		25.5	0.62
Nervonic(24:1)	0.00	0.00	0.29		0.1	0.10

Fatty Acid Indices

Saturates	43.4	51.1	48.1		47.5	2.24
Monoenes	13.8	11.1	12.6		12.5	0.80
PUFA	42.8	37.9	39.3		40.0	1.46
n6	9.4	8.3	8.1		8.6	0.43
n3	32.7	29.0	30.6		30.8	1.07
n3 HUFA	30.9	28.0	29.4		29.4	0.86
n3/n6	3.5	3.5	3.8		3.6	0.11

Treatment: Warm Morts
Tissue: Muscle
Fraction: Neutral

Name	1=Run 328	2=Run 343	3=Run 371		Mean	SE
Myristic(14:0)	6.07	6.23	5.78		6.0	0.13
Myristolic(14:1)	0.11	0.12	0.13		0.1	0.01
Palmitic(16:0)	17.03	15.93	16.72		16.6	0.33
Palmitol(16:1)	8.01	8.86	7.72		8.2	0.34
(16:2n4)	0.42	0.45	0.43		0.4	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.41	0.74	0.43		0.5	0.11
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	1.89	2.39	2.07		2.1	0.15
Oleic(18:1)	19.67	16.74	20.78		19.1	1.20
Vaccenic(18:1)	4.25	3.75	3.80		3.9	0.16
Linoleic(18:2)	4.65	5.14	4.78		4.9	0.15
gLinolen(18:3)	0.08	0.08	0.07		0.1	0.00
(18:3n4)	0.10	0.10	0.09		0.1	0.00
aLinolen(18:3)	5.90	7.86	6.03		6.6	0.63
Stearidon(18:4)	3.13	3.98	3.44		3.5	0.25
(18:4n1)	0.00	0.05	0.00		0.0	0.02
Arachidic(20:0)	0.31	0.33	0.31		0.3	0.01
Eicosen(20:1)	2.17	0.28	0.30		0.9	0.63
Eicosad(20:2)	1.04	0.96	0.96		1.0	0.03
(20:3n6)	0.31	0.29	0.31		0.3	0.01
Arachidon(20:4)	2.46	2.74	2.80		2.7	0.10
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	1.90	1.83	2.05		1.9	0.06
EPA(20:5)	6.73	8.09	7.51		7.4	0.39
Behenic(22:0)	0.16	0.18	0.16		0.2	0.01
Erucic(22:1)	0.40	0.33	0.41		0.4	0.02
(21:5n3)	0.35	0.40	0.40		0.4	0.02
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.48	0.48	0.53		0.5	0.02
(22:5n6)	1.38	1.27	1.02		1.2	0.11
DPA(22:5)	2.08	2.31	2.19		2.2	0.07
Lignocer(24:0)	0.00	0.10	0.00		0.0	0.03
DHA(22:6)	7.96	7.56	8.19		7.9	0.18
Nervonic(24:1)	0.55	0.43	0.59		0.5	0.05

Fatty Acid Indices

Saturates	25.5	25.2	25.0		25.2	0.12
Monoenes	35.2	30.5	33.7		33.1	1.37
PUFA	39.4	44.3	41.2		41.7	1.44
n6	10.4	11.0	10.5		10.6	0.18
n3	28.1	32.0	29.8		30.0	1.15
n3 HUFA	19.0	20.2	20.3		19.9	0.42
n3/n6	2.7	2.9	2.8		2.8	0.07

Treatment: Warm Morts
Tissue: Muscle
Fraction: Polar

Name	1=Run 310	2=Run 344	3=Run 373		Mean	SE
Myristic(14:0)	1.09	1.16	1.19		1.1	0.03
Myristolic(14:1)	0.00	0.00	0.00		0.0	0.00
Palmitic(16:0)	28.08	28.74	29.93		28.9	0.54
Palmitol(16:1)	1.22	1.50	1.42		1.4	0.08
(16:2n4)	0.21	0.25	0.22		0.2	0.01
(17:0)	N/A	N/A	N/A		N/A	N/A
(16:3n4)	0.17	0.16	0.18		0.2	0.01
(16:4n1)	N/A	N/A	N/A		N/A	N/A
Stearic(18:0)	6.45	6.71	6.32		6.5	0.11
Oleic(18:1)	6.63	6.85	8.06		7.2	0.44
Vaccenic(18:1)	2.09	2.12	2.31		2.2	0.07
Linoleic(18:2)	0.99	1.12	1.16		1.1	0.05
gLinolen(18:3)	0.12	0.14	0.00		0.1	0.04
(18:3n4)	0.00	0.00	0.00		0.0	0.00
aLinolen(18:3)	0.67	0.95	0.83		0.8	0.08
Stearidon(18:4)	0.25	0.33	0.34		0.3	0.03
(18:4n1)	0.00	0.00	0.00		0.0	0.00
Arachidic(20:0)	0.10	0.14	0.00		0.1	0.04
Eicosen(20:1)	0.11	0.41	0.52		0.3	0.12
Eicosad(20:2)	0.52	0.56	0.61		0.6	0.02
(20:3n6)	0.19	0.19	0.20		0.2	0.00
Arachidon(20:4)	6.58	6.64	5.88		6.4	0.24
Eicosatri(20:3)	N/A	N/A	N/A		N/A	N/A
(20:4n3)	0.44	0.39	0.51		0.4	0.03
EPA(20:5)	7.16	8.08	6.86		7.4	0.37
Behenic(22:0)	0.00	0.80	0.00		0.3	0.27
Erucic(22:1)	0.00	0.00	0.00		0.0	0.00
(21:5n3)	0.00	0.00	0.00		0.0	0.00
Tricosa(23:0)	N/A	N/A	N/A		N/A	N/A
Docosatet(22:4)	0.62	0.52	0.64		0.6	0.04
(22:5n6)	2.61	2.38	2.61		2.5	0.08
DPA(22:5)	1.52	1.50	1.52		1.5	0.01
Lignocer(24:0)	0.00	0.00	0.00		0.0	0.00
DHA(22:6)	32.19	28.36	28.68		29.7	1.23
Nervonic(24:1)	0.00	0.00	0.00		0.0	0.00

Fatty Acid Indices

Saturates	35.7	37.5	37.4		36.9	0.59
Monoenes	10.1	10.9	12.3		11.1	0.66
PUFA	54.2	51.6	50.2		52.0	1.17
n6	11.6	11.5	11.1		11.4	0.17
n3	42.2	39.6	38.7		40.2	1.04
n3 HUFA	41.3	38.3	37.6		39.1	1.14
n3/n6	3.6	3.4	3.5		3.5	0.06