Forget Dad, its Mom Who Brings Home the Bacon: Maternal Provision of Fatty Acids to the Eggs of Walleye, *Sander vitreus* (Mitchill 1888)

Robin Lee Szmadyla

Supervisor: Dr. Murray Wiegand

A thesis submitted in partial fulfillment of
the Honours Thesis 05.4111/6 Course
Department of Biology
The University of Winnipeg
2007

Abstract

Two of the factors which influence the provision of fatty acids by a female walleye to her eggs are the constitution of the maternal diet and female tissue reserves. Iteroparous fish must provide sufficient nutrition for the developing offspring while conserving enough for maternal maintenance in subsequent spawning years. Previous studies have examined egg fatty acid composition in wild fish, including walleye, but few studies exist which compare somatic reserves to what is found within the egg. In this study, I analyzed the fatty acid profiles of eggs and somatic tissues in two populations of walleye, one from Lake Nipissing and the other from Lake Ontario. Fatty acid profiles of eggs, liver, muscle and visceral fat were determined using gas chromatography. For each population, I compared profiles among tissues within sexes, and proportions of individual fatty acids between sexes. As expected, there were substantial differences in fatty acid profiles among tissues in both sexes. I also found significant differences in liver fatty acid profiles between sexes in both populations. Importantly, females in both populations were found to contain higher proportions of arachidonic acid (AA) in their livers, where egg yolk is manufactured. This is consistent with the importance of AA in eggs.

Acknowledgements

Most importantly I thank Dr. Murray Wiegand for his time, his patience, his understanding, his guidance and his strange sense of humour.

To Dr. Tom Johnston from the Ontario Ministry of Natural Resources, thank you for providing me with the samples needed to make this project possible.

I would like to thank Dr. Ric Moodie for his encouragement and for helping me keep on top of deadlines.

Members of my committee Dr. Jens Franck and Nancy Loadman also deserve my thanks for their help, insight and encouragement along the way.

Thanks to my family for helping me to deal with my stress and for their support.

Many, many thanks to all.

Table of Contents

Abstract ii

Acknowledgements iii

Table of Contents iv

List of Tables v

List of Figures vi

Introduction 1

Methods 6

Field Sampling 6

- Extraction 7
- Saponification and Methylation 8
- Chromatography 10
- Statistical Analysis 12

Results 14

- Population Comparison 14
- Tissue Comparisons within Sexes 15
- Sex Comparisons 21
- Egg Comparison 22

Discussion 23

Conclusions 30

References 31

Appendix A 34

Appendix B 35

List of Tables

Table 1: Female trait summary for Lake Nipissing and Lake Ontario spawning walleye populations in 2005. 14

Table 2: Comparison of Fulton's Condition Factor for female and male spawning walleye from Lake Ontario and Lake Nipissing populations in 2005. 15

Table 3: Relative percentages of tissue fatty acids for a 2005 spawning walleye population from the Bay of Quinte in Lake Ontario. 34

Table 4: Relative percentages of tissue fatty acids for a 2005 spawning walleye population from Lake Nipissing. 35

List of Figures

Figure 1: Relative abundance of specific fatty acids (% of total fatty acids) ± standard deviation in female Lake Ontario walleye tissues in 2005. Letter labels of bars indicate transformed percentages of fatty acids that are significantly different among tissues. * indicates a significant difference between sexes for the indicated fatty acid in that tissue. $ indicates a significant difference between proportions of that fatty acid in Lake Ontario walleye eggs and the same fatty acid in Lake Nipissing walleye eggs. In all cases, significance is accepted at $p<0.05$. 17

Figure 2: Lake Ontario male walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1. 18

Figure 3: Lake Nipissing female walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1. 19

Figure 4: Lake Nipissing male walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1. 20

Introduction

The organic nutrient content of an egg consists mainly of lipids and proteins, each of which serves a function for the developing embryo. The lipid reserve of an egg serves the critical function of providing nutrients used in every aspect of embryonic development. This includes providing substrates for energy metabolism, providing the precursors for chemical messengers and acting as structural components used in membrane biogenesis (Rainuzzo *et al*. 1997, Finn 1994). The lipids in an egg can come from 3 possible sources; directly from the diet, mobilized from maternal reserves and *de novo* synthesis.

There are 4 major categories of fatty acids, namely, the saturated fatty acids (SFA), the monounsaturated fatty acids (MUFA), the omega-3 polyunsaturated fatty acids (PUFA) and the omega-6 PUFAs. All of these categories have roles as sources of fuel as well as in the proper development of cell membrane structure. Among the omega-3 (*n*-3) fatty acids, docosahexanoic acid (22:6(*n*-3), DHA)[1] is found in all tissues, but is especially important in neural tissue including brain and retina (Sargent *et al*. 1993 and Bell and Dick 1991). Eicosapentaenoic acid (20:5(*n*-3), EPA), also belonging to the omega-3 family and arachidonic acid (20:4(*n*-6), AA) of the omega-6 family both have known roles as the precursors of eicosanoids, which are intra- and extracellular messengers (Shea and Del Poeta 2006).

[1] Fatty acids are designated a:b (n-x), where a is the number of carbon atoms, b is the number of methylene-interrupted double bonds, and (n-x) indicates the position of the first double bond relative to the methyl end of the molecule.

While many fatty acids can be synthesized *de novo* by vertebrate animals, some, such as the omega-3 and omega-6 family of polyunsaturated fatty acids (PUFA), cannot. These are called essential fatty acids (EFA) because vertebrates have no known mechanism by which to synthesize them, although they are especially important to the fish (Wiegand *et al*. 2004). Thus, these EFAs must be taken up in the diet. Dietary fatty acids are ruled out as the primary source of egg lipids as many fish species have been shown to reduce their food intake during sexual maturation (Lal and Singh 1987). In addition, it has been shown that in another species, the gilthead seabream (*Sparus aurata*), a change in diet for the two month period before the onset of spawning had no effect on egg fatty acid profiles (Almansa *et al*. 1998). This furthers the notion that dietary fatty acids are not directly incorporated into eggs, leaving those fatty acids which are mobilized from maternal reserves as the primary source for egg lipid.

The chemical composition of an egg is directly related to the egg quality as the nutritional needs for development and growth of the embryo must be met (Harel *et al*. 1994). In addition to containing sufficient quantities of fatty acids, the relative ratio of some fatty acids has been shown to have a large impact on egg quality. Work on one species, the gilthead seabream has demonstrated the importance of maintaining the ratios of α-linolenic acid and oleic acid to the omega-3 PUFAs (Almansa *et al*. 1998). An increase in this ratio causes detrimental effects to the egg membranes, and is responsible for low egg quality and reduced hatching success. A previous study has demonstrated that in Lake

Ontario walleye, the relative percentages of AA and EPA, as well as the EPA:AA ratio of an egg fatty acid profile is related to maternal size. A similar trend existed in Lake Nipissing concerning maternal age. With increasing maternal size and/or age, levels of AA increase, while EPA and the EPA:AA ratio decrease in the eggs (Wiegand *et al*. in press). Higher levels of AA have been associated with increased resistance to infection, greater egg quality and growth (Bell and Sargent 2003). Conversely, low EPA:AA ratios have been associated with certain pathologies (Sargent 1995). An optimal ratio of some fatty acids must exist, and it is speculated that this ratio is related to the life history of the fish (Sargent *et al*. 1999).

Walleye (*Sander vitreus*) was chosen as my target species. One population of walleye came from the Bay of Quinte in Lake Ontario and another from Lake Nipissing. Walleye are a piscivorous species, feeding mainly on other fish, but also on crayfish, snails, insects, frogs, mudpuppies and even small mammals. The species extends over a large geographic range, from southern United States to the Mackenzie River delta in northern Canada (Scott and Crossman 1973). Over this range, the species exhibits variability in reproductive characteristics from egg quantity (Baccante and Reid 1988), egg size (Johnston and Leggett 2002), egg survival in relation to maternal age and size (Johnston 1997) and fecundity in relation to environmental conditions (Baccante and Colby 1996: Johnston and Leggett 2002). Walleye from both populations are known to exhibit similar strategies concerning nutrient provision to the eggs, despite the

fact that Lake Nipissing fish are under greater stress due to resource limitation (Wiegand *et al*. in press). Some walleye populations have been known to skip reproduction in years where sufficient body lipid stores have are not present (Henderson *et al*. 1996). Whole lipids or some fraction of them may be a limiting factor of egg production in walleye (Wiegand *et al*. in press).

The fatty acid profiles of walleye eggs have been shown to be correlated with maternal size in Lake Ontario and age in Lake Nipissing. Larger, older females tend to produce eggs higher in DHA and AA, and have lower ratios of EPA/AA (Wiegand *et al*. 2004). This suggests that when resources are abundant, walleye will preferentially incorporate DHA and AA into the egg, while EPA is not as important. From what is known about the function of these fatty acids, this strategy would pack the egg with as much material for the optimal development of the embryo as possible. It is known that while both EPA and AA have roles as precursors to eicosanoids. AA may be preferentially incorporated to reach the desired ratio for the eicosanoid-producing pathway.

The provision of sufficient quantities of fatty acids in the proper ratios to eggs is beneficial to the offspring, but an iteroparous fish needs to maintain sufficient quantities of fatty acids to survive through the winter until subsequent spawning seasons. The seasonal imbalance that a north-temperate fish experiences from the winter months to the summer months necessitates a trade-off between maternal maintenance and egg quality. Thus, the fatty acid composition of fish

eggs is influenced by the physiological requirements of the parent (Schwalme 1994).

I sought to evaluate several predictions concerning the strategy of lipid provision to eggs in walleye. To do so I compared the fatty acid profiles within sexes, among tissues and within tissues, between sexes of two populations of walleye. The main goal was to demonstrate how the lipid allocation to walleye eggs varies with respect to maternal characteristics, thus leading to a further understanding of the reproductive strategy of walleye. For the purpose of this study, five fatty acids were analyzed in detail based on other studies illustrating their importance. These included palmitoleic acid (16:1 (n-7), PLA), oleic acid (18:1 (n-9). OA), AA, EPA and DHA. It was expected that female tissues of post-recrudescent walleye from Lake Ontario would be more depleted in EFAs compared to Lake Nipissing walleye. I expected the female Lake Ontario walleye to produce higher quality eggs at the expense of her own tissue, because there is a higher likelihood of replenishing her essential fatty acids before the winter. In both populations, I expected that male tissues would contain higher levels of EFAs which correlates with the assumption that they allocate very little EFA to their gametes. Because of previous work demonstrating the optimum EPA:AA ratios in eggs, I expected Lake Ontario eggs to be closer to this ratio then Lake Nipissing eggs, due to the nutrient limitation in Lake Nipissing.

Methods

Field Sampling:

Walleye eggs and tissue samples in this study were obtained by Dr. T. A. Johnston. Samples were stored at -70˚C until shipped to the University of Winnipeg on dry ice. Once at the University of Winnipeg samples were stored at -70˚C until use.

Spawning walleye females as well as males were captured from the Bay of Quinte in Lake Ontario in early to late April 2005 and walleye from Lake Nipissing in early May 2005. Upon capture, somatic characteristics were measured including fork length, total body mass and somatic mass (total body mass less gonad mass). The age at capture was determined by the opercles (Wiegand *et al*. 2007).

A total of 15 Bay of Quinte walleye were captured, consisting of nine males and six females. A total of 53 Lake Nipissing walleye were captured, consisting of 30 males and 23 females. Six males and six females were randomly chosen from each population, giving a total of 12 fish per lake. For analysis of fatty acid profiles muscle, visceral fat and liver samples were taken from each fish in addition to ova from females.

Extraction:

Extraction of lipids from raw tissue samples was accomplished by following the procedure of Folch *et al*. (1957). Frozen subsamples of 0.4-0.5g of eggs, liver and muscle tissues and 0.2-0.3g of visceral fat were used for analysis. Each subsample was immediately placed into a 15mL Ten Broeck homogenizing tube containing 10mL of homogenizing solution (2:1 chloroform:methanol). Homogenization of tissues was accomplished by a minimum of 20 strokes of the homogenizer and until no discernable tissue clumps were visible.

Homogenized samples were poured through a chloroform-rinsed filter paper into a 125mL or 60mL separatory funnel. The homogenizing tube was rinsed with a further 10mL of homogenizing solution and this was also poured through the filter paper into the separatory funnel. The filtrate was washed with 5mL of 0.88% potassium chloride solution. The separatory funnel was shaken vigorously for a minimum of one minute before being covered with aluminum foil. Each sample was left overnight or for a minimum of 6 hours to ensure complete separation of the two phases.

The lower phase contained the lipid extract and was collected into a 15mL stoppered test tube. One milliliter of 0.2% butylated hydroxytoluene (BHT) solution was added to prevent reactions with oxygen. The tube was topped up with chloroform and filled with nitrogen to remove as much oxygen as possible.

Once capped, it was stored in the freezer for a minimum of four hours so that any water contained in the sample could form a layer on top and could be removed and discarded.

Saponification and Methylation:

Three milliliters of the total lipid extract was transferred to a 40mL stoppered glass tube. This fraction was dried under a stream of nitrogen gas. At the moment of total solvent evaporation, 6 drops of toluene and 2.5mL of ethanol were added. After mixing, 130µL of a 33% KOH solution was added. Tubes were left in a 37˚C water bath overnight so hydrolysis of the ester linkages (saponification) occurred.

The following morning samples were removed from the water bath and 2.5mL of distilled water added. pH was tested on pH strips to ensure alkalinity. Five milliliters of 30-60°C petroleum ether (Pet Ether) was added to each tube and each tube was vortexed for 1 minute. Two layers then formed and the top layer, consisting of Pet Ether and non-saponifiables, was removed and discarded. This was repeated four times. The extract was then acidified by the addition of 0.4mL 6M hydrochloric acid (HCl). pH was again tested on pH strips to ensure acidity. A further four washings with Pet Ether was done. This time the top layer was retained after each washing and transferred to a 15mL stoppered tube.

Each sample was dried down completely under nitrogen and 3mL of 14% boron-trifluoride added. Tubes were stoppered and placed in a 60˚C water bath for 9 minutes to allow methylation to occur. Once cool, methylated samples were poured into 125mL separatory funnels.

Tubes were washed with 10mL of hexanes and also poured into the separatory funnels. A further 20mL of hexanes was then added. Two washings with 20mL of a saturated sodium chloride solution were carried out. The separatory funnel was shaken vigorously for a minimum of 1 minute. The bottom layer was discarded after each washing. After the second washing, the samples were collected into 40ml tubes. A small scoop of anhydrous sodium sulphate was added to each and the tubes vortexed to dry the samples. Samples were then quantitatively transferred and concentrated under nitrogen into new 15mL tubes. When about 3mL of sample remained, 1mL of a 0.2% BHT solution was added and tubes topped up with chloroform. Nitrogen was bubbled into the tubes before they were stoppered to eliminate the presence of oxygen. Prepared fatty acid methyl ester (FAME) samples were stored in a freezer.

An additional step was performed by L. Porteous on Lake Nipissing visceral fat samples. Neutral and polar lipids were separated prior to analysis by silica gel column chromatography as outlined in Wiegand *et al*. (2004).

Chromatography:

Verification of the purity of the FAME samples was accomplished using thin-layer chromatography (TLC). One hundred microlitres of the sample was dried under nitrogen and 6 drops of a spotting solution composed of 4:1 dichloromethane:methanol added. This small sample was then spotted onto a Whatman LHPKF Silica gel 60A thin layer plate. The plate was run with a 80:20:1 hexanes:ether:acetic acid solvent in a sealed glass chamber. Once removed and dry, plates were placed into another closed glass chamber containing iodine and left to develop. Each sample's lane was visually inspected for the presence of free fatty acids and desirable lipids to ensure the preparation procedure was a success.

The entire FAME sample was concentrated for gas chromatography (GC) analysis. The majority of it was dried down in the original 15mL tube. When approximately 1mL remained, the sample was transferred to an 0.3mL amber Reactivial. The sample was then completely dried down to remove all traces of chloroform. The sample was re-suspended in about 400µL of a 3:1 hexanes:ethanol solution. The Reactivials were filled with nitrogen, capped and stored in cold until several chromatography analyses could be done on them. The exception to this procedure is the Bay of Quinte visceral fat samples. Because they contained a large volume of fatty acids, only 3mL of the prepared sample was used for GC analysis. In addition, because fatty acid was scarce in

muscle of both populations, the prepared GC sample in the Reactivial was further reduced to 150µL immediately prior to injection.

GC analysis was conducted according to the procedure first developed by Moodie *et al*. (1989). The author and M. D. Wiegand performed the injection procedures in order to make effective use of the time the GC machine was on. A Varian 330 flame ionization gas chromatography system, linked to a personal computer equipped with the Varian Star Chromatography version 5.5 data system was used to separate fatty acid methyl esters on a 30m, 0.25mm bore capillary column coated with a 0.25µm film of Durabond 225. The temperature program for the analysis of all samples consisted of a hold at 150˚C for 5 minutes, a climb to 215˚C at a rate of 2.5˚C/min. and a hold at 215˚C for 17 minutes. The hydrogen carrier flow was 35cm/s and the injector split ratio was 40:1. Injection volumes varied from 0.2µL for visceral fat to 0.4-0.6µL for egg and liver to 0.8µL for muscle. Injection volumes were determined largely through trial and error and several runs had to be discarded due to inadequate injection volume (Wiegand *et al*. 2004).

Fatty acids were identified using a cod liver oil standard in which the retention times of most fatty acids were previously identified. Walleye fatty acids were identified by a comparison of retention times to the standard. The area under the peak represents the proportional amount of specific fatty acids present. The relative amount of each fatty acid was calculated by the data system and each

value was normalized to 100%. Two to six chromatograms were obtained for each sample.

Statistical Analysis:

The data for 28 fatty acids was entered into a Microsoft Excel spreadsheet for each sample. Once normalized to 100%, means and standard deviations were calculated. Arcsine transformed means of the relative proportions of fatty acids of interest were regressed against known maternal characteristics using the Statistical Package for the Social Sciences software (SPSS). Preliminary analysis of covariance (ANCOVA) was followed by analysis of variance (ANOVA) and Student-Newman-Kuels (SNK) tests. The dependant variables of interest were lipid fatty acid composition. The independent variables were population and sex. In all cases the level of significance was accepted at 0.05. Age (years), somatic mass (g wet) and fork length (mm) were examined as covariates in some cases while only fork length was used in others. Somatic condition was estimated by using the calculation for Fulton's Condition Factor. The somatic mass (g wet) was divided by the cube of the length (mm) and multiplied by 100,000. A Students t-test was performed comparing the Condition Factor of females to males within each population and comparing within each sex between the two populations.

Overall, 12 fish from each of the two populations (6 of each sex) were analyzed for their fatty acid profile. Three tissues were analyzed for each male and four for each female. A total of 84 samples were included in this study. Thirty of these samples, including ova, liver and visceral fat, were previously analyzed by Lindsay Porteous under the supervision of M.D. Wiegand. I began reanalysis of the Lake Nipissing visceral fat samples done by L. Porteous as the method used to analyze the two populations differed slightly. I was unable to complete this due to breakdown of the gas chromatograph, so the data collected by L. Porteous for the three Lake Nipissing tissues were used for statistical analysis.

Results

Population Comparison

Population characteristics were provided by Dr. Tom Johnston from the Ontario Ministry of Natural Resources (OMNR) (Table 1). Fish from Lake Ontario were older and larger (longer fork length) than those from Lake Nipissing. While Lake Ontario fish had a longer fork length and greater somatic mass, the Condition Factor of the two populations was similar. Both liver and gonad lipid (% dry mass) was slightly greater for the Lake Ontario fish. Lake Ontario walleye produced larger eggs.

Table 1: Female trait summary for Lake Nipissing and Lake Ontario spawning walleye populations in 2005.

Trait	**Population**	
	Ontario	Nipissing
Age (years)	13 ± 4.8	6.5 ± 1.4
Fork Length (mm)	667 ± 66	436 ± 35
Somatic Mass (g wet)	3225 ± 917	955 ± 264
Somatic Condition	1.07 ± 0.14	1.12 ± .065
Somatic Lipid (% dry mass)*		23 ± 5.2
Liver Lipid (% dry mass)	15 ± 3.3	11 ± 2.5
Gonad Lipid (% dry mass)	38 ± 1.8	34 ± 1.1
Egg Size (mg dry)	1.00 ± 0.049	0.87 ± 0.13

* No data was available for the somatic lipid dry mass of Lake Ontario female walleye.

Student's t-tests performed on Fulton's Condition Factor for males and females showed no significant difference in condition between the sexes in either lake (Table 2). This is an indication that there is no sex difference in the extent of somatic depletion due to recrudescence.

Table 2: Comparison of Fulton's Condition Factor for female and male spawning walleye from Lake Ontario and Lake Nipissing populations in 2005.

Lake	Female Condition ± SD	Male Condition ± SD
Ontario	1.071 ± 0.142	1.203 ± 0.151
Nipissing	1.122 ± 0.645	1.169 ± 0.054

Tissue Comparisons within Sexes

The mean percentages and standard deviations of the 28 fatty acids identified for each sample are provided in Appendix A1 and A2. Figures 1 through 4 illustrate a tissue comparison of the five fatty acids of greatest importance within each sex for each population. ANOVA followed by SNK tests confirmed expectations that several significant differences exist among tissues.

Because fork length was previously found to be the best predictor of egg fatty acid composition (Wiegand *et al*. 2007), ANCOVA was done using this characteristic as a covariate. The purpose of this was to see if fork length explained any variation in fatty acid abundance among tissues. The only correlation between fork length and tissue fatty acids was EPA in Lake Ontario

male walleye. The covariate was then removed and ANOVA performed using tissue as a fixed factor and fatty acid as the dependant variable.

In the Lake Ontario population (Figures 1 and 2), visceral fat was consistently statistically higher in the monounsaturated fatty acids (MUFAs) PLA and OA, while containing relatively low amounts of polyunsaturated fatty acids (PUFAs). Muscle had the lowest MUFA concentrations of all tissues, and had the greatest amount of DHA. Liver contained mid-range amounts of both MUFAs, and PUFAs. These results are consistent throughout both populations and each sex (Figures 1, 2, 3 and 4).

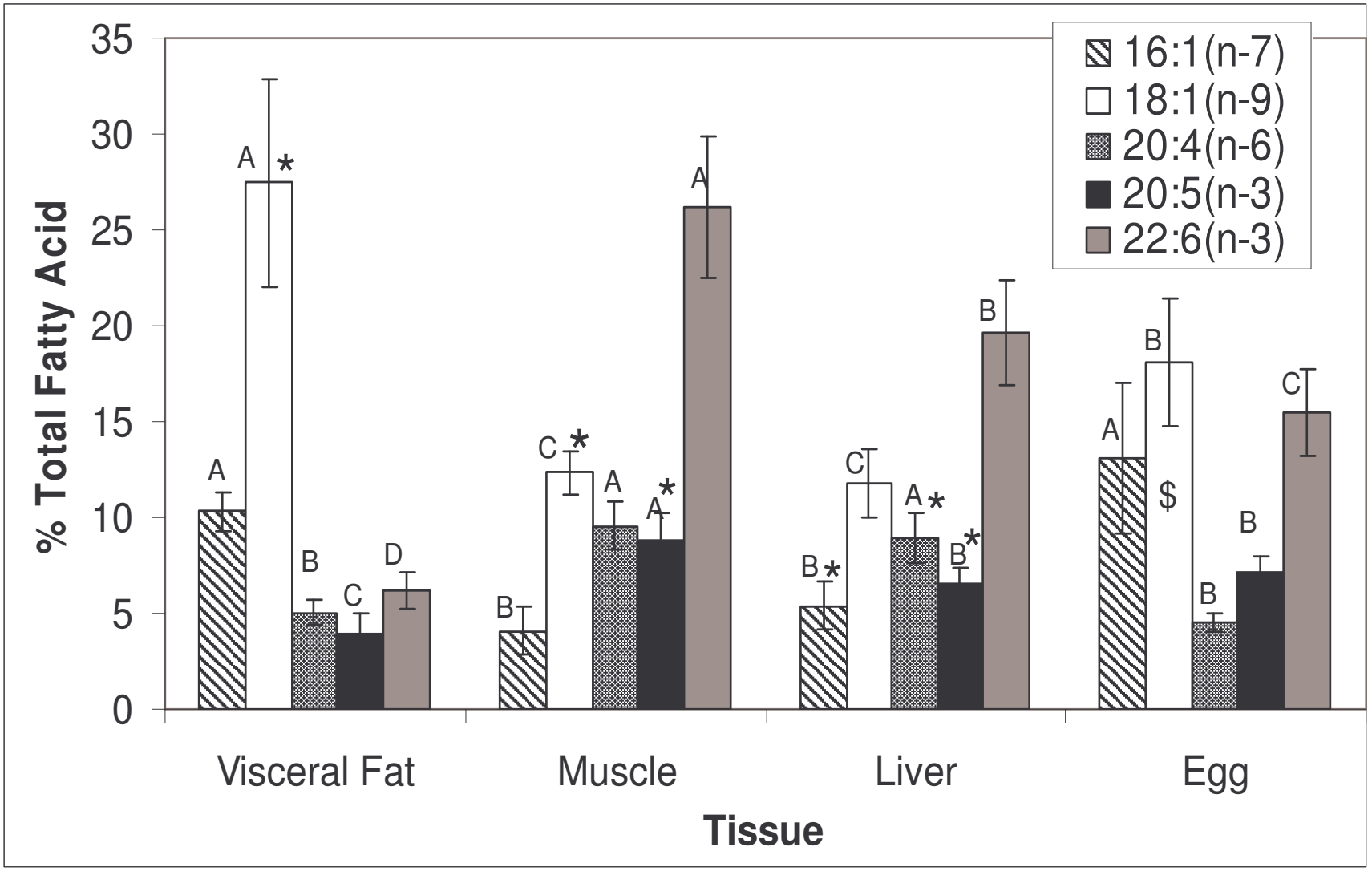

Figure 1: Relative abundance of specific fatty acids (% of total fatty acids) ± standard deviation in female Lake Ontario walleye tissues in 2005. Letter labels of bars indicate transformed percentages of fatty acids that are significantly different among tissues. * indicates a significant difference between sexes for the indicated fatty acid in that tissue. $ indicates a significant difference between proportions of that fatty acid in Lake Ontario walleye eggs and the same fatty acid in Lake Nipissing walleye eggs. In all cases, significance is accepted at $p<0.05$.

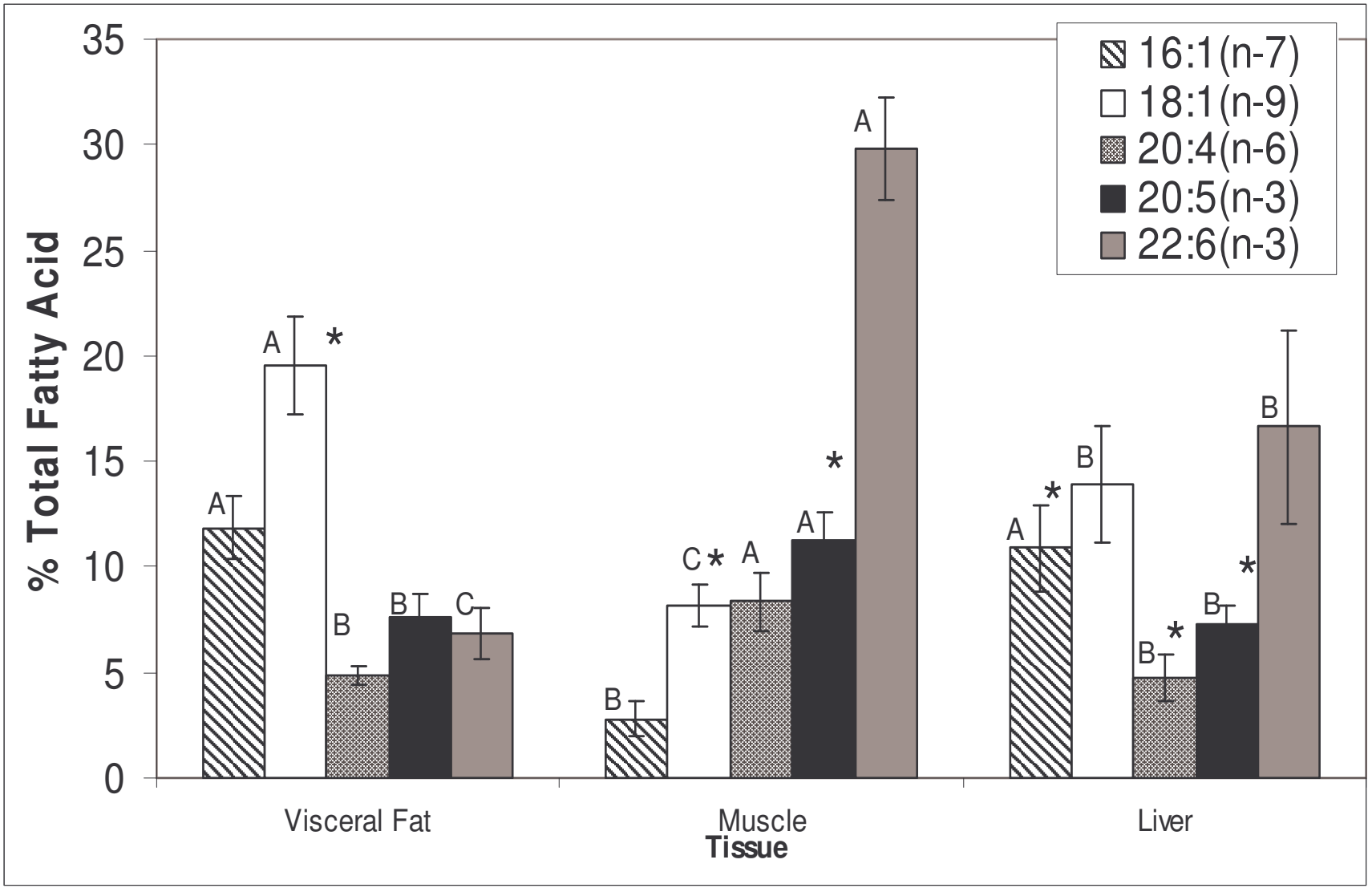

Figure 2: Lake Ontario male walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1.

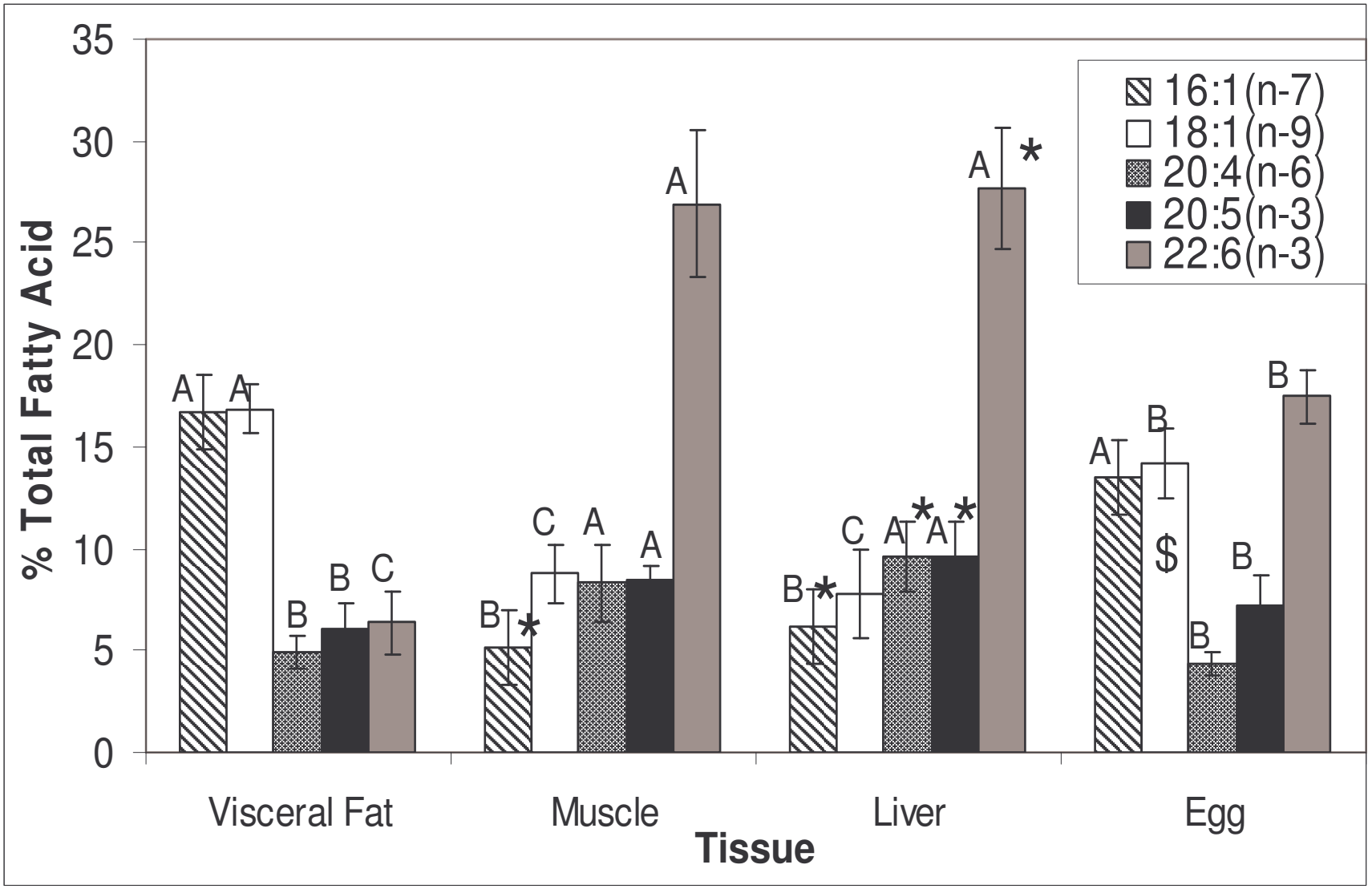

Figure 3: Lake Nipissing female walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1.

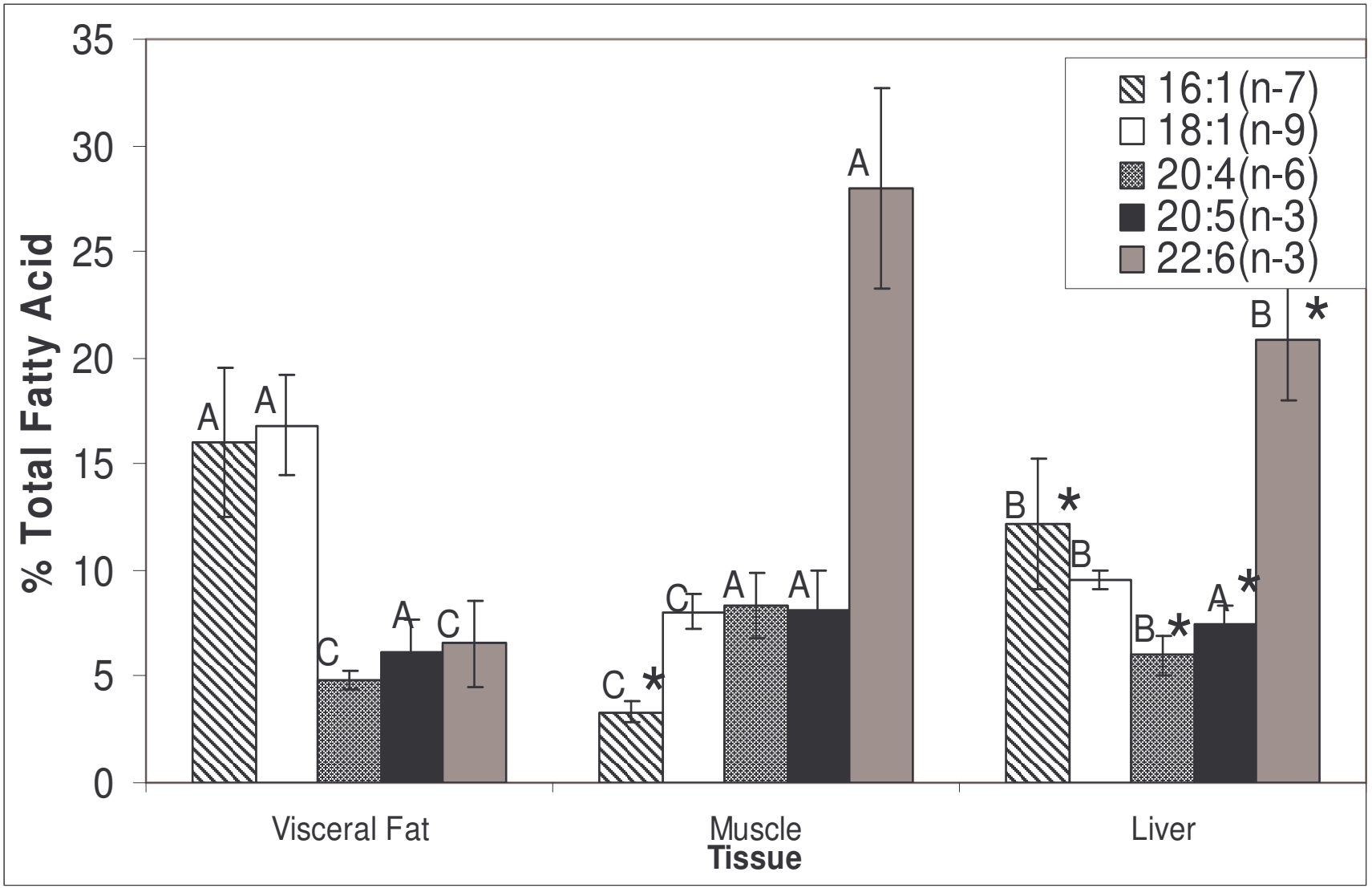

Figure 4: Lake Nipissing male walleye tissue and sex comparison for % relative abundance of fatty acids (% total fatty acid) ± standard deviation for a 2005 population. Symbols as in Figure 1.

Sex Comparison

Preliminary ANCOVA was performed for each fatty acid with fork length as the covariate for both lakes, but no significant relationships were found. The covariate was then removed and ANOVA performed using sex as a fixed factor and fatty acid as the dependant variable.

In the Lake Ontario population, female visceral fat had a significantly greater proportion of OA then male visceral fat as well as lower EPA. The only significant difference in PLA existed in the liver, where males had a greater relative abundance compared to females. AA was significantly higher in female liver. DHA was at similar levels between the sexes in all tissues. Males contained significantly higher levels of muscle EPA than females, but lower amounts of OA (Figures 1 and 2).

Females contained significantly more PLA in their muscle compared to males in Lake Nipissing. No sex differences existed between fatty acid profiles of visceral fat for the Lake Nipissing population. The greatest difference between the sexes was found in the liver, where all female fatty acid percentages differed statistically from those of the male except OA. AA, EPA and DHA were more significantly abundant in females then in males, while PLA was less abundant in females (Figures 3 and 4).

Egg Comparison

Fatty acid percentages were compared for the eggs of the Lake Nipissing and Lake Ontario population. Preliminary ANCOVA using three maternal characteristics as covariates (age, fork length and somatic mass) showed no significant effect on egg fatty acid composition for the five fatty acids in question. Removal of covariates showed only one difference between the two populations. Lake Ontario walleye eggs contained a significantly greater proportion of OA compared to the Lake Nipissing walleye eggs (Figures 1 and 3).

Discussion

The somatic differences between Lake Ontario and Lake Nipissing walleye indicate that Lake Nipissing fish were less stressed in 2005 than previously reported for 2004 (Wiegand *et al*. 2007). This led to the expectation that less of a difference would exist in the fatty acid composition of eggs between the two populations. However, a comparison of the population characteristics shows a difference in egg size. Although it seems plausible that egg size could be a good indicator of egg quality in terms of lipid content, it is generally not a good predictor (Heyer *et al*. 2001). My results concur with previous findings as the fatty acid profiles of the eggs from each population were very similar, even though Lake Ontario Walleye produced larger eggs.

I examined the tissue fatty acid composition of two isolated populations of walleye. My original hypothesis that maternal somatic tissues would be depleted of important PUFAs in order to produce higher quality offspring was not supported. Few differences existed between the two populations, one of which was assumed to be resource-stressed, and the other which was not. A marked difference should have existed between the sexes within the populations had somatic depletion from maternal tissues been occurring.

The one difference in egg composition between the two populations was that of OA. The double bond of OA is in the ideal position to provide maximum fluidity

to a membrane and is thus key in acclimatization for overwintering fish (Wiegand 1996). In nutrient limited Lake Nipissing, selective retention in maternal tissues as opposed to provisioning of OA to eggs may be a maternal maintenance strategy. In addition, vertebrates have the ability to synthesize OA *de novo* (Wiegand 1996), so its presence in an egg may be less significant.

The size of somatic stores at spawning are influenced by three factors: the size of reserves at the beginning of recrudescence, dietary intake of resources during recrudescence, and the amount of stored reserved mobilized during recrudescence (Wiegand *et al*. in press). If preferential and greater mobilization of either total fatty acid or PUFAs from maternal somatic reserves occurred as in Atlantic salmon (Ackman and Takeuchi 1986), lower concentrations of fatty acids in females than in males would be expected. This was not the case for either the Lake Ontario or the Lake Nipissing walleye population. Salmon are a semelparous species, thought Atlantic salmon are not as rigorous as Pacific salmon in this regard. They mostly breed only once; therefore have no need for self conservation. The depletion of their tissues to the greatest extent possible in order to make the healthiest offspring possible is necessary as they only get one opportunity to reproduce (Ackman and Takeuchi 1986). In an iteroparous species such as walleye, retaining sufficient concentrations of tissue fatty acids for maintenance and energy in a time of potential starvation is critical to the survival of the fish until the next spawning season.

If a female walleye can conserve enough nutrients in her tissues so that several breeding events are possible throughout her lifetime, she greatly enhances her reproductive fitness. Participation in several reproductive seasons will allow her genes to be passed on, even in the event that a catastrophe wipes out her offspring in one of those years. It also allows her to make potentially poorer offspring in times of nutrient deprivation and better offspring in times of nutrient abundance. Iteroparity, while advantageous in that it allows for more then one breeding attempt, introduces into the species the necessity for a trade-off between the reproductive investment in any given year and maternal maintenance (Adams 1999). Walleye appear to be following the strategy followed by the northern pike as reported by Schwalme (1994). In that species, maternal maintenance is more important than any one reproductive event.

The importance of DHA not only as a source of fuel but also as a structural component in neural tissue is well documented (Wiegand 1996, Tocher 2003). While DHA is important for maternal maintenance, it is also important in embryonic development. DHA deficiencies in developing fish larvae have been associated with behavioral impairment (Bell *et al*. 1995, Masuda *et al*. 1998, Ishizaki *et al*. 2001). DHA is important for both the mother and her developing offspring. Some evidence exists that mature female walleye will skip a reproductive season if sufficient fatty acid body reserves are not present (Henderson *et al*. 1996). This implies that maternal maintenance is valued over provision to offspring as in pike (Schwalme 1994).

AA and EPA are important in membrane structure and also as the precursors for eicosanoids. Eicosanoids are a group of intra- and extracellular messengers which have important roles in many processes from the production of steroids to the development of gonads (Tocher 2003). These two fatty acids may therefore be of great importance in some step of development to a state of reproductive maturity. It's possible providing the optimal ratios of AA and EPA to developing embryos will allow superior and quicker attainment of maturity.

Analysis of covariance (ANCOVA) was performed for maternal characteristics because a previous study has shown certain characteristics to be of consequence to the fatty acid profile of the ova (Wiegand *et al*. 2007). These observations were not supported by the results the present study. The one possible reason for the inconsistency in results could be due to the small sample size used in this study. Sample sizes exceeded 20 fish when covariates were found to have an effect. Wiegand *et al*. (2007) found a negative relationship at the time of spawning between somatic lipid reserves and female age and fork length. This relationship makes sense in the context of an iteroparous strategy. As the female gets older her opportunities for reproduction are dwindling. While young, conservation of fatty acids is essential for maintenance and affords the female a distinctive advantage in helping her survive until future reproductive seasons, despite seasonal low temperatures and low potential nutrient availability. As a iteroparous female ages, providing greater amounts of specific fatty acids in the

right ratios, even at the expense of her own survival, increases the chance of survival of her offspring. From an evolutionary perspective, this increases the likelihood of passing on the females genes as the likelihood of spawning events decreases.

The diet of walleye may be of greater importance during recrudescence then with other species, meaning that female walleye may be incorporating more EFAs directly into their eggs as opposed to deposition in fat stores for future mobilization. Walleye are known to feed throughout the winter (Henderson *et al*. 1996), as well as to go through a period of gonad quiescence in between spawning cycles so it is unlikely that the investment a female makes in her eggs is a continuous process (Malison *et al*. 1994). The high levels of DHA remaining in the liver implied that walleye continue to make yolk at the end of vitellogenesis.

Female muscle was no more depleted of PUFAs than male muscle tissue in either population. This suggests one of two things. Either males are putting just as great of an investment into sperm as females are putting into eggs, or muscle is not targeted as a source of fatty acid during recrudescence. The latter is more likely as this would increase the chances of maternal survival. Depletion of maternal muscle reserves would hinder foraging by limiting the range over which a female could search for food, as well as decrease the rate of winter survival because of the important roles of PUFAs and OA in the homeoviscious adaptation

to cold. Muscle has a low lipid content to begin with, so its use as a source of PUFA is limited. In addition, the lipid component of muscle is mainly phospholipids used in membrane structure. Mobilization of these fatty acids would result in cellular destruction and tissue breakdown.

The lack of a sex difference in visceral fat for both the Lake Nipissing and Lake Ontario populations was surprising. Visceral fat stored in adipose tissue acts as an energy store. These lipids are much more labile compared to the lipids in muscle. If preferential mobilization of certain fatty acids in spawning females was occurring, a sex difference would be present specifically in visceral fat. The lack of a difference indicates that there is no preferential mobilization of PUFAs for provision to the offspring. Results for visceral fat compared with other tissues were typical in that they were rich in MUFAs.

The understanding of how maternal somatic characteristics influence offspring quality is important both for ecological research and fish culture. *Sander vitreus* has long been a significant recreational and commercial target species in central North America (Johnston *et al*. 2006). Fish culture aimed at replenishing depleted populations and ensuring that native species continue to thrive will benefit from knowledge gained concerning this species. The similarities between the sexes suggest that females preferentially incorporate nutrients into their own tissues before providing for their offspring. The overall strategy of walleye in both the Lake Nipissing and Lake Ontario populations seems to be to conserve

their somatic reserves at the expense of their offspring. This conservation of maternal fatty acids increases the likelihood of spawning events in the future when conditions may be more suitable for production of higher quality eggs.

Conclusions

1. Female somatic tissues are not depleted of essential fatty acids in order to supply them to their offspring.

2. Fork length is correlated with the EPA composition of male somatic tissues in Lake Nipissing.

3. Visceral fat is high in MUFA and low in PUFA in both Lake Nipissing and Lake Ontario populations.

4. Both muscle and liver are high in PUFA in the Lake Nipissing and Lake Ontario populations.

5. Levels of AA do not appear to be as closely regulated in eggs as other fatty acids.

6. The greatest variability between the sexes in both lakes exists in liver.

References

Ackman, R.G. and Takeuchi, T. 1986. Comparison of fatty acids and lipids of molting hatchery-fed and wild Atlantic Salmon, *Salmo salar*. Lipids, **21**: 117-120.

Adams, S.M. 1999. Ecological role of lipids in the health and success of fish populations. *In* Lipids in freshwater ecosystems. *Edited by* M.T. Arts and B.C. Wainman. Springer-Verlag, New York. pp. 132-160.

Almansa, E., Jose Perez, M., Cejas, J.R., Badia, P., Villamandos, J.E. and Lorenzo, A., 1999. Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality fatty acid composition throughout the spawning season. Aquaculture, **170**: 323-336.

Bell, J.G. and Sargent, J.R. 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture, **218**: 491-499.

Bell, M.V. and Dick, J.R. Molecular species composition of the major diacyl glycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morhua*). Lipids, **26**: 565-573.

Colby, P.J. and Nepszy, S.J. 1981. Variation among stocks of walleye (*Stizostedion vitreum*): management implications. Can. J. Fish. Aquat. Sci., **38**: 1814-1831.

Finn, R.N., 1994. Physiological energetics of developing marine fish embryos and larvae. Dr. Scient. Thesis, University of Bergen, Norway.

Folch J., Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, **226**: 497-509.

Fulton, T. 1902. Rate of growth of seas fishes. Sci. Invest. Fish. Div. Scot. Rept.

Harel, M., Tandler, A., Kissil, G.W. 1994. The kinetics of nutrient incorporation into body tissues of gilthead seabream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. Br. J. Nutr. **72**: 45-48.

Henderson, B.A., Wong, J.L. and Nepszy, S.J. 1996. Reproduction of walleye in Lake Erie: allocation of energy. Can. J. Fish. Aquat. Sci., **53**: 127-133.

Heyer, C.J., Miller, T.J., Binkowski, F.P., Caldarone, E.M. and Rice, J.A. 2001. Maternal effects as a recruitment mechanism in Lake Michigen yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci., **58**: 1477-1487.

Ishizaki, Y., Masuda, R., Uemtasu, K., Shimizu, K., Arimoto, M. and Takeuchi, T. 2001. The effect of dietary decosahexenoic acid on schooling behavior and brain development in larval yellowtail. J. Fish Biol., **58**: 1691-1703.

Johnston, T.A. 1997. Within-population variability in egg characteristics of walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*). Can. J. Fish. Aquat. Sci., **54**: 1006-1014.

Johnston, T.A., Wiegand, M.D., Leggett, W.C., Pronyk, R.J., Dyal S.D., Watchorn, K.E., Kollar, S. and Casselman, J.M. 2006. Hatching success of walleye embryos in relation to maternal and ova characteristics. Ecology of Freshwater Fish, In review

Malison, J.A., Procarione, L.S., Barry, T.P., Kapuscinski, A.R. and Kayes, T.B. Endocrine and gonadal changes during the annual reproductive cycle of the freshwater teleost, *Stizostedion vitreum*. Fish Physiology and Biochemistry, **13**(6): 473-484.

Masuda, R., Takeuchi, T., Tsukamoto, K., Ishizaki, Y., Kanematu, M. and Imaizumi, K. 1998. Critical involvement of dietary docohexaenoic acid in the ontogeny of schooling behavior in the yellowtail. J. Fish Biol., **53**: 471-484.

Moodie, G.E., Loadman, N.L., Wiegand, M.D. and Mathias, J.A. 1989. Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizostedion vitreum*). Can. J. Fish. Aquat. Sci., **46**: 516-521.

Rainuzzo, R.J., Reitan, K.I. and Olsen, Y.1997. The significance of lipids in early stages of marine fish: A review. Aquaculture, **177**(1): 103-115.

Schwalme, K. 1992. A quantitive comparison between diet and body fatty acid composition in wild northern pike (*Esox lucius* L.). Fish Physiology and Biochemistry, **10**(2): 91-98.

Schwalme, K. 1994. Reproductive and overwintering adaptations in northern pike (*Esox, lucius* L.): Balancing essential fatty acid requirements with dietary supply. Physiological Zoology, **67**(6): 1507-1522.

Scott, W.B. and Crossman, E.J. 1973. Freshwater Fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Sargent, J.R., Bell, L., McEnvoy, D., Tocher, D., Estevez, A. 1999. Recent developments in the essential fatty acid nutrition in fish. Aquaculture, **177**: 191-199.

Sargent, J.R., Bell, M.V. and Tocher, D.R. 1993. Docosahexaenoic acid and the development of brain and retina in marine fish. *In* Omega-3 fatty acids: Metabolism and biological effects. *Edited by* C.A. Drecon, I. Balksaas, and H.E. Kroken. Birkhauser, Verlag, Basel, Switzerland. pp. 139-149.

Shea, J.M. and Del Poeta, M. 2006. Lipid signaling in pathogenic fungi. Current Opinion in Microbiology, **9**(4): 352-358.

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science, **11**(2): 107-184.

Wiegand, M.D. 1996. Composition, assimilation and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries, **6**: 259-286.

Wiegand, M.D., Idler, D.R. 1982. Synthesis of lipids by the rainbow trout (*Salmo gairdneri*) ovary *in vitro*. Can. J. Zoology, **60**(11): 2683-2693.

Wiegand, M.D., Idler, D.R. 1985. Ovarian neutral lipid fatty acid compositions varies with state of ovarian growth in landlocked Atlantic salmon. Can. J. Zoology, **63**(12): 2775-2777.

Wiegand, M.D., Johnston, T.A., Leggett, W.C., Watchorn, K.E., Ballevona, A.J., Porteous, L.R. and Casselman, J.M. 2007. Contrasting strategies of ova lipid provisioning in relation to maternal characteristics in three walleye populations. Can. J. Fish. Aquat. Sci., **64**: 700-712.

Wiegand, M.D., Johnston, T.A., Martin, J. and Leggett, W.C. 2004. Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can. J. Fish. Aquat. Sci., **61**: 110-121.

Appendix A

Tissue	Egg	Liver		Visceral Fat		Muscle	
	Female	Male	Female	Male	Female	Male	Female
Fatty Acid							
14:0	1.0 ± 0.3	1.6 ± 0.4	0.8 ± 0.2	2.3 ± 0.8	2.9 ± 1.2	0.3 ± 0.1	0.4 ± 0.2
15:0	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
16:0	10.5 ± 1.3	16.3 ± 1.2	19.1 ± 3.0	11.5 ± 1.0	12.1 ± 1.1	13.6 ± 1.0	11.3 ± 2.2
16:1(n-9)	2.9 ± 0.3	2.1 ± 0.3	2.7 ± 0.6	1.6 ± 0.3	2.3 ± 0.5	0.9 ± 0.3	1.2 ± 0.2
16:1(n-7)	13.1 ± 3.9	10.9 ± 2.0	5.4 ± 1.3	11.8 ± 1.5	10.3 ± 1.0	2.8 ± 0.8	4.1 ± 1.3
16:1(n-5)	0.1 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.8 ± 0.4	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
17:0	0.3 ± 0.2	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
17:1	0.9 ± 0.1	0.9 ± 0.3	0.6 ± 0.1	1.0 ± 0.2	0.7 ± 0.3	0.3 ± 0.1	0.5 ± 0.1
18:0	2.2 ± 0.5	3.9 ± 0.6	5.6 ± 1.2	2.1 ± 0.3	2.0 ± 0.3	4.1 ± 0.2	4.2 ± 2.0
18:1(n-9)	18.1 ± 3.4	13.9 ± 2.7	11.8 ± 1.8	19.5 ± 2.3	27.5 ± 5.4	8.2 ± 1.0	12.3 ± 1.2
18:1(n-7)	4.7 ± 1.0	4.5 ± 0.7	5.3 ± 0.7	5.3 ± 1.0	4.5 ± 0.5	3.2 ± 0.4	3.4 ± 0.5
18:2(n-6)	4.5 ± 1.1	2.8 ± 1.0	1.7 ± 0.3	4.7 ± 0.9	5.5 ± 1.0	1.4 ± 0.2	2.5 ± 0.5
18:3(n-6)	0.7 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
18:3(n-3)	4.3 ± 1.5	3.7 ± 0.8	1.0 ± 0.5	5.8 ± 0.7	3.6 ± 1.2	1.7 ± 0.2	2.0 ± 0.7
18:4(n-3)	1.7 ± 0.4	1.7 ± 0.6	0.5 ± 0.3	2.9 ± 0.6	1.3 ± 0.4	0.9 ± 0.2	0.7 ± 0.3
20:0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	0.3 ± 0.1
20:1(n-9)	0.7 ± 0.1	0.8 ± 0.3	1.3 ± 0.2	1.4 ± 0.2	2.1 ± 0.2	0.6 ± 0.1	0.9 ± 0.1
20:1(n-7)	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.3	0.3 ± 0.1
20:2(n-6)	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.0
20:3(n-6)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
20:4(n-6)	4.3 ± 0.5	4.7 ± 1.1	8.9 ± 1.3	4.9 ± 0.5	5.1 ± 0.7	8.3 ± 1.3	9.6 ± 1.3
20:3(n-3)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4(n-3)	1.3 ± 0.4	0.9 ± 0.3	0.4 ± 0.1	1.5 ± 0.3	1.0 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
20:5(n-3)	7.1 ± 0.9	7.3 ± 0.9	6.5 ± 1.5	7.6 ± 1.1	3.9 ± 1.1	11.3 ± 1.3	8.8 ± 0.8
22:4(n-6)	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
22:5(n-6)	1.4 ± 0.4	0.8 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	2.4 ± 0.4	3.0 ± 0.6	3.3 ± 0.6
22:5(n-3)	3.0 ± 0.5	2.8 ± 0.6	1.8 ± 0.1	3.2 ± 0.5	2.9 ± 0.4	5.7 ± 0.7	4.7 ± 0.8
22:6(n-3)	15.5 ± 2.3	16.6 ± 4.6	19.6 ± 2.7	6.8 ± 1.2	6.2 ± 0.9	29.8 ± 2.5	26.2 ± 3.7
sum sat'd	14.3 ± 2.1	22.9 ± 1.8	26.6 ± 4.1	16.6 ± 1.8	17.5 ± 2.3	18.7 ± 1.3	16.8 ± 3.6
sum mono	40.8 ± 4.8	34.2 ± 5.2	27.9 ± 3.1	41.7 ± 3.7	48.1 ± 5.3	17.0 ± 1.8	23.0 ± 2.8
sum (n-6)	11.7 ± 1.5	9.5 ± 1.7	13.1 ± 1.6	13.6 ± 1.4	15.2 ± 1.5	14.1 ± 2.1	17.0 ± 1.2
sum (n-3)	33.2 ± 4.7	33.4 ± 4.2	30.0 ± 4.3	28.0 ± 2.3	19.3 ± 2.4	50.2 ± 0.6	43.2 ± 3.4
(n-3)/(n-6)	2.8 ± 0.2	3.6 ± 0.7	2.3 ± 0.4	2.1 ± 0.3	1.3 ± 0.1	3.6 ± 0.5	2.5 ± 0.2
EPA/AA	1.7 ± 0.3	1.6 ± 0.4	0.7 ± 0.2	1.6 ± 0.3	0.8 ± 0.2	1.4 ± 0.3	0.9 ± 0.1
DHA/EPA	2.2 ± 0.4	2.3 ± 0.5	3.1 ± 0.4	0.9 ± 0.3	1.8 ± 0.8	2.7 ± 0.6	3.0 ± 0.5

Table 3 – Relative percentages of tissue fatty acids for a 2005 spawning walleye population from the Bay of Quinte in Lake Ontario.

Appendix B

Tissue	Egg	Liver		Visceral Fat		Muscle	
	Female	Male	Female	Male	Female	Male	Female
Fatty Acid							
14:0	1.8 ± 0.4	2.5 ± 0.5	1.5 ± 0.4	3.9 ± 1.1	4.0 ± 0.8	0.9 ± 0.5	1.1 ± 0.4
15:0	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
16:0	10.2 ± 0.8	17.7 ± 0.5	14.7 ± 1.2	11.6 ± 1.0	11.1 ± 1.5	19.0 ± 5.0	17.2 ± 2.1
16:1(n-9)	3.1 ± 0.5	1.5 ± 0.3	1.7 ± 0.4	1.7 ± 0.3	1.7 ± 0.3	0.5 ± 0.3	1.0 ± 0.2
16:1(n-7)	13.5 ± 1.8	12.1 ± 3.1	6.1 ± 2.5	16.1 ± 3.5	16.7 ± 3.7	3.3 ± 0.5	5.1 ± 1.8
16:1(n-5)	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.7	0.9 ± 0.7	0.9 ± 0.7	0.4 ± 0.3
17:0	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
17:1	1.3 ± 0.2	0.9 ± 0.1	0.6 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	0.5 ± 0.1	0.6 ± 0.2
18:0	1.8 ± 0.4	2.6 ± 1.0	4.0 ± 0.8	1.9 ± 0.3	1.8 ± 0.6	5.1 ± 1.1	4.0 ± 0.2
18:1(n-9)	14.2 ± 1.7	9.5 ± 0.4	7.8 ± 2.2	16.8 ± 2.3	16.9 ± 1.2	8.0 ± 0.8	8.8 ± 1.5
18:1(n-7)	4.4 ± 1.0	3.3 ± 0.4	4.8 ± 0.6	5.4 ± 0.6	5.2 ± 1.0	3.1 ± 0.5	3.3 ± 0.6
18:2(n-6)	5.1 ± 0.6	4.3 ± 0.5	1.7 ± 0.4	6.4 ± 0.7	6.0 ± 0.5	2.1 ± 0.3	2.4 ± 0.3
18:3(n-6)	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
18:3(n-3)	5.6 ± 1.6	4.8 ± 0.9	2.0 ± 0.4	6.0 ± 1.0	6.0 ± 1.2	1.7 ± 0.3	2.0 ± 0.4
18:4(n-3)	1.8 ± 0.9	1.5 ± 0.8	1.2 ± 0.4	2.1 ± 0.8	2.4 ± 1.4	0.6 ± 0.3	0.8 ± 0.4
20:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.2 ± 0.0
20:1(n-9)	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
20:1(n-7)	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:2(n-6)	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
20:3(n-6)	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4(n-6)	4.3 ± 0.6	6.0 ± 0.9	9.6 ± 1.7	4.8 ± 0.4	4.9 ± 0.8	8.3 ± 1.6	8.3 ± 1.9
20:3(n-3)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
20:4(n-3)	1.0 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	1.1 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
20:5(n-3)	7.2 ± 1.5	6.6 ± 0.9	9.6 ± 1.8	6.2 ± 1.5	6.1 ± 1.2	8.1 ± 1.9	8.5 ± 0.7
22:4(n-6)	0.5 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	1.1 ± 1.0	0.6 ± 0.2
22:5(n-6)	1.3 ± 0.3	0.7 ± 0.1	1.3 ± 0.2	1.8 ± 0.6	2.1 ± 0.6	2.7 ± 0.6	3.2 ± 0.6
22:5(n-3)	2.5 ± 0.4	1.4 ± 0.3	1.7 ± 0.4	2.0 ± 0.2	1.9 ± 0.5	3.1 ± 0.8	3.0 ± 0.8
22:6(n-3)	17.4 ± 1.4	20.9 ± 2.8	27.7 ± 3.0	6.5 ± 2.0	6.4 ± 1.5	28.0 ± 4.7	26.9 ± 3.6
sum sat'd	14.5 ± 1.0	23.7 ± 0.8	21.1 ± 1.3	18.5 ± 1.3	18.0 ± 1.5	26.0 ± 6.9	23.4 ± 2.5
sum mono	37.4 ± 3.6	28.5 ± 2.7	22.1 ± 4.7	42.7 ± 5.3	43.5 ± 3.5	16.9 ± 1.9	19.7 ± 4.3
sum (n-6)	12.2 ± 1.1	11.8 ± 0.9	13.6 ± 1.8	14.6 ± 0.9	14.5 ± 1.6	14.8 ± 1.3	15.1 ± 2.1
sum (n-3)	35.8 ± 4.4	36.0 ± 2.4	43.1 ± 4.7	24.2 ± 5.0	24.0 ± 4.1	42.3 ± 6.7	41.8 ± 3.8
(n-3)/(n-6)	3.0 ± 0.5	3.1 ± 0.4	3.2 ± 0.6	1.7 ± 0.3	1.7 ± 0.4	2.9 ± 0.5	2.8 ± 0.6
EPA/AA	1.7 ± 0.5	1.1 ± 0.3	1.0 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	1.0 ± 0.2	1.1 ± 0.3
DHA/EPA	2.5 ± 0.6	3.2 ± 0.7	2.9 ± 0.4	1.1 ± 0.2	1.1 ± 0.2	3.5 ± 0.5	3.2 ± 0.4

Table 4 - Relative percentages of tissue fatty acids for a 2005 spawning walleye population from Lake Nipissing.