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## Dietary Effects of Non-native Species on Yellow Perch, *Perca flavescens*, Reproductive Performance

Christina Ann Cappelli

*The College at Brockport*, cappelli7911@gmail.com

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**Dietary Effects of Non-native Species on Yellow Perch, *Perca flavescens*,  
Reproductive Performance**

by

Christina Ann Cappelli

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

December 2012

## **Biographical Sketch**

Christina Ann Cappelli was born in Elmira, NY on June 18, 1983. Upon graduation from Horseheads High School in 2001, she attended Corning Community College, Corning, NY and received an Associate of Science in 2003. Following her associate's degree Christina entered The University of South Carolina, Columbia, SC in 2005 where she attained a Bachelor of Science in Marine Science. In 2007 she attended The College at Brockport, SUNY where she pursued her Master of Science in Environmental Science and Biology. While at Brockport she worked as a Research Assistant under Jacques Rinchard, Ph.D.

## **Abstract**

In Lake Michigan, native yellow perch (*Perca flavescens*) have experienced poor recruitment since 1989. With the introduction of non-native species yellow perch prey fish have changed dramatically, which could affect successful recruitment of these fish. Therefore, in the present study I investigated dietary effects of two non-native species (alewife and round goby) on female yellow perch reproduction using lipid and fatty acid composition of their eggs, liver, muscle, and visceral fat. Two-year-old yellow perch were fed two diets, representing two distinct fatty acid signatures. These two diets were significantly different, in terms of fatty acid composition, with alewife containing higher concentrations of SAFA and MUFA and round goby having higher concentrations of PUFA. Unexpectedly, the entire dietary fatty acid composition was not reflected in tissues of yellow perch, but some individual dietary fatty acids were incorporated. Biosynthesis of linoleic acid into ARA and linolenic acid into EPA and DHA was also clearly observed in all tissues of yellow perch. Yellow perch fed round goby yielded a significantly higher body mass than yellow perch fed alewife ( $p < 0.05$ ), but there were no significant differences in fecundity, embryo survival at pigmented eyed stage, HSI, or pseudo-GSI ( $p > 0.05$ ). In conclusion, although round goby and alewife have high levels of n-3 and n-6 fatty acids, (which are general requirements for successful reproduction and recruitment by fish) successful reproduction of all female yellow perch was not observed in the present study. This observation could have been due to poor quality of egg ribbons; indicated by the ribbon separating in several places, and females spawning in tanks, thus limiting our ability to successfully fertilize eggs by hand.

## **Acknowledgements**

I would like to thank my thesis advisor, Dr. Jacques Rinchar for his many years of support, advice and understanding, as well as my thesis committee, Dr. James Haynes and Dr. Sergiusz Czesny, for their patience and helpful feedback on this experiment. I would also like to thank Dr. John Sweka for his guidance on some of the statistical analysis. I am also grateful to Blake Snyder for his help with feeding the fish for this project. Most importantly, I would like to thank my mother who has given me tremendous motivation my whole life and my husband Chris who has supported me throughout this process.

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## 1. Introduction

The Laurentian Great Lakes have a long history of invasions by non-indigenous aquatic species. To date, more than 170 invaders are well established in the Great Lakes (Mills et al. 1993, Ricciardi 2001, Holeck et al. 2004) and strong evidence suggests that these species are having dramatic and damaging impacts on ecosystem functioning. They compete for habitats and overlap diets with native species (French and Jude 2001, Janssen and Jude 2001, Lauer and McComish, 2001), alter nutrient pathways (Pothoven et al. 2001, Hecky et al. 2004, Parker-Stetter et al. 2005), increase bioaccumulation of contaminants (Kuhns and Berg 1999, Morrison et al. 2000), and cause declines of native species through predation (Mason and Brandt 1996, Nalepa et al. 1998, Chotkowski and Marsden 1999, Kuhns and Berg 1999). In addition to economic costs (Pimentel et al. 2000), these invaders have the potential to decrease biodiversity (Sala et al. 2000) and cause extinction of native species (Ricciardi and Rasmussen 1999). Therefore, a better understanding of the effects that invasions have on food webs is essential for adequate fishery management responses.

In Lake Michigan, native yellow perch (*Perca flavescens*) have experienced poor recruitment since 1989. Although the mechanisms driving its poor recruitment have not been identified (Dettmers et al. 2003) several factors have been proposed to potentially influence yellow perch recruitment, including predation (Shroyer and McComish 2000), zooplankton availability (Bremigan et al. 2003; Dettmers et al. 2003), water movement (Dettmers et al. 2005), and spawning stock characteristics (Heyer et al. 2001).

In 1949, alewife (*Alosa pseudoharengus*) was introduced to Lake Michigan causing the yellow perch population to decline along with the populations of other native species

(e.g., lake trout *Salvelinus namaycush*) (Madenjian et al. 2008). Alewives have contributed to a change in the zooplankton community due to their ability to switch feeding techniques between filter and particulate feeding and therefore allowing them to prey on both small and large zooplankton (Janssen 1976). By reducing the abundance of small zooplankton, alewives have directly impacted the ability of yellow perch larvae to survive as they have a small gape size limiting them to smaller zooplankton (Bremigan et al. 2003). The consumption of yellow perch eggs by alewives is another factor that has been associated with the poor recruitment of yellow perch in Lake Michigan (Brandt et al. 1987, Bremigan et al. 2003, Dettmers et al. 2003). Alewife population in Lake Michigan declined between 1965 and 1990 due to the control of the sea lamprey *Petromyzon marinus*, which brought back salmonine populations that prey on alewives (Madenjian et al. 2008). This reduction of alewife abundance led to a yellow perch population recovery from 1983-1985, but it never returned to its pre-alewife-introduction level. (Madenjian et al. 2008).

The yellow perch is a generalist and opportunistic omnivorous species (Tyson and Knight 2001). Adult forage broadly upon benthic invertebrates (e.g., chironomids, Sphaeriidae), pelagic fish such as alewife, rainbow smelt *Osmerus mordax*, white perch *Morone americana*, spottail shiner *Notropis hudsonius* and benthic fish such as johnny darter *Etheostoma nigrum* and mottled sculpin *Cottus bairdi* depending on prey availability (Parrish and Margraf 1994, Schaeffer et al. 1999, Graeb et al. 2005, Truemper et al. 2006). Yellow perch will switch their diet to utilize the most readily available prey. Thus, Truemper and Lauer (2005) reported that the round goby *Neogobius melanostomus* (a benthic non-native species introduced in the 1990s in the Great Lakes) has become a relevant component of adult yellow perch > 100 mm diets (ranging from 7 to 47% of the

total diet) in southern Lake Michigan (Michigan City, MI and Gary, IN). This change in yellow perch is indicative of increasing abundance of round goby in this system.

Although the quantity of food affects growth in fish, its quality is also an important factor. Fatty acids are key nutrients that influence physiological performance of aquatic organisms. They are required to ensure growth, survival and reproduction of zooplankton, pelagic and benthic invertebrates, and fish. They play major roles as a source of metabolic energy ( $\beta$ -oxydation of fatty acids which provides ATP), as components of cellular membranes (supporting membrane fluidity and permeability), and as precursors of eicosanoids (controlling e.g., immune responses, ovulation, embryonic development, hatching and early larval performance) (Tocher 2003). Essential fatty acids cannot be synthesized in appreciable quantities by animals and therefore must be obtained through diet. In most animals, linoleic (18:2n-6) and linolenic (18:3n-3) are essential because organisms without chlorophyll lack  $\Delta$ 12 and  $\Delta$ 15 desaturases required for their formations from oleic acid (18:1n-9). These dietary fatty acids can be desaturated and elongated to form the physiologically essential polyunsaturated fatty acids (PUFA – Figure 1). The degree to which an animal can perform these conversions is dependent on the relative activities of fatty acid elongases and desaturases in their tissues. The activities of these enzymes depend on the extent to which the species can or cannot readily obtain the end products of these conversions from their natural diets (Tocher 2003). Carnivores and omnivores lack the ability to efficiently make the above described conversions and mostly rely on their diet as a source of arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). Considerable research in aquaculture has demonstrated the importance of essential fatty acids in fish. Essential fatty acid deficiencies result in lordosis or curvature of the

spine (Geurden et al. 1995), fin erosion (Higashi et al. 1966), erratic swimming (Castell et al. 1972), bleeding gills (Lochmann and Gatlin 1993), vision impairment (Bell et al. 1995), swim bladder non-inflation (Kitajima et al. 1994, Tandler et al. 1995), improper pigmentation (Kanazawa 1993, Rainuzzo et al. 1994), brain damage (Navarro et al. 1993), susceptibility to stress (Tago et al. 1999), impaired ontogeny of schooling behavior (Ishizaki et al. 2001, Masuda et al. 1998), decreased fecundity and reduced viability (Furuita et al. 2000), and impaired growth and decreased survival (Bell et al. 1999, Smith et al. 2004).

Along with their role as key nutrients, fatty acid signatures (the entire array of fatty acids in a species) have been used to trace and confirm predator-prey interactions for more than 30 years (Napolitano 1999, Dalsgaard et al. 2003). This approach is based on the concept, originally proposed by Lovern (1935), that fatty acids are conservatively transferred from prey to predator and therefore infer diet in accordance to the principle “you are what you eat.” During digestion dietary fatty acids pass into the circulation of monogastric animals, and those of carbon chain length greater than 14, remain largely intact during digestion process (Smith et al. 1997). They are retained within the predator, thus it is possible to distinguish between those fatty acids that could be biosynthesized by the animal and those that can only come via prey from the diet (Iverson 1993). Fatty acids have been used successfully as trophic markers both at the bottom (Fraser et al. 1989, Cripps and Atkinson 2000, Graeve et al. 1994, 2002, Goedkoop et al. 2000, Jeffs et al. 2004) and at the top (Iverson 1993, Iverson et al. 1997, Smith et al. 1997, Kirsch et al. 1998, Raclot et al. 1998, Barrado et al. 2003, Dwyer et al. 2003, Käkälä et al. 2006) of the food web. The fatty acid method integrates not only spatial and temporal variations in diets, but is also reliable for rapid detection of diet shifts in fish, because the time

needed to influence fatty acid signature is relatively short, e.g., 3 weeks (Kirsch et al. 1998).

A prerequisite to this powerful analytical method is that each prey species has a distinct fatty acid signature. Most of the fatty acid data sets currently available are limited to marine species and lower trophic levels (Kirsch et al. 1998, Auel et al. 2002, Budge et al. 2002, Graeve et al. 2002, Phillips et al. 2002, Reuss and Poulsen 2002, Staniland and Pond 2005, Käkälä et al. 2006). Recently, lipid concentration and fatty acid signatures were described for nine species of fish (alewife, johnny darter, rainbow smelt, round goby, spottail shiner, ninespine stickleback *Pungitius pungitius*, threespine stickleback *Gasterosteus aculeatus*, white sucker *Catostomus commersonii*, and yellow perch) and several different invertebrates (amphipods, arthropods, crayfish spp., dreissenid mussels, isopods, *Mysis diluviana*, and zooplankton) collected in Lake Michigan (Czesny et al. 2011). These authors found that alewife and round goby have two distinct fatty acid signatures with alewife associated with pelagic species and round goby with benthic species. A non-metric multidimensional scaling (nMDS) plot revealed two distinct clusters, where zooplankton, dreissenid mussels, rainbow smelt and alewife made one cluster and the other consisted of benthic invertebrates (i.e., amphipods, arthropods, isopods, and *Mysis*), round goby, johnny darter, spottail shiner, adult yellow perch, and stickleback species (Figure 2). One can observe from these results that dreissenid mussels are clustered with pelagic species'. Since mussels are filter feeders their diet consists of mainly zooplankton. This conclusion could also be suggested for spottail shiner, which may consume benthic invertebrates clustering them with benthic species. In a feeding trial conducted by Weber et al. (2010) yellow perch favored alewife over round goby, however, in Lake Michigan they had a benthic fatty acid signature indicating

they are taking advantage of the exploding round goby population in the lake (Czesny et al. 2011).

With the introduction of these non-native species yellow perch prey fish have changed dramatically, which could affect successful recruitment of yellow perch in Lake Michigan. Therefore, the purpose of this study was to compare the effects of two prey species (alewife and round goby) representing two distinct fatty acid signatures on growth, survival, egg quality, and fatty acid composition of adult yellow perch.

## **2. Methods**

### **2.1. Experimental fish and husbandry**

On April 7, 2008, two-hundred and twenty-six (226) two-year-old yellow perch (74 males and 152 females) were purchased from Coolwater Fish Farm in Geneseo, NY. Fish were bagged (~30 fish/bag) and transported to The College at Brockport, State University of New York (SUNY). The bags were placed in several coolers on ice to reduce fish stress during transport. Upon arrival the bags were placed in a 450 L tank for 20 minutes so fish could acclimate to water temperature. Fish were then sorted and distributed into 4-450 L circular, fiberglass tanks maintained as a flow-through water system. Tanks were supplied with flow-through, dechlorinated city water. Dechlorination was attained using a carbon filter system (Siemens Water Technologies, Warrendale, PA). Females were placed into three tanks (51 fish/tank) and males in the remaining tank. Each tank was supplied with continuous aeration and a mesh screen was placed on top of each tank to prevent fish escapement.

Fish were acclimated for one week and then individually weighed and reassigned into four tanks based on weight to maintain similar biomass per tank (~ 3.8 kg/tank). The

four tanks contained 38 females each. Water was continuously aerated using air stones. Throughout the experiment, fish were reared at ambient water temperature and a natural photoperiod was provided by the use of fluorescent daylight tubes; suspended above the tanks. Water temperature was recorded and tanks were cleaned daily. Water quality measurements including chlorine and ammonia were recorded for each tank throughout the experiment using a colorimetric method (Smart Water Lab, Model SCL-04, LaMotte, Chestertown, MD). Dissolved oxygen was measured using a waterproof dissolved oxygen meter (Hanna Instruments, Woonsocket, RI). These measurements were initially taken every two-weeks and then monthly. Prior to the feeding experiment, eight fish (2 per tank) were randomly selected and sacrificed. Fish were individually weighed, measured and dissected. Gonads and liver were excised and weighed, whereas a sample of visceral fat and muscle were sampled, but not weighed. All samples were immediately frozen at  $-80^{\circ}\text{C}$  until lipid and fatty acid analysis.

## 2.2. Diet

At the Coolwater fish farm, yellow perch were reared on a 3.0 mm floating dry diet from Melick Aquafeed Incorporated (Catawissa, PA). During the experiment, which started on April 12, 2008, yellow perch were fed alewife or round goby (2 tanks/dietary treatment). Both prey fish were collected in the nearshore water of Lake Michigan at Waukegan, IL by the Illinois Natural History Survey personnel. Fish were collected using gill nets, trap nets, and bottom trawl. Nets were set up for 24 hours, parallel to the shore at the 5 m depth contour line. Large gill nets consisted of 100 ft panels with 2.0, 2.5, 3.0 and 3.5 inch (in.) stretched mesh and small gill nets having 33 ft panels with 0.31, 0.5, 0.75 and 1.0 in. stretched mesh. Fyke nets were used with a 4 X 6 ft double-



end with a 100 ft. leader between the double-throated pockets. A 16-ft. semi-balloon otter trawl was used for bottom trawling. Upon collection, fish were immediately placed on dry ice and stored at  $-80^{\circ}\text{C}$ . Fish were shipped on dry ice to The College at Brockport, NY and then immediately stored at  $-80^{\circ}\text{C}$  until feeding.

Prior to feeding, alewife and round goby were removed from the  $-80^{\circ}\text{C}$  biofreezer, they were partially thawed and chopped into 1-2 cm pieces. Starting on April 15, 2008, fish were fed 15 g/tank of alewife or round goby (duplicates/dietary treatment), which was gradually increased until fish accepted 35 g or 1% of their total body weight about three and half months later and for the remainder of the experiment (i.e., until spawning began).

A composite of alewife and round goby were analyzed for lipid contents and fatty acid signatures. Each composite consisted of three to four fish. Whole body alewife and round goby were measured, weighed, and homogenized in a commercial blender (Waring, Torrington, CT), then they were immediately stored at  $-80^{\circ}\text{C}$  until biochemical analysis.

### 2.3. Yellow perch reproduction and survival

In March and April 2009, females were observed closely for signs of egg ovulation, which include roundness of the abdomen and a distended genital papilla. When signs of ovulation were apparent the length and weight of each fish were recorded and eggs were stripped by hand. Yellow perch produce a ribbon or skein of eggs when they spawn. The entire egg mass was weighed and recorded, then a subsample was taken to determine the fecundity of each fish and another subsample for lipid and fatty acid analysis.

Approximately 2 g of eggs were fertilized using the dry method of fertilization (Czesny

and Dabrowski 1998) by placing 200 µl of sperm on eggs, adding water, swirling the mixture and allowing fertilization to take place for one minute. Eggs were rinsed several times with clean dechlorinated water and placed in baskets made of polyvinyl chloride (PVC) approximately 110 mm in diameter with 1.0 mm mesh glued to the bottom to hold the eggs. The baskets were placed in trays inside a hatching system for incubation until hatching. The hatching system was a recirculating system, supplied with dechlorinated city water maintained at a temperature of 9.0-9.5°C.

Embryo survival at the pigmented eyed stage (fertilization rate) was determined one week after fertilization from three subsamples of eggs for each female. The total number of eggs and the number of eggs containing embryos at pigmented eyed stage were counted for each subsample under a dissecting microscope. Survival, expressed in percent, was determined by dividing the number of eggs containing embryos at the pigmented eyed stage by the total number of eggs. The average of the three subsamples was then calculated and considered as the percentage of survival for each female.

Absolute fecundity (total number of eggs in gonad) and relative fecundity (total number of eggs per g of fish) were calculated for each female. A subsample of egg from each female was weighed, eggs were counted and the number of eggs per g of weight was determined. Absolute fecundity was calculated by multiplying the total weight of eggs by the number of eggs per g. Relative fecundity was determined as the number of eggs produced per g of body weight.

Some females were sacrificed just after spawning. Each sacrificed female was weighed, measured, and dissected. Liver, visceral fat, and a sample of muscle were removed and frozen for further biochemical analysis. Liver was individually weighed.

The hepatosomatic index (HSI) and pseudo-gonadosomatic index (pseudo-GSI) were calculated for female yellow perch. The HSI was determined as the ratio of the liver weight to body weight indicating the energy reserve in the fish; a larger liver designates a larger energy reserve. The pseudo-GSI was determined as the ratio of the weight of eggs to the body weight, which is used to approximate the reproductive effort in the fish (Kjesbu et al. 1991).

#### 2.4. Lipid analysis

Lipids in diet (alewife and round goby), liver, muscle, visceral fat, and eggs were extracted following the procedure described by Folch et al. (1957). Approximately 1 g of wet fish tissue or eggs was homogenized in a 20 mL mixture of chloroform and methanol (2:1, v/v) with 0.01% butylated hydroxytoluene as an antioxidant. The homogenate was then filtered through a Büchner funnel using a 42.5 mm Whatman filter (Whatman, England). Then the solvent containing lipids was washed with a 4 mL  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  solution. At this stage two phases are visible; a  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  solution on top and an organic phase on the bottom. Nitrogen was added and the two phases were left overnight for complete separation. Then the bottom solvent was extracted using a Pasteur pipet and evaporated under nitrogen. The total lipid concentration (TL) was determined gravimetrically.

Total lipids from eggs and liver were separated into phospholipids and neutral lipids using a Sep-Pak silica cartridges (Waters Corporation, Milford, MA). Chloroform was used to extract the neutral lipids and methanol for the phospholipids (Juaneda and Rocquelin 1985). Neutral lipid and phospholipid levels were then determined

gravimetrically. All samples (total lipids, neutral lipids, and phospholipids) were stored at  $-80^{\circ}\text{C}$  until transmethylation of fatty acids.

## 2.5. Transmethylation of fatty acids

Transmethylation of fatty acids (FA) followed the method developed by Metcalfe and Schmitz (1961). A known amount of nonadecanoic acid (19:0) was added as an internal standard (IS) to the lipids, which is correlated with amount of total and neutral lipids or phospholipids (8 mg of 19:0 per mL). The IS was used for quantification of the fatty acids. After the IS was evaporated using nitrogen, total lipids and neutral lipids were saponified using sodium hydroxide (NaOH) in methanol at  $80^{\circ}\text{C}$  for 1 h. Fatty acid methyl esters (FAMES) were then prepared by transesterification with borontrifluoride in methanol at  $80^{\circ}\text{C}$  for 30 min. The same procedure was used for phospholipids, without saponification.

FAMES were quantified by the Agilent 7890A Gas Chromatograph (Agilent Technologies, Santa Clara CA) equipped with an Agilent 7693 Series Automatic Liquid Sampler and mass spectrometer detector (ID). The column was an Omegawax 250 fused silica capillary column, 30 m, 0.25 mm, and 0.25  $\mu\text{m}$  film thickness (SUPELCO, Bellefonte, PA, USA). Helium was used as carrier gas, with the flow rate set to 1.8 mL/min and the injection volume of 2.0  $\mu\text{L}$ . The oven temperature was programmed initially for  $175^{\circ}\text{C}$  for 26 min; it was increased at a rate of  $2^{\circ}\text{C}/\text{min}$  to  $205^{\circ}\text{C}$ , and finally held at  $205^{\circ}\text{C}$  for 24 min. The source and analyzer temperature for the mass spectrometer was set to  $230^{\circ}\text{C}$ . The fatty acids were integrated by their retention times from the standard mix to identify all the peaks. Each fatty acid is expressed in % of total fatty acid detected.

## 2.6. Statistics

Data are expressed as mean  $\pm$  standard deviation. Percent data were arcsine transformed before analysis. All data were checked to ensure normality and homogeneity of variance prior to choice of specific statistical analysis techniques.

When normality and homogeneity of variance assumptions were met, independent t-test and one-way analysis of variance (ANOVA) were used to determine statistical differences between (two dietary treatments) or among groups (eight fish sampled at the start of the experiment and two dietary treatments), respectively. When statistical differences were observed using ANOVA, a post hoc Tukey's test was performed to determine which groups differed. The Bonferroni correction factor (BCF), which accounts for a large number of comparisons by reducing the alpha level to decrease the probability of obtaining a type I error, was used to distinguish means after t-tests. The BCF was calculated by dividing the alpha level (0.05) by the number of fatty acids analyzed. Since 26 individual fatty acids were analyzed, the new alpha value used for individual fatty acids was 0.002 ( $\alpha = 0.05/26$ ). The alpha value used for summation groups and the ratio of n-3/n-6 was 0.008 ( $\alpha = 0.05/6$ ).

If data failed to meet the normality requirements, Mann-Whitney and Kruskal-Wallis tests were used. When statistical differences were observed using the Kruskal-Wallis test, a post hoc Tamhane's test, which does not assume equal variances was applied to determine differences among groups. Bonferroni correction factor was also applied as previously described for t-tests. Differences were considered statistically significant if  $p < 0.05$ , except for fatty acids, which used the alpha values (0.002 for

individual fatty acids and 0.008 for fatty acid groups) calculated from the Bonferroni correction.

Classification and Regression Tree (CART) analysis, a non-parametric analysis, was used to determine if the tissues from fish fed two different diets were similar to their corresponding diet. CART does not require the data be distributed normally or homogeneity of variance. CART narrows variables down to the most important ones that influence the dataset. Classification was used to separate eggs and liver by neutral and phospholipid fraction and to separate visceral fat and muscle by total lipids. The diet was used as the target variable and 26 different fatty acids were used as the predictors of diet. CART produces nodes depending on the number of variables that influence the dataset. Generally, the first node represents how many rows (N) were placed in this particular node, the target variable, and misclassification percent, whereas the subsequent nodes also include the predictor variable (diet) used for generating the split from the first node.

Linear regression analysis was used to determine relationship between fish length and fecundity. Statistical data were analyzed using IBM SPSS 20.0 (independent t-test, Mann-Whitney, ANOVA, and Kruskal-Wallis) and DTREG (CART).

### **3. Results**

#### 3.1. Experimental fish and husbandry

All water quality parameters were appropriate throughout the experiment (Table 1). However, starting in May 2008, signs of stress (i.e., decreased appetite, lordosis, lethargy, hemorrhaging) and fungus were noticed on some fishes. Salt treatments (10%) were administered to each tank from August 2008 to February 2009 to reduce stress and

prevent disease development (see arrows on Figure 3). As a result, seven fish died during the experiment, three fed alewife and four fed round goby.

### 3.2. Diet

Whole body lipid was significantly higher in alewife ( $8.6 \pm 0.1\%$ ) than in round goby ( $2.9 \pm 0.0\%$ ) (independent t-test,  $t = 61.07$ ,  $df = 4$ ,  $p < 0.001$ ). The fatty acid composition of the two different diets is presented in Table 2.

The percentage of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and the ratio of n-3/n-6 for the diet were higher in alewife than in round goby (Table 2; independent t-test,  $t = 58.82$ ,  $df = 4$ ,  $p < 0.001$ ,  $t = 26.29$ ,  $df = 4$ ,  $p < 0.001$ ,  $t = 20.00$ ,  $df = 4$ ,  $p < 0.001$ , respectively). In contrast, the sum of n-3 and n-6 as well as the percentage of PUFA were higher in round goby (Table 2; independent t-test,  $t = 12.30$ ,  $df = 4$ ,  $p < 0.001$ ;  $t = 4.17$ ,  $df = 4$ ,  $p < 0.05$ ;  $t = 9.88$ ,  $df = 4$ ,  $p < 0.01$ , respectively). Hexadecanoic acid (16:0) was the dominant SAFA in both species but was significantly at a higher concentration in alewife (Table 2; independent t-test,  $t = 31.24$ ,  $df = 4$ ,  $p < 0.001$ ). The main MUFA consisted of 16:1n-7 and 18:1n-9, however alewife had the higher concentration of 16:1n-7 (Table 2; independent t-test,  $t = -111.73$ ,  $df = 4$ ,  $p < 0.001$ ) and round goby had the higher concentration of 18:1n-9 (Table 2; independent t-test,  $t = 46.97$ ,  $df = 4$ ,  $p < 0.001$ ). The dominant fatty acids from the n-6 family in both species were 18:2n-6, ARA, and 22:5n-6. Round goby exhibited the higher level of ARA (Table 2; independent t-test,  $t = -46.86$ ,  $df = 4$ ,  $p < 0.001$ ) and 22:5n-6 (Table 2; independent t-test,  $t = -63.27$ ,  $df = 4$ ,  $p < 0.001$ ), whereas alewife contained the higher level of 18:2n-6 (Table 2; independent t-test,  $t = 50.36$ ,  $df = 4$ ,  $p < 0.001$ ). The main fatty acids from the n-3 family were 18:3n-3, EPA, and DHA. The concentrations of EPA and

DHA were higher in round goby compared to alewife (Table 2; independent t-test,  $t = -46.42$ ,  $df = 4$ ,  $p < 0.001$ ;  $t = -33.79$ ,  $df = 2$ ,  $p < 0.001$ , respectively), whereas 18:3n-3 was higher in alewife (Table 2: independent t-test,  $t = 29.54$ ,  $df = 4$ ,  $p < 0.001$ ). The only fatty acids that were not significantly different between both species were 15:0 and 18:1n-7.

### 3.3. Yellow perch reproduction and survival

Spawning of yellow perch occurred early morning or late afternoon. Although efforts were made to strip eggs by hand from each female, some females started to spawn in tanks early morning prior to hand stripping. Overall, eggs from 31 females (12 fed the alewife diet and 19 fed the round goby diet) were collected for lipids and fatty acid analysis.

Females fed round goby had a significant increase in body weight of 18.3 g in comparison to fish fed alewife with an increase of 6.0 g (Table 3; Kruskal-Wallis, Chi-Square = 16.97,  $df = 2$ ,  $p < 0.05$ , Bonferroni post hoc test,  $p < 0.001$ ).

Absolute fecundity and relative fecundity were not significantly different between the two dietary treatments (Table 4; independent t-test,  $t = -0.65$ ,  $df = 17$ ,  $p = 0.52$  and  $t = 0.44$ ,  $df = 3.37$ ,  $p = 0.69$ , respectively). Regardless of the dietary treatment, there was no relationship between yellow perch length and relative fecundity (Figure 4; fish fed alewife:  $R^2 = 0.77$ ,  $F = 6.68$ ,  $df = 1$ ,  $p > 0.05$  and fish fed round goby:  $R^2 = 0.03$ ,  $F = 0.39$ ,  $df = 1$ ,  $p > 0.05$ ).

Embryo survival at the pigmented eyed stage for both alewife-fed ( $n = 7$ ) and round goby-fed ( $n = 3$ ) fish was very poor, 10.4% and 30.3 %, respectively and did not differ significantly (Table 4; independent t-test,  $F = 6.77$ ,  $df = 2.3$ ,  $p > 0.05$ ). Once again



many fish spawned in the tank, which impacted our ability to successfully fertilize the eggs.

Female HSI was not statistically different between yellow perch fed the alewife diet (1.8%) and round goby diet (1.9%; Table 4; independent t-test,  $t = 0.42$ ,  $df = 8$ ,  $p = 0.69$ ). Pseudo-GSI (alewife-fed fish 31% and round goby-fed fish 29%) also did not differ significantly (Table 4; independent t-test,  $t = 0.28$ ,  $df = 3.28$ ,  $p = 0.79$ ).

#### 3.4. Lipid concentration and fatty acid signatures in yellow perch tissues

Lipids were extracted from all yellow perch tissues sampled at the beginning ( $n = 8$ ) and at the end of the experiment. Twenty six fatty acids were consistently identified in all lipid fractions (total, neutral, and phospholipids) from each tissue examined.

##### 3.4.1. Eggs

###### 3.4.1.1. Total, neutral, and phospholipids

The concentration of total lipid in yellow perch eggs was significantly higher in fish fed alewife compared to fish fed round goby (Table 5; Mann-Whitney,  $U = 61.5$ ,  $p < 0.05$ ). The eggs of fish fed the alewife diet had a significantly higher percentage of neutral lipids (Table 5; Mann-Whitney,  $U = 36.5$ ,  $p < 0.05$ ), while eggs of fish fed the round goby diet had a significantly higher percent of phospholipids (Table 5; Mann-Whitney,  $U = 36.5$ ,  $p < 0.05$ ).

###### 3.4.1.2. Fatty acids in neutral lipids of eggs

The sum of SAFA in the neutral lipid fraction of yellow perch eggs was not significantly different between dietary treatments (Table 6; Mann-Whitney,  $U = 104.0$ ,  $p = 0.685$ ). The dominant fatty acid among SAFA was 16:0 but, its concentration did not

differ significantly between dietary treatments (Table 6; Mann-Whitney,  $U = 105.0$ ,  $p = 0.715$ ). The only SAFA difference between the two dietary treatments was 14:0, with eggs of the fish fed alewife having a significantly higher concentration (Table 6; Mann-Whitney,  $U = 33.0$ ,  $p < 0.01$ ).

The sum of MUFA was significantly higher in eggs of fish fed round goby when compared to the eggs of fish fed alewife (Table 6; Mann-Whitney,  $U = 6.0$ ,  $p < 0.01$ ).

The major fatty acid found in MUFA was 18:1n-9, which also was significantly higher in eggs of yellow perch fed alewife compared to eggs of fish fed round goby (Table 6; Mann-Whitney,  $U = 65.5$ ,  $p < 0.05$ ). Palmitoleic acid (16:1n-7) was a second major fatty acid of MUFA, which was significantly higher in eggs of round goby-fed fish (Table 6; Mann-Whitney,  $U = 0.0$ ,  $p < 0.01$ ).

In the neutral lipid fraction of eggs in fish fed round goby only linoleic acid, total PUFA, and ratio of n-3 to n-6 was similar to the diet (Table 6). The sum of PUFA was significantly higher in eggs of fish fed alewife when compared to eggs of fish fed round goby (Table 6; Mann-Whitney,  $U = 10.0$ ,  $p < 0.01$ ). The sum of n-6 was similar in the two dietary treatments (Table 6; Mann-Whitney,  $U = 68.0$ ,  $p = 0.062$ ). The major fatty acid from the n-6 family was 18:2n-6. There was a higher concentration of linoleic acid (12.8%) in neutral lipid fraction of eggs in fish fed round goby compared to the diet (3% - Table 6; Mann-Whitney,  $U = 62.0$ ,  $p < 0.05$ ). The sum of n-3 was significantly higher in the alewife-fed fish (Table 6; Mann-Whitney,  $U = 8.0$ ,  $p < 0.01$ ). DHA was the main fatty acid from the n-3 family and also was significantly higher in the fish fed alewife (Table 6; Mann-Whitney,  $U = 24.0$ ,  $p < 0.01$ ). The ratio of n-3 to n-6 was significantly higher in the fish fed alewife (Table 6; Mann-Whitney,  $U = 31.0$ ,  $p < 0.01$ ).

CART analysis performed using fatty acids in the neutral lipid fraction of eggs revealed that 18:0 was the most important fatty acid discriminating between the two dietary treatments (Figure 5). If the percentage of 18:0 in yellow perch eggs was greater than 13.14% then the fish were fed alewife. Misclassification was 38.71% on the first node and 0% on the second and third nodes.

#### 3.4.1.3. Fatty acids in phospholipids of eggs

The sum of SAFA in the phospholipid fraction was nearly seven times higher than neutral lipid fractions for eggs, but was not significantly different between dietary treatments (Table 6; Mann-Whitney,  $U = 110.5$ ,  $p = 0.89$ ). The major fatty acid was 16:0, which was not significantly different between treatments (Table 6; Mann-Whitney,  $U = 97.0$ ,  $p = 0.49$ ). Stearic acid (18:0) was also abundant in SAFA and was significantly higher in fish fed round goby (Table 6; Mann-Whitney,  $U = 53.5$ ,  $p < 0.05$ ).

The sum of MUFA in the phospholipid fraction of eggs was nearly half the concentration in comparison to the neutral lipid fraction of eggs and was significantly higher in eggs of fish fed round goby (Table 6; Mann-Whitney,  $U = 19.0$ ,  $p < 0.01$ ). The main fatty acid was 18:1n-9, which was similar in dietary treatments (Table 6; Mann-Whitney,  $U = 111.5$ ,  $p = 0.92$ ). The concentration of 16:1n-7 was almost two times higher in eggs of fish fed round goby compared to eggs of fish fed alewife (Table 6; Mann-Whitney,  $U = 0.0$ ,  $p < 0.01$ ).

In the phospholipid fraction linoleic acid, ARA, EPA, and total PUFA were retained (Table 6). The sum of PUFA and n-3 fatty acids were significantly higher in eggs of fish fed alewife compared to the ones fed round goby (Table 6; Mann-Whitney,  $U = 56.0$ ,  $p < 0.05$  and  $U = 35.0$ ,  $p < 0.01$ , respectively). The major n-3 fatty acids were

EPA and DHA. EPA was not significantly different between dietary treatments, but DHA was significantly higher in eggs of fish fed alewife compared to eggs of fish fed round goby (Table 6; Mann-Whitney,  $U = 100.5$ ,  $p = 0.58$  and  $U = 36.0$ ,  $p < 0.01$ , respectively). The sum of n-6 was similar to for both dietary treatments (Table 6; Mann-Whitney,  $U = 93.5$ ,  $p = 0.41$ ) and consisted mainly of 18:2n-6 and ARA. Linoleic acid was significantly higher in eggs of fish fed round goby (Table 6; Mann-Whitney,  $U = 29.0$ ,  $p < 0.01$ ) and ARA was significantly higher in eggs of fish fed alewife (Table 6; Mann-Whitney,  $U = 37.5$ ,  $p < 0.01$ ). The ratio of n-3 to n-6 was not significantly different (Table 6; Mann-Whitney,  $U = 103.0$ ,  $p = 0.65$ ).

CART analysis performed using fatty acids of eggs from the phospholipid fraction revealed that only 18:0 was the most important fatty acid for distinguishing between the two dietary treatments (Figure 6). If the percentage of 18:0 was greater than 2.17% then fish were fed round goby. Misclassification was 38.71% on the first node and 0% on the second and third nodes.

### 3.4.2. Liver

#### 3.4.2.1. Total, neutral, and phospholipids

Although, total lipids and neutral lipid fractions in liver were much higher in fish at the start of the experiment (7.6% and 72.6%, respectively) compared to the results after dietary treatments (round goby-fed fish 3.1% and alewife-fed fish 3.8% and round goby-fed fish 34.5% and alewife-fed fish 36.7%, respectively), there were no significant differences (Table 5; Kruskal-Wallis, Chi-Square = 13.05,  $df = 2$ ,  $p < 0.01$ , Tamhanes post hoc test,  $p > 0.05$  and Chi-Square = 7.41,  $df = 2$ ,  $p < 0.05$ , Tamhanes post hoc test,  $p > 0.05$ , respectively). There was also no significant difference in the phospholipid

fraction of liver among groups, even though females at the start of the experiment had a much lower concentration (27.4%) than the ones (round goby-fed fish 65.5% and alewife-fed fish 63.3 %) fed both diets (Table 5; Kruskal-Wallis, Chi-Square = 7.41, df = 2,  $p < 0.05$ , Tamhanes post hoc test,  $p > 0.05$ ).

#### 3.4.2.2. Fatty acids in neutral lipids of liver

The sum of SAFA in the neutral lipid fraction of liver was significantly higher in fish fed alewife when compared to fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 9.93, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.005$ ). SAFA was made up mainly of 16:0, which was also higher in liver of fish fed alewife, but no significant difference was found between the two dietary treatments (Table 7; Kruskal-Wallis, Chi-Square = 9.82, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ).

The sum of MUFA for the neutral lipid fraction of liver was the highest in fish at the start of the experiment, but did not differ statistically due to high variation (Table 7; Kruskal-Wallis, Chi-Square = 10.94, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p > 0.008$ ). MUFAs consisted primarily of 16:1n-7 and 18:1n-9. Both of these fatty acids were at least twofold higher in fish at the start of the experiment, but only 18:1n-9 was statistically different when compared to liver in fish fed round goby (Table 7; Kruskal-Wallis, Chi-Square = 15.20, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.002$ ). The concentration of 16:1n-7 was not significantly different among groups (Table 7; Kruskal-Wallis, Chi-Square = 11.92, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ).

The sum of PUFA for the neutral lipid fraction of liver was the highest in fish at the start of the experiment, but was not significantly different in comparison to liver of fish fed the two dietary treatments (Table 7; Kruskal-Wallis, Chi-Square = 6.79, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p > 0.008$ ). The sum of n-6 was also significantly the highest in liver of fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 15.56, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.002$ ). Linoleic acid was the main n-6 fatty acid, initially, the concentration in yellow perch liver was 13.2% and after the experiment the concentration in fish fed alewife and round goby was 5.2% and 4.4%, respectively (Table 7; Kruskal-Wallis, Chi-Square = 17.80, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). ARA was relatively similar in liver of fish fed the two dietary treatments as well as four times higher than fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 17.49, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). The sum of n-3 was the highest in liver of fish fed alewife, but was not significantly different among groups (Table 7; Kruskal-Wallis, Chi-Square = 0.89, df = 2,  $p = 0.641$ , BCF  $\alpha = 0.008$ ). Regardless of dietary treatment, linolenic acid in the neutral lipid fraction of liver remained low throughout the experiment ( $< 1.5\%$ ) especially compared to the concentration in both round goby (2.5%) and alewife (4.5% - Table 7; Kruskal-Wallis, Chi-Square = 11.15, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ). The liver in fish fed alewife also had the highest concentration of DHA, but was also not significantly different from the other groups (Table 7; Kruskal-Wallis, Chi-Square = 1.94, df = 2,  $p = 0.38$ , BCF  $\alpha = 0.002$ ). EPA was the second major fatty acid in the sum of n-3 and contained similar concentrations among the three groups (Table 7; Kruskal-Wallis, Chi-Square = 2.33, df = 2,  $p = 0.31$ , BCF  $\alpha = 0.002$ ). Liver of fish fed the two dietary

treatments had significantly higher ratios of n-3 to n-6 compared to fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 15.52, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.002$ ).

CART analysis performed using fatty acids in the neutral lipid fraction of liver revealed that 22:5n-6 was the most important fatty acid for distinguishing between the dietary treatments (Figure 7). If the percentage of 22:5n-6 was less than or equal to 0.17% then fish were fed round goby and if it was greater than 0.17% then fish were fed alewife. Misclassification was 33.33% on the first node and 0% for the second and third node.

#### 3.4.2.3. Fatty acids in phospholipids of liver

The sum of SAFA for the phospholipid fraction of liver was similar among groups (Table 7; Kruskal-Wallis, Chi-Square = 2.51, df = 2,  $p = 0.29$ , BCF  $\alpha = 0.008$ ). The major fatty acid, 16:0 was the highest in liver of fish at the start of the experiment compared to the two dietary treatments, but was not statistically different (Table 7; Kruskal-Wallis, Chi-Square = 8.68, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ). Stearic acid (18:0) was statistically higher in liver of fish fed round goby compared to liver of fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 13.44, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). The sum of MUFA for the phospholipid fraction of liver was highest in fish at the start of the experiment, while the liver of the two dietary treatments was similar (Table 7; Kruskal-Wallis, Chi-Square = 3.17, df = 2,  $p = 0.21$ , BCF  $\alpha = 0.008$ ). The three major MUFA were 18:1n-9, 18:1n-7, and 16:1n-7. Liver in fish at the start of the experiment had the highest concentration of 16:1n-7 compared to the two dietary treatments (Table 7;

Kruskal-Wallis, Chi-Square = 11.09, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ), whereas 18:1n-9 and 18:1n-7 were not significantly different among groups (Table 7; Kruskal-Wallis, Chi-Square = 6.55, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$  and Chi-Square = 9.90, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ , respectively).

The sum of PUFA was twofold higher in the phospholipid fraction of liver in comparison to the neutral lipid fraction of liver and were similar throughout the three groups (Table 7; Kruskal-Wallis, Chi-Square = 3.20, df = 2,  $p = 0.20$ , BCF  $\alpha = 0.008$ ). For the sum of n-6, liver in fish fed the two dietary treatments were significantly higher than fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 14.19, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.005$ ). ARA was the main fatty acid found in the n-6 group and was considerably higher in liver of fish fed the two dietary treatments (Table 7; Kruskal-Wallis, Chi-Square = 15.27, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). Linoleic acid was the secondary fatty acid that made up the n-6 family. This fatty acid was twofold higher in liver of fish at the start of the experiment compared to liver of fish fed round goby (Table 7; Kruskal-Wallis, Chi-Square = 15.05, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). The sum of n-3 was not significantly different among the three groups (Table 7; Kruskal-Wallis, Chi-Square = 3.84, df = 2,  $p = 0.20$ , BCF  $\alpha = 0.008$ ). Regardless of dietary treatment, linolenic acid in the phospholipid fraction of liver remained low throughout the experiment ( $< 1.0\%$ ) especially compared to the concentration in both round goby (2.5%) and alewife (4.5% - Table 7; Kruskal-Wallis, Chi-Square = 14.17, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ). DHA was the primary fatty acid of the n-3 family and concentrations (initial 33.2%, fish fed round goby 30.4% and



fish fed alewife 32.7%) were similar throughout the three groups (Table 7; Kruskal-Wallis, Chi-Square = 2.14, df = 2, p = 0.34, BCF  $\alpha$  = 0.002). EPA was the second main n-3 fatty acid, where the concentration was similar among the groups (Table 7; Kruskal-Wallis, Chi-Square = 1.55, p = 0.46, BCF  $\alpha$  = 0.008). The ratio, sum of n-3 to sum of n-6, was significantly lower in liver of fish fed the two dietary treatments; almost half when compared to liver of fish at the start of the experiment (Table 7; Kruskal-Wallis, Chi-Square = 14.50, df = 2, p < 0.01, BCF  $\alpha$  = 0.008, Tamhanes post hoc test, p < 0.001).

CART analysis performed using fatty acids in the phospholipid fraction of liver revealed that only 22:1n-11 was the most important fatty acid between the dietary treatments (Figure 8). If the percentage of 22:1n-11 was less than or equal to 0.33% then fish were fed round goby and if it was less than 0.33% then fish were fed alewife. Misclassification for the first node was 30.77% and 0% for the second and third node.

### 3.4.3. Muscle

#### 3.4.3.1. Total lipids

Lipid content in muscle of fish was significantly different among groups with fish fed round goby having significantly lower total lipid content than fish at the start of the experiment (Table 5; Kruskal-Wallis, Chi-Square = 6.43, df = 2, p < 0.05, BCF  $\alpha$  = 0.05, Tamhanes post hoc test, p < 0.05).

#### 3.4.3.2. Fatty acids in total lipids of muscle

Fish at the start of the experiment had a significantly higher sum of SAFA in total lipid of muscle than muscle of fish fed alewife (Table 8; Kruskal-Wallis, Chi-Square = 10.30, df = 2, p < 0.05, BCF  $\alpha$  = 0.008, Tamhanes post hoc test, p < 0.005). Once again, SAFA consisted of mostly 16:0 with the highest concentration in muscle of fish at the

start of the experiment, but was not statistically different (Table 8; Kruskal-Wallis, Chi-Square = 10.42, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ).

The other main fatty acid of SAFA was 18:0, which was similar in muscle among the three groups of fish (Table 8; Kruskal-Wallis, Chi-Square = 8.00, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ).

Fish sampled at the start also had a significantly higher sum of MUFA in total lipid of muscle than fish fed alewife, with the lowest concentration in muscle of fish fed round goby (Table 8; Kruskal-Wallis, Chi-Square = 12.20, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.005$ ). Oleic acid was the main fatty acid in MUFA and was statistically higher in muscle of fish at the start of the experiment (Table 8; Kruskal-Wallis, Chi-Square = 16.42, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). Palmitoleic acid was a second major fatty acid of MUFA and was higher in muscle of both fish at the start of the experiment and fish fed round goby, but was not significantly higher in muscle of fish fed alewife (Table 8; Kruskal-Wallis, Chi-Square = 11.10, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ).

The lowest sum of PUFA was in muscle of fish at the start of the experiment with the highest in muscle of fish fed round goby (Table 8; Kruskal-Wallis, Chi-Square = 17.30, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.001$ ). For the sum of n-6, muscle of fish at the start of the experiment was significantly lower than in muscle of fish fed the two dietary treatments (Table 8; Kruskal-Wallis, Chi-Square = 17.67, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.001$ ). Linoleic acid was the primary fatty acid of the n-6 family, with the highest concentration found in muscle of fish at the start of the experiment (Table 8; Kruskal-Wallis, Chi-Square = 13.58, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.002$ ). ARA was the secondary

fatty acid of the n-6 family and had a lower concentration in muscle of fish at the start of the experiment compared to muscle of fish fed the two dietary treatments (Table 8; Kruskal-Wallis, Chi-Square = 19.50, df = 2,  $p < 0.001$ , BCF adjustment  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). The sum of n-3 was not significantly different among muscle of the three groups (Table 8; Kruskal-Wallis, Chi-Square = 4.52, df = 2,  $p = 0.11$ , BCF  $\alpha = 0.008$ ). DHA was the major fatty acid in n-3 and had the highest concentration in fish fed round goby and was significantly different compared to fish at the start of the experiment (Table 8; Kruskal-Wallis, Chi-Square = 5.47, df = 2,  $p = 0.07$ , BCF  $\alpha = 0.002$ ). EPA was the other major fatty acid in n-3. The concentration of EPA (7.8%) in the muscle of fish fed alewife was similar to the concentration of EPA (7.2%) in their diet, whereas muscle in fish fed round goby (8.7%) had a slightly lower concentration than the diet fish (10.4%). Although, the concentration of EPA in muscle of fish fed round goby was lower than the diet fish themselves, it was higher than the initial concentration (7.4%) of yellow perch (Table 8; Kruskal-Wallis, Chi-Square = 11.78, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). The ratio of n-3 to n-6 was significantly higher in muscle of fish at the start of the experiment in comparison to muscle of fish fed the two dietary treatments (Table 8; Kruskal-Wallis, Chi-Square = 16.04, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.005$ ).

CART analysis performed using fatty acids from total lipid of muscle revealed that 20:2n-6 was the most important fatty to distinguish between the dietary treatments (Figure 9). If the percentage of 20:2n-6 was less than or equal to 0.29% then fish were fed round goby. The second most important fatty acid that was responsible for distinguishing between the dietary treatments was 17:0. If the percentage of 17:0 was

greater than or equal to 1.22% then fish were fed round goby and if it was greater, then fish were fed alewife. Misclassification was 33.33%, 6.25%, 12.50%, 0% and 0% for node 1, node 2, node 3, and node 4 and 5, respectively.

#### 3.4.4. Fat

##### 3.4.4.1. Total lipids

Lipid content in visceral fat of fish was significantly higher at the start of the experiment compared to visceral fat of fish fed the two dietary treatments (Table 5; Kruskal-Wallis, Chi-Square = 8.85, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.05$ , Tamhanes post hoc test,  $p < 0.05$ ).

##### 3.4.4.2. Fatty acids in total lipids of fat

The sum of SAFA in visceral fat was similar throughout all three groups of fish (Table 9; Kruskal-Wallis, Chi-Square = 3.12, df = 2,  $p = 0.21$ , BCF  $\alpha = 0.008$ ). The main fatty acid in SAFA of visceral fat was 16:0 and was not significantly different among the three groups of fish (Table 9; Kruskal-Wallis, Chi-Square = 3.77, df = 2,  $p = 0.15$ , BCF  $\alpha = 0.002$ ).

The sum of MUFA was statistically higher in visceral fat of fish fed round goby fish when compared to visceral fat of fish at the start of the experiment (Table 9; Kruskal-Wallis, Chi-Square = 15.33, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p < 0.001$ ). The main MUFA was 18:1n-9, which was the highest in visceral fat of fish at the start of the experiment, but was not significantly different due to high variation in visceral fat of fish fed the two dietary treatments (Table 9; Kruskal-Wallis, Chi-Square = 7.35, df = 2,  $p < 0.05$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p > 0.002$ ). The second MUFA was 16:1n-7, which was significantly higher in visceral fat of both fish at the start

of the experiment and fish fed round goby in comparison to visceral fat of fish fed alewife (Table 9; Kruskal-Wallis, Chi-Square = 14.82, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.002$ ).

For the PUFA, visceral fat of fish fed alewife had the highest concentration compared to visceral fat of both fish at the start of the experiment and fish fed round goby, but was not significantly different due to high variation among all three groups (Table 9; Kruskal-Wallis, Chi-Square = 13.84, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p > 0.008$ ). The sum of n-6 was also not statistically different among visceral fat of the three groups (Table 9; Kruskal-Wallis, Chi-Square = 4.64, df = 2,  $p = 0.10$ , BCF adjustment  $\alpha = 0.008$ ). The most abundant n-6 fatty acid was 18:2n-6. The concentration of linoleic acid in both round goby (3.0%) and alewife (5.0%) was notably lower than the initial concentration found in fat of yellow perch (16.3%). Furthermore, the concentration of linoleic acid in fat of both dietary treatments was lower than that of the initial concentration of yellow perch (Table 9; Kruskal-Wallis, Chi-Square = 15.07, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.002$ ). ARA was the second fatty acid and was significantly higher in visceral fat of fish fed round goby in comparison to visceral fat of fish at the start of the experiment (Table 9; Kruskal-Wallis, Chi-Square = 16.14, df = 2,  $p < 0.001$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.001$ ). For the sum of n-3, there were no statistical differences among visceral fat of the three groups due to high variation in visceral fat of fish fed alewife (Table 9; Kruskal-Wallis, Chi-Square = 10.89, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.008$ , Tamhanes post hoc test,  $p > 0.008$ ). DHA was the most abundant fatty acid in the n-3 family and the concentration in visceral fat of fish fed alewife was significantly higher than visceral fat of both fish at the start of the experiment as well as fish fed round goby

(Table 9; Kruskal-Wallis, Chi-Square = 11.38, df = 2,  $p < 0.01$ , BCF  $\alpha = 0.002$ , Tamhanes post hoc test,  $p < 0.002$ ). EPA was the second main fatty acid, but the concentration in visceral fat among the three groups was similar (Table 9; Kruskal-Wallis, Chi-Square = 0.19, df = 2,  $p = 0.91$ , BCF  $\alpha = 0.002$ ).

CART analysis performed using fatty acids in total lipid of fat revealed that 18:0 was the most important fatty acid responsible for distinguishing between the dietary treatments (Figure 10). If the percentage of 18:0 was less than or equal to 11.21% then the yellow perch were fed alewife. Misclassification was 33.33% on the first node and 0% on the second and third nodes.

#### **4. Discussion**

The yellow perch is an important species both ecologically and economically in the Laurentian Great Lakes and it is thus imperative to better understand its decline. I investigated the effects of consuming two non-native species (alewife and round goby) on female yellow perch reproduction using lipid and fatty acid composition of their diets, eggs, liver, muscle, and visceral fat.

It is known that a diet deficient in essential fatty acids (EFA) can inhibit growth. Reduced growth rates were found in juvenile turbot, *Scophthalmus maximus*, fed a diet consisting of linseed oil or olive oil in replacement of fish oil, which increased linoleic and linolenic acid and reduced ARA, EPA, and DHA (Bell et al. 1999). Depressed growth was also observed in rainbow trout juveniles, *Onchorynchus mykiss*, fed a diet with 18:1n-9 as the only source of lipid (Rinchard et al. 2007).

##### **4.1. Growth, fecundity, embryo survival at eyed stage, HSI and pseudo-GSI**

In my study, female yellow perch fed the two different diets exhibited a higher body mass at the end of the experiment compared to the weight of yellow perch at the start. Females fed round goby had a significantly higher body weight at the end of the experiment than the weight of fish at the start. However, there were no significant differences detected in fecundity, embryo survival at eyed stage, HSI or pseudo-GSI. Therefore, despite the lower weights of fish fed alewife, both diets appeared to satisfy the EFA requirements for reproduction by yellow perch.

The sample size for yellow perch, especially group fed alewife ( $n = 4$ ), was small; this could have been a possible reason that no relationship was found between length of fish and absolute fecundity. Yellow perch embryo survival rate for both dietary treatments was low. Similar results were found in wild Eurasian perch embryos at eyed stage under controlled reproduction, where ribbons were categorized into four groups with the lowest grade having an embryo survival rate of 9.71% (Zarski et al. 2011). In the present study, several fish started to spawn in tanks prior to spawning them by hand, which prevented obtaining the entire egg mass to estimate the fecundity and survival of yellow perch embryos at pigmented eyed stage. When the yellow perch were spawned by hand several of the egg ribbons were of low quality, indicated by the ribbon separating in several places. This could explain the non-significant, but reduced rate of embryo survival. It would be interesting to examine the oil droplet fragmentation in ovulating eggs of yellow perch as was done in the study by Zarski et al. (2011) on Eurasian perch. They found that highly fragmented oil droplets in eggs of perch were correlated with a low rate of embryo survival (Zarski et al. 2011).

Although the percentage of lipids found in the liver of females fed alewife was slightly higher, yet not significant compared to fish fed round goby their HSI was lower

than for fish fed round goby. Higher lipid content was also observed in eggs of fish fed alewife in comparison to fish fed round goby. These results reflected the lipid composition of their diets, with alewife presenting a higher lipid concentration than round goby. Similarly, Sheikh-Eldin et al. (1996) found that wild Macquarie perch *Macquaria australasica*, fed a higher lipid content than cultured perch, exhibited a higher percentage of lipids (28.3%) in their liver compared to cultured perch (22.5%), but their HSI (0.6%) was lower than the ones reported for cultured perch (1.2%). The authors attributed this higher percentage of lipids in liver of wild perch to the increase of DHA from 10.6% in dietary lipids to over 16% in their liver (Sheikh-Eldin et al. 1996).

The pseudo-GSI values found in my yellow perches' diet were comparable to GSI values found in mature female Eurasian perch collected in Sweden (22%; Noaksson et al. 2005) and France (25%; Sulistyo et al. 1998). According to the GSI values found in the present study, eggs would be considered viable, however, more research will have to be done to examine why embryo survival was low. Possible reasons for this could be related to insufficient accumulation of lipids in the eggs or increased oil droplet fragmentation in ovulated eggs (Zarski et al. 2011).

#### 4.2. Fatty acid compositions in yellow perch, round goby and alewife

Previous studies have shown that alewife in Lake Michigan are rich in 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, linoleic acid, linolenic acid, ARA, EPA, and DHA (Honeyfield et al. 2009, Czesny et al. 2011). In this study, concentrations of 14:0, 16:1n-7, 18:0, and EPA were similar to the previous studies. The concentrations of 16:0, 18:1n-9, linoleic, and linolenic acid were higher in the alewife of this study, whereas ARA was lower when compared to both previous studies (Table 3). Another difference found between the



present study and previous studies was in the concentration of DHA, which was almost twofold lower in this study. Since linolenic acid concentration in my study was higher and DHA was lower it could be suggested that desaturase and elongase activity was lower in the alewife used in my study compared to enzyme activity in alewife of previous studies. Another explanation of these differences could be due to seasonal variation in fatty acids. In the present study, alewife were collected in early spring, whereas in previous studies they were collected in fall (Honeyfield et al. 2009) as well as in all three seasons (spring, summer, and fall – Czesny et al. 2011). Lipid concentrations in alewife can be highly variable in the spring compared to the summer and summer concentrations tend to be lower than in the fall (Czesny et al. 2011).

Czesny et al. (2011) also quantified the fatty acid composition of round goby from Lake Michigan and found that 16:0, 16:1n-7, 18:1n-9, 18:1n-7, ARA, 22:5n-6, EPA, 22:5n-3, and DHA were the most abundant fatty acids. This study indicated concentrations of 16:0, ARA, and EPA (Table 3) were similar to my study, whereas the concentrations of 18:1n-9 and 18:1n-7 were slightly lower. Concentrations of the remaining fatty acids, 16:1n-7, 22:5n-6, 22:5n-3, and DHA, were significantly higher in my round goby compared to the previous study by Czesny et al. (2011). As a results of this relatively higher concentrations of ARA, EPA, DHA, linoleic, and linolenic acid (i.e., essential fatty acids) in both alewife and round goby suggest that these species are sufficient prey for higher food chain fish (e.g., yellow perch, lake trout).

In most experiments dietary fatty compositions have been reflected in the tissues of consumers. Kirsch et al. (1998) found that only 3 weeks were required for Atlantic cod, *Gadus morhua*, to completely reflect their dietary fatty acids. Although, similar results were found in Eurasian perch, the time necessary for dietary fatty acid reflection

in fish carcass, liver, and muscle was 76 days (Blanchard et al. 2008). These results indicate that dietary fatty acid reflection in fish tissues is species specific. On the other hand tissues of some fish species do not reflect the dietary fatty acid composition (Tocher 2003). Honeyfield et al. (2009) found this when lake trout were fed two different fish species (alewife and bloater) at different concentrations for approximately 2 years. The authors measured the fatty acid composition in lake trout eggs and suggested that lake trout regulate the fatty acid composition found in their eggs physiologically. Some fatty acids may be oxidized, where they are present in high concentration in the parent, but are low in their eggs (Henderson et al. 1984a, Tocher 2003).

Since yellow perch are closely related to Eurasian perch, it was expected that dietary fatty acids would be incorporated in their tissues rapidly. Although, yellow perch were fed exclusively on round goby or alewife for a year, a complete reflection of the diet in yellow perch tissues was not observed, especially in muscle and visceral fat tissue. The tissue that by and large reflected the dietary fatty acids was the liver. The fatty acids found in the liver that were similar between round goby and the dietary treated yellow perch were 14:0, 16:0, 18:0, 18:1n-9, 18:1n-7, linoleic acid, ARA, 22:5n-6, and DHA. In alewife the similar fatty acids in liver samples were 18:0, 16:1n-7, linoleic acid, ARA, 22:5n-6, linolenic acid, 22:5n-3, and DHA (Table 7). One possible reason for this incomplete reflection of dietary fatty acid composition could be due to the metabolism of fatty acids. It is known that both 16:0 and 18:1n-9 are metabolized in fish for energy (Tocher 2003). In the livers of yellow perch fed alewife 16:0 and 18:1n-9 had a significantly lower concentration than the dietary fish, which suggests the metabolism of these fatty acids. The visceral fat tissue also had specific fatty acids that were reflected in the dietary treatments. Concentrations of 16:0, 18:0, 18:1n-7, linoleic acid, ARA, 22:5n-

6, linolenic acid, 22:5n-3, and DHA were similar in round goby and the yellow perch to which they were fed. Concentrations of 14:0, 16:1n-7, 18:1n-9, 18:1n-7, linoleic acid, ARA, 22:5n-6, linolenic acid, 22:5n-3, and DHA were similar in alewife and the yellow perch to which they were fed.

In most experiments, fatty acid composition of total lipids is investigated, however, in this experiment I wanted to examine specifically which dietary fatty acids were being incorporated in the eggs and liver. Muscle tissue had low lipid content and it is known that fat tissue is mostly comprised of neutral lipids (Tocher 2003). Therefore, total lipids were divided into neutral and phospholipid fractions and then compared to the diet. Since samples of eggs were not collected before the experiment, fatty acid compositions of yellow perch eggs were compared to dietary fatty acid composition. It was apparent that in the phospholipid fraction of eggs in fish fed alewife synthesis of linoleic acid into ARA occurred due to the lower concentration of linoleic acid in the diet and higher concentration of ARA in eggs. However, in neutral lipid fraction, linoleic acid was accumulating in eggs of fish fed round goby. Synthesis of linolenic acid into DHA appeared to occur in the neutral lipid fraction of eggs in fish fed alewife as well as in the phospholipid fraction of eggs regardless of dietary treatment due to the lower linolenic acid concentration and higher DHA concentration compared to their corresponding diet (Table 6).

Incorporation of dietary ARA was observed in the neutral lipid fraction of liver. There was a significant increase in ARA concentration for fish fed both diets, which could have been a reason for the decrease in linoleic acid. These results suggest that there was an incorporation of dietary fatty acids due to the increase of ARA in both fish. As mentioned previously, linoleic acid can transform into longer chain fatty acids such as

ARA by elongase and desaturase enzymes. In the phospholipid fraction of liver there was also an increase in ARA for fish fed both diets.

The lower concentrations of linolenic acid found in neutral lipid and phospholipid fractions of liver in comparison to both diets could be explained by the high DHA concentration initially in yellow perch liver and in both dietary treatments (Table 7). The main product of linolenic acid desaturation and elongation is DHA. Similar results were found in a study conducted by Bell et al. (2001), in which they investigated the fatty acid composition of liver and muscle tissues in Atlantic salmon, *Salmo salar*, fed five diets with different concentrations of fatty acids. Bell et al. (2001) found that the concentration of DHA in liver and muscle of salmon were higher than in their dietary lipid. In the current study, only the phospholipid fraction of liver for both dietary treatments had a higher concentration of DHA than the diet concentrations. A higher concentration of DHA was also found in the total lipid of muscle for both dietary treatments compared to that found in dietary lipid. Although, DHA concentrations were higher in the phospholipid fraction of liver and total lipid of muscle in comparison to dietary lipids, initial concentrations in both tissues were also high. It is well documented that DHA is retained in tissues due to its low rate of oxidation, which follows the peroxisomal pathway and not the mitochondrial  $\beta$ -oxidation pathway (Bell et al. 2001). Menoyo et al. (2007) showed similar results for DHA concentrations in Atlantic salmon. The DHA concentration was retained in neutral lipid fraction of muscle in Atlantic salmon, in which the concentration in muscle was similar to that found in the diet (Menoyo et al. 2007).

Henderson (1996) found that dietary polyunsaturated fatty acids were incorporated and esterified in muscle of freshwater fish. In this study both ARA and

EPA were incorporated into the muscle of both dietary treatments; due to the significant drop in concentration of linoleic acid from muscle of fish at the start of the experiment to muscle of fish at the conclusion, regardless of dietary treatment (Table 8). The result of EPA concentration in muscle of fish fed round goby lower than their diet was similar to what Menoyo et al. (2007) found in the muscle of Atlantic salmon, whereas the muscle in fish fed alewife did not follow this pattern. In the study by Menoyo et al. (2007) salmon were fed eight different diets containing different concentrations of SAFA, n-3 and n-6 fatty acids, such that one diet (0% fish oil) had the highest SAFA, higher total n-3, and the lowest total n-6, whereas another diet (75% linseed oil) had the lowest SAFA, lower total n-3, and the highest n-6. EPA concentration of both neutral and phospholipid fractions in muscle of fish fed 0% fish oil was lower than the diet, whereas the concentration in neutral and phospholipid fractions of fish fed 75% linseed oil remained similar to dietary EPA (Menoyo et al. 2007).

Specific dietary fatty acids were being incorporated in the fatty acid composition of fat in my yellow perch (i.e., linoleic acid). Since the concentrations of linoleic acid in both dietary treatments was lower than the initial concentration, (most likely due to the low concentrations found in round goby and alewife) then one can suggest that dietary linoleic acid was being incorporated into the fatty acids of yellow perch fat. Synthesis of 16:0 into 16:1n-7 and 18:0 into 18:1n-9 was evident in visceral fat samples (Tocher 2003). Incorporation of 16:0 and 18:0 was observed in the higher concentrations found in round goby and alewife fats (Table 3) compared to the fat in dietary treatments (Table 9). When comparing concentrations of 16:1n-7 and 18:1n-9 results showed that the concentration of these fatty acids were higher in fat of dietary treatments compared to

round goby and alewife, indicating synthesis of 16:1n-7 and 18:1n-9 from 16:0 and 18:0, respectively.

#### 4.3. CART analysis

I applied CART analysis, a non-parametric technique because data were not normally distributed and the sample size among groups was not equal. CART analysis revealed that one fatty acid in eggs (18:0 for both neutral and phospholipids), liver (22:5n-6 for neutral lipids and 22:1n-11 for phospholipids), and fat (18:0 for total lipids) of yellow perch could distinguish between dietary treatments of yellow perch. For muscle tissue CART analysis indicated two fatty acids (20:2n-6 and 17:0 for total lipids) could distinguish between dietary treatments of yellow perch. It was apparent that each tissue had different concentrations of fatty acids in order for the analysis to produce such a small number of nodes for each tissue. Since there were only one or two fatty acids that distinguished which diet was fed to yellow perch one can suggest that most of the fatty acids concentrations in alewife and round goby were different. These different fatty acid concentrations in alewife and round goby were then incorporated in the tissues of yellow perch. If these concentrations weren't distinct then CART would reveal more than one or two distinguishing fatty acids.

The high percentage of misclassification was likely due to small sample size, which varied among tissues and dietary treatment with a range of 5 to 16. Budge et al. (2002) used CART analysis to investigate differences within fatty acid composition of 28 marine species. The authors found that if the species sample size was below 20 then it was more likely to misclassify that individual at a rate greater than 20% in comparison to a larger sample size (Budge et al. 2002). Another explanation could be large variance

within fatty acid concentrations of tissues for each dietary treatment resulting in incorrect identification.

#### 4.4. Non-dietary factors that may have influenced the results

My study fish were certified pathogen free prior to the experiment by Coolwater Fish Farm for the following pathogens: viral hemorrhagic septicemia (VHS), furunculosis (*Aeromonas salmonicida*), enteric redmouth (*Yersinia ruckeri*), infectious pancreatic necrosis (IPN), and spring viremia carp (SVC). Within a few months of beginning the experiment fish began to express signs of stress, which include decreased appetite, lordosis, lethargy, hemorrhaging, and fungi on head. These symptoms could be attributed to anything from disease to diet deficiency. Several fish were found moribund or dead throughout the experiment, but it is unlikely that disease was the cause due to environmental conditions (i.e., overcrowding, water flow, and temperature).

While ammonia levels were not at zero ppm the concentrations in the tanks were not considered lethal to the fish. Signs of ammonia toxicity in fish are gulping for air at the surface, erratic swimming, and decreased appetite (Israeli-Weinstein & Kimmel 1998, Suski et al. 2007). The fish in this experiment did not exhibit all of these symptoms; this indicates that high ammonia levels were not the cause for fish showing signs of stress.

Another possible cause for fish to display signs of stress and fungus could be related to a decrease in food uptake or a diet deficiency. According to Ruyter et al. (2000) an increase in the percentage of DHA and a decrease in 18:1n-9 in phospholipid fractions of liver can be signs observed in starved animals; these conditions were both noted in the liver of yellow perch regardless of dietary treatment compared to initial concentrations in yellow perch. Diet deficiency has also been associated with skeletal

deformities (Ahlgren et al. 1999), such as the ones observed in the fish of the present study (e.g., lordosis). Ahlgren et al. (1999) found that a high concentration of DHA associated with a low concentration of ARA can explain deformities in freshwater fish. Skeletal deformities are a result from oxidation of PUFA. In the present study, the ratio of n-3 to n-6 increased in the neutral lipid fraction of liver as well as the total lipid in fat, likely caused by an increase in the concentration of DHA and decrease in ARA, which according to Ahlgren et al. (1999) could explain skeletal deformities found in the yellow perch. However, skeletal deformities can also be induced by a nutrient deficiency, particularly vitamin C and E. Lee and Dabrowski (2004) found that after 20 weeks, reared on a diet with no supplementation of vitamins C and E, juvenile yellow perch exhibited signs of diet deficiency (scoliosis, lordosis, and lens cataract). These vitamins are antioxidants protecting tissue lipids as well as the lipid bilayer in bone cells from free radical attack (Santosh and Lewis-McCrea 2007). Although, vitamins C and E were not considered in this study it would be interesting to measure these two vitamins in alewife and round goby in a future study.

Diet deficiency is also associated with poor reproduction (Furuita et al. 2000); although yellow perch reproduced, the quality of their ribbons and corresponding eggs, regardless of dietary treatment, was poor, as evidenced by low embryo survival rate. A balance of n-3 and n-6 fatty acids is important to support development in fish (Bell et al. 1995, Tocher 2003, Furuita et al. 2000). Furuita et al. (2000) found that a higher level of ARA in the diet was associated with a higher egg quality. However, these studies involve marine fish, which have different EFA requirements than freshwater fish because their desaturase and elongase enzyme activity are limited compared to freshwater fish. Due to the relatively higher concentrations of ARA, EPA, and DHA the n-3 and n-6 fatty



acids in the current study meet the requirements for yellow perch reproduction. As mentioned previously, further research should be conducted to investigate oil droplet fragmentation in ovulating eggs of yellow perch.

#### 4.5. Conclusion

Dietary fatty acids were not completely reflected in yellow perch tissues, but specific fatty acids were influenced by the diet. Biosynthesis of linoleic and linolenic into ARA, EPA, and DHA was evident among all tissues due to the higher levels of the latter three EFAs. Further experiments and analysis need to be conducted to include whole body lipid content of yellow perch at the end of the experiment. Whole body lipid content is important because neutral lipids can be associated with growth in fish due to higher lipid retention (Peng et al. 2003, Tocher et al. 2008). Further examination of whole body lipid content might provide a possible explanation for the lack of growth in yellow perch.

Although, round goby and alewife have high levels of n-3 and n-6 fatty acids (which are general requirements for successful reproduction and recruitment in fish), successful reproduction of all female yellow perch was not observed in the present study. The next step would be to investigate the specific n-3 and n-6 requirements of yellow perch for successful reproduction by incorporating different concentrations of linoleic acid and linolenic acid in their diet. For comparative purposes, one could also examine wild yellow perch reproduction in terms of egg quality, embryo survival at eyed stage, and larval quality.

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Table 1. Water quality tests were conducted for each tank throughout the experiment. Ammonia was not taken after November due to instrument malfunction. N/A = not applicable.

Date	Tank #	Diet	Dissolved				Chlorine (ppm)
			Oxygen (mg/L)	Ammon (ppm)	NH <sub>3</sub> (ppm)	NH <sub>4</sub> <sup>+</sup> (ppm)	
4/23/2008	1	alewife	9.6	0.14	0.17	0.18	0.02
	4	alewife	9.9	0.11	0.13	0.14	0.00
	2	round goby	9.6	0.10	0.12	0.13	0.00
	3	round goby	9.9	0.14	0.17	0.18	0.00
4/29/2008	1	alewife	9.6	0.12	0.14	0.16	0.02
	4	alewife	9.7	0.12	0.14	0.16	0.01
	2	round goby	9.6	0.09	0.11	0.12	0.02
	3	round goby	9.8	0.12	0.14	0.16	0.00
6/6/2008	1	alewife	NA	0.12	0.14	0.16	NA
	4	alewife	NA	0.20	0.24	0.26	NA
	2	round goby	NA	0.17	0.20	0.22	NA
	3	round goby	NA	0.14	0.17	0.18	NA
7/3/2008	1	alewife	5.6	NA	NA	NA	NA
	4	alewife	5.9	NA	NA	NA	NA
	2	round goby	5.0	NA	NA	NA	NA
	3	round goby	5.5	NA	NA	NA	NA
7/8/2008	1	alewife	6.3	0.20	0.24	0.26	0.00
	4	alewife	6.9	0.22	0.26	0.29	0.00
	2	round goby	6.2	0.28	0.34	0.36	0.01
	3	round goby	6.3	0.22	0.26	0.29	0.03
8/5/2008	1	alewife	5.9	0.22	0.26	0.29	0.00
	4	alewife	5.8	0.30	0.36	0.39	0.04
	2	round goby	5.7	0.24	0.29	0.31	0.03
	3	round goby	5.7	0.26	0.31	0.34	0.00
9/9/2008	1	alewife	5.4	0.23	0.28	0.30	0.01
	4	alewife	4.8	0.25	0.30	0.33	0.05
	2	round goby	4.7	0.22	0.26	0.29	0.00
	3	round goby	5.2	0.22	0.26	0.29	0.02
10/8/2008	1	alewife	6.5	0.26	0.31	0.34	0.02
	4	alewife	6.6	0.24	0.29	0.31	0.02
	2	round goby	5.8	0.24	0.29	0.31	0.02
	3	round goby	6.5	0.32	0.38	0.42	0.01
11/4/2008	1	alewife	NA	0.27	0.32	0.35	0.02
	4	alewife	NA	0.24	0.29	0.31	0.02
	2	round goby	NA	0.28	0.34	0.36	0.02
	3	round goby	NA	0.29	0.35	0.38	0.03
12/5/2008	1	alewife	NA	NA	NA	NA	0.02
	4	alewife	NA	NA	NA	NA	0.03
	2	round goby	NA	NA	NA	NA	0.01
	3	round goby	NA	NA	NA	NA	0.01

2/25/2009	1	alewife	8.6	NA	NA	NA	NA
	4	alewife	8.1	NA	NA	NA	NA
	2	round goby	8.3	NA	NA	NA	NA
	3	round goby	8.3	NA	NA	NA	NA

Table 2. Whole body fatty acid composition (% of total fatty acids detected) of the two experimental diets (alewife and round goby). Results are expressed as the average of three pools with three fish for each diet  $\pm$  standard deviation. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters. nd= not detected.

Fatty acids	Dietary Treatments	
	Round goby	Alewife
<i>Saturated</i>		
14:0	$1.6 \pm 0.1^b$	$4.5 \pm 0.1^a$
15:0	$0.5 \pm 0.0$	$0.6 \pm 0.0$
16:0	$12.8 \pm 0.1^b$	$20.0 \pm 0.4^a$
17:0	$0.3 \pm 0.0^b$	$0.5 \pm 0.0^a$
18:0	$2.9 \pm 0.0^b$	$3.3 \pm 0.0^a$
Sum Saturated	$18.0 \pm 0.2^b$	$28.8 \pm 0.3^a$
<i>Monounsaturated</i>		
16:1n-9	$0.4 \pm 0.0^b$	$0.9 \pm 0.0^a$
16:1n-7	$11.9 \pm 0.1^a$	$5.1 \pm 0.1^b$
17:1	$0.5 \pm 0.1^a$	$0.3 \pm 0.0^b$
18:1n-9	$9.6 \pm 0.1^b$	$19.9 \pm 0.4^a$
18:1n-7	$4.7 \pm 0.0$	$4.7 \pm 0.0$
20:1	$1.4 \pm 0.0^b$	$1.8 \pm 0.1^a$
22:1n-11	$0.1 \pm 0.0^b$	$0.3 \pm 0.0^a$
22:1n-9	$0.3 \pm 0.0^a$	$0.1 \pm 0.0^b$
Sum Monounsaturated	$28.9 \pm 0.3^b$	$33.1 \pm 0.2^a$
<i>Polyunsaturated</i>		
18:2n-6	$3.0 \pm 0.1^b$	$5.0 \pm 0.0^a$
20:2n-6	$0.2 \pm 0.0^b$	$1.0 \pm 0.1^a$
20:3n-6	$0.1 \pm 0.0^b$	$0.3 \pm 0.0^a$
20:4n-6	$7.5 \pm 0.1^a$	$3.3 \pm 0.0^b$
22:4n-6	$1.4 \pm 0.0^a$	$0.4 \pm 0.0^b$
22:5n-6	$6.4 \pm 0.1^a$	$1.6 \pm 0.0^b$
Sum n-6	$18.6 \pm 0.2^a$	$11.6 \pm 0.2^b$
18:3n-3	$2.5 \pm 0.1^b$	$4.5 \pm 0.1^a$
18:4n-3	$1.5 \pm 0.1^b$	$2.7 \pm 0.1^a$
20:3n-3	$0.1 \pm 0.0^b$	$1.1 \pm 0.0^a$
20:4n-3	$0.3 \pm 0.0^b$	$1.8 \pm 0.1^a$

20:5n-3	$10.4 \pm 0.1^a$	$7.2 \pm 0.1^b$
22:5n-3	$6.9 \pm 0.1^a$	$1.9 \pm 0.1^b$
22:6n-3	$12.9 \pm 0.3^a$	$7.3 \pm 0.1^b$
Sum n-3	$34.5 \pm 0.2^a$	$26.5 \pm 0.3^b$
Sum Polyunsaturated	$53.2 \pm 0.4^a$	$38.1 \pm 0.4^b$
Sum n-3/Sum n-6	$1.9 \pm 0.0^b$	$2.3 \pm 0.0^a$

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Table 3. Weight (mean  $\pm$  standard deviation) of yellow perch females at the start and the end of the experiment. Weight of females after spawning was used. Means with different superscript letters are significantly different ( $p < 0.05$ ).

	<b>n</b>	<b>Weight (g)</b>
Females		
Initial	152	75.7 $\pm$ 18.4 <sup>b</sup>
Fed alewife	12	81.7 $\pm$ 18.1 <sup>ab</sup>
Fed round goby	19	94.0 $\pm$ 14.9 <sup>a</sup>

Table 4. Description of spawning yellow perch; fecundity, survival of eggs at pigmented-eyed stage, hepatosomatic index and pseudo-gonadosomatic index fed two dietary treatments (alewife and round goby). Fish were fed one of the two diets for approximately 387 days.

	<b>Dietary Treatments</b>	
	<b>Round goby</b>	<b>Alewife</b>
<i>Number of females</i>	19	12
Stripped by hand	13	4
Partially in tank	6	8
<i>Fecundity</i>		
n	15	4
Absolute fecundity (# eggs per gonad)	22809 ± 7169	19767 ± 12235
Relative fecundity (# eggs per g of fish)	176 ± 51	200 ± 107
<i>Embryo survival</i>		
n	3	7
Survival at eyed stage (%)	30.3 ± 23.7	10.4 ± 9.3
<i>Reproductive parameters</i>		
n	15	7
HSI (%)	1.9 ± 0.6	1.8 ± 0.9
n	15	4
pseudo-GSI (%)	29.2 ± 5.6	31.2 ± 13.6

Table 5. Composition of lipids (means  $\pm$  standard deviation) among female yellow perch at the start of the experiment and fed the two dietary treatments (alewife and round goby). Abbreviations are as follows: neutral lipid fraction (NL), phospholipid fraction (PL), and total lipids (TL). NA = not applicable. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters.

			<b>Dietary Treatments</b>	
	<b>Lipids</b>	<b>Initial</b>	<b>Round goby</b>	<b>Alewife</b>
n		4	19	12
Eggs (%)	TL	NA	5.4 $\pm$ 0.7 <sup>b</sup>	6.0 $\pm$ 1.0 <sup>a</sup>
	NL	NA	80.9 $\pm$ 2.1 <sup>b</sup>	83.3 $\pm$ 1.1 <sup>a</sup>
	PL	NA	19.1 $\pm$ 2.1 <sup>a</sup>	16.7 $\pm$ 1.1 <sup>b</sup>
n		4	16	8
Liver (%)	TL	7.6 $\pm$ 2.6	3.1 $\pm$ 0.5	3.8 $\pm$ 0.9
	NL	72.6 $\pm$ 18.4	34.5 $\pm$ 10.1	36.7 $\pm$ 11.0
	PL	27.4 $\pm$ 18.4	65.5 $\pm$ 10.1	63.3 $\pm$ 11.0
n		4	16	8
Muscle (%)	TL	1.4 $\pm$ 0.0 <sup>a</sup>	1.1 $\pm$ 0.2 <sup>b</sup>	1.2 $\pm$ 0.2 <sup>ab</sup>
n		4	9	5
Fat (%)	TL	93.4 $\pm$ 1.3 <sup>a</sup>	68.9 $\pm$ 12.9 <sup>b</sup>	68.6 $\pm$ 10.8 <sup>b</sup>



Table 6. Fatty acid composition of neutral lipid fractions and phospholipid fractions in eggs of yellow perch fed two diets. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters. nd = not detected.

Fatty acids	Neutral lipids		Phospholipids	
	Dietary Treatments			
	Round goby	Alewife	Round goby	Alewife
n	19	12	19	12
<i>Saturated</i>				
14:0	0.8 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	1.1 ± 0.1 <sup>a</sup>
15:0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
16:0	2.8 ± 0.4	2.8 ± 0.4	20.5 ± 1.0	20.5 ± 0.7
17:0	0.2 ± 0.2	0.1 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
18:0	0.3 ± 0.2	0.2 ± 0.0	5.5 ± 0.4 <sup>a</sup>	5.1 ± 0.3 <sup>b</sup>
Sum Saturated	4.1 ± 0.6	4.2 ± 0.6	27.6 ± 1.1	27.3 ± 0.9
<i>Monounsaturated</i>				
16:1n-9	3.3 ± 0.4	3.3 ± 0.3	1.8 ± 0.5	1.9 ± 0.2
16:1n-7	17.4 ± 1.5 <sup>a</sup>	10.5 ± 0.9 <sup>b</sup>	3.0 ± 0.4 <sup>a</sup>	1.7 ± 0.2 <sup>b</sup>
17:1	0.6 ± 0.3	0.5 ± 0.1	0.3 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>b</sup>
18:1n-9	16.5 ± 1.2 <sup>b</sup>	17.3 ± 1.1 <sup>a</sup>	8.5 ± 0.9	8.3 ± 0.8
18:1n-7	3.0 ± 0.4 <sup>a</sup>	2.6 ± 0.3 <sup>b</sup>	3.4 ± 0.4 <sup>a</sup>	3.3 ± 0.2 <sup>b</sup>
20:1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1 <sup>b</sup>	0.5 ± 0.2 <sup>a</sup>
22:1n-11	nd	nd	nd	nd
22:1n-9	nd	nd	nd	nd
Sum Monounsaturated	41.2 ± 2.5 <sup>a</sup>	34.6 ± 2.1 <sup>b</sup>	17.9 ± 1.1 <sup>a</sup>	15.9 ± 1.2 <sup>b</sup>
<i>Polyunsaturated</i>				
18:2n-6	12.8 ± 1.7 <sup>a</sup>	11.7 ± 1.2 <sup>b</sup>	2.1 ± 0.1 <sup>a</sup>	1.6 ± 0.1 <sup>b</sup>
20:2n-6	0.1 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>a</sup>	0.0 ± 0.0	0.1 ± 0.1
20:3n-6	0.3 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>b</sup>
20:4n-6	3.0 ± 0.3 <sup>b</sup>	3.9 ± 0.4 <sup>a</sup>	9.7 ± 0.1 <sup>b</sup>	10.5 ± 0.2 <sup>a</sup>
22:4n-6	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>b</sup>
22:5n-6	1.4 ± 0.2 <sup>b</sup>	2.2 ± 0.3 <sup>a</sup>	1.3 ± 0.1	1.5 ± 0.1
Sum n-6	18.3 ± 1.4	18.9 ± 0.9	13.9 ± 0.2	14.2 ± 0.3
18:3n-3	2.3 ± 0.5 <sup>b</sup>	3.7 ± 0.4 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.5 ± 0.0 <sup>b</sup>
18:4n-3	0.2 ± 0.1 <sup>b</sup>	0.6 ± 0.2 <sup>a</sup>	nd	nd

20:3n-3	$0.1 \pm 0.0^b$	$0.4 \pm 0.1^a$	$0.1 \pm 0.0^b$	$0.5 \pm 0.0^a$
20:4n-3	$0.4 \pm 0.1^b$	$1.8 \pm 0.2^a$	$0.1 \pm 0.0^b$	$0.5 \pm 0.1^a$
20:5n-3	$5.8 \pm 0.6$	$5.9 \pm 0.5$	$8.3 \pm 0.1$	$8.1 \pm 0.2$
22:5n-3	$3.2 \pm 0.3^a$	$2.7 \pm 0.2^b$	$2.7 \pm 0.1^a$	$1.7 \pm 0.1^b$
22:6n-3	$24.3 \pm 2.1^b$	$27.3 \pm 1.4^a$	$29.2 \pm 0.5^b$	$31.3 \pm 0.3^a$
Sum n-3	$36.4 \pm 2.1^b$	$42.2 \pm 2.0^a$	$40.6 \pm 0.5^b$	$42.6 \pm 0.3^a$
Sum Polyunsaturated	$54.7 \pm 2.9^b$	$61.2 \pm 2.1^a$	$54.5 \pm 0.4^b$	$56.8 \pm 0.4^a$
Sum n-3/Sum n-6	$2.0 \pm 0.2^b$	$2.2 \pm 0.1^a$	$2.9 \pm 0.1$	$3.0 \pm 0.1$

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Table 7. Fatty acid composition of neutral lipid fractions and phospholipid fractions in liver of yellow perch at the start of the experiment and fed the two diets. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters. nd= not detected.

Fatty acids	Neutral lipids			Phospholipids		
	Initial	Diets		Initial	Diets	
		Round goby	Alewife		Round goby	Alewife
n	8	16	8	8	9	4
<i>Saturated</i>						
14:0	2.1 ± 0.3 <sup>a</sup>	1.1 ± 0.4 <sup>b</sup>	1.4 ± 0.6 <sup>ab</sup>	0.8 ± 0.2	0.6 ± 0.2	0.7 ± 0.1
15:0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	10.8 ± 1.5	11.0 ± 3.1	14.7 ± 2.1	15.8 ± 1.3	13.7 ± 0.9	14.5 ± 1.1
17:0	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
18:0	0.6 ± 0.2 <sup>b</sup>	1.8 ± 0.6 <sup>a</sup>	1.8 ± 0.5 <sup>a</sup>	2.7 ± 0.9 <sup>b</sup>	5.6 ± 0.5 <sup>a</sup>	5.1 ± 0.8 <sup>ab</sup>
Sum Saturated	13.9 ± 1.6 <sup>b</sup>	14.4 ± 3.5 <sup>ab</sup>	18.4 ± 2.4 <sup>a</sup>	19.7 ± 0.6	20.1 ± 0.5	20.9 ± 0.6
<i>Monounsaturated</i>						
16:1n-9	1.4 ± 0.6	1.2 ± 0.5	1.4 ± 0.4	1.3 ± 0.4	1.0 ± 0.2	0.9 ± 0.1
16:1n-7	15.6 ± 6.4	7.1 ± 3.7	4.8 ± 1.7	2.2 ± 0.5 <sup>a</sup>	1.7 ± 0.5 <sup>ab</sup>	1.0 ± 0.0 <sup>b</sup>
17:1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
18:1n-9	20.4 ± 6.0 <sup>a</sup>	8.3 ± 3.4 <sup>b</sup>	9.8 ± 3.4 <sup>ab</sup>	6.4 ± 1.4	4.7 ± 0.8	4.8 ± 0.4
18:1n-7	2.3 ± 0.7	1.8 ± 0.7	2.1 ± 0.6	2.3 ± 0.5	3.4 ± 0.6	3.2 ± 0.1
20:1	nd	nd	nd	nd	nd	nd
22:1n-11	nd	nd	nd	nd	nd	nd
22:1n-9	nd	nd	nd	nd	nd	nd

Sum Monounsaturated	39.7 ± 13.6	18.7 ± 8.0	18.3 ± 6.0	12.3 ± 0.9	10.9 ± 0.5	10.0 ± 0.2
<i>Polyunsaturated</i>						
18:2n-6	13.2 ± 2.2 <sup>a</sup>	4.4 ± 2.5 <sup>b</sup>	5.2 ± 1.6 <sup>b</sup>	3.0 ± 0.3 <sup>a</sup>	1.4 ± 0.4 <sup>b</sup>	1.4 ± 0.3 <sup>ab</sup>
20:2n-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
20:3n-6	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.8	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
20:4n-6	0.5 ± 0.1 <sup>b</sup>	2.2 ± 0.6 <sup>a</sup>	2.1 ± 0.5 <sup>a</sup>	2.9 ± 0.5 <sup>b</sup>	6.8 ± 1.0 <sup>a</sup>	6.4 ± 0.3 <sup>a</sup>
22:4n-6	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	nd	0.3 ± 0.1	0.2 ± 0.1
22:5n-6	0.2 ± 0.1 <sup>b</sup>	0.6 ± 0.2 <sup>a</sup>	0.9 ± 0.4 <sup>ab</sup>	0.2 ± 0.0 <sup>b</sup>	1.4 ± 0.3 <sup>a</sup>	1.5 ± 0.5 <sup>ab</sup>
Sum n-6	14.4 ± 2.5 <sup>a</sup>	7.5 ± 2.9 <sup>b</sup>	8.6 ± 2.5 <sup>b</sup>	6.4 ± 0.3 <sup>b</sup>	10.3 ± 0.4 <sup>a</sup>	10.1 ± 0.5 <sup>a</sup>
18:3n-3	0.7 ± 0.3	0.6 ± 0.2	1.1 ± 0.8	0.2 ± 0.0	0.1 ± 0.1	0.6 ± 0.1
18:4n-3	0.1 ± 0.2	0.1 ± 0.1	0.5 ± 0.5	nd	nd	0.1 ± 0.1
20:3n-3	nd	nd	0.2 ± 0.1	nd	nd	0.6 ± 0.1 <sup>a</sup>
20:4n-3	0.3 ± 0.2	0.1 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	nd	nd
20:5n-3	2.3 ± 0.8	1.7 ± 0.6	1.8 ± 0.8	4.3 ± 1.1	3.8 ± 0.6	4.2 ± 0.9
22:5n-3	1.2 ± 0.6	0.8 ± 0.4	0.8 ± 0.5	0.8 ± 0.2	1.4 ± 0.3	1.2 ± 0.1
22:6n-3	7.4 ± 2.1	8.7 ± 2.4	9.5 ± 3.8	34.4 ± 2.5	32.7 ± 0.8	32.7 ± 2.8
Sum n-3	12.0 ± 3.4	11.8 ± 3.4	14.5 ± 6.3	39.9 ± 1.0	38.1 ± 0.2	39.4 ± 1.1
Sum Polyunsaturated	26.4 ± 5.3	19.2 ± 5.5	23.1 ± 8.6	46.3 ± 1.3	48.3 ± 0.5	49.5 ± 1.4
Sum n-3/Sum n-6	0.8 ± 0.2 <sup>b</sup>	1.7 ± 0.5 <sup>a</sup>	1.6 ± 0.4 <sup>a</sup>	6.2 ± 0.1 <sup>a</sup>	3.7 ± 0.1 <sup>b</sup>	4.0 ± 0.2 <sup>b</sup>

Table 8. Fatty acid composition of total lipids in muscle of yellow perch at the start of the experiment and fed the two diets. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters. nd= not detected.

Fatty acids	Diets		
	Initial	Round goby	Alewife
n	8	10	5
<i>Saturated</i>			
14:0	1.2 ± 0.3	1.1 ± 0.3	1.0 ± 0.2
15:0	0.2 ± 0.0 <sup>b</sup>	0.3 ± 0.04 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>
16:0	18.7 ± 0.5	17.3 ± 1.4	17.5 ± 0.7
17:0	0.2 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>ab</sup>
18:0	3.9 ± 0.2	3.8 ± 0.6	3.2 ± 0.5
Sum Saturated	24.2 ± 0.2 <sup>a</sup>	22.2 ± 0.4 <sup>ab</sup>	22.7 ± 0.5 <sup>b</sup>
<i>Monounsaturated</i>			
16:1n-9	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.2
16:1n-7	4.6 ± 0.6	4.0 ± 0.9	2.8 ± 1.1
17:1	0.2 ± 0.0 <sup>b</sup>	0.3 ± 0.1 <sup>a</sup>	0.2 ± 0.0 <sup>b</sup>
18:1n-9	12.0 ± 1.2 <sup>a</sup>	9.1 ± 1.4 <sup>b</sup>	8.5 ± 1.1 <sup>b</sup>
18:1n-7	2.3 ± 0.1 <sup>b</sup>	3.3 ± 0.3 <sup>a</sup>	3.0 ± 0.3 <sup>a</sup>
20:1	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
22:1n-11	nd	nd	nd
22:1n-9	nd	nd	nd
Sum Monounsaturated	20.3 ± 0.6 <sup>a</sup>	15.9 ± 0.8 <sup>ab</sup>	18.0 ± 0.5 <sup>b</sup>
<i>Polyunsaturated</i>			
18:2n-6	7.5 ± 0.9 <sup>a</sup>	5.9 ± 1.2 <sup>ab</sup>	4.9 ± 1.1 <sup>b</sup>
20:2n-6	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.1
20:3n-6	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
20:4n-6	2.8 ± 0.2 <sup>b</sup>	6.9 ± 0.8 <sup>a</sup>	7.7 ± 1.0 <sup>a</sup>
22:4n-6	0.3 ± 0.0 <sup>b</sup>	0.4 ± 0.1 <sup>a</sup>	0.4 ± 0.1 <sup>ab</sup>
22:5n-6	0.7 ± 0.1 <sup>b</sup>	2.0 ± 0.4 <sup>a</sup>	2.9 ± 0.6 <sup>a</sup>
Sum n-6	11.8 ± 0.3 <sup>b</sup>	16.6 ± 0.7 <sup>a</sup>	15.9 ± 0.5 <sup>a</sup>
18:3n-3	0.5 ± 0.1	0.7 ± 0.3	0.9 ± 0.3
18:4n-3	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.1
20:3n-3	0.7 ± 0.0	0.1 ± 0.2	0.3 ± 0.1
20:4n-3	0.4 ± 0.0	0.3 ± 0.2	0.7 ± 0.3
20:5n-3	7.4 ± 0.5 <sup>b</sup>	8.7 ± 0.7 <sup>a</sup>	7.8 ± 1.0 <sup>ab</sup>
22:5n-3	2.0 ± 0.2 <sup>b</sup>	3.2 ± 0.4 <sup>a</sup>	2.6 ± 0.4 <sup>ab</sup>
22:6n-3	33.2 ± 2.5	30.4 ± 2.4	32.7 ± 3.1

Sum n-3	$43.7 \pm 0.8$	$45.3 \pm 1.0$	$43.5 \pm 0.5$
Sum Polyunsaturated	$55.4 \pm 0.6^b$	$61.9 \pm 0.5^a$	$59.3 \pm 0.6^a$
Sum n-3/Sum n-6	$3.7 \pm 0.2^a$	$2.8 \pm 0.2^b$	$2.8 \pm 0.1^b$

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Table 9. Fatty acid composition of total lipids in visceral fat of yellow perch at the start of the experiment and fed the two diets. Means with significant differences ( $p < 0.05$ ) are denoted with different superscript letters.

Fatty acids	Diets		
	Initial	Round goby	Alewife
n	8	10	5
<i>Saturated</i>			
14:0	4.3 ± 0.2	4.3 ± 0.6	4.7 ± 0.5
15:0	0.3 ± 0.0 <sup>b</sup>	0.4 ± 0.1 <sup>a</sup>	0.4 ± 0.0
16:0	13.7 ± 1.0	12.6 ± 1.9	12.1 ± 1.2
17:0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
18:0	1.5 ± 0.2	1.6 ± 0.2	1.2 ± 0.2
Sum Saturated	19.9 ± 1.1	19.3 ± 1.6	18.7 ± 0.9
<i>Monounsaturated</i>			
16:1n-9	0.8 ± 0.1 <sup>b</sup>	1.8 ± 0.3 <sup>a</sup>	1.8 ± 0.2 <sup>a</sup>
16:1n-7	12.8 ± 0.9 <sup>a</sup>	15.3 ± 2.5 <sup>a</sup>	8.8 ± 1.0 <sup>b</sup>
17:1	0.3 ± 0.0 <sup>b</sup>	0.5 ± 0.1 <sup>a</sup>	0.4 ± 0.1 <sup>ab</sup>
18:1n-9	29.2 ± 0.6	25.4 ± 3.3	25.2 ± 2.9
18:1n-7	2.7 ± 0.1 <sup>b</sup>	4.5 ± 0.5 <sup>a</sup>	4.0 ± 0.3 <sup>ab</sup>
20:1	1.1 ± 0.2	1.6 ± 0.3	1.8 ± 0.3
22:1n-11	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
22:1n-9	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Sum Monounsaturated	47.1 ± 1.0 <sup>a</sup>	49.3 ± 1.5 <sup>a</sup>	42.1 ± 3.7 <sup>b</sup>
<i>Polyunsaturated</i>			
18:2n-6	16.3 ± 0.9 <sup>a</sup>	11.0 ± 2.2 <sup>b</sup>	10.4 ± 1.7 <sup>b</sup>
20:2n-6	0.2 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>b</sup>	0.6 ± 0.1 <sup>ab</sup>
20:3n-6	0.3 ± 0.0	0.4 ± 0.2	0.4 ± 0.1
20:4n-6	0.7 ± 0.0 <sup>b</sup>	2.3 ± 0.4 <sup>a</sup>	2.8 ± 0.6 <sup>ab</sup>
22:4n-6	0.2 ± 0.0 <sup>b</sup>	0.6 ± 0.1 <sup>a</sup>	0.5 ± 0.0 <sup>a</sup>
22:5n-6	0.3 ± 0.0 <sup>b</sup>	1.4 ± 0.4 <sup>a</sup>	2.5 ± 0.4 <sup>a</sup>
Sum n-6	18.0 ± 0.9	15.9 ± 2.3	17.2 ± 1.1
18:3n-3	1.4 ± 0.1	1.6 ± 0.5	3.1 ± 1.3
18:4n-3	0.7 ± 0.1	0.4 ± 0.2	1.4 ± 0.7
20:3n-3	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.2
20:4n-3	0.6 ± 0.0 <sup>a</sup>	0.4 ± 0.1 <sup>b</sup>	1.4 ± 0.5 <sup>ab</sup>
20:5n-3	3.5 ± 0.5	3.4 ± 1.0	3.5 ± 1.1

22:5n-3	$1.3 \pm 0.1^b$	$2.4 \pm 0.4^a$	$2.1 \pm 0.2^a$
22:6n-3	$7.4 \pm 0.8^b$	$7.2 \pm 0.6^b$	$10.1 \pm 0.7^a$
Sum n-3	$14.9 \pm 0.8$	$15.5 \pm 1.9$	$22.0 \pm 4.1$
Sum Polyunsaturated	$32.9 \pm 1.1$	$31.4 \pm 1.6$	$39.2 \pm 3.5$
Sum n-3/Sum n-6	$0.8 \pm 0.1$	$1.0 \pm 0.3$	$1.3 \pm 0.3$

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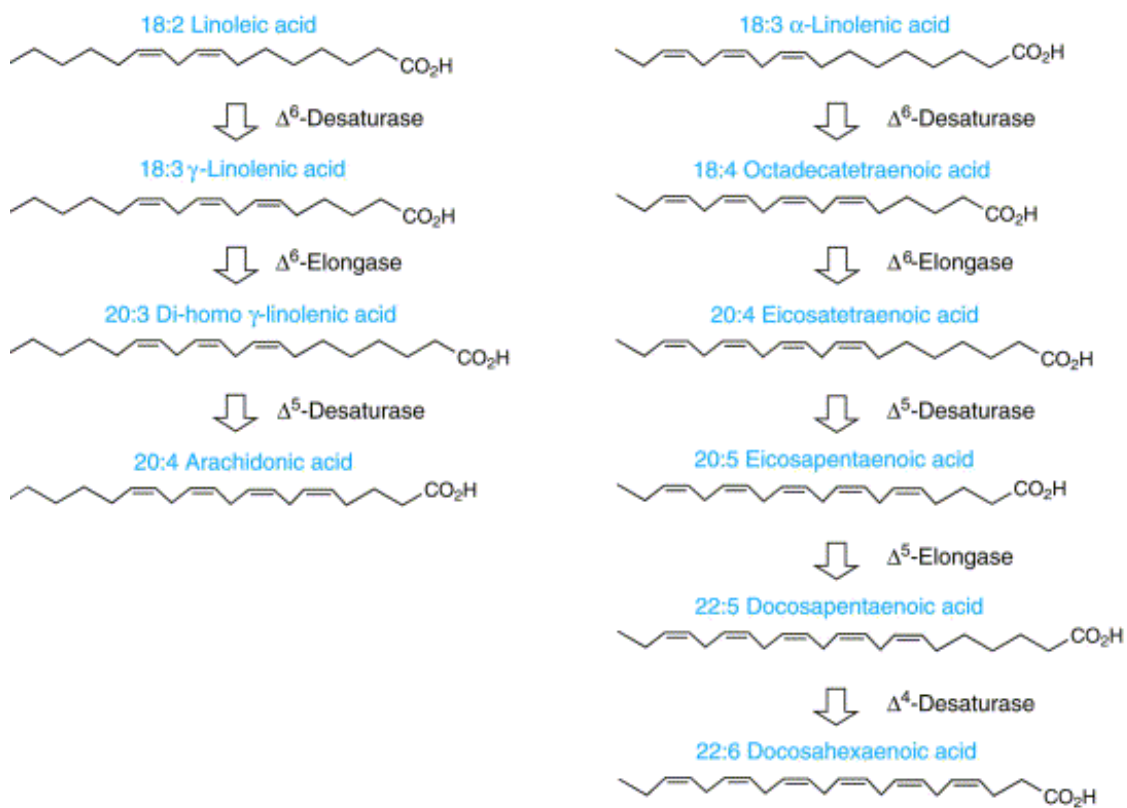


Figure 1. Biosynthesis pathways of linoleic acid (18:2n-6) and linolenic acid (18:3n-3) to arachidonic acid (ARA; 20:4n-6) and docosahexaenoic acid (DHA; 20:5n-3), respectively (Napier 2002).

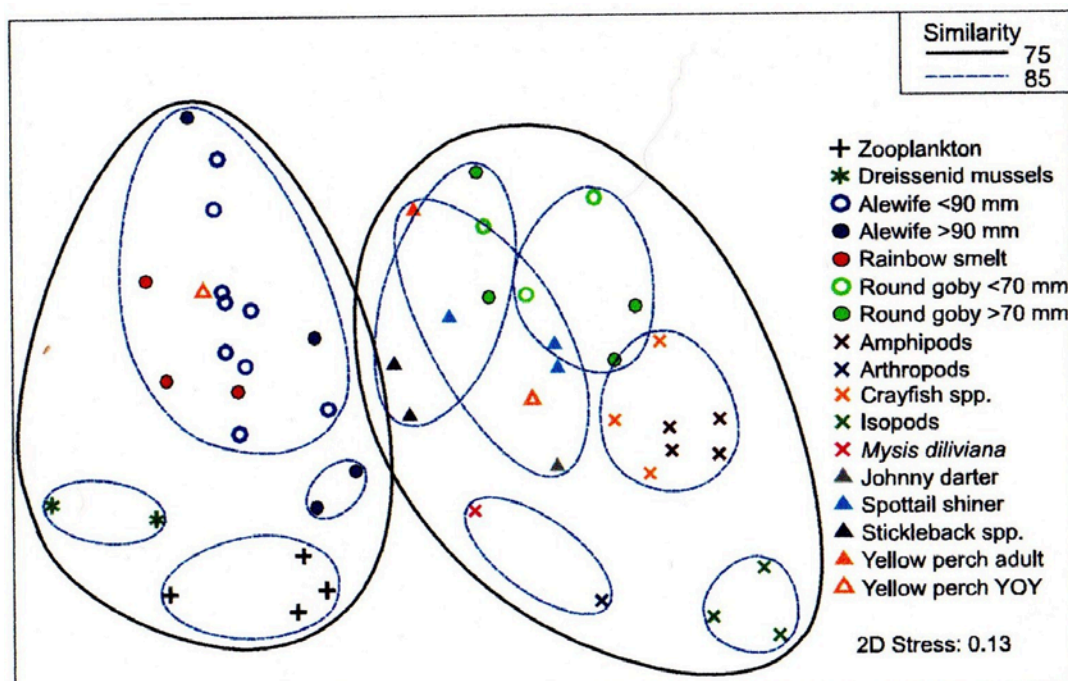


Figure 2. nMDS plot of group-averaged fatty acid signature data for samples from the Illinois management district. Alewife and round goby data were averaged by season–year and size class; all other data were averaged by species or taxa group and year. Solid and dashed ovals enclose clusters of group means with 75% and 85% similarities, respectively (Czesny et al. 2011).

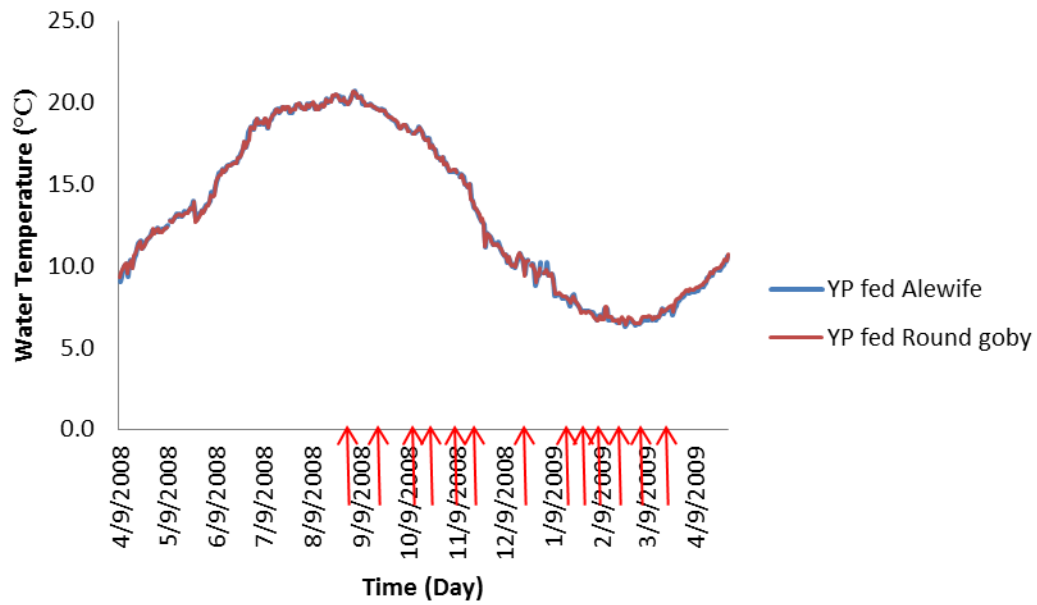


Figure 3. Change in average daily water temperature for each dietary treatment (n = 2). The arrows indicate when a salt treatment was administered to each tank.

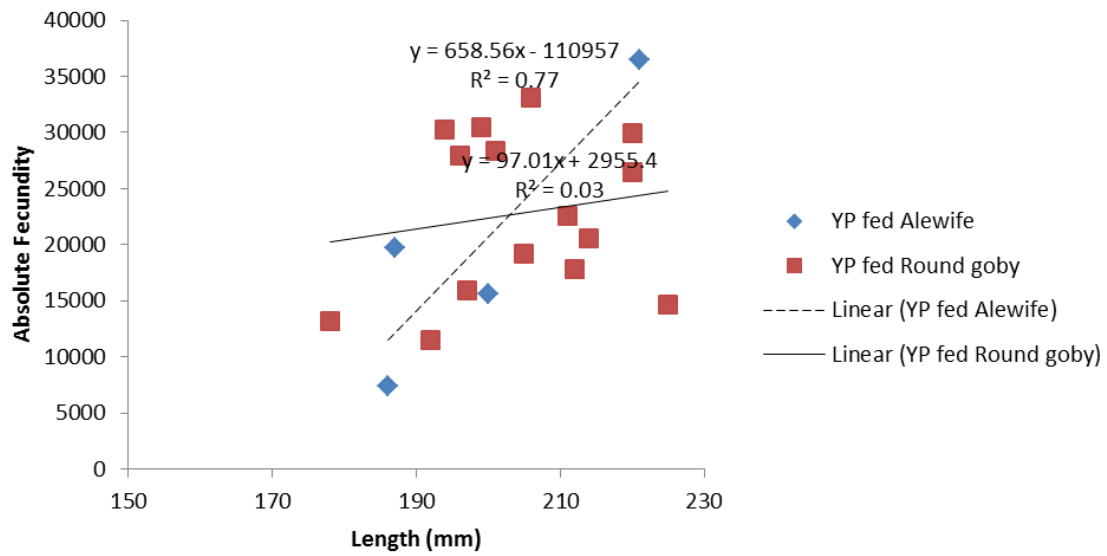


Figure 4. Relationship between female yellow perch length fed two dietary treatments and their absolute fecundity (number of eggs).

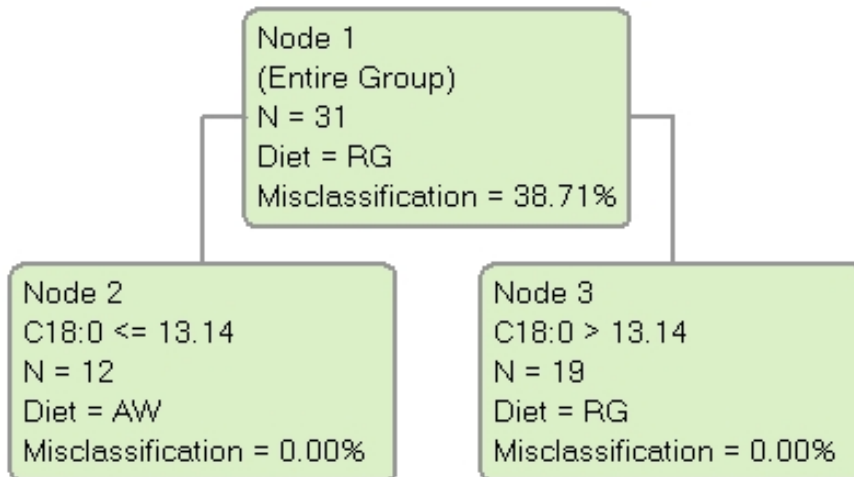


Figure 5. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART analysis revealed three nodes for neutral lipid fractions in eggs, where one fatty acid (18:0) distinguished between the two diets. Abbreviations are as follows: RG is round goby and AW is alewife.

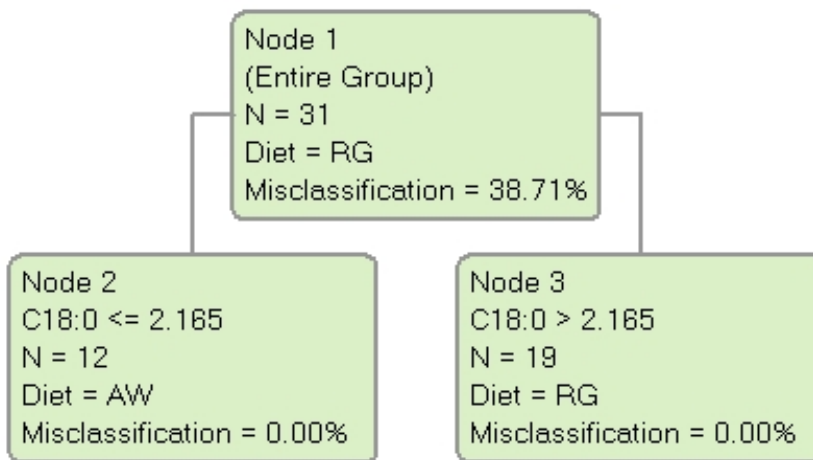


Figure 6. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART analysis revealed three nodes for phospholipid fractions in eggs, where one fatty acid (18:0) distinguished between the two diets. RG is round goby and AW is alewife.

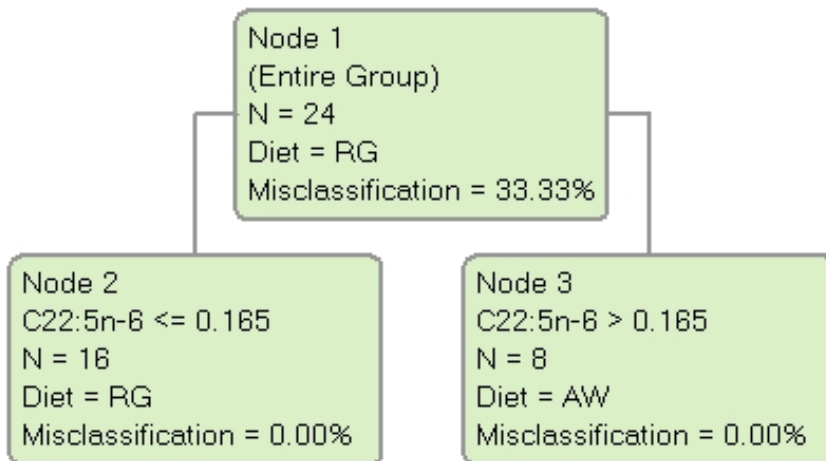


Figure 7. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART analysis revealed three nodes for neutral lipid fractions in liver, where one fatty acid (22:5n-6) distinguished between the two diets. RG is round goby and AW is alewife.

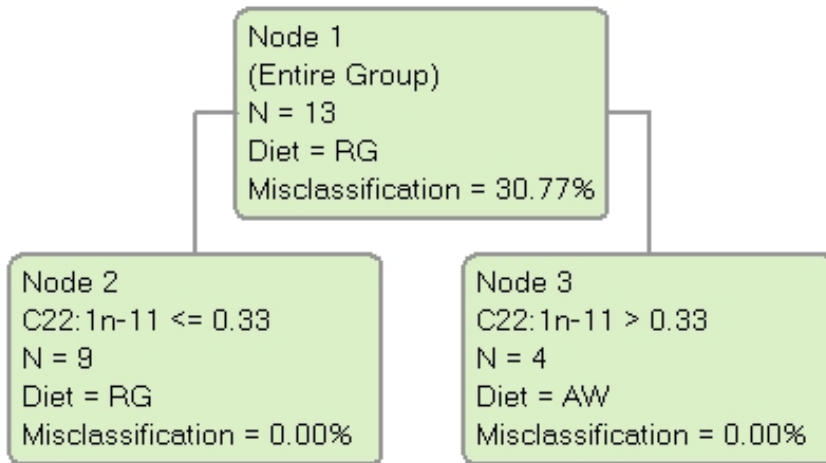


Figure 8. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART analysis revealed three nodes for phospholipid fractions in liver, where one fatty acid (22:1n-11) distinguished between the two diets. RG is round goby and AW is alewife.



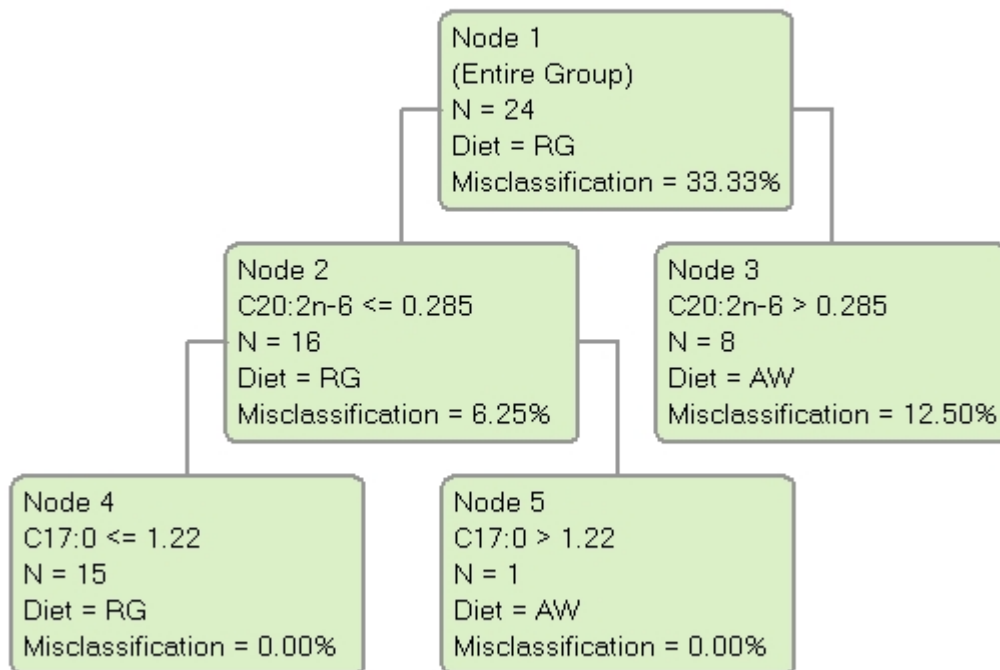


Figure 9. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART analysis revealed five nodes for total lipids in muscle, where two fatty acids (20:2n-6 and 17:0) distinguished between the two diets. RG is round goby and AW is alewife.

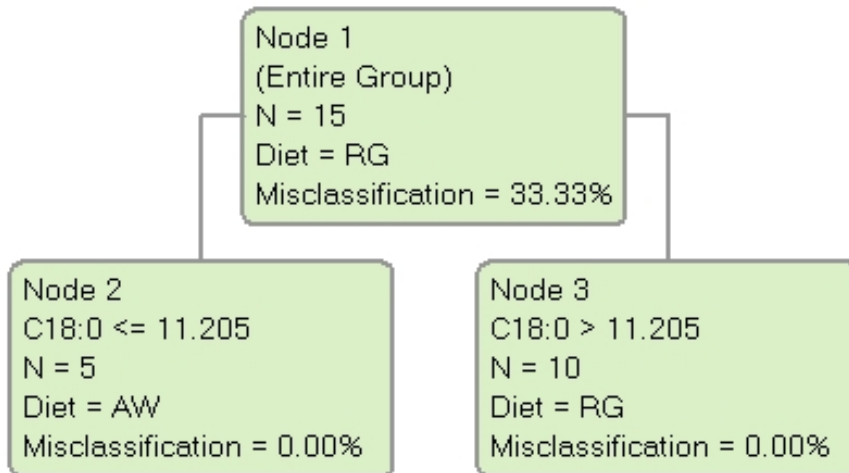


Figure 10. Yellow perch were fed two different diets, alewife and round goby for 387 days and CART revealed three nodes for total lipids in visceral fat, where one fatty acid (18:0) distinguished between the two diets. RG is round goby and AW is alewife.