# Thiamine and Fatty Acid Content of Lake Michigan Chinook Salmon

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ABSTRACT. Nutritional status of Lake Michigan Chinook salmon (Oncorhynchus tshawytscha) is inadequately documented. An investigation was conducted to determine muscle and liver thiamine content and whole body fatty acid composition in small, medium and large Chinook salmon. Muscle and liver thiamine concentrations were highest in small salmon, and tended to decrease with increasing fish size. Muscle thiamine was higher in fall than spring in large salmon. The high percentage of Chinook salmon (24–32% in fall and 58–71% in spring) with muscle thiamine concentration below 500 pmol/g, which has been associated with loss of equilibrium and death in other Great Lake salmonines, suggest that Chinook appear to rely less on thiamine than other Great Lakes species for which such low concentrations would be associated with thiamine deficiency (Brown et al. 2005b). A positive correlation was observed between liver total thiamine and percent liver lipids (r = 0.53, P < 0.0001, n = 119). In medium and large salmon, liver lipids were observed to be low in fish with less than 4,000 pmol/g liver total thiamine. In individuals with greater than 4,000 pmol/g liver thiamine, liver lipid increased with thiamine concentration. Individual fatty acids declined between fall and spring. Essential omega-3 fatty acids appear to be conserved as lipid content declined. Arachidonic acid (C20:4n6), an essential omega-6 fatty acid was not different between fall and spring, although the sum of omega-6 (Sw6) fatty acids declined over winter. Elevated concentrations of saturated fatty acids (sum) were observed in whole body tissue lipid. In summary, thiamine, a dietary essential vitamin, and individual fatty acids were found to vary in Lake Michigan Chinook salmon by fish size and season of the year.

**INDEX WORDS:** Early mortality syndrome, fatty acids, energy metabolism, nutrient deficiency, season.

## INTRODUCTION

Since their introduction into the Great Lakes in large numbers in the 1960s, Chinook salmon (*Oncorhynchus tshawytscha*) have become an important part the fishery (Tanner and Tody 2002). In the late 1980s a significant population decline was observed in association with an epizootic disease outbreak (Benjamin and Bence 2003, Holey *et al.* 1998). Although the underlying cause for the disease incident was thought to be nutritionally related, this was not confirmed. Beginning in the early 1990s, reproduction of several Great Lakes salmonids, including

Chinook salmon, was adversely affected by early mortality syndrome, a malady linked to a deficiency of thiamine, an essential dietary vitamin (Marcquenski and Brown 1997, McDonald *et al.* 1998, Wolgamood *et al.* 2005). A strong argument for linking alewife consumption and reproductive failure of salmonids in the Great Lakes (Brown *et al.* 2005a) was made based on the available evidence (Blazer and Brown 2005, McDonald *et al.* 1998). Thiaminase-rich alewife consumption has been shown to cause thiamine deficiency in Great Lakes salmonid fry (Honeyfield *et al.* 2005). Thiamine deficiency has been documented in adults of several species (Brown *et al.* 2005b) but there are no pub-

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TABLE 1. The number of Chinook salmon collected within respective season and size that were male, sexually mature from western or southern Lake Michigan and evaluated for thiamine and fatty acid content.

| Assay (n)        | Season | (n)   | Size a          | (n)        | Male <sup>b</sup> | Life stage<br>Mature <sup>c</sup> | Location<br>Western d | Southern e |
|------------------|--------|-------|-----------------|------------|-------------------|-----------------------------------|-----------------------|------------|
| Thiamine (196)   | Fall   | (124) | Small           | (37)       | 27                | 5                                 | 9                     | 0          |
| , ,              |        | ` ,   | Medium          | (49)       | 31                | 27                                | 15                    | 10         |
|                  |        |       | Large           | (38)       | 19                | 36                                | 13                    | 10         |
|                  | Spring | (72)  | Small           | (10)       | 6                 | 0                                 | 2                     | 4          |
|                  | 1 0    | . ,   | Medium          | (38)       | 16                | 8                                 | 4                     | 24         |
|                  |        |       | Large           | (24)       | 15                | 18                                | 2                     | 15         |
| E-44 A -11- (20) | E-11   | (12)  | C 11            | (4)        | 2                 | 2                                 | 0                     | 0          |
| Fatty Acids (29) | Fall   | (12)  | Small<br>Medium | (4)        | 2<br>4            | 2<br>3                            | 0                     | 0          |
|                  |        |       | Large           | (6)<br>(2) | 1                 | 1                                 | 0                     | 0          |
|                  | Spring | (17)  | Small           | (4)        | 3                 | 2                                 | 1                     | 4          |
|                  |        |       | medium          | (10)       | 4                 | 2                                 | 2                     | 10         |
|                  |        |       | Large           | (3)        | 1                 | 0                                 | 0                     | 3          |

<sup>&</sup>lt;sup>a</sup> Fish were categorized as small if they measured < 495 mm in the spring or < 550 mm in fall. Fish were categorized as large if they measured > 700 mm in spring or >775 mm in the fall.

lished data on the thiamine status of juvenile Chinook salmon.

Thiamine plays an important role as a co-factor in the enzyme reactions of intermediate metabolism. In muscle and liver free or unphosphorylated thiamine (T) is converted to thiamine pyrophosphate (TPP) which is the active form of thiamine used by enzymes requiring thiamine (Gubler 1991). Thiamine deficiency adversely affects activity of thiamine-requiring enzymes including those involved in energy metabolism. Because liver is a key metabolic tissue for many metabolic processes, liver thiamine tends to be conserved more so than muscle tissue. Thus relative concentrations in muscle and liver can be used to assess the severity of thiamine deficiency. Brown et al. (2005c) reported differences in Chinook salmon egg fatty acid content between fish with adequate and deficient thiamine. These differences in fatty acid content may be due to diet or to the effect of thiamine deficiency on metabolic pathways. Fatty acid data for Lake Michigan Chinook salmon are limited, with the most current data provided by Iverson (1972). The

objectives of this work were to investigate the thiamine status of lake-dwelling juvenile and adult Chinook salmon and to report preliminary findings on the fatty acid composition of fall- and springcaptured Chinook salmon from Lake Michigan.

# **METHODS**

Lake Michigan Chinook salmon used in these studies are a subset of a larger study reported by Peters *et al.* (2007). All fish were caught by angling or by suspended, short-set gillnets in the open waters of Lake Michigan between 2000 and 2002. All captured fish were assigned to one of three size categories to correspond approximately to ages 1, 2, or 3. Three size categories were based on length using the following criteria: small (< 490 mm spring, < 550 mm fall), intermediate (490–700 mm spring, 550–775 mm fall) and large (> 700 mm spring, > 775 mm fall) based on prior observations of length-age relationships in Lake Michigan Chinook salmon (R. Elliott, USFWS, Green Bay, WI, unpublished data). Spring samples were collected from 15

<sup>&</sup>lt;sup>b</sup> For the number of females, subtract number of males from (n) within a row.

<sup>&</sup>lt;sup>c</sup> For the number of immature fish, subtract number of mature fish from (n) within a row.

<sup>&</sup>lt;sup>d</sup> For the number of fish from eastern Lake Michigan, subtract number of western Lake Michigan fish from (n) within a row.

<sup>&</sup>lt;sup>e</sup> For the number of northern fish, subtract number of fish for southern Lake Michigan from (n) within a row.

|                 |       |     |       |    |         | • •   |       |           |      |       |           | -           |           |
|-----------------|-------|-----|-------|----|---------|-------|-------|-----------|------|-------|-----------|-------------|-----------|
| Muscle (pmol/g) |       |     |       |    |         |       |       |           |      |       |           |             |           |
| Size            | N     | TPP | (±SE) |    | P value | TP    | (±SE) | Pvalue    | T    | (±SE) | P value   | Total (±SE) | P value   |
| Small           | 47    | 377 | (32)  | a  | < .0073 | 691   | (53)  | a < .0001 | 201  | (17)  | a < .0001 | 1,268 (85)  | a < .0001 |
| Medium          | 87    | 258 | (24)  | b  |         | 235   | (39)  | b         | 89   | (13)  | b         | 582 (62)    | b         |
| Large           | 62    | 258 | (28)  | b  |         | 220   | (46)  | b         | 103  | (15)  | b         | 582 (74)    | b         |
| Liver (pm       | ol/g) |     |       |    |         |       |       |           |      |       |           |             |           |
| Small           | 35    | 748 | (51)  | a  | < .0044 | 1,712 | (197) | a < .0083 | 6950 | (411) | a < .0001 | 9,416 (508) | a < .0001 |
| Medium          | 59    | 645 | (39)  | ab |         | 1,570 | (152) | a         | 4797 | (317) | b         | 7,012 (391) | b         |
| Large           | 53    | 531 | (41)  | b  |         | 1,004 | (160) | b         | 3408 | (334) | c         | 4,943 (413) | c         |

TABLE 2. Effect of Chinook size category on muscle and liver thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free thiamine (T), and sum of the three forms of thiamine (Total).

abc Means in a column with different superscript letters are significantly different (P < 0.05); Duncan multiple range test. Fish were categorized as small if they measured < 495 mm in the spring or < 550 mm in fall. Fish were categorized as large if they measured > 700 mm in spring or > 775 mm in the fall.

April to 15 June and fall samples were collected from 15 August to 15 September. Methods used for whole body lipid, muscle lipid, and liver lipid have been reported (Peters *et al.* 2007). For thiamine and fatty acid analysis, a small muscle sample (10 g) and the entire liver were removed from each fish before the whole fish was ground. Muscle and liver samples were immediately frozen and shipped to Wellsboro PA on dry ice. Tissue samples that arrived unfrozen were discarded. All samples remained at –80°C prior to analysis.

Muscle and liver thiamine were determined using the method of Brown *et al.* (1998). The chromatography (HPLC) method separates the three forms of thiamine which were quantified using commercially available authentic standards. Samples were analyzed in duplicate. Sample analysis was repeated if the mean value of duplicates was greater than 10 percent.

Fatty acid analysis was conducted on 29 ground, whole body tissue samples collected in the fall of 2000 and spring of 2001. Whole fish were transferred to the lab on ice and immediately processed. Sub-samples of the ground fish were collected and frozen for shipment to Wellsboro on dry ice. Samples were stored at -80°C. Although three fish sizes were present (Table 1), the number of whole fish within a size was limited and therefore only the effect of season was evaluated. Samples for fatty acid analysis were lyophilized prior to lipid extraction with chloroform and methanol (Bligh and Dyer 1959). Extracted lipid was treated with boron trifluoride in methanol to produce fatty acyl methyl esters (FAME) of the fatty acids present. Individual fatty acids were separated using gas chromatography and peaks calibrated with known standards as described in Brown et al. (2005c).

## **DATA ANALYSIS**

Thiamine and fatty acid data were analyzed with a main effects ANOVA, using season and size (age) as the fixed effects. Higher-order effects were either not estimable due to small sample sizes or were not significant. Differences among means were determined using Duncan multiple range test. Statistical analyses were conducted using Statistical Analysis System software (SAS version 9.1, 2003), using a level of significance of  $\alpha = 0.05$ .

# **RESULTS**

All forms of muscle thiamine were higher in the small Chinook than in medium or large fish (Table 2). There were no differences in thiamine in medium and large fish for any form of muscle thiamine. Thiamine pyrophosphate (TPP; P < 0.0044) and thiamine monophosphate (TP; P < 0.0083) were higher in the liver of small salmon compared to large salmon. Liver free and total thiamine were significantly different among the three fish sizes (P < 0.0001). All forms of liver thiamine decreased with increase in fish size.

Spring collected medium and large salmon had lower muscle TPP, TP, and total thiamine than fish caught in the fall. Muscle TPP, TP, and total thiamine did not change in small salmon between fall and spring (Table 3). In contrast, muscle free thiamine was higher in spring caught fish in small and medium but not in large fish. Liver free and total thiamine were lower in spring for medium fish

TABLE 3. Effect of season on muscle and liver thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free thiamine (T), and sum of the three forms of thiamine (Total) for three fish sizes of Lake Michigan Chinook salmon.

|             |          |    | Season |       |    |        |            |          |
|-------------|----------|----|--------|-------|----|--------|------------|----------|
|             |          |    | Fall   |       |    | Spring |            | ANOVA    |
| Small Fish  | Thiamine | N  | pmol/g | (±SE) | N  | pmol/g | $(\pm SE)$ | P Value  |
| Muscle      | TPP      | 37 | 368    | (53)  | 10 | 407    | (103)      | < 0.7416 |
|             | TP       |    | 726    | (116) |    | 564    | (224)      | < 0.5237 |
|             | T        |    | 158    | (34)  |    | 358    | (66)       | < 0.0097 |
|             | Total    |    | 1,252  | (178) |    | 1,329  | (342)      | < 0.8432 |
| Liver       | TPP      | 35 | 748    | (57)  | 0  |        |            |          |
|             | TP       |    | 1,712  | (270) |    | _      | _          | _        |
|             | T        |    | 6,950  | (582) |    | _      | _          | _        |
|             | Total    |    | 9,416  | (704) |    |        |            |          |
| Medium Fish |          |    |        |       |    |        |            |          |
| Muscle      | TPP      | 49 | 320    | (25)  | 38 | 178    | (28)       | < 0.0003 |
|             | TP       |    | 268    | (17)  |    | 191    | (20)       | < 0.0039 |
|             | T        |    | 79     | (7)   |    | 101    | (8)        | < 0.0351 |
|             | Total    |    | 668    | (39)  |    | 471    | (44)       | < 0.0013 |
| Liver       | TPP      | 47 | 664    | (45)  | 12 | 568    | (89)       | < 0.3422 |
|             | TP       |    | 1,673  | (174) |    | 1,170  | (343)      | < 0.1963 |
|             | T        |    | 5,225  | (324) |    | 3,123  | (641)      | < 0.0049 |
|             | Total    |    | 7,561  | (381) |    | 4,861  | (754)      | < 0.0023 |
| Large Fish  |          |    |        |       |    |        |            |          |
| Muscle      | TPP      | 38 | 293    | (27)  | 24 | 205    | (34)       | < 0.0482 |
|             | TP       |    | 265    | (20)  |    | 150    | (25)       | < 0.0007 |
|             | T        |    | 105    | (9)   |    | 99     | (12)       | < 0.6998 |
|             | Total    |    | 662    | (43)  |    | 454    | (54)       | < 0.0037 |
| Liver       | TPP      | 37 | 519    | (43)  | 16 | 560    | (66)       | < 0.6087 |
|             | TP       |    | 970    | (116) |    | 1,081  | (177)      | < 0.6039 |
|             | T        |    | 3,516  | (257) |    | 3,157  | (3,910)    | < 0.4455 |
|             | Total    |    | 5,006  | (365) |    | 4,797  | (556)      | < 0.7550 |

Fish were categorized as small if they measured < 495 mm in the spring or < 550 mm in fall. Fish were categorized as large if they measured > 700 mm in spring or > 775 mm in the fall.

while no seasonal differences in thiamine levels were observed for large fish. No liver samples were available for small spring caught salmon.

Median muscle total thiamine values were 864 (fall, small fish), 878 (spring, small fish), 582 (fall, medium fish), 464 (spring medium fish), 619 (fall, large fish), and 360 pmol/g (spring, large fish). Mean values for muscle total thiamine (Table 3) were numerically higher than the median values. None of the spring and only 5% of the small fall caught fish had muscle concentrations of total thiamine below 500 pmol/g. Within the medium size

Chinook salmon, the percentage of fish with less than 500 pmol/g was 35% in the fall and 61% in the spring. In the largest fish, the percentages were 26% and 71% in fall and spring fish, respectively.

On dry matter basis, combined average % lipid across for all fish was 6.9% in whole fish, 4.4% in muscle, and 3.1% in liver. Whole fish % lipid was higher in medium fish than in small or large fish whereas liver lipid decreased with increasing fish size (Table 4). Pearson correlations between tissue thiamine and tissue lipid were weak and generally not significant for muscle tissue. Stronger correla-

TABLE 4. Descriptive summary of the percent lipid (% dry weight) in the three fish sizes of Chinook salmon used in this publication<sup>1</sup>.

| Variable            | N  | Mean | Std Dev | Minimum | Maximum |
|---------------------|----|------|---------|---------|---------|
| Small Fish          |    |      |         |         |         |
| Whole Fish Lipid %  | 34 | 6.49 | 2.21    | 1.73    | 11.08   |
| Muscle Lipid, %     | 30 | 4.49 | 2.49    | 1.21    | 11.07   |
| Liver Lipid, %      | 26 | 5.17 | 2.73    | 1.13    | 11.44   |
| Medium Fish         |    |      |         |         |         |
| Whole Fish Lipid, % | 56 | 8.28 | 2.52    | 1.21    | 13.78   |
| Muscle Lipid, %     | 58 | 5.52 | 4.40    | 0.08    | 18.88   |
| Liver Lipid, %      | 50 | 3.16 | 2.34    | 0.59    | 9.99    |
| Large Fish          |    |      |         |         |         |
| Whole Fish Lipid, % | 53 | 6.55 | 2.49    | 2.44    | 14.31   |
| Muscle Lipid, %     | 52 | 3.98 | 2.96    | 0.52    | 13.37   |
| Liver Lipid, %      | 51 | 1.92 | 1.35    | 0.61    | 6.55    |

<sup>1</sup>Complete data set found in Peters *et al.* (2007). Fish were categorized as small if they measured < 495 mm in the spring or < 550 mm in fall. Fish were categorized as large if they measured > 700 mm in spring or > 775 mm in the fall.

tions were observed for the liver (Table 5). The highest correlations were found between percent liver lipid and total thiamine (Fig. 1) or liver free thiamine. A similar pattern was found when all sizes of Chinook were pooled together or when medium and large fish were considered separately. For small fish the data appeared more random (Fig. 1). In addition, liver total thiamine increased as whole fish lipid increased (r = 0.23, P < 0.006) and percent muscle lipid increased as liver free (r = 0.24, P < 0.0045) and total thiamine (r = 0.23, P < 0.006) increased.

Whole fish fatty acid composition varied between

fall and spring (Table 6), but not among size categories (fall and spring combined). The majority of the fatty acids ( $\mu$ g/mg) were higher in the fall than in the spring. Within the omega-3 fatty acids, total omega-3 fatty acids (Sw3; P < 0.0218), C20:3n3 (P < 0.0001), C20:5n3 (P < 0.0565), C22:5n3c (P < 0.0955) and C22:6n3 (P < 0.0014) were higher in the spring than in the fall. In contrast total omega-6 fatty acids (Sw6; P < 0.0302) and C18:2n6c (P < 0.0304) were lower in the spring than in the fall caught Chinook salmon. Total mono-unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) had similar concentrations in fall and

TABLE 5. Pearson correlation (r) and probability value (P<) between liver thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free thiamine (T), sum (Total) of the three forms of tissue thiamine and percent lipid content.

| Tissue             | N   |         | TPP            | Liver<br>TP    | Т               | Total           |
|--------------------|-----|---------|----------------|----------------|-----------------|-----------------|
| % Whole Fish Lipid | 143 | r<br>P< | 0.24<br>0.0035 | 0.30<br>0.0003 | 0.13<br>0.1188  | 0.23<br>0.0058  |
| % Muscle Lipid     | 140 | r<br>P< | 0.05<br>0.5671 | 0.09<br>0.3002 | 0.24<br>0.0045  | 0.23<br>0.0059  |
| % Liver Lipid      | 127 | r<br>P< | 0.23<br>0.0103 | 0.26<br>0.0035 | 0.50<br>< .0001 | 0.53<br>< .0001 |

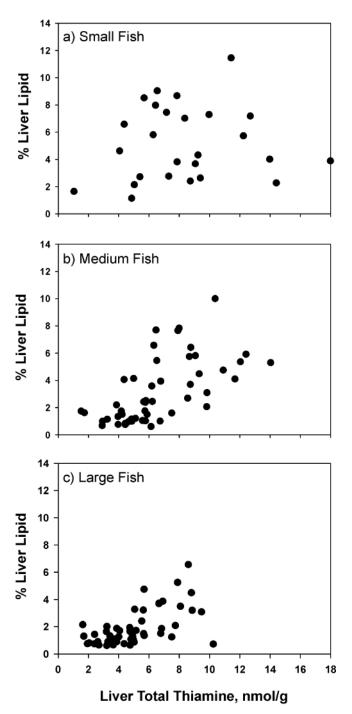


FIG. 1. Relationship in Chinook salmon between liver total thiamine and percent liver lipid within (a) small fish (r = 0.07, P < 0.71), (b) medium fish (r = 0.61, P < 0.0001) and (c) large fish (r = 0.60, P < 0.0001). Fish were categorized as small if they measured <495mm in the spring or < 550mm in fall. Fish were categorized as large if they measured > 700 mm in spring or > 775 mm in the fall.

spring. Total saturated fatty acids (SAFA; P < 0.0050) were lower in the spring than in the fall.

### **DISCUSSION**

A loss of equilibrium and mortality has been observed in adult lake trout, coho and steelhead when muscle thiamine drops below 500 pmol/g (Brown et al. 2005b). Large size Lake Michigan Chinook salmon showed an overall loss of thiamine over winter whereas the small category fish did not. Overall there were a limited number (< 5%) of small Chinook salmon had muscle thiamine below 500 pmol/g. In contrast, 60-70% of the large fish and 26-35% of medium sized fish were below 500 pmol thiamine/g muscle. There were no field reports that the fish in the present study had lethargy or loss of equilibrium at capture suggesting they were not suffering from thiamine deficiency. Fitzsimons et al. (2007) reported that the EC20 (effective concentration for greater than 20% fry mortality) for egg thiamine concentration in Chinook salmon was lower (1.52 nmol/g) than that of lake trout (2.63 nmol/g) or coho salmon (2.99 nmol/g). Together these findings suggest that Chinook have a lower metabolic requirement for thiamine compared with other salmonid species. Additional work with Chinook and other Great Lake salmonids may provide insight as to why Chinook appear to rely less on thiamine than other species.

The importance of thiamine in energy metabolism is suggested in the relationship observed between liver free thiamine and liver lipid content (Fig. 1). A negative relation between thiamine and body size (weight or length) has been noted previously in adult Chinook salmon captured at spawning weirs (Wolgamood et al. 2005). Fatty acid content of eggs from Chinook and coho salmon fry that developed EMS differed from eggs that produced normal fry without EMS (Brown et al. 2005c). Although we can identify differences in fatty acids and thiamine concentrations in the present and in other studies, the metabolic interrelationship between thiamine and tissue fatty acid (or lipid) content remains to be determined. Results from laboratory studies focusing on lipid synthesis and catabolism at varying thiamine concentrations would be important information for explaining the results shown in Figure 1.

The higher muscle and liver thiamine content found in the small Chinook salmon compared to the medium and large salmon is likely the result of the smaller fish eating a more diverse diet with less thi-

TABLE 6. Fatty acid content of whole Lake Michigan Chinook salmon from fall 2000 and spring 2001. (µg/mg freeze dried whole body tissue).

|          |                                  | Fal    | 1 2000  | Sprir  | ng 2001 |
|----------|----------------------------------|--------|---------|--------|---------|
|          |                                  | Mean   |         | Mean   |         |
| Variable | N                                | 12     | (±SE)   | 17     | (±SE)   |
| C8:0     | Caprylic acid                    | 0.011  | (0.008) | 0.007  | (0.007) |
| C10:0    | Capric acid                      | 0.028  | (0.007) | 0.006  | (0.006) |
| C11:0    | Undecanoic acid                  | 0.054  | (0.006) | 0.006  | (0.005) |
| C12:0    | Lauric acid                      | 0.207  | (0.021) | 0.089  | (0.018) |
| C13:0    | Tridecanoic acid                 | 0.065  | (0.009) | 0.031  | (0.008) |
| C14:0    | Myristic acid                    | 8.740  | (0.875) | 4.938  | (0.735) |
| C14:1n5  | Myristoleic acid                 | 0.067  | (0.010) | 0.037  | (0.008) |
| C15:0    | Pentadecanoic acid               | 0.928  | (0.098) | 0.622  | (0.082) |
| C16:0    | Palmitic acid                    | 30.978 | (2.755) | 20.554 | (2.315) |
| C16:1n7  | Palmitoleic acid                 | 8.160  | (1.061) | 5.926  | (0.892) |
| C17:0    | Heptadecanoic acid               | 0.853  | (0.073) | 0.549  | (0.061) |
| C18:0    | Stearic acid                     | 8.062  | (0.662) | 5.363  | (0.557) |
| C18:1n9t | Elaidic acid                     | 0.371  | (0.059) | 0.372  | (0.050) |
| C18:1n9c | Oleic acid                       | 23.290 | (2.598) | 16.927 | (2.183) |
| C18:2n6t | Linolelaidic acid                | 0.011  | (0.007) | 0.000  | (0.006) |
| C18:2n6c | Linoleic acid                    | 2.247  | (0.258) | 1.476  | (0.217) |
| C20:0    | Arachidic acid                   | 0.524  | (0.051) | 0.305  | (0.042) |
| C18:3n6  | γ-linolenic acid                 | 0.143  | (0.020) | 0.097  | (0.017) |
| C20:1n9  | Eicosenoic acid                  | 1.557  | (0.185) | 1.174  | (0.155) |
| C18:3n3  | α-Linolenic acid (ALA)           | 1.081  | (0.181) | 0.976  | (0.152) |
| C20:2    | cis-11,14-eicosadienoic acid     | 0.259  | (0.035) | 0.139  | (0.030) |
| C22:0    | Behenic acid                     | 0.000  | (0.007) | 0.008  | (0.006) |
| C20:3n6  | Homo-γ-linolenic acid            | 0.086  | (0.014) | 0.064  | (0.012) |
| C22:1n9  | Eucic acid                       | 0.293  | (0.032) | 0.177  | (0.027) |
| C20:3n3  | Eicosatrienoic acid              | 0.084  | (0.007) | 0.025  | (0.006) |
| C20:4n6  | Arachidonic acid (ARA)           | 0.211  | (0.033) | 0.153  | (0.028) |
| C23:0    | Tricosanoic acid                 | 0.608  | (0.120) | 0.906  | (0.101) |
| C22:2    | cis-13, 16-docosadienoic acid    | 0.092  | (0.009) | 0.048  | (0.007) |
| C24:0    | Ligocericic acid                 | 0.149  | (0.015) | 0.078  | (0.013) |
| C20:5n3  | Eicosapentaenoic acid            | 0.669  | (0.149) | 1.057  | (0.125) |
| C24:1n9  | Nervonic acid                    | 1.254  | (0.094) | 0.823  | (0.079) |
| C22:5n3c | Docosapentaenoic acid (DPA)      | 0.267  | (0.069) | 0.423  | (0.058) |
| C22:6n3  | Docosaĥexaenoic acid (DHA)       | 1.471  | (0.471) | 3.657  | (0.396) |
| Sw3      | Sum omega-3 fatty acids          | 3.573  | (0.807) | 6.138  | (0.678) |
| Sw6      | Sum omega-6 fatty acids          | 2.698  | (0.304) | 1.791  | (0.255) |
| SAFA     | Sum saturated fatty acids        | 51.206 | (4.544) | 33.460 | (3.818) |
| MUFA     | Sum mono-unsaturated fatty acids | 34.992 | (3.984) | 25.436 | (3.347) |
| PUFA     | Sum poly-unsaturated fatty acids | 6.621  | (1.013) | 8.115  | (0.851) |
| Total    | Sum of total fatty acids         | 92.819 | (8.746) | 67.010 | (7.348) |

aminase positive prey in their diet. Preliminary results based on stable isotope analysis of muscle tissue, show that comparable small fish in Lake Ontario consume fewer alewives than larger salmonids (Fitzsimons unpublished data). Consumption of alewife has been found to cause thiamine deficiency in salmonids (Honeyfield *et al.* 2005).

The results from this study raise biological as

well as technical questions. Could sample handling and processing prior to freezing have affected the results? Sample handling and processing may have affected individual forms of thiamine but not total thiamine. Total thiamine measured in alewife held at 4°C for 5 hours were found to be similar to thiamine content of flash frozen alewives (Wright *et al.* 2005). For fatty acid data, chromatographs of improperly handled samples will show an increase

in peak areas for short chain fatty acids (< 10 carbon length) and a loss of highly unsaturated fatty acids. This was not seen in the chromatograms. In both thiamine and fatty analysis improper sample handling results in significant sample to sample variation but again this was not observed among samples used in the present data.

As lipid stores decreased between fall and spring, there appeared to be selectivity in fatty acid catabolism for some fatty acids while certain essential fatty acids appear to have been conserved. When individual fatty acids data are expressed as percentage of the total fatty acids in the sample, C20:3n3 was found to be higher in the fall (0.10%) than in the spring (0.03%). Furthermore, C22:6n3 was found to be much higher in the spring (6.47%) as compared to the fall (1.96%). When fatty acids are compared by concentration (µg/mg), four fatty acids (C20:5n3, C22:5n3c, C20:3n3, C22:6n3) and the sum of omega-3 fatty acids (Sw3) were found to higher in the spring than in the fall (Table 6). In contrast the sum of omega-6 fatty acids (Sw6) and C18:2n6c were significantly lower in the spring than in the fall. Based on concentration, arachidonic acid (C20:4n6) was numerically higher in the fall than in the spring but the percentages of C20:4n6 were similar between fall (0.24%) and spring (0.22%).

The concentration of total saturated fatty acids (SAFA) found in these fish was high when compared to previously published Chinook salmon data (Iverson 1972). The significance of elevated saturated fatty acids is unknown although higher saturated fatty acid content may indicate a greater reliance on bacterial sources of fatty acids in the food web (Arts and Weinman 1998). The seasonal change that was observed with SAFA could have occurred by two mechanisms. First, as overall whole-fish lipids decreased, SAFA may have declined with the catabolism of lipids for energy. Alternatively, a decline in SAFA might reflect an enzymatic transformation to unsaturated fatty acids as an adjustment or adaptation to the colder water temperatures in winter. It's been noted that fish species in the southern part of their range contain more saturated fatty acids than fish at higher latitudes (Glemet et al. 1997), but because both the MUFA and total fatty acid content declined as well as the SAFA this would suggest that catabolism probably was the main effecter of change.

In summary we found seasonal and size differences in Lake Michigan Chinook salmon thiamine and fatty acid content. The data lend support to the

idea of a lower thiamine requirement in Chinook salmon than other Great Lakes salmonids.

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