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Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species

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ABSTRACT

Thiamine deficiency is an impediment to salmonine reproduction in the Great Lakes, but little is known about other measures of dietary quality, such as lipid-soluble vitamins or fatty acids in prey fish. The objective of the present research was to measure selected essential nutrients and thiaminase activity in five Lake Ontario prey fish species (alewife *Alosa psuedoharengus*, rainbow smelt *Osmerus mordax*, slimy sculpin *Cottus cognatus*, threespine stickleback *Gasterosteus aculeatus* and round goby *Neogobius melanostomus*). Total thiamine was greater in alewife (13.6 nmol/g) than in the other species (6.2–9.0 nmol/g). In 2006, thiaminase activity was unexpectedly high in goby (12.49 nmol/g/min), sculpin (1.99 nmol/g/min) and smelt (9.24 nmol/g/min). In 2007, thiaminase activity in goby (0.99 nmol/g/min) and smelt (4.94 nmol/g/min) was low compared to 2006, whereas sculpin thiaminase activity was greatest (6.01 nmol/g/min). The causes for this variability are unknown. Thiaminase activity was within the expected range for alewife (4.31–6.31 nmol/g/min) and stickleback (0.06 nmol/g/min). Concentrations of retinoids, carotenoids, vitamin E (tocopherol) and fatty acids also differed among prey fish species. Tocopherol concentrations in goby (12.74 ng/mg), sculpin (25.29 ng/mg), and smelt (22.81 ng/mg) were greater than in alewife (1.59 ng/mg). Goby had the lowest $\sum$ ω-3 to $\sum$ ω-6 fatty acid ratio (1.44) when compared to sculpin (2.97) and smelt (2.85). Thiaminase concentrations in alewife and smelt (and possibly goby) suggest that they have the potential to adversely affect natural reproduction in salmonines. Concentrations of carotenoids, retinoids and tocopherol in prey fish appear to be lower than salmonine dietary requirements.

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Introduction

Both diet quantity and quality can influence health and well-being (e. g. growth, survival and reproduction) of fish. While concerted efforts are expended annually to determine the quantity of food available for Great Lakes top predators, evaluation of dietary quality is limited. Percent lipid, a measure of energy, is the most commonly measured and reported parameter related to nutritional quality of fish diets. Numerous factors can affect the dietary requirements of essential nutrients. Essential nutrients are compounds that must be in the diet because they either cannot be synthesized at all or else their rate of synthesis is below that required for optimal physiological function.

To evaluate the ecological impacts of diet quality on Great Lakes trout and salmon, we need to know the concentration of essential nutrients in their diets. However, these concentrations are largely unknown for key Great Lakes prey fish. Implications of nutrient deficiencies (other than caloric energy and thiamine) have generally not been considered in the management of Great Lakes fish populations. Great Lakes trout and salmon support highly-valued recreational fisheries, which contribute significantly to regional and local economies. They also play an important ecological role in controlling the abundance of non-native prey fishes, such as alewife *Alosa pseudoharengus* and rainbow smelt *Osmerus mordax*. In order to restore self-sustaining populations of native salmonines, such as lake trout *Salvelinus namaycush* and Atlantic salmon *Salmo salar*, fish must be able to successfully complete their life cycles in Great Lakes waters.

Thiamine deficiency in Great Lakes salmonines is a well-studied example of an essential nutrient deficiency affecting multiple life stages (Brown et al., 2005a, 2005b; Honeyfield et al., 2005). Thiamine,

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an essential nutrient, cannot be synthesized by animals and thiamine deficiency can be the result of consuming thiaminase I, an enzyme found in certain fish, bacteria and plants that is known to destroy dietary thiamine (Evans, 1975; Honeyfield et al., 2002; NRC, 1983). The requirement for thiamine in trout and salmon diets has been set at 0.6 nmol/g (wet weight basis, NRC, 1993). While thiamine concentrations of common prey fish species (Fitzsimons et al., 1998; Tillitt et al., 2005) exceed trout and salmon dietary requirements (NRC, 1993), the presence of thiaminase in the prey species often results in thiamine deficiency. Available data for Lake Ontario prey fish thiamine and thiaminase activity are limited to alewife and rainbow smelt (Fitzsimons et al., 1998, 2005; Tillitt et al., 2005).

The potential role of other essential nutrients (e.g. vitamin A family and vitamin E) associated with thiamine deficiency in Great Lakes predators is unknown, in part, because these important nutrients have not been reported in Great Lakes prey fish. Observed concentrations of these vitamins in salmon eggs have been found to differ between fry with and without thiamine deficiency (Brown et al., 2005b; Pettersson and Lignell, 1998; Pickova et al., 1998, 2003). There is no evidence, however, that anti-nutrients (analogous to thiaminase) exist that could affect these lipid-soluble vitamins (NRC, 2011). Although each vitamin has a unique biological role, thiamine, vitamin E and members of the vitamin A family share a common role as antioxidants. Cellular damage caused by oxygen free radicals and lipid peroxides is mediated or prevented by lipid-soluble antioxidants (Saito, 1990; Surai, 1999). Tocopherol (vitamin E) has been reported to ameliorate the oxidative stress associated with the reactive oxygen species induced by cytochrome P450 - enzymes during PCB intoxication (Saito, 1990; Traber, et al., 1993). Susceptibility to oxidative stress may be further exacerbated in thiamine deficient states if tocopherol concentration is low; thiamine deficiency and oxidative stress have been linked in Baltic salmon *S. salar* (Vuori and Nikinmaa, 2007; Vuori et al., 2008). Thus, if thiamine is low, the demand for vitamin E or another antioxidant vitamin is increased. The effects of vitamin A deficiency have been described in the laboratory (Kitamura et al., 1967). According to NRC (1993), the dietary requirements of Pacific salmon and rainbow trout *Oncorhynchus mykiss) are* ~6.7 ng/mg and 60 ng/mg for vitamins E and A, respectively (wet weight basis). There are no data on the concentrations of these lipid-soluble vitamins for Great Lakes prey fish. Therefore, prey fish essential nutrient concentrations represent important information required to assess the nutritional status of top fish predators.

Essential fatty acids are building blocks for the eicosanoids involved in reproduction, immune function and other physiological roles (Kinsella et al., 1990). Two families of essential fatty acids, omega-3 (ω-3) and omega-6 (ω-6), are required by fish and other animals (NRC, 1993; Parrish, 2009). Fatty acid composition, particularly the degree of saturation, plays an important role in the general phenomenon of membrane fluidity which provides an adaptive response to changing water temperature (Arts and Kohler, 2009). Fish residing in warm water have a higher proportion of saturated fatty acids compared to fish in cold water. If the fatty acid composition of the prey does not closely resemble that of the predator's, with respect to the degree of saturation required for optimal membrane fluidity levels in relation to environmental temperature, then metabolic energy must be expended to alter fatty acids that otherwise could have been used for growth and reproduction. No fatty acid data are published for prey fish from Lake Ontario and only limited fatty acid data from Lake Michigan prey fish are available (Honeyfield et al., 2009, 2010; Sergiusz et al., 2011; Wagner et al., 2010).

The objectives of this study were to: 1) document thiamine and thiaminase activity in five fish prey species important to Lake Ontario salmonines, 2) survey prey fish status with respect to concentrations of vitamin E and the vitamin A family of compounds (retinoids and carotenoids), and 3) evaluate fatty acid concentrations both as a general measure of the health of prey fish and as an indicator of diet quality for their predators.

Methods

Field collections

Fish were collected in the fall so as to provide an estimate of prey fish nutrient stores going into winter. Winter is a time when feeding stops or is limited and therefore it is unlikely that fish will acquire additional nutrients until spring. Thus, measurements of energy and nutrients at this time represent an integration of what was accumulated in the preceding growing season and provide a synopsis on the quantity and quality of food in that season relative to other seasons or locales. In the fall of 2006, five prey fish species, alewife; rainbow smelt; slimy sculpin, *Cottus cognatus*; threespine stickleback, *Gasterosteus aculeatus*; and round goby, *Neogobius melanostomus* (henceforth, smelt, sculpin, stickleback and goby, respectively) were collected from four areas in Lake Ontario (Fig. 1): northern (off of Cobourg, Ontario), southwestern (near Olcott, New York), southcentral (off Rochester and Smoky Point, New York) and southeastern (off Fair Haven and Oswego, New York). Fish were collected opportunistically at these sites, as part of ongoing research and assessment activities. Sites were chosen to represent a diversity of lake areas, not to evaluate differences among them. Fish were captured with bottom trawls and sorted by species and size. Live fish were selected, measured (mm total length, TL) and immediately frozen on dry ice to preserve their biochemical integrity. Samples were stored at −80 °C until analysis. In the laboratory, fish were individually ground while still frozen, using methods described by Zajicek et al. (2005). Thiamine, thiaminase, lipid-soluble vitamins and fatty acids were measured in prey fish collected in the fall of 2006. Additional samples to repeat analyses of thiaminase activity were collected and measured in the same prey fish species in the fall of 2007.

Chemical component analyses

Thiamine and thiaminase activity

Concentrations of thiamine pyrophosphate, thiamine monophosphate and unphosphorylated (free) thiamine were determined by high-performance liquid chromatography (Brown et al., 1998a). Total thiamine is the sum of the three forms of thiamine measured. The activity of the thiamine degrading enzyme, thiaminase, was

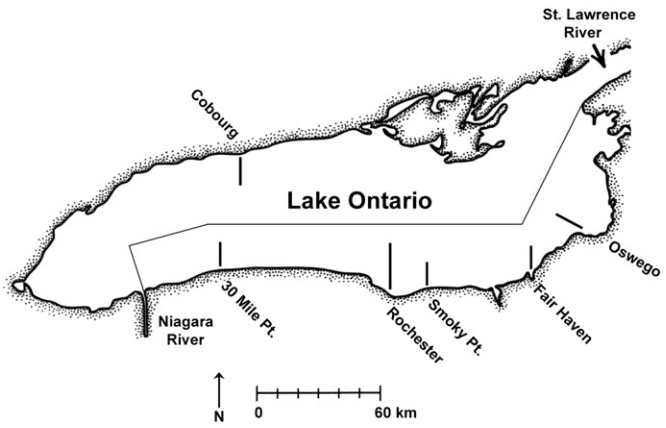

Fig. 1. Map showing prey fish collection transects. Sites were in northern (Cobourg), southwest (30 Mile Point), southcentral (Rochester and Smoky Point), and southeastern (Fair Haven and Oswego) areas of Lake Ontario.

determined using standard ^{14}C labeled thiamine assay used to report thiaminase activity in Lake Michigan prey fish (Zajicek et al., 2005).

Lipid-soluble vitamins

Tissue extraction procedures were that of Brown and Vandenbyllaardt (1996) and were conducted under reduced light. Extracts were protected from light and stored at −80 °C. Concentrations of carotenoids, retinoids and tocopherol compounds were determined using the methods of Palace and Brown (1994) and Brown et al. (2005b). Three major carotenoid peaks (astaxanthin, carotenoid A and carotenoid B) were measured. Although carotenoids A (unknown 1) and B (unknown 2) were unidentified, they were characterized by their absorbance spectra (maximum near 450 nm). Because we did not have authentic standards for these unidentified carotenoids, quantification was relative to authentic beta-carotene. Dehydroretinyl esters were identified based on their characteristic absorbance spectrum, which is similar to that of dehydroretinyl (absorption maxima at 280 and 340 nm). The concentration of an individual dehydroretinyl ester was determined relative to chromatographic response, using authentic retinyl palmitate.

Fatty acids

Fatty acids were quantified as described in Brown et al. (2005b) and reported as μg per mg tissue dry weight (DW). The gas chromatographic method separates fatty acid methyl ester derivatives. Concentration of each fatty acid was based on chromatographic response calculated from a four point standard curve. Fatty acid identifications were confirmed by comparing the retention times of individual fatty acids in the samples to those of authentic standards (Supelco FAME mix, Catalog# 47885-U). Percent total lipid in freeze-dried tissues (i.e. on a DW basis) was determined gravimetrically from duplicate aliquots removed from the chloroform:methanol extracts.

Results

Fish collections in the fall of 2006 from among the four lake areas were: 48 alewife, 12 goby, 24 sculpin, 32 smelt and 8 stickleback. Mean fish lengths and weights were those available in trawl catches and targeted to minimize variation in size among individuals of a given species (Table 1). On dry matter (DW) basis, alewife (45.2%) had at least two times the caloric lipid energy as sculpin (24.8%), smelt (27.7%), stickleback (24.7%) or goby (16.9%). In 2007, an additional 12 alewife, 18 goby, 18 sculpin, and 31 smelt were collected solely to repeat the analyses of thiaminase activity (see below). Mean lengths ± SE (mm) and weights ± SE (g) in 2007 were similar to those in 2006: alewife 135 ± 4 mm, 23.3 ± 1.6 g, goby 114 ± 6 mm, 19.4 ± 3.1 g, sculpin 103 ± 4 mm, 12.2 ± 1.6 g, and smelt 109 ± 2 mm, 7.9 ± 0.3 g.

Chemical component analyses

Thiamine and thiaminase activity

Total thiamine was greater in alewife than in the other four species; there were no differences in thiamine among the other prey fish species (Fig. 2). The three forms of thiamine partitioned differently among the species (on-line supplemental Appendix). Thiamine pyrophosphate (TPP), as a percentage of total thiamine, was the greatest in alewife and sculpin (75–76%) and intermediate in smelt (57%). Goby (38%) and stickleback (41%) had the lowest percentage of TPP. Total thiamine in these prey fish was more than adequate to meet salmonine dietary requirements, provided thiaminase was absent. Total thiamine was variable among species and sites (Appendix). Thiaminase activity in prey fish collected in 2006 differed among species (Fig. 2) with goby > smelt > alewife > sculpin. To verify these results, additional prey fish were collected and analyzed in 2007 (numbers reported above). Stickleback thiaminase activity was very low in 2006 (none collected in 2007) and probably is not biologically relevant with regard to thiamine deficiency in top predators. Thiaminase activity was found in all goby collected in 2006 with activity ranging from 1.35 to 57.70 nmol/g/min. Thiaminase activity was again found in goby in the fall of 2007, but activity was lower than that found in 2006. In 2007, goby thiaminase activity ranged from 0 to 4.97 nmol/g/min; three goby had no thiaminase, eight had low activity of unknown biological significance (0.08–0.24 nmol/g/min), five had activity of 0.90–1.40 nmol/g/min and four had activity of 2.00–4.97 nmol/g/min. Smelt thiaminase was highly variable (range 0.46–56.65 nmol/g/min) and thiaminase activity in the 2007 samples was approximately half (5.05 nmol/g/min) the activity observed in 2006. Sculpin thiaminase activity in the fall of 2007 was 3 times greater than was observed in the fall of 2006 (Fig. 2) and thiaminase activity varied among sites (Appendix). Alewife and smelt thiaminase activity was similar between years.

Lipid-soluble vitamins

Vitamin E concentration was the greatest in sculpin (25.3 ng/mg) and smelt (22.8 ng/mg; Fig. 3). Goby contained approximately half

Table 1
Mean ± SE fish length, weight, percent lipid on dry weight (DW) basis, concentration of ω-3 and ω-6 fatty acids in Lake Ontario prey fish collected in 2006. Common names for Omega-3 fatty acids: 18:3n3 = α-linolenic acid (ALA), 20:3n3 = Eicosatrienoic acid (ETA), 20:5n3 = Eicosapentaenoic acid (EPA), 22:5n3c = Docosapentaenoic acid (DPA), 22:6n3 = Docosahexaenoic acid (DHA). Common names for Omega-6 fatty acids: 18:2n6 = Linolelaidic acid, 18:3n6 = γ-linolenic acid, 20:3n6 = homo-γ-linolenic acid, 20:4n6 = Arachidonic acid (ARA), 22:4n6 = 7,10,13,16-docosatetraenoic acid, 22:5n6 = 4,7,10,13,16-docosapentaenoic acid.

	Alewife	Goby	Sculpin	Smelt	Stickleback
N	48	12	24	32	8
Length, mm	116 ± 5	103 ± 9	95 ± 5	96 ± 5	57 ± 2
Weight, g	19.3 ± 2	16.8 ± 3.6	9.5 ± 1.3	6.8 ± 0.8	1.5 ± 0.2
Lipid, % of DMW	45.24 ± 1.21	16.89 ± 1.49	24.79 ± 1.4	27.74 ± 2.24	24.7 ± 4.41
Omega-3 fatty acids, μg/mg dry tissue					
18:3n3	19.63 ± 0.92	3.74 ± 0.41	5.63 ± 0.49	7.17 ± 0.74	6.75 ± 1.53
20:3n3	2.17 ± 0.21	0.38 ± 0.05	0.78 ± 0.07	1.53 ± 0.24	2.70 ± 0.97
20:5n3	21.38 ± 0.83	10.6 ± 1.08	20.17 ± 1.20	19.908 ± 2.61	7.81 ± 1.30
22:5n3c	5.73 ± 0.20	4.98 ± 0.39	6.77 ± 0.49	1.81 ± 0.15	7.34 ± 1.31
22:6n3	18.00 ± 0.66	9.56 ± 0.78	17.17 ± 0.76	14.43 ± 0.93	16.81 ± 3.04
Omega-6 fatty acids, μg/mg dry tissue					
18:2n6c	14.97 ± 0.67	4.23 ± 0.57	6.00 ± 0.45	6.90 ± 0.70	7.27 ± 1.32
18:3n6	1.75 ± 0.09	0.28 ± 0.04	0.71 ± 0.06	0.68 ± 0.09	0.46 ± 0.09
20:3n6	0.84 ± 0.04	0.27 ± 0.06	0.22 ± 0.02	0.23 ± 0.03	0.19 ± 0.04
20:4n6	10.36 ± 0.41	5.59 ± 0.31	6.09 ± 0.20	5.56 ± 0.39	5.98 ± 0.36
22:4n6	0.92 ± 0.13	2.68 ± 0.38	0.77 ± 0.11	0.37 ± 0.04	1.08 ± 0.17
22:5n6	3.00 ± 0.49	7.25 ± 1.02	3.23 ± 0.47	1.97 ± 0.24	2.43 ± 0.58

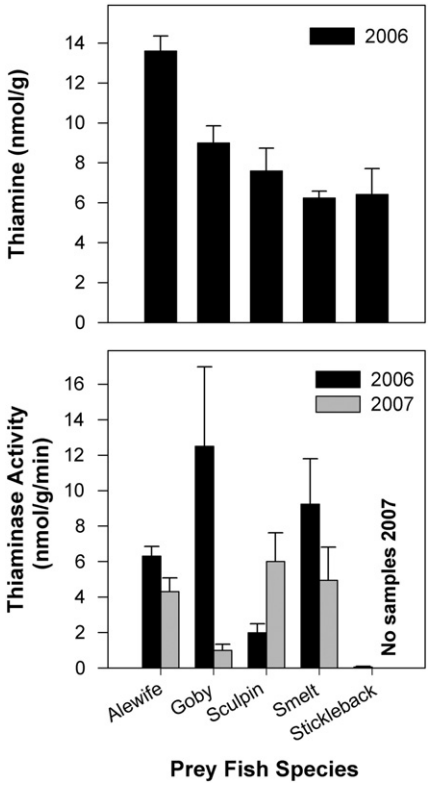

Fig. 2. Total thiamine concentration and thiaminase activity (mean ± SE) in five Lake Ontario prey fish species collected in 2006–2007.

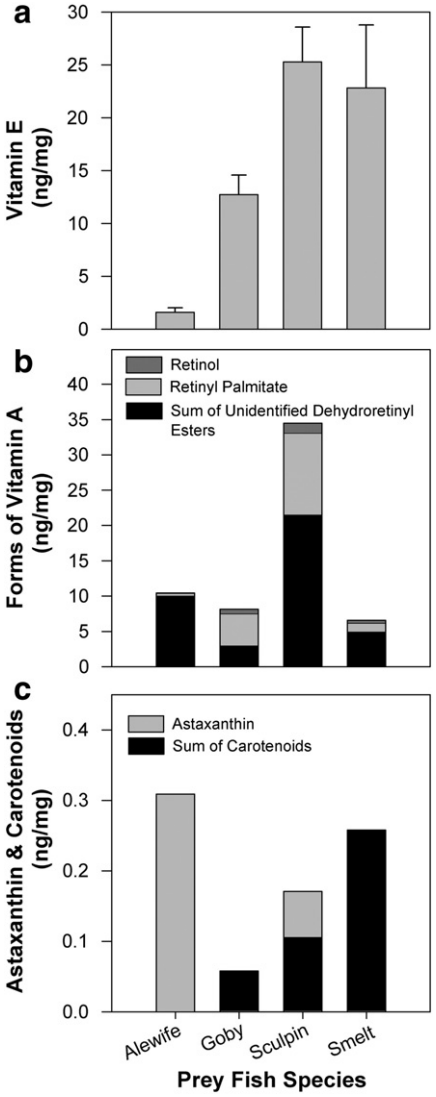

Fig. 3. Concentrations of vitamin E (tocopherol, panel a), vitamin A forms (retinoids, panel b), and carotenoids (panel c) found in Lake Ontario prey fish collected in the fall of 2006.

the tocopherol content of sculpin and smelt. Most importantly, alewife tocopherol concentration (1.6 ng/mg) was very low and at the Northern or Cobourg site, alewife (Fig. 1) contained no measurable amounts of vitamin E (Appendix). Retinoids found in prey species tissue included retinol, retinol acetate, di-dehydroretinol, retinyl palmitate and 12 unidentified dehydroretinyl esters. This family of vitamin A derivatives varied in concentration among prey fish species (Fig. 3; Appendix). Sculpin had the greatest concentration of retinol (1.4 ng/mg). Retinyl palmitate was the greatest in sculpin (11.6 ng/mg), intermediate in goby (4.5 ng/mg) and least in smelt (1.2 ng/mg) and alewife (0.4 ng/mg). Smelt contained greater concentrations of di-dehydroretinol (ng/mg) than sculpin and alewife. Goby (0.6 ng/mg) and smelt (0.4 ng/mg) were intermediate and alewife (0.03 ng/mg) had the lowest concentration of di-dehydroretinol. Retinol acetate was low in smelt and was not found in alewife, goby or sculpin. The sum of the twelve unknown dehydroretinyl esters was significantly higher in sculpin than in alewife, goby or smelt. Dehydroretinyl esters 1, 2, 11 and 12 were absent in goby (Appendix). Dehydroretinyl ester 11 was also absent in alewife and smelt tissue.

Carotenoids, including astaxanthin, were either very low or absent in these prey fish. In Fig. 3, note that the carotenoid y-axis scale is 100-fold lower than the y-axis of the retinoid graph. Alewife had no detectable unknown carotenoids. The sum of the unknown carotenoids in smelt, sculpin and goby was 0.258, 0.106 and 0.058 ng/mg, respectively. Astaxanthin was present at very low concentrations in alewife (0.31 ng/mg) and sculpin (0.07 ng/g). Insufficient stickleback sample remained for carotenoid-astaxanthin analysis.

Fatty acids

Alewife, because of their high lipid contents, had the greatest saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) concentrations compared to the other four prey fish species (Fig. 4). The concentration of these fatty acid classes was similar among goby, sculpin, smelt and stickleback. Alewife had the greatest concentration of total ω-3 PUFA (66.9 μg/mg) DW and goby had the lowest concentration (29.3 μg/mg DW; Fig. 4). Sculpin (50.5 μg/mg DW) and smelt (44.8 μg/mg DW) had intermediate concentrations. Alewife had the highest concentrations of α-linolenic acid (ALA, 18:3n − 3) whereas ALA concentrations did not differ among the other four prey fish species (Table 1). This fatty acid is a precursor for longer-chain ω-3 PUFA. Alewife and stickleback had higher concentrations of eicosatrienoic acid (20:3n − 3) than goby and sculpin. Alewife, sculpin and smelt had higher concentrations of eicosapentaenoic acid (EPA, 20:5n − 3) than goby and stickleback. Sculpin and stickleback had higher concentrations of ω-3 docosapentaenoic acid (DPA, 22:5n − 3) than goby or smelt; smelt had the lowest concentration of this fatty acid. Goby had the lowest concentration of docosahexaenoic acid (DHA, 22:6n − 3). Within the ω-6 fatty acids (Table 1), concentrations of linoleic acid (LIN, 18:2n − 6), homo-γ-linolenic acid (20:3n − 6) and arachidonic acid (ARA, 20:4n − 6) were greater in alewife than in goby, sculpin, smelt or stickleback. Goby had the highest concentration of adrenic acid (ADA, 22:4n − 6), smelt had the lowest concentration and stickleback was intermediate. Three fatty acids (15:0i, 15:0ai, 16:0i), considered to be primarily of bacterial origin, differed between and

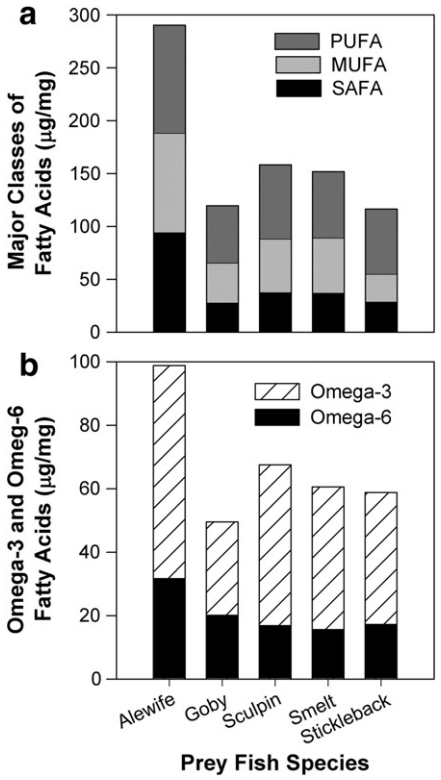

Fig. 4. Concentrations (µg/mg dry tissue weight) of fatty acids groupings mesured in Lake Ontario prey fish species. In panel a, are the polyunsaturated fatty acids (PUFA) monounsaturated fatty acids (MUFA), and saturated fatty acids (SAFA). In panel b, omega-3 fatty acids (∑ ω-3), and omega-6 fatty acids (∑ ω-6) (panel b) are shown.

within goby and smelt. This difference was not seen in alewife and sculpin. Concentrations of fatty acids that were measured but not reported in Table 1 are found in the Appendix.

Discussion

Over the past 15 years, the consequences of low thiamine concentrations have been documented and the implications on the health and well-being of predators feeding on thiaminase-positive prey fish reported (Brown et al., 2005c). This study adds to the limited data available for thiamine, thiaminase activity and fatty acid content in Great Lakes prey fish and provides new data on vitamins A and E and fatty acid content of prey fishes. Thiaminase activity was found to be elevated and highly variable. Furthermore, alewife contained little or no vitamin E and Lake Ontario species in this study had only low concentrations of the vitamin A family of retinoids.

Thiamine concentrations were more than adequate to meet the dietary requirements of predators consuming Lake Ontario prey fish. Thiamine requirement of trout and salmon is ~0.6 nmol/g (NRC, 1993). Lake Ontario alewife total thiamine concentration in 2006 (13.6 nmol/g) was marginally greater than previously reported in 1998 (10.0 nmol/g; Fitzsimons et al., 1998). Alewife thiamine concentration reported from the New York Finger Lakes was 22.4 nmol/g (Fitzsimons et al., 2005) and 15.5 nmol/g from Lake Michigan (Tillitt et al., 2005). Similar to other studies, thiamine content was found to vary among and within the prey species in the present study, but overall thiamine concentrations were more than sufficient to meet the dietary requirements of trout and salmon, provided thiaminase was not present.

The role and behavior of thiaminase in the aquatic food web are not well understood. The source, its biological function and the factors which lead to variability in thiaminase activity have not been fully described. *De novo* protein synthesis of thiaminase by fish has been assumed, but not confirmed experimentally. Thiaminase has been reported in isolated alewife gut bacteria (Honeyfield et al., 2002) and in cyanobacteria (Grigor et al., 1977), but the definitive source of thiaminase in prey fish has not been identified. Thiaminase activities among and within prey fish species can be highly variable. Tillitt et al. (2005) found no thiaminase activity in Lake Michigan goby, which is in contrast to the present study. Dreissenid mussels are an important food item of goby (Walsh et al., 2007) and have been reported to have very high levels of thiaminase activity, ranging from 33.6 to 148.1 nmol/g/min (Tillitt et al., 2009). Studies to evaluate the biochemical properties of thiaminase in goby, and its potential to cause thiamine deficiency in predators, are warranted. Thiaminase activity in Lake Ontario smelt was two to three times greater than that reported in Lake Michigan smelt (2.64 nmol/g/min, Tillitt et al., 2005). Lake Ontario alewife thiaminase activity was similar to those previously reported in New York Finger Lakes and Lake Michigan (Fitzsimons et al., 2005; Tillitt et al., 2005). Thiaminase activity in Lake Ontario sculpin and stickleback also was similar to enzyme activity observed in the same species from Lake Michigan (Tillitt et al., 2005).

A striking finding in the survey of lipid-soluble vitamins in Lake Ontario prey fish was the very low vitamin E concentration observed in alewife (1.6 ng/mg), but not in sculpin (25.3 ng/mg), smelt (22.8 ng/mg) or goby (12.7 ng/mg). Sculpin, smelt and goby contain two to three times the dietary requirements of vitamin E for trout or salmon. Low vitamin E in alewife has negative implications for alewife (Snyder and Murray, 2009) and for the health of their predators. Trout and salmon require a dietary intake of ~6.7 ng/mg (WW, NRC, 1993). Adequate vitamin E may have a conserving effect on thiamine reserves because both vitamins are known to function as cellular antioxidants (Lukienko, et al., 2000; Portari et al., 2008). Low vitamin E concentration in lake trout fry hatched from thiamine deficient eggs has been observed (Lee et al., 2008). Alewife vitamin E requirement has not been reported but a concentration of 0-2.9 ng/mg is very low. Snyder and Murray (2009) reported enhanced survival in alewife treated with vitamin E. This raises a question: could low concentrations of vitamin E be a factor in population declines of alewife reported in Lake Ontario (Murry et al., 2010), Lake Michigan and Lake Huron (Fitzsimons et al., 2010; Riley et al., 2008)?

The potential pool of vitamin A from the retinoids, carotenoids and astaxanthin measured in this study is less than the published requirements for trout and salmon (60 ng/mg WW; NRC, 1993). However, not all forms of vitamin A were measured in this study. Retinal is an important biological form of vitamin A that was not considered during laboratory analysis. Only during data analysis did the absence of retinal become apparent. In retrospect, we recommend that future studies measure additional forms of vitamin A including protein bound retinal (Ire and Seki, 2002). Differences in retinoid and carotenoid concentrations have previously been noted in eggs from females with and without thiamine deficient fry mortality (Brown et al., 2005b; Pettersson and Lignell, 1998; Pickova et al., 1998, 2003). Lake Ontario alewife, goby, sculpin and smelt had very low concentrations of astaxanthin. Based on recent publications (Nie et al., 2011; Ytrestøyl and Bjerkeng, 2007), the two unidentified carotenoid peaks in the present study may be the 9 and 13 cis-isomers of astaxanthin. We have no explanation as to why astaxanthin or the carotenoids are low or absent in the prey fish examined, but our results are supportive of the suggestion by the authors above that the differences observed in retinoids and carotenoids in thiamine deficient salmon eggs were related to the prey species consumed.

Fatty acids are the principle components of total body lipid. They have several biological roles and have been used as tracers in food webs (Budge et al., 2006; Hebert et al., 2009; Napolitano, 1998). Two long-chain unsaturated fatty acids (EPA and ARA) are precursors of eicosanoids which function as signaling molecules. The ω-6 derived eicosanoid family

tends to be pro-inflammatory compared to the ω-3 derived eicosanoids. Within the eicosanoids, there are four sub-groups consisting of prostaglandins, prostacyclins, thromboxanes and leukotrienes, each of which has specific biological roles (De Caterina and Basta, 2001; Funk, 2001; Klurfeld et al., 2008; Soberman and Peter, 2003). Because synthesis of long-chain highly unsaturated fatty acids utilizes the same enzymes for both ω-3 and ω-6 fatty acids, the ratio of the ω-3 and ω-6 families will ultimately affect the ratio of eicosanoids produced and this will impact physiological responses. Goby had the lowest ω-3/ω-6 fatty acid ratio while sculpin and smelt had the highest. The ω-3/ω-6 ratio was intermediate for stickleback and alewife. Goby populations have expanded and if trout and salmon consume more goby at the expense of other prey species, changes in salmonid fatty acid profiles can be expected. However, the health and reproductive consequences of such a shift in ω-3 to ω-6 fatty acid ratios have yet to be determined.

The range in the $\sum$ ω–3 fatty acid concentrations among the prey fish species was over twice that of the $\sum$ ω-6 fatty acids. Goby contained the lowest amount of $\sum$ ω-3 fatty acids, with low concentrations in four of the five long-chain ω-3 fatty acids, including ALA. ALA cannot be synthesized by fish. It must come from the diet. Therefore, differences in the concentration of ALA reflect not only the underlying difference in prey fish's choice of diet items, but also the quality of the underlying food web. The sum of ω-6 fatty acids was the highest in alewife, while the other prey fish had similar, but lower concentrations. In alewife, the concentrations of two individual ω-6 fatty acids, 22:4n6 and 22:5n6, were more than 2 times higher than concentrations measured in the other four prey fish. Differences in the concentrations of three fatty acids (15:0i, 15:0ai, 16:0i) primarily of bacterial origin were observed between and within goby and smelt (Appendix). This supports conclusions that there are subtle differences in food items consumed by prey fish within Lake Ontario food web (Walsh et al., 2007).

Our study of four essential nutrients is only a snap shot of these biologically important compounds and more data should be collected. We were limited in the number of samples we could collect and analyze, therefore we were unable to properly evaluate annual or site-specific variation in these nutrients. However, even without knowing all the sources of variation, the present data suggests that precursors (carotinoids, retinoids) of vitamin A in these prey fish are below published nutrient requirements for salmonines. Alewife contained unusually low concentrations of the antioxidant vitamin E. Thiaminase was elevated and more variable in these fish compared to similar species in other lakes. Knowledge about these nutrients (anti-nutrients) has important implications for the health of prey fish and their predators. In summary, the measured compound concentrations were highly variable in Lake Ontario prey fish. A low concentration of vitamin E in alewife, the presence of thiaminase activity in goby, and the low concentrations of vitamin A family (retinoids and carotenoids) in four of the species examined warrant further investigation. Salmonid thiamine deficiency is a known problem in the Great Lakes and the data suggest that other essential nutrient deficiencies may exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jglr.2011.11.008.

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