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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

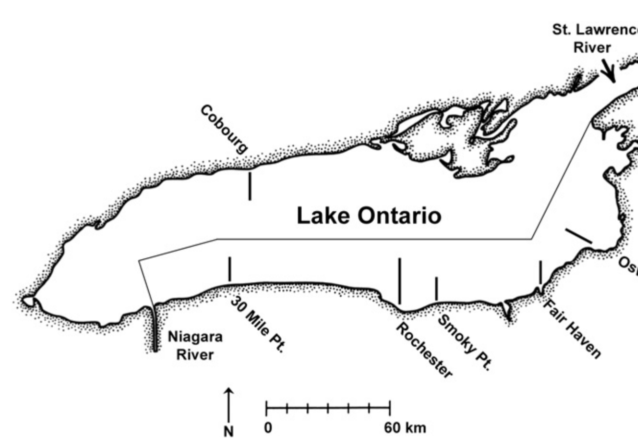

Fig. 1. Map showing prey fish collection transects. Sites were in northern (Cobourg), southwest (30 Mile Point), southcentral (Rochester and Smoky Point), and southeastern (Fair Haven and Oswego) areas of Lake Ontario.