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Five year trends in the egg-thiamine status of Atlantic salmon from the St. Marys River, Michigan

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ABSTRACT

Early mortality syndrome (EMS) is the term used to describe the mortality in early life stages of salmonids due to thiamine deficiency within the Great Lakes basin. Since 1985, Lake Superior State University (LSSU) has raised Atlantic salmon *Salmo salar* for stocking in the St. Marys River region near Sault Ste. Marie, Michigan. In 2000, the Atlantic salmon raised at LSSU experienced 99% mortality due to EMS related symptoms. In order to better understand and document the incidence of EMS in the St. Marys River population of Atlantic salmon, egg-thiamine content has been measured in this population since 2003. Egg-thiamine levels were measured using a rapid reversed-phase solid-phase extraction (RP–SPE) method of thiamine analysis. This paper presents the egg-thiamine data for this population of Atlantic salmon for the years 2003–2007. Over this five-year period, the egg-thiamine content of eggs from 2005 saw a significant increase. This one year shift may have larger ecological implications related to changes in the diet of this population of Atlantic salmon.

Introduction

Since the mid-1990s Great Lakes fisheries biologists and managers have become interested in a disease affecting salmonids due to a deficiency of vitamin B_1 (thiamine). This disease, analogous to beriberi in humans, was identified in the 1970s in the Baltic Sea (termed M74), and later recognized in the late early 1990s as the same malady in Lake Ontario lake trout and in Cayuga Lake Atlantic salmon (termed Cayuga syndrome). Subsequently, other salmonid populations in the Great Lakes began to exhibit the same disease and the deficiency was termed early mortality syndrome (EMS). For a more complete historical description please refer to Honeyfield et al. (1998).

In addition to Atlantic salmon *Salmo salar*, EMS has been identified in other Great Lakes piscivores (i.e. lake trout *Salvelinus namaycush*, coho salmon *Oncorhynchus kisutch* and chinook salmon *Oncorhynchus tshawytscha*) (Marcqenski and Brown, 1997; Honeyfield et al., 2005a). Research has demonstrated that EMS is due to a deficiency of the essential vitamin thiamine and was experimentally induced in Atlantic salmon and rainbow trout *Oncorhnchus mykiss* (Saunders and Henderson, 1974; Lehmitz and Spannhof, 1977; Honeyfield et al., 2005b). Fisher et al. (1996) demonstrated that the incidence of EMS was inversely correlated with concentrations of thiamine in eggs from gravid females. Subsequently, it was demonstrated that EMS in larvae and in eggs can be reversed in a hatchery setting by treating gravid females or the resulting eggs and sac-fry with thiamine immersion (Fitzsimons, 1995; Brown et al., 2005; Fitzsimons et al., 2005). During the course of these studies, researchers have identified what appears to be a "threshold" level of egg-thiamine below which significant mortality will occur. While this threshold varies by fish species, significant mortality occurs when egg-thiamine is below one to three nanomoles total thiamine per gram of egg (Fitzsimons et al., 2007).

One of the presumed causes of EMS is the enzyme thiaminase, a biological toxin that degrades vitamin B_1 (Begley, 1996). There are two types of this enzyme, type I thiaminase requires a nucleophilic substrate (i.e. amine or sulfhydryl) to cleave thiamine, while type II thiaminase only requires water as a co-substrate. The biological role of type II thiaminase appears to be as part of a thiamine salvage pathway (Toms et al., 2005; Bettendorff, 2007), however, the role of type I thiaminase is uncertain. Thiaminase I has been found in certain bacteria, plants, mollusks, and fish species, most notably those in the clupeid family, while thiaminase II is primarily found in yeast and bacteria. Dietary presence of thiaminase can cause either adult, juvenile or larval mortality in piscivorous fish such as lake trout, walleye, chinook salmon, and Atlantic salmon. Mortality in fish is brought about by depletion of available thiamine resulting in early mortality syndrome (EMS) (Honeyfield et al., 1998, 2005b). Thus, dietary thiaminase from alewife consumption has been suggested as a major cause of fry mortality in salmonid culture, reproductive failure by stocked lake trout, and extirpation of landlocked Atlantic salmon in Lake Ontario after alewife invasion (Ketola et al., 2000). Recent work has helped strengthen the connection between the affect of alewife consumption and the incidence of EMS in Great Lakes salmonids (Riley et al., 2007, 2008).

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The presence of thiaminase and its role in the development of EMS as a major impediment to native fish restoration has been highlighted by the Great Lakes Fishery Commission (GLFC) as a specific concern for fish health research priorities (Commission, 2006). As a result of concern surrounding EMS, several state and federal agencies have begun to monitor the "thiamine status" of fishery stocks throughout the Great Lakes by examining the thiamine content of eggs from various fish species. This monitoring has primarily been used to determine if fish culture facilities need to treat their eggs with thiamine immersion baths in order to prevent EMS.

For the past several years we have monitored the egg-thiamine status and juvenile mortality in a population of Atlantic salmon reared and released on the St. Marys River, the connecting waterway between Lake Superior and Lake Huron. The linkage between egg-thiamine levels and the resulting prevalence of EMS for this population have been previously published (Werner et al., 2006). Here we present the five-year trend in egg-thiamine status for this unique population of Atlantic salmon.

Methods

Egg collection

Atlantic salmon have been stocked by the Lake Superior State University Aquatic Research Laboratory (LSSU ARL) since 1985 and are released as year old fingerlings. Atlantic salmon broodstock consist of the West Grand Lake (ME) strain. Beginning in 2001, returning Atlantic salmon females have supplied sufficient eggs to supply the LSSU ARL such that additional purchased broodstock eggs were no longer required. Returning females are typically between two to four years of age and begin to appear in the St. Marys rapids in late May or early June. Fin clips are utilized to track which year classes are caught upon returning to the St. Marys Rapids. Atlantic salmon eggs for this study were collected during the first two weeks of November in 2003, 2004, 2005, 2006, and 2007. Eggs were collected at the LSSU ARL (located on the St. Marys River just below the St. Marys rapids in Sault Ste. Marie, Michigan). Atlantic salmon eggs for this study were stripped from gravid Atlantic salmon females returning to the St. Marys rapids. A subsample of each individual female's eggs (approx. 10 g, 100 eggs) were placed on ice for thiamine analysis before fertilization, and then stored at −80 °C within 4 h of collection.

Thiamine analysis

Approximately 1 g of this sample was subsequently homogenized for thiamine analysis as previously described (Werner et al., 2006). The RP–SPE method for thiamine analysis of fish eggs was used to measure the egg-concentration of phosphorylated thiamine, non-phosphorylated thiamine, and total thiamine. The method used was essentially that of Zajicek et al. (2005) but with several published modifications (Werner et al., 2006). This technique relies on liquid phase extraction of thiamine containing compounds from disposable solid-phase columns followed by oxidation and conversion of the eluted compounds to a fluorescent thiochrome. The relative fluorescence of the resulting thiochrome is then measured using a fluorescent microplate reader and thiamine concentrations are calculated for each sample. Reversed-phase solid-phase extraction (RP–SPE) analysis was accomplished using 1 mL Strata RP–SPE syringe columns (Phenomenex, Torrance, CA, part #8B-S001-EAK). One column was used for each individual sample or standard. Following oxidation, a fluorescent microplate reader (Biotek FLX-800 TBI, Biotek Instruments, Winooski, VT) was used to determine the relative fluorescence of samples and standards using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Each individual microplate well was read 3 times and a mean was calculated. Duplicates were then averaged and standard errors were calculated (data presented in Table 1).

Results

Thiamine analysis

Non-phosphorylated thiamine accounted for the majority of total egg-thiamine in all years except 2005 (Fig. 1). The average total egg-thiamine for all years except 2005 was 3.12 nmol thiamine per gram (avg. standard error 0.25). Total egg-thiamine remained relatively constant from 2003–2007 with the exception of eggs taken in 2005. For that year, egg-thiamine concentrations were significantly higher than other years, with a mean concentration of 9.41 nmol/g (Table 1). Eggs sampled in 2005 had significantly more phosphorylated than non-phosphorylated thiamine. Egg samples in 2007 also had slightly higher levels of phosphorylated than non-phosphorylated thiamine although this difference was not statistically significant. The eggs sampled in 2005 showed significant variability and a wide range of thiamine values (Table 1).

Discussion

Egg-thiamine trends and significance

Atlantic salmon are similar to other salmonids in their susceptibility to EMS due to the effects of low egg-thiamine. Recently, Fitzsimons et al. (2007) established ED50 values that represent the mean egg-thiamine concentration below which 50% fry mortality was observed. The ED50s were 1.03, 1.57 and 2.38 nmol/g egg for Chinook salmon *O. tshawytscha*, lake trout *S. namaycush,* and coho salmon *O. kisutch*, respectively. The ED50 for the St. Marys River Atlantic salmon population was approximately 1.0 nmol/g egg (Werner et al., 2006). This result is similar to those found by other groups working with Atlantic salmon (Marcqenski and Brown, 1997; Amcoff et al., 1998, Fisher et al., 1998). Thus, Atlantic salmon, along with Chinook salmon, appear to be on the lower end of this "threshold effect" due to low thiamine than either lake trout or coho salmon above listed species. The detailed reasons for this lower threshold are unclear. As stated previously, the St. Marys River population of Atlantic salmon has been shown to be susceptible to the effects of EMS due to low levels of egg-thiamine (Werner et al., 2006).

During four of the five years of this study period, the St. Marys River population of Atlantic salmon exhibited an average of 3.12 nmol total thiamine per gram of egg (avg. standard error 0.25). However, in 2005, a mean total egg-thiamine concentration of 9.41 nmol/g was observed (Table 1). The sample variance for 2005 was quite high exhibiting a wide range of values between 1.09 and 24.76 nmol total thiamine per gram of egg. Note also that the mean total thiamine value for all other years was at or above the established ED50 of 1.0 nmol total thiamine per gram of egg for this population. It has been suggested, and now convincingly demonstrated, that egg-thiamine concentrations above the ED50 between 2 and 4 nmol/g of egg for most salmonids may cause sub-lethal effects such as reduced predator avoidance or neurologic problems (Fitzsimons et al., 2007, 2009; Carvalho et al., 2009). The mean total egg-thiamine concentrations observed (2003, 2004 and 2006) fall in this range, with 2007 having slightly higher than 4.0 nmol total thiamine per gram of egg. The egg-thiamine concentrations for 2005 are well above this presumed sub-lethal threshold.

The ratio of non-phosphorylated (NPT) to phosphorylated thiamine (PT) found in eggs is also of interest. In general, salmonids have an NPT to PT ratio greater than one, meaning they typically have more non-phosphorylated thiamine (Brown et al., 1998). This is unlike walleye *Sander vitreum* that typically have an NPT to PT ratio less than one (Zajicek et al., 2005; Honeyfield et al., 2007). In 2005, the NPT to