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Early Feeding In Lake Trout Fry (salvelinus Namaycush) As A Mechanism For Ameliorating Thiamine Deficiency Complex

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EARLY FEEDING IN LAKE TROUT FRY (*SALVELINUS NAMAYCUSH*) AS A MECHANISM FOR AMELIORATING THIAMINE DEFICIENCY COMPLEX

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ABSTRACT

Recruitment failure of lake trout (*Salvelinus namaycush*) in the Great Lakes has been attributed in part to the consumption of alewife (*Alosa pseudoharengus*) by adult lake trout, leading to Thiamine Deficiency Complex (TDC) and early mortality in fry. The current understanding of thiamine deficiency in lake trout fry is based on information from culture and hatchery settings, which do not represent conditions fry experience in the wild and may influence the occurrence of TDC. In the wild, lake trout fry have access to zooplankton immediately following hatching; previous studies found that wild fry begin feeding before complete yolk-sac absorption. However, hatchery-raised fry are not provided with food until after yolk-sac absorption, long after the development of TDC. Zooplankton are a potential source of dietary thiamine for wild fry in the early life stages that has not previously been considered in the occurrence of thiamine deficiency. We postulated that wild-hatched fry could mitigate thiamine deficiency through early feeding on natural prey. Specifically, we hypothesized 1) feeding should increase thiamine concentrations relative to unfed fry and 2) feeding should increase survival relative to unfed fry. Feeding experiments were conducted on lake trout fry reared from eggs collected from Lake Champlain in 2014 and Cayuga Lake in 2015. A fully crossed experimental design was used to determine the effect of early feeding by lake trout fry in thiamine replete and thiamine deplete treatments before and after feeding. Overall, thiamine concentrations and survival did not significantly differ between fed and unfed fry. Thiamine concentrations increased from egg stage to hatching in both years, suggesting a potential source of thiamine, which had not previously been considered, was available to the lake trout eggs during development.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Lake trout (*Salvelinus namaycush*) is a top predator in North American aquatic systems and were once an important part of the commercial fishing industry in the Laurentian Great Lakes (Koelz 1926; Berst and Spangler 1973). The species is a priority for restoration where populations have declined or been extirpated (Krueger et al. 1995a; Muir et al. 2012). Populations in the Great Lakes crashed in the 1940s as a result of overfishing and predation by invasive sea lamprey, *Petromyzon marinus* (Coble et al. 1990; Eshenroder 1992; Eshenroder et al. 1995). Restoration programs were established and stocking began as early as the 1950s, but these programs have failed to fully restore lake trout populations nearly 65 years later (Muir et al. 2012a). Currently, self-sustaining populations are only present in Lake Superior and a few localized areas in Lake Huron (Bronte et al. 1995; Reid et al. 2001; Riley et al. 2007). Natural recruitment is increasing in Lake Michigan, but populations are still supplemented with stocking; natural recruitment has not been documented in Lake Erie or Lake Ontario (Markham et al. 2008; Hanson et al. 2013).

Restoration of lake trout is also a management focus in Lake Champlain, which forms the border between New York and Vermont. Lake trout were extirpated from Lake Champlain in the early 1900s, although the cause of decline is not understood (Marsden and Langdon 2012; Plosila and Anderson 1985). Similar to the Great Lakes, a restoration program was developed in the 1970s, but almost no natural recruitment occurs and the population is currently supported almost entirely through

stocking (Ellrott and Marsden 2004). The similarity of the Great Lakes and the Lake Champlain ecosystems provides an interesting opportunity to investigate the challenges of achieving successful restoration of lake trout and compare trophic dynamics in each system.

Lake trout life history

Lake trout is the largest of the charr species, with a native range that extends from the northern boundaries of North America in Alaska, through Canada, to the southern region of the Great Lakes and as far east as Maine and portions of Quebec (Martin and Olver 1980; Scott and Crossman 1973). Introduced lake trout in the western region of the United States have recently become problematic in several lakes, threatening native salmonids (Crossman 1995; Martinez et al. 2009). Lake trout occupy the pelagic zone of lakes, spending the majority of their life below the thermocline (Berst and Spangler 1973; Scott and Crossman 1973).

Lake trout is an extremely plastic species, with variability in morphology and behavior. At least 10 morphotypes were once recognizable, primarily in Canadian waters of Lake Superior, but only four morphotypes remain: siscowet, humper, lean, and most recently identified, redfin (Goodier 1981; Hansen et al. 2016; Muir et al. 2014). Siscowet characteristically has higher body fat content compared to other morphs, a robust body shape, and a blunt snout, and typically occupy depths greater than 100 m (Eschmeyer 1955; Moore and Bronte 2001; Pycha and King 1975). Humper has intermediate body fat and large eyes and occupy midwater shoal areas. Lean lake trout are the most common morph throughout the Great Lakes and have the lowest body

fat content, have a slender body shape with a straight, pointed snout, and typically occupy depths less than 70 m (Moore and Bronte 2001). Redfin was historically described in the Great Lakes by commercial fishermen and naturalists but have only recently been identified as a separate morphotype; they are characterized by a robust body shape, large head and eyes, and red paired fins (Rakestraw 1968; Muir et al. 2014). Differences in body forms of lake trout may be the result of phenotypic plasticity related to environment and feeding habits (Moore and Bronte 2001).

Lake trout reach maturity in six to seven years. The spawning behavior of lake trout is distinguishable from other members of the Salmonidae family in that the species displays no sexual dimorphism, spawns in lakes, and spawns typically at night (Martin and Olver 1980; Esteve et al. 2008). A few populations are known to move into rivers to spawn but generally spawning occurs in lakes at depths ranging from 3 to 80 m (Loftus 1958; Scott and Crossman 1973; Marsden et al. 1995a; Janssen et al. 2006). Siscowet are believed to spawn even deeper than 80 m in Lake Superior but sampling challenges make their spawning behavior difficult to study; no information is available on humpers or redfins (Goodier 1981; Hansen et al. 1995; Lawrie and Rahrer 1973). Spawning typically occurs during the fall, although spawning can begin earlier in northern latitudes and last until January in southern latitudes (Royce 1951; Eschmeyer 1955; Bronte 1993).

Several cues have been associated with the onset of spawning including photoperiod, water temperature, and wind direction (Balon 1980; Martin and Olver 1980; Gunn 1995; Muir et al. 2012). Photoperiod likely initiates gonadal maturation

while temperatures cue the specific timing of spawning (Bromage et al. 2001; Davies and Bromage 2002; Janssen et al. 2007). When water temperatures approach 10°C adult lake trout move onto spawning reefs, with males arriving before females (DeRoche 1969). Clean substrate, free of sediments and organic matter, is critical for egg survival. Lake trout do not construct redds like other Salmonidae. Instead, interstitial spaces are used as a passive form of parental care; after oviposition, negatively buoyant eggs fall between the rocks out of the reach of most predators (Royce et al. 1951; Eschmeyer 1964; Martin and Olver 1980; Gunn 1995; Marsden et al. 1995b). Most spawning behavior takes place at night, after a brief courtship period that includes nudging of sides, nibbling, and swimming in circles (Martin and Olver 1980; Esteve et al. 2008; Muir et al. 2012). Lake trout is a broadcast spawner, with multiple males spawning with each female. The spawning period lasts between 10-20 days and repetitive oviposition may occur (Gunn 1995). The number of eggs produced by a gravid female is dependent upon size and health and ranges from 880 to 2640 eggs/kg of body weight (Scott and Crossman 1973). Differences in spawning among various morphotypes are poorly understood and the majority of information regarding lake trout spawning is based on observations of lean lake trout. Recent research suggests that lake trout spawning behavior may also be plastic; for example, spawning in Yellowstone Lake occurs on substrate that was previously regarded as poor habitat for egg deposition and development, yet the population is increasing exponentially (Beauchamp et al. 1992; Simard et al. *in. prep*.)

The incubation period of lake trout eggs is a function of water temperature and can vary from two to seven months (Royce 1951; Loftus 1958; DeRoche 1969; Bronte et al. 1995; Perkins and Krueger 1995). Dissolved oxygen, light, and egg size also influence development (Kamler 2002). Eleutheroembryos (fry hereafter), the stage following hatching and prior to filling of the swim bladder, hatch in the spring and range from 13-17 mm in length (Balon 1980). Lake trout fry hatch with a large yolk-sac of maternally-deposited nutrients from which they initially feed. Fry are photophobic following hatching and remain within the interstitial spaces of the reef until they reach approximately 25 mm in length. When fry have used up lipid reserves in the yolk sac they move to the surface to fill their swim bladder (Balon 1980). Young of river-spawning salmonids exit redds shortly after filling their swim bladders; however, lake trout fry remain on the reef for several weeks following swim bladder inflation, making nightly migrations from the substrate into the water column to feed (Krueger et al. 1995). Laboratory and culture studies demonstrate that first feeding on exogenous sources begins when the swim bladder is filled and when the yolk sac is still present, allowing for mixed feeding from endogenous and exogenous food sources (Tait 1960; Balon 1980, 1986). Overlap in feeding modes gives young lake trout the ability to practice feeding while still having access to a highly nutritious internal food source (Balon 1986; Jaroszewska and Dabrowski 2011). Once yolk sac absorption is complete, lake trout move off the spawning reefs. In the Great Lakes, fry are typically found at depths less than 20 m during the first two months, moving deeper as the season progresses (Bronte et al. 1995). The juvenile stage begins with completion of

calcification of the skeleton and scales, typically at 50 mm, and lasts until ages 5-7 years (Balon 1980). There are no data available describing potential differences in ontogeny of fry from different morphotypes.

The diet of lake trout changes throughout ontogeny and can vary among lake systems depending on the prey species composition. Lake trout begin their lives as planktivores, feeding primarily on zooplankton, and then shift to piscivory as juveniles when they are no longer gape limited (Martin and Olver 1980; Nunn et al. 2012). Little information is available on the diets of young-of-the year juvenile lake trout in the Great Lakes because of the difficulty of sampling early life stages and previously low natural recruitment. Recent natural recruitment in a few locations has helped fill in information gaps; important prey species for young lake trout include *Mysis diluviana,* Chironomidae, yearling slimly sculpin (*Cottus cognatus*), alewife (*Alosa pseudoharengus*), and rainbow smelt (*Osmerus mordax*) (Elrod and O'Gorman 1991; Roseman 2009). Adult lake trout are primarily piscivorous and feed on the most available prey items. In the Great Lakes, adult lake trout feed on cisco (*Coregonus artedi*), deepwater sculpin (*Myoxocephalus thompsoni*), slimy sculpin, alewife, rainbow smelt, and the young of several species, including lake trout (Van Oosten and Deason 1938; Martin and Olver 1980; Elrod and O'Gorman 1991; Jacobs et al. 2010). However, adults will remain planktivores in fishless lakes and exhibit slower growth and reach significantly smaller maximum sizes (Martin 1952; Paterson 1968). Many changes in the Great Lakes food web have occurred with the decline of lake trout and the invasion of non-native species, causing shifts in diets for lake trout (Christie 1974;

Eck and Wells 1987; McDonald et al. 1990; Knight and Vondracek 1993; Bronte et al. 2003; Riley et al. 2008; Bunnell et al. 2014; Rudstam et al. 2015).

Lake trout in the Great Lakes: causes of decline, restoration challenges, and current status

Lake trout is an important species in the Great Lakes ecologically, culturally, and economically, and were once highly sought after in the commercial fishery. Historically, lake trout were abundant throughout lakes Superior, Michigan, Huron, and Ontario but were only found in the eastern basin of Lake Erie; multiple sub-populations of morphs were reported by fishermen in Michigan, Superior, and Huron (Koelz 1926; Cornelius et al. 1995; Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). Limited information is available about lake trout population dynamics in the Great Lakes prior to population crashes. Lakes Ontario and Erie suffered population declines earlier than other lakes. Lake Erie catches peaked during the late 1800s and decreased in the early1900s (Kozelz 1926). Lake trout populations in Lake Ontario were degraded as early as 1860. The population rebounded briefly, but crashed again in the 1930s (Christie 1973; Baldwin et al. 1979). Catches in lakes Michigan and Huron peaked in the late 1800s and declined throughout the 1900s until collapsing in the 1930-1940s. The fishery in Lake Superior developed more slowly than the rest of the lakes, and the nearshore population did not collapse until the 1950s (Muir et al. 2012). Lake trout were completely extirpated from lakes Erie, Ontario, and Michigan, while remaining populations in lakes Superior and Huron were severely degraded.

Population crashes throughout the Great Lakes have been attributed to over-harvest and sea lamprey predation (Coble et al. 1990; Eshenroder 1992; Eshenroder et al. 1995). A commercial fishery targeting lake trout began in the early 1800s with the settlement of the area, and as fishing technology improved, harvest increased (Koelz 1926). The early fishery was only loosely regulated, and regulations generally did not protect fish during the spawning season. Lake trout harvest ranged from 1.8 to 2.7 million kg annually in each lake prior collapse in the 1940s, with the largest catches from Lake Michigan (Koelz 1926; Cornelius et al. 1995; Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). Stocks were likely declining before the 1940s but the decrease may not have been accurately reflected in harvest rates; as sub-populations in a lake were depleted, fishermen would move further offshore to new grounds, targeting new stocks (Koelz 1926; Eshenroder 1992).

Increased fishing pressure coupled with increasing sea lamprey abundance ultimately led to the basin-wide decline of lake trout. Sea lamprey were historically found in Lake Ontario, but were blocked from the other Great Lakes by Niagara Falls (Applegate 1950). The construction of the Welland Canal, first opened in 1829 and completed in 1932, allowed sea lamprey to invade the rest of the Great Lakes basin. Sea lamprey were first detected in Lake Erie in 1921 and by 1945 they had spread throughout the system (Zimmerman and Bright 1942; Applegate 1950). With an abundance of suitable spawning habitat throughout the basin and no natural predators, the sea lamprey population quickly exploded and lake trout were heavily targeted as prey. Large, adult lake trout have higher likelihood of survival from a sea lamprey

attack than younger, smaller fish (Madenjian et al. 2008), and it was likely combination of the commercial fishery removing large, adult fish and sea lamprey predation on younger fish that led to population crashes in the basin (Schneider et al. 1996; Sitar et al. 1999; Bergstedt et al. 2001; Madenjian et al. 2008a).

In response to the declining lake trout populations, the Great Lakes Fishery Commission was formed in 1955 with the main goals of creating a sea lamprey suppression program and a lake trout restoration plan (Smith and Tibbles 1980). Currently, millions of dollars are spent each year on sea lamprey research and suppression, using lampricides, physical and electric barriers, traps, and pheromones to control populations (Christie and Goddard 2003). Lake trout restoration efforts have been mainly supported through stocking, which began in the 1950s in the Great Lakes and continues in all lakes except Lake Superior, which currently has a self-sustaining population (Lawrie and Rahrer 1972; Ebener et al. 2007). Each fall during the spawning season, eggs and milt are harvested from adult lake trout captured in each lake, which are generally of hatchery origin. Young are raised in hatcheries for one year until reaching approximately 15 cm in total length before being stocked into lakes. Lake trout stocked in the Great Lakes are marked with either a clipped fin or a coded-wire tag inserted in the snout so that they can be identified as hatchery-origin (Elrod and Schneider 1986). Initial stocking was below target levels in most lakes due to challenges of rearing the desired number of lake trout. Fry and fingerlings were initially used for stocking but stocking fry proved to be less effective for increasing abundance than stocking yearlings (Schneider et al 1983).

Lake Superior is currently the only lake considered restored and able to support a commercial harvest. The Lake Superior population was the least depleted of all of the Great Lakes populations and lake trout abundance did not begin to decline until the 1950s (Lawrie and Rahrer 1973; Muir et al. 2012). The population began to increase in 1961 as a result of sea lamprey suppression, stocking to boost remnant populations, and regulations on the commercial fishery (Dryer and King 1968; Curtis 1990; Hansen et al. 1995). Wild recruitment finally became sustainable, and stocking ceased in Michigan waters in 1997, when restoration objectives had been met (Hansen et al. 1995; Hansen 1996; Ebener 2007). The current commercial fishery is regulated using catch quotas (Bronte et al. 2003).

In Lake Huron, following the decline in the main basin, only two small populations remained, in North Bay and in Parry Sound, Georgian Bay, although both populations were severely reduced (Berst and Spangler 1972, Ebener 1998). Lake-wide stocking began in the 1970s but natural reproduction from stocked lake trout was not reported until 1982 in Thunder Bay, near Alpena, MI (Nester and Poe 1984; Weber and Clark 1984; Eshenroder et al. 1995). Trawl surveys during the early 1990s captured wild yearling lake trout in South Bay and juveniles in the Thunder Bay area for the first time since the collapse, indicating that natural recruitment was beginning to occur (Anderson and Collins 1995; Johnson and VanAmberg 1995). The Parry Sound population was considered restored by 1997; its success was attributed to decreased sea lamprey predation, concentrated stocking, and restricted harvests (Reid et al. 2001). Increased juvenile capture rates in U.S. Geological Survey trawls from 2004 to 2006

suggest natural reproduction was also occurring throughout the western basin of Lake Huron (Riley et al. 2007). Increases in lake trout natural recruitment coincided with population crashes of alewife, a species that is believed to have muliple negative impacts on lake trout (Riley et al. 2007; Roseman et al. 2007). Sea lamprey suppression efforts have decreased lamprey wounding rates since 2000 while the relative abundance of wild lake trout in Lake Huron has increased (He et al. 2012). Populations in Lake Huron appear to be headed towards restoration with evidence of widespread natural recruitment in the main lake, and the Great Lakes Fishery Commission's Lake Huron Committee is considering reducing stocking efforts in the main basin (Lake Huron Committee 2016).

Restoration efforts in Lake Michigan began in 1965 with a stocking regime but progress was initially slow. High mortality from sea lamprey predation and harvest resulted in low adult abundances in Lake Michigan at the outset of restoration efforts. Reductions in sea lamprey wounding and increased stocking resulted in greater abundances of adult lake trout. Natural recruitment in Lake Michigan has slowly been increasing although wild (unclipped) lake trout still comprise <50% of the adult population (LMLTWG 2015). Natural reproduction in Lake Michigan has been recorded at multiple sites but very few lake trout have been collected past the early life stages (Marsden 1994; Holey et al. 1995). In 2013, juveniles believed to be wild recruits from the 2007 year-class were captured (Hanson et al. 2013). The Lake Michigan Lake Trout Task Group reported gillnet catches in assessment surveys of up

to 45% wild adult lake trout in southern Lake Michigan in 2015, but catches of wild fish remained low (~3%) in the northern lake near Grand Traverse Bay (LMLTWG 2015).

Progress toward rehabilitation of self-sustaining populations has been slow in both Lake Erie and Lake Ontario. Stocking in Lake Ontario began in 1953 but had little success early on due to high mortality from sea lamprey predation and high exploitation rates (Lantry et al. 2014). Increased stocking, sea lamprey control, and restricted harvest limits resulted in an increase in mature lake trout by the 1990s, and although fry were collected on spawning reefs, no naturally recruited lake trout were collected in assessment surveys (Marsden et al. 1988; Marsden and Krueger 1991; Lantry et al. 2014). Naturally produced lake trout were first detected in Lake Ontario in the mid-1990s, and since then 19 year classes of naturally reproduced lake trout have been recorded but abundances remain low (Lantry and Lantry 2014). Restoration efforts in Lake Erie began in 1969 with stocking, but stocking numbers were below target levels (Markham et al. 2008). Increased stocking and sea lamprey control through the 1980s was able to establish an adult population. Similar to Lake Ontario, eggs and emergent fry have been collected, but the low capture rate of juveniles suggests that lake trout are not surviving past early life stages (Fitzsimons and Williston 2000; Muir et al 2012).

Lake trout are also native to Lake Champlain and historically supported a small commercial fishery, but the population was completely eliminated by 1900 due to unknown causes (Plosila and Anderson 1985; Marsden and Langdon 2012). Historical population dynamics are not well documented; nearshore commercial harvest of lake trout using seines occurred during the fall, often on spawning grounds, during the late

1800s but there are no comprehensive records of catches (Marsden and Langdon 2012). Sea lamprey have been identified as a main cause of lake trout decline in the Great Lakes but their presence in Lake Champlain as a native or exotic species within the lake is disputed (Plosila and Anderson 1985; Bryan et al. 2005; Eshenroder 2014; Marsden and Langdon 2012). Stocking of lake trout began in 1972, and the population is currently sustained entirely through stocking. Hatchery-raised lake trout are fin-clipped to identify them as hatchery origin; a sequence of five fin clips, one of the four paired fins or the adipose fin, is used in a five-year rotation to assist in identifying the year in which an individual was stocked. Evidence of spawning in Lake Champlain has been found at multiple sites, and eggs and emergent fry have been routinely collected, but little to no recruitment has been documented in fall surveys (Marsden et al. 2005; Riley and Marsden 2009).

Several factors may be hindering successful restoration in the Great Lakes and Lake Champlain including low spawner abundance, loss of genetic diversity, non-native species, habitat degradation, and low natural recruitment (Krueger et al. 1995; Bronte et al. 2003; Muir et al. 2012). Although stocking programs have been successful in restoring lake trout populations in lakes Superior and Huron, stocking numbers remain too low in other lakes to adequately boost populations (Bronte et al. 2005; Markham et al. 2008; Lantry et al. 2014). Hatcheries are unable to produce the required number of lake trout due to facility and resource limitations (Hansen et al. 1999; Markham et al. 2008). New understanding of lake trout behavior, especially among different morphs, at different depths and habitats, suggests that stocking locations should be spread out

across each lake and across a gradient of depths (Markham et al. 2008). Although sea lamprey numbers in the Great Lakes have decreased since control programs began, sea lamprey still cause losses of adult lake trout that could impact restoration where adult lake trout numbers are low (Madenjian et al. 2008a; He et al. 2012). Stress from lamprey parasitism may also decrease lake trout growth and reproductive output (Edsall and Swink 2001).

Loss of genetic variation in lake trout populations is also cited as one of the major impediments to restoration (Evans and Willox 1991; Burnham-Curtis et al. 1995; Krueger et al. 1995a). Historic populations have been eliminated from most lakes, causing concern over the loss of genetic variation and limiting genetic resources available for stocking (Krueger and Ihssen 1995). However, only anecdotal evidence of historic morphotypes is available (Eshenroder 1995). Morphotypes may vary in distribution and behavior that could be pertinent to successful restoration. Historically, all broodstocks used for stocking programs in the Great Lakes were derived from the lean morphotype (Page et al. 2004). Genetic strains used for stocking may not reflect historic populations and may not be well adapted to specific systems, resulting in little or slow recovery (Marsden et al. 1989). More recent management plans call for stocking other morphotypes, such as in Lake Erie were the Klondike strain is being stocked (Horns et al. 2003; Markham et al. 2008).

The role of habitat degradation as an impediment to restoration has been debated, but there is no strong evidence in the literature that indicates whether spawning habitat is limited (Elrod et al. 1995; Hansen 1996; Horns et al. 2003; Markham et al.

2008). Anthropogenic stressors, such as contaminants, can lead to increased fry mortality. High levels of TCDD in Lake Ontario have been reported to lead to blue sacs disease and death in lake trout fry (Cook et al. 2003). The invasion of zebra and quagga mussels (*Dreissena polymorpha, D. rostriformis bugensis*) is believed to have negatively impacted quality of spawning reefs, filling in critical interstitial spaces (Marsden and Chotkowski 2001, Bronte et al. 2008). Human activities, such as the construction of breakwalls, have increased available spawning habitat for lake trout (Jude et al. 1981; Nester and Poe 1984; Marsden et al. 1995b). Work is also being done to create artificial reefs and to potentially increase spawning habitat availability (Fitzsimons 1996; Marsden and Chotkowski 2001, Marsden et al. 2016).

All of the Great Lakes and Lake Champlain have experienced changes in ecosystem structure with the decline of native species and the arrival of non-natives (Bronte et al. 1991; Bronte et al. 2003). Over 180 nonidigenous species have been identified in the Great Lakes basin since 2012, and although the impacts of many invaders are not fully understood, several recent aquatic invaders have had direct and indirect negative impacts on lake trout (Mills et al. 2005, Ricciardi 2006). Sea lamprey was one of the main causes of the decline of lake trout. Invasive mussels degrade spawning habitat and cause shifts in the lower food web which can negatively impact lake trout fry (Hall et al. 2003; Barbiero et al. 2012; Pothoven and Fahnenstiel 2015). Alewife have replaced cisco and bloater (*Coregonus hoyi*) as the main diet item for adult lake trout. Alewife were first recorded in Lake Ontario in 1873 and by 1959 they had spread throughout the upper Great Lakes (Smith 1972). Their presence overlapped

both with the lake trout collapse and failed restoration efforts. Increased natural recruitment of lake trout in Lake Huron corresponds with the decline of alewife abundance in the main basin; Lake Michigan alewife populations are similarly declinging, which will may lead to increased natural recruitment of lake trout (Fitzsimons et al. 2010; Riley et al. 2011; Madenjian et al. 2012).

Factors affecting lake trout recruitment

Restoration still has not been fully achieved despite decreased lake trout adult mortality and increased adult numbers. Successful spawning has been documented in each of the Great Lakes and Lake Champlain, but recruitment is limited to lakes Superior and Huron. Eggs have been collected on lake trout spawning sites, and lake trout fry have been captured in the spring in all lakes, but juvenile capture rates remain low in most lakes, suggesting that a bottleneck occurs in the early life stages (Marsden et al. 1988; Fitzsimons and Williston 2000; Riley et al. 2007). The recruitment of early life stages plays a critical role in determining abundances of adult fish (Hjort 1914, Cushing 1974). The lack of natural recruitment of early life stages is one of the biggest challenges to restoring lake trout throughout the Great Lakes. The three major factors that may affect lake trout early life stages are starvation, predation and disease (Jones et al. 1995; Krueger et al. 1995b; Jonas et al. 2005; Riley and Marsden 2009; Zimmerman and Krueger 2009).

Starvation appears to be an unlikely impediment to young lake trout survival (Edsall et al. 2003). Lake trout fry hatch with a yolk sac from which they are able to feed from for several weeks (Balon 1980). First feeding on exogenous food begins

when the yolk sac is still present, allowing for development in foraging skills and variations in temporal food overlap (Balon 1986; Jaroszewska and Dabrowski 2011; Ladago et al. 2016). Zooplankton are generally abundant on spawning reefs when fry begin feeding. For starvation to become a major impediment, a shift in the predator-prey temporal overlap would need to occur. However, although overt starvation may not be occurring, decreasing prey populations may be affecting lake trout recruitment. The burrowing amphipod *Diporeia*, an important food source for young lake trout, declined throughout the Great Lakes in the 1990s and the abundance of zooplankton has also declined over time coincident with expansion of quagga mussels (Nalepa et al. 2000; Scharold et al. 2004; Barbiero et al. 2012; Pothoven and Fahnenstiel 2015). Slimy sculpin, another important prey species for juvenile lake trout, have also declined with the decrease of *Diporeia; Diporeia* are a prey item of slimy sculpin. (Elrod 1983; Christie et al. 1987; Lozano et al. 2001; Owens and Dittman 2003; Mills et al. 2005; Hoyle 2005; Watkins et al. 2007). No research has been done on how declines in these food sources have affected lake trout but decreased food availability could negatively impact survival and fitness.

Lake trout fry are highly vulnerable to predation; native predators in the Great Lakes include burbot (*Lota lota*), sculpin, and crayfish; recent invasive predators include round goby (*Neogobius melanostomus*) and alewife (Jones et al. 1995; Krueger et al. 1995a). Alewife negatively impact lake trout fry by predation as well as competition for zooplankton (Krueger et al. 1995b; Madenjian et al. 2008b; Krueger et al. 2014). Alewife move inshore in the spring with the warming water temperatures,

corresponding to the emergence of lake trout fry from the interstitial spaces of the reef to fill their swim bladder (Krueger et al. 1995b). Fry activity on the reef peaks during the night; alewife activity increases over reefs at night when alewife are feeding (Krueger et al. 1995b; Krueger et al. 1995c; Madenjian et al. 2008b). The fastest recovery of lake trout occurred in Lake Superior, where alewife never became fully established (Miller 1957; Smith 1970; Bronte et al. 1991). Interestingly, alewife did not invade Lake Champlain until the early 2000s but natural recruitment was not occurring prior to their invasion, suggesting that other factors may also be impacting recruitment (Ellrott and Marsden 2004).

The frequency and severity of diseases that have been detected in wild lake trout to date are not sufficient to substantially impact recruitment, but a nutritional deficiency can also cause severe mortality (Fitzsimons et al. 1995a). Unusual levels of mortality among early life stage salmonids were first noted in Great Lakes hatcheries in 1968 (Marcquenski and Brown 1997). Initially, early mortality was overcome by increasing egg collections, but when mortality reached 60-90% the possible cause of high mortality was investigated. Mortality occurring in the early life stages became known as Early Mortality Syndrome (EMS) or swim-up syndrome. Although pollution was initially a primary suspect, environmental contaminants such as PCBs and DDT were eliminated as probable causes for EMS (Mac and Edsall 1991; Mac et al. 1993; Fitzsimons 1995a). Treating eggs with thiamine (vitamin B1) increased survival and reduced the occurrence of swim-up syndrome (Fitzsimons 1995). This was the first study to suggest that EMS could be a result of a vitamin deficiency; many subsequent

studies supported the use of thiamine to treat EMS (Amcoff et al. 1998; Fisher et al. 1996; Wooster et al. 2000; Brown et al. 2005a). Early Mortality Syndrome, subsequently renamed Thiamine Deficiency Complex (TDC), is considered to be one of the major impediments to lake trout restoration (Fisher et al. 1996; Marcquenski and Brown 1997; Brown et al. 2005; Bronte et al. 2008; Muir et al. 2012).

Thiamine, thiamine deficiency, and thiaminase

Thiamine is a water-soluble B-vitamin that is an essential nutrient for all animal species, functioning as a cofactor in metabolic processes for energy metabolism. Thiamine is necessary for organ function, tissue regeneration, and neuronal activity (Manzetti et al. 2014). Thiamine is a coenzyme for several enzymes involved in oxidation-reduction reactions, dehydrogenation, carbohydrate metabolism, as well as the regulation of cellular metabolism; enzymes that require thiamine for energy production include transketolase, pyruvate dehydrogenase, and α-ketoglutarate (Manzetti et al. 2014). There are several forms of thiamine including thiamine pyrophosphate (TPP), thiamine monophosphate (TMP), thiamine triphosphate (TTP) and free thiamine. Thiamine pyrophosphate is the active form used in the pyruvate dehydrogenase complex, which is the first component of the citric acid cycle and involved in the generation of cellular energy (Lonsdale 2006). The metabolic role of other forms of thiamine are debated and still poorly understood. Thiamine is also critical for brain development, function, maintenance, and neuron communication; a lack of thiamine most severely affects the nervous system (Combs 2012). Pyruvate dehydrogenase requires thiamine in the production of the neurotransmitter,

acetylcholine (Heinrich et al. 1973; Elnageh and Gaitonde 1988). Nerve cells also rely heavily on glucose oxidation for their energy needs. Thiamine transport across cell membranes is a relatively slow process, especially across the blood-brain barrier, which is likely why the nervous system is most severely affected by a thiamine deficiency. When thiamine is not present, a build-up of lactic acid occurs, causing a decrease in pH and eventually leading to cell death (Manzetti et al. 2014). Recent research in humans has revealed that thiamine also plays a role in immune system function and inflammatory response (Molina et al. 1994; Yadav et al. 2010; Shoeb and Ramana 2012; Manzetti et al. 2014).

Thiamine is only synthesized by plants, fungi, and bacteria, and therefore must be acquired from the diet for all other taxa (Bender 1999). All forms of thiamine are dephosphorylated in the intestinal tract when ingested and then rephosphorylated by thiamine pyrophosphokinase for use in the cell (Rindi and Laforensa 2000; Settembre et al. 2003; De Jong et al. 2004). The uptake mechanism of thiamine in the intestine is determined by the concentration of thiamine in the gut; at low concentrations (< 2 µM), thiamine is actively transported across the intestinal membrane by organic cation transporters, alkaline phosphatase transporters, and solute carrier proteins, while at high concentrations, thiamine spontaneously diffuses across the membrane (Said et al. 1999; Martel and Keating 2000; Manzetti et al. 2014). Excess thiamine is excreted rather than stored in the body of an organism because it is water soluble, and thus a continuous supply of thiamine is required in the diet. Sources of dietary thiamine for wild fish include phytoplankton and zooplankton (Nishijima et al. 1979; van der Meeren et al.

2008). Several species of freshwater phytoplankton have been shown to produce thiamine, secreting excess thiamine into the water (Nishijima et al. 1979). Thiamine is transferred to higher trophic levels through grazing and predation (Niimi et al. 1997; Fitzsimons et al. 1998). The recommended amount of dietary thiamine for adult fish in culture is at least 3.0 nmol/g for salmonines (Halver 1989; Brown et al. 2005b). Thiamine is not limiting in most aquatic systems, except for deficiencies caused by lack of thiamine or the ingestion of thiaminase, an enzyme that degrades thiamine (Niimi et al. 1997). Plankton and prey fish in the Great Lakes contain thiamine levels high enough to fulfill lake trout thiamine requirements, suggesting that thiamine deficiencies in lake trout are the result of other factors (Fitzsimons et al. 1998; Tillitt et al. 2005).

Aside from a lack of thiamine in the diet, thiamine deficiencies can result from the presence of thiamine antagonists such as oxythiamine, pyrithiamine, and amprolium, or the enzyme thiaminase. Thiamine antagonists have been used in laboratory studies to produce symptoms of thiamine deficiency, producing a deficiency by competing with thiamine for binding sites in enzyme complexes (Rogers 1962; Fynn-Aikins et al. 1998; Honeyfield et al. 1998; Fitzsimons et al. 2001). Fujita (1952) first discovered a thiamine-degrading enzyme while studying shellfish in 1941; two types of enzymes that are known to degrade thiamine have since been identified (Fujita et al. 1952; Fujita et al. 1954). Thiaminase I differs from antagonists in that it destroys thiamine by catalyzing the cleavage between the pyrimidine ring and thiazole moiety of the molecule through an exchange reaction with an organic base (Lonsdale 2006). The exchange reaction results in a product that is no longer thiamine and therefore cannot be

used as a substitute for thiamine by thiamine-requiring enzymes. Thiaminase II degrades thiamine by simple hydrolysis, resulting in the separation of the pyrimidine and thiazole moieties (Toms et al. 2005). Less information is available regarding thiaminase II but it appears to be less common than thiaminase I (Evans 1976, Toms et al. 2005). Thiaminase I (thiaminase hereafter) has been documented in bacteria, plants, dreissenids, and fish, although there is disagreement about the source of thiaminase within these tissues (Honeyfield et al. 2002; Richter et al. 2012).

Thiamine deficiency resulting from the consumption of thiaminase was first characterized in silver fox (*Vulpes vulpes*) when captive individuals fed a diet of raw fish or artificial feed made from fish exhibited symptoms associated with thiamine deficiency (Green et al. 1941; Evans et al. 1942). Thiamine deficiency produces symptoms including ataxia, loss of coordination, paralysis, and eventually death in severe cases (Green et al. 1941; Gnaedinger 1964; Evans et al. 1975; Aulerich et al. 1995). The consumption of fish containing thiaminase has also been linked to thiamine deficiency in other mammals and reptiles, such as river otters (*Lutra Canadensis*) fed a thiaminase-rich diet and Florida alligators (*Alligator mississippienssis)* feeding on shad (Aulerich et al. 1995; Honeyfield et al. 2008; Ross et al. 2009).

The importance of thiamine in fish culture, especially in salmonid rearing, has been noted since the 1940s (Wolf 1942; Woodbury 1943; Phillips 1949). Signs of thiamine deficiency in fish include loss of equilibrium, lethargy, muscle atrophy, whirling behavior, loss of appetite, anorexia, dark discoloration, and ultimately death if left untreated (Woodbury 1943). Low thiamine has also been shown to negatively

impact growth and corneal development (Aoe 1969). Thiamine deficiency complex leads to increased mortality in lake trout fry between hatching and the start of exogenous feeding. Signs of TDC in lake trout fry include loss of equilibrium, lethargy, whirling, hemorrhaging, hyper-excitability, and ultimately death (Fisher et al. 1996).

Thiamine deficiency in lake trout in the Great Lakes has been linked to the consumption of alewife, which contain thiaminase (Gnaedinger 1964; Ji and Adelman 1998; Honeyfield et al. 2005b). The connection between the consumption of alewife and thiamine deficiency was first noted in landlocked Atlantic salmon (*Salmo salar*) in Cayuga Lake (Fisher et al. 1995). Laboratory studies have shown that the consumption of alewife by adult lake trout induces thiamine deficiency in adults and low thiamine levels in eggs (Honeyfield et al. 2005b). Salmonids appear to be especially sensitive to thiamine deficiency, as thiamine deficiency has also been documented in other salmonids in the Great Lakes including steelhead (*Oncorhynchus mykiss*), coho salmon (*Oncorhyncus kisutch*), chinook salmon *(Oncorhynchus tshawytscha*), and brown trout (*Salmo trutta*; Marcquenski and Brown 1997). Atlantic salmon and brown trout in the Baltic Sea also suffer from a thiamine deficiency, termed M74-Syndrome, after adults consume herring (*Clupea harengus membras*) or sprat (*Sprattus sprattus*), species low in thiamine and high levels of thiaminase (Amcoff et al. 2000; Amcoff et al. 1999; Keinänen et al. 2012; Lundstrom et al. 1998; Norrgren et al. 1993). Thiaminase is also present in rainbow smelt, dreissenids, and round gobies, but does not always cause thiamine deficiency for predators of these species (Coble 1965; Tillitt et al. 2009; Tillitt et al. 2005). Interestingly, species associated with the cause of TDC in fishes are all

non-native and most belong to the Clupeidae family (Fitzsimons et al. 2012). Some native species, such as spottail shiner (*Notropis hudsonius*) contain high levels of thiaminase, however, because these species are not heavily targeted as prey by adult lake trout, they are not associated with TDC (Riley and Evans 2008). Cisco and bloater, species that do not contain thiaminase, were once the main forage base for lake trout and other salmonids in the Great Lakes, but foraging has shifted to primarily alewife (Eck and Wells 1987; Bronte et al. 2003;). Studies have shown that fish contain adequate thiamine levels to support thiamine requirements of lake trout, however, consumption of prey containing thiaminase degrades any thiamine from the prey. A diverse diet could mitigate the effects of consuming alewife because less thiaminase would be present to break down thiamine (Fitzsimons et al. 1998; Honeyfield et al. 2005b; Tillitt et al. 2005).

The source of thiaminase in alewife and other fish species is poorly understood. The bacteria *P. thiaminolyticus* found in the gut of alewife was thought to be the source of thiaminase, however, this has been refuted (Richter et al. 2012). Recent research suggests that interactions between fatty acids and thiamine may influence the occurrence of thiamine deficiency (Pickova et al. 1998; Czensy et al. 2009; Czensy et al. 2012). An unbalanced diet of prey rich in fat by adult salmonids has been proposed as the cause of thiamine deficiency in the Baltic Sea, rather than solely the consumption of thiaminase-rich prey (Mikkonen et al. 2011). Further investigation of the source of thiaminase is required to determine the source of thiaminase.

The early life stages of lake trout are especially sensitive to thiamine deficiency and TDC has been identified as a factor limiting natural recruitment (Bronte et al. 2008). Adult lake trout are able to mitigate the effects of a thiamine deficiency by feeding on a diverse diet if a diverse forage base is available. However, if the diet consists primarily of thiaminase-containing fish, upon reaching maturity, adult lake do not allocate an adequate amount of thiamine in their eggs (Brown et al. 1998; Amcoff et al. 1999; Honeyfield et al. 2005b). Fry affected by TDC die between hatching and the swim-up stage. To avoid overt mortality, eggs require a minimum concentration of 1.5 nmol/g thiamine (Brown et al. 1998; Honeyfield et al. 2005a; Fitzsimons et al. 2009). Individuals from eggs with thiamine levels above the minimum requirement but below 8 nmol/g may exhibit secondary effects of thiamine deficiency which can impact larval growth, feeding efficiency, and predator avoidance (Ojanguren and Brana 2003; Carvalho et al. 2009; Fitzsimons et al. 2009). Eggs collected from wild broodstock receive a thiamine treatment in the hatchery to prevent the occurrence of TDC. Restoration plans for the Great Lakes list increasing egg thiamine levels as a goal toward rehabilitation; in Lake Michigan, managers have set a target level of egg thiamine concentrations greater than 4 nmol/g (Bronte 2008).

Evidence of TDC in wild lake trout fry is not well documented; early life stages are challenging to study in the wild because lake trout fry can be difficult to locate and collect, and wild lake trout fry affected by TDC may not be susceptible to traditional capture methods. The current understanding of thiamine deficiency in lake trout fry is based on culture and hatchery settings which do not fully mimic the conditions this life

stage experiences in the wild. Hatcheries and culture studies use artificial feed formulated to meet nutritional requirements, but because fry are not responsive to immobile food until they have used up internal food reserves, food is withheld from fry until after yolk-sac absorption (Atkins 1905; Skoglund and Barlaup 2006). The effects of TDC manifest before yolk-sac absorption is complete and prior to feeding in the hatchery. However, previous research indicates that wild fry begin feeding before the yolk sac is fully absorbed. At the onset of initial feeding by lake trout fry, the yolk sac is still present, allowing for mixed feeding from endogenous and exogenous sources (Balon 1986). Studies have shown mixed feeding is advantageous and results in increased growth and survival (Wallace and Aasjord 1984; Koss and Bromage 1990). Wild fry are exposed to a diverse community of prey items that contain thiamine immediately following hatching that could allow fry to acquire sufficient thiamine to satisfy metabolic needs (Atkins 1905; Hutchinson 1943; Leitritz and Lewis 1976; Balon 1980; Hardy and Barrows 2003; Skoglund and Barlaup 2006; van der Meeren et al. 2008; Waagbo 2010; Ladago et al. 2016). Copepods, cladocerans, and rotifers are important prey items for lake trout fry. Marine copepods are reported to contain 7.6 nmol/g thiamine for the average wet weight (van der Meeren et al. 2008); no data are available for freshwater copepods. Daphnia is known to contain thiamine, however concentrations were reported in volume rather than dry weight, and no recent studies report thiamine concentrations (Hutchinson 1943). As fry grow in size and transition to the juvenile stage, *Mysis* and *Diporeia* become important food sources. *Mysis* contain

0.3 nmol/g thiamine and while *Diporeia* contain 0.64 nmol/g thiamine (Niimi et al. 1997).

Research is needed to determine whether TDC occurs in wild fry, and whether early consumption of thiamine-rich prey mitigates thiamine deficiency. The purpose of this thesis was to determine if early feeding on natural diet items by thiamine-deficient lake trout could sufficiently replenish thiamine concentrations. If wild fry are able to restore thiamine levels by early feeding on zooplankton, then relationship between egg thiamine levels and mortality would need to be revised to account for the thiamine acquired during early feeding. If even a very thiamine-deplete fry could restore thiamine levels by early feeding, then TDC may not limit successful restoration in the Great Lakes and Lake Champlain and other factors impeding recruitment need to be further investigated.

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CHAPTER 2: THE EFFECT OF EARLY FEEDING ON THIAMINE CONCENTRATIONS IN LAKE TROUT FRY

Introduction

Lake trout were an important part of the commercial fishing industry in the Great Lakes until populations crashed in the 1940s, likely caused by a combination of overfishing and sea lamprey predation (Coble et al. 1990; Eshenroder 1992; Eshenroder et al. 1995). Restoration efforts were implemented through sea lamprey control and stocking, beginning in the 1950s and continuing in all lakes except Lake Superior, where self-sustaining populations have been restored (Krueger et al. 1995; Muir et al. 2012). Despite extensive stocking and evidence of spawning at many sites, natural recruitment remains low in the lower four Great Lakes, except for a few localized areas in Lake Huron (Reid et al. 2001; Riley et al. 2007). Poor recruitment of lake trout has been attributed to habitat degradation, genetic incompatibility of stocked strains, alewife predation on emergent fry, and, most recently, early life stage mortality caused by thiamine deficiency (Jones et al. 1995; Krueger et al. 1995; Bronte et al. 2003; Muir et al. 2012).

Thiamine deficiency, caused by consumption of thiaminase-containing prey, emerged as an impediment to lake trout (*Salvelinus namaycush*) restoration efforts in the early 1990s. Acute thiamine deficiency results in high mortality of fry at emergence (also referred to as swim up stage), prior to first feeding, in the absence of live prey. However, incidence of mortality due to thiamine deficiency in the wild has not been well documented. We postulated that early feeding by wild fry on zooplankton may

restore thiamine concentrations to offset a thiamine deficiency, and we used laboratory experiments to test our hypothesis.

Thiamine deficiency complex (TDC), previously termed Early Mortality Syndrome, leads to increased mortality in lake trout fry between hatching and the start of exogenous feeding (Fitzsimons 1995b; Fisher et al. 1996; Brown et al. 2005). Thiamine is an essential B-vitamin that is required as a co-factor in energy metabolism (Lonsdale 2006). Thiamine is rarely limited in aquatic environments, and a deficiency typically results from presence of thiaminase in the diet, an enzyme that degrades the molecular structure of thiamine (Fujita et al. 1952; Niimi et al. 1997). New research also suggests lipid-rich prey are an important factor in the occurrence of TDC (Czensy et al. 2009; Czensy et al. 2012). Thiamine deficiency in lake trout has been linked to the consumption of alewife (*Alosa pseudoharengus*), an invasive species in the Great Lakes that contains thiaminase (Fisher et al. 1995; Amcoff et al. 2000; Honeyfield et al. 2002; Fitzsimons et al. 2005; Honeyfield et al. 2005; Keinänen et al. 2012). Adult lake trout that have consumed a thiaminase-rich diet do not allocate enough thiamine to their eggs during oogenesis, such that fry hatch with insufficient thiamine levels (Amcoff et al. 1999). Thiamine deficiency most severely affects the nervous system; signs of TDC in lake trout fry include lethargy, poor swimming ability, loss of equilibrium, and whirling behavior, and acutely low deficiency can result in death (Fitzsimons 1995b; Fisher et al. 1996; Marcquenski and Brown 1997; Brown et al. 1998; Carvalho et al. 2009). Thiamine treatments are used in hatchery production during the egg stage to increase survival (Fitzsimons 1995b). Eggs collected for stocking programs are placed in an

aqueous thiamine solution following fertilization and thiamine is absorbed by the embryos. This method reduces mortality associated with TDC in hatcheries.

Most information about TDC in lake trout is derived from hatchery and laboratory culture settings but evidence of TDC in wild lake trout fry is not well documented. Culture and feeding practices in hatcheries and laboratories differ from natural settings, and may influence the occurrence of TDC. In hatcheries, food is withheld from fry until yolk-sac absorption is complete because fry are unresponsive to non-moving prey, i.e., hatchery feeds (Atkins 1905 ; Leitritz and Lewis 1976; Hardy and Barrows 2003). Wild fry begin feeding on exogenous food sources within two weeks after hatching, which is substantially prior to complete yolk sac absorption (Ladago et al. 2016). At this stage, fry experience a period of mixed feeding on both internal and external food sources (Balon 1986; Ladago et al. 2016). Mixed feeding provides an opportunity for fry to practice prey capture while accessing a highly nutritious endogenous resource (Balon 1986; Jaroszewska and Dabrowski 2011). The yolk sac acts as insurance against starvation that may result from fluctuations in prey availability and newly developing prey capture abilities. Temporal overlap in feeding types also allows fry to mediate mild nutritional deficiencies and may allow lake trout fry to acquire sufficient thiamine from exogenous food sources to offset low yolk-sac thiamine levels (Jaroszewska and Dabrowski 2011). Upon hatching, wild fry are exposed to a diverse community of prey items, including copeopods, rotifers, *Mysis*, and small benthic macroinvertebrates (DeRoche 1969; Ladago et al. 2016). Zooplankton provide an available source of thiamine to lake trout fry in the wild

(Hutchinson 1943; van der Meeren et al. 2008; Waagbo 2010), whereas dietary thiamine is not available to cultured fry until after TDC has manifested. Although there is no data available for thiamine concentrations in freshwater copepods, marine copepods are reported to contain 7.6 nmol/g thiamine for the average wet weight (van der Meeren et al. 2008)). *Daphnia* contain thiamine, however concentrations were reported in volume rather than dry weight, and no recent studies report thiamine concentrations (Hutchinson 1943). As fry grow in size and transition to the juvenile stage, *Mysis* and *Diporeia* become important food sources (Martin and Olver 1980; Elrod and O'Gorman 1991; Roseman 2009; Nunn et al. 2012). *Mysis* contain 0.3 nmol/g thiamine and while *Diporeia* contain 0.64 nmol/g thiamine (Niimi et al. 1997).

We postulated that wild-hatched lake trout fry can mitigate thiamine deficiency through early feeding on natural prey. We tested two hypotheses in laboratory culture experiments: 1) fry fed zooplankton will have higher thiamine concentrations relative to unfed fry (Figure 1a), and 2) fry fed zooplankton will have higher survivorship relative to unfed fry (Figure 1b). If our hypotheses are supported, future research can focus on the effects of zooplankton community composition and prey preferences on the rate of thiamine uptake by fry.

We tested these hypotheses using lake trout from Lake Champlain, VT and Cayuga Lake, NY. Thiamine deficiency complex connected with the consumption of alewife was first reported in Cayuga Lake in Atlantic salmon, *Salmo salar* (Fisher et al. 1995; Fisher et al. 1996). Lake trout in Cayuga Lake also suffer from critically low thiamine levels and no natural recruitment is occurring (Rinchard et al. *in prep*.). No

diet data are available for adult lake trout in Cayuga Lake but alewife are present and likely a major prey item for lake trout. Lake trout are native to Lake Champlain but were extirpated in 1900 due to unknown causes (Plosila and Anderson 1985; Marsden and Langdon 2012). Stocking of lake trout began in 1972 and the population is currently sustained entirely through stocking (Ellrott and Marsden 2004). While the lack of successful restoration in the Great Lakes is attributed to thiamine deficiency linked to the consumption of alewife (Fisher et al. 1995; Honeyfield et al. 2005), the factors impeding lake trout restoration in Lake Champlain are less understood. Alewife were not found in Lake Champlain until 2003, but natural recruitment was not evident prior to the arrival of alewife. Thiamine concentrations in lake trout eggs were above 4 nmol/g, the target concentration set by managers in the Great Lakes as a restoration objective, prior to the presence of alewife and decreased following the invasion (Bronte 2008; Ladago et al. *in review*.).

Methods

A fully crossed experimental design was used to determine the effect of early feeding by lake trout fry in thiamine replete and thiamine deplete conditions in the presence or absence of feeding.

Lake trout eggs were collected from mature lake trout from Gordons Landing, Grand Isle, VT, on Lake Champlain early November 2014. Adults were collected in trapnets by the Vermont Department of Fish and Wildlife as a part of their annual sea lamprey wounding survey. Eggs were collected by manually stripping 53 ovulating females into plastic containers. A 5 g sub-sample of eggs from each female was

collected and frozen immediately on dry ice for subsequent thiamine analysis. Milt from 4-5 male lake trout was collected into 50 mL vials. Lengths and weights of females were recorded, and all fish were released. Collected eggs and milt were stored separately in a cooler for approximately two hours at 10°C during transportation to the Rubenstein Ecosystem Science Laboratory in Burlington, VT. Sperm was checked for motility and eggs were fertilized with pooled milt from three males using the dry method and rinsed with dechlorinated water after 5 min. Each egg family was split in two and kept separately in 10.2 cm diameter polyvinyl chloride (PVC) containers (schedule 40 sewer drain caps), each with a screen bottom and a removable screen top (2 mm^2 mesh). One group from each family was treated with an aqueous solution of 5,000 ppm thiamine monophosphate and 50 ppm betadine bath for 1 hour for disinfection (hereafter referred to as "treated"). The corresponding group of eggs from each family was not treated with thiamine but was treated with betadine (hereafter referred to as "untreated"). Eggs were rinsed with dechlorinated water (Burlington, VT city water) and placed in Heath tray incubators on a recirculating water system. Incubator flow rates were adjusted to 13-15 L/min, the temperature was set to 10°C, and incubators were covered with black plastic sheeting to exclude light; temperature was slowly decreased to 6°C over several weeks. Eggs were checked daily until hatching and all dead or unfertilized eggs were removed. Following the results of the initial egg thiamine analysis, families were split into two groups. Families with initial thiamine concentrations below 4.0 nmol/g were designated as low thiamine and families with thiamine above 4.0 nmol/g were designated as high thiamine. Families with similar

thiamine concentrations at the egg stage were reduced so that no duplicate concentrations remained; 16 families were removed, leaving 25 low thiamine families and 12 high thiamine families. The fully crossed design was used in each of the low and high initial thiamine families.

Six 151 L fish tanks, three replicate tanks for each feeding treatment, were set up for feeding experiments (Figure 2a). Tanks were placed inside a chilled water bath to maintain water temperature at 8° C and filled with clean, dechlorinated water; water in tanks was neither flow through nor re-circulating and was changed frequently to maintain water quality. Water quality was assessed by measuring the pH and ammonia levels. A pH of 7.0 and an ammonia level of 0 ppm was considered good water quality. Hatching began at 448 degree days (dd). When at least 50% of the eggs had hatched in a family, 400 fry from the family were randomly selected, split in half again, and transferred to a 15.2 cm x 12.7 cm x 10.2 cm mesh box constructed of #10 plastic canvas mesh (2.5 mm^2 mesh). One box from each family was placed in a "fed" treatment tank, the other in the "unfed" tank. The fed group was given a mixture of zooplankton daily and the unfed group was not presented with exogenous food. The zooplankton mixture consisted of copepods, *Moina* sp., and *Daphnia magna*. Zooplankton were sourced from Sachs Systems Aquaculture Inc. in Augustine, Florida, and cultured in 151 L tanks, separated by species, filled with dechlorinated water at 25 °C. Zooplankton cultures were fed an algal culture, *Ankistrodesmus*; algae was cultured in Modified Bold 3N media. Fry began feeding on zooplankton at 748 dd (37 days post-hatch). The temperature of the tanks was slowly increased from 8° to 11°C throughout

the treatment period to mimic the natural temperatures fry would experience in the wild following hatching. Each week, thirty fry from each family of each treatment group were randomly selected, euthanized using Aqui-S, rinsed with de-ionized water, then spread out between sheets of Glad Press-n-Seal and stored at -80°C until thiamine analysis was conducted. Fry were observed for signs of thiamine deficiency, such as lethargy, lordosis, loss of equilibrium, and poor swimming (Fitzsimons 1995b; Fisher et al. 1996). The experiment lasted until 981 degree days (61 days post-hatch); any remaining fry were euthanized and discarded.

In 2015, lake trout eggs were collected in early October from Cayuga Lake, NY at Taughannock Falls State Park by the New York State Department of Environmental Conservation as a part of their annual stocking program. Unfertilized eggs were collected from 21 ovulating females, and stored in separate plastic containers at 4°C; a 5g subsample of eggs from each female was collected for initial thiamine analysis. Milt was collected from 10 males and stored on ice in individual plastic cups. Eggs were placed in a cooler with ice to maintain temperature; a layer of newspaper was placed between the containers and ice to prevent eggs from coming in direct contact with ice. Sperm containers were placed directly on ice. Gametes were transported to The College at Brockport-State University of New York for fertilization. Sperm from three males was added to each container of eggs, after one minute, eggs were rinsed with water to remove excess sperm. One group from each family was treated with an aqueous solution of 1,000 ppm thiamine mononitrate for two hours and the corresponding group was immersed in a dechlorinated water bath for an equal time for control. Each group

was then placed in a 50 ppm betadine bath for 1 hour for disinfection (hereafter referred to as "treated"). Eggs were reared at The College at Brockport for approximately one week at 9°C before being transport to Burlington, Vermont. For transportation, fertilized eggs were placed in separate 10.2 cm diameter polyvinyl chloride (PVC) containers (schedule 40 sewer drain caps), each with a screen bottom and a removable screen top (2 mm^2 mesh). Containers were placed inside coolers with chilled dechlorinated water and an air bubbler; thiamine treated and untreated families were transported in separate coolers to prevent any contamination. Ice was added periodically to maintain temperature at 9°C. Upon arrival, containers were transferred to Heath tray incubators. Treated and untreated families were kept in separate incubators and maintained on separate flow-through water systems. Eggs were checked routinely and any dead were removed. Temperature was slowly decreased to 4°C to further delay development. Hatching occurred at 478 dd.

Two 150 µm Nitex mesh cages with an open top, 60.9 x 40.6 x 53.3 cm, were designed to keep zooplankton inside the appropriate feeding regime. Cages were submerged in each of two 416 L re-circulating tanks with the top of each cage remaining above the water level for access. In each tank, one cage received zooplankton and the other did not (Figure 2b). When at least 50% of the eggs had hatched in a family, 400 fry from the family were randomly selected, split in half, and each half was transferred to a 15.2 cm x 12.7 cm x 10.2 cm mesh box constructed of #10 plastic canvas mesh (2.5 mm^2 mesh). One box from each family was placed in a "fed" treatment cage, the other in the "unfed" cage. The fed group was given a mixture of

zooplankton daily, consisting of copepods, *Moina* sp., and *Daphnia magna*; the unfed group was not presented with exogenous food throughout the experiment. Zooplankton were sourced from Sachs Systems Aquaculture Inc. in Augustine, Florida, and cultured in 151 L tanks, separated by species, filled with dechlorinated water at 25 ℃. Zooplankton cultures were fed an algal culture, *Ankistrodesmus*; algae was cultured in Modified Bold 3N media. Zooplankton were added to the fed tank through the open top. Thirty fry from all lake trout families from each treatment were sampled when fry were transferred to the boxes to serve as a hatching reference point. Weekly sampling of fry began one week after feeding was confirmed, which occurred at 650 dd (26 days post-hatch). Thereafter, families were examined weekly for signs of thiamine deficiency, i.e. lethargy, lordosis, loss of equilibrium, and poor swimming ability (Fitzsimons 1995a; Fisher et al. 1996). Thirty fry from each family from each treatment were randomly selected, euthanized using Aqui-S, rinsed with de-ionized water, then spread out between sheets of Glad Press-n-Seal and stored at -80°C until thiamine analysis was conducted. The experiment lasted until 1098 dd (68 days post-hatch); any remaining fry were euthanized and discarded.

Thiamine analysis

Unfertilized eggs were analyzed in two duplicates from each female fish before eggs were split in to treatment groups. Fry in each treatment comparison (i.e., fed/unfed, and thiamine treated/untreated) were analyzed by family group. Any remaining yolk was removed from fry prior to analysis to avoid potential interference of the yolk lipids in the analytical process; sample weights were recorded after yolk-sac removal. To test

the assumption that all thiamine in the hatched fry was contained in the fry body, i.e., no thiamine was contained in the remaining yolk sac, we performed thiamine analysis on four families with and without the yolk-sac.

Two batches of ten fry from each treatment comparison were used as replicates to evaluate analytical error; if replicates differed from one another by more than 20%, the samples were re-run. If the error was not reduced, the samples were omitted from the data set. Thiamine analysis of 2014 samples was completed on fry samples at week 3 (587 dd) and week 6 (981 dd), any difference between treatments that occurred throughout the experiment would have been apparent at these two time points. Thiamine analysis of 2015 samples was completed on fry samples at hatching (478 dd), weeks three (855 dd) and six (1098 dd). Samples at hatching were collected before families were split into unfed and fed groups, resulting in one value for untreated and one for treated.

Thiamine analysis was performed at The College at Brockport-State University of New York. Individual thiamine vitamers (free thiamine, thiamine monophosphate, and thiamine pyrophosphate) were extracted from lake trout eggs using 6 mL of trichloracetic acid (2%) and 4 mL trichloracetic acid (2%) for fry, and quantified using a high-performance liquid chromatography (HPLC) system as described by Brown et al. (1998) with modifications. The HPLC system was an Agilent 1200 series which includes a delivery pump, an automatic sample injector, a Hamilton PRP-HI column (150 x 4.1 mm: 5 µm mesh size) with attached guard column (25 x 2.3 mm; 12 to 20-mm mesh size), and a fluorometric detector (375-nm excitation wavelength and 433-nm

emission wavelength for thiochrome detection). The column thermostat was set to 30°C. The mobile phase comprised 25 mM potassium phosphate buffer (pH 8.4) and was applied for the first minute. After one minute, the mobile phased was changed to 0.5% N,N-dimethylformamide (DFM), 25 mM potassium phosphate (pH 8.4) and this was run until five minutes. After five minutes, the mobile phase was changed to 10% DFM, 25 mM potassium phosphate (pH 8.4) and ran to nine minutes. At nine minutes, the mobile phase was changed to 30% DFM, 25 mM postassium phosphate (pH 8.4) and ran to 11 minutes. At 11 minutes, the mobile phase was changed to 35% DFM, 25 mM potassium phosphate (pH 8.4) and was ran to 11.1 minutes. Afterwards, the mobile phase returned to the initial conditions, equilibrating the column for the next sample. Flow rate was 1.0 mL/minute and total run time was 25 minutes. A six-point standard curved was performed (0, 1, 2.5, 5, 10, 30 nmol/g) at the start of each group of samples run, and blank was ran as well. Based on the data from the standard curve, the levels of each vitamer were calculated and expressed as nmol/g.

Statistical analysis

The difference in thiamine concentrations in fed and unfed fry in the untreated group was calculated to test the hypothesis that fry fed zooplankton differ in thiamine concentration relative to unfed fry. In 2014, thiamine content of fry at week 6 (981 dd) was initially compared between fed and unfed fry from the untreated group with low initial thiamine. A paired t-test ($\alpha = 0.05$) was used to compare 5 untreated families that had sufficient surviving unfed and fed fry at week 6. This analysis was repeated with 5 families from the thiamine-treated fed and unfed fry of the low initial thiamine group at

6 weeks. The same analysis was completed on 14 families of untreated and 5 families of thiamine treated, fed and unfed fry of the high initial thiamine group at week 6. In 2015, thiamine content of fry at week 6 (1098 dd) was initially compared between fed and unfed fry for 16 untreated families using a paired t-test ($\alpha = 0.05$). This analysis was repeated with 12 thiamine treated families. A paired t-test was also used to compare the individual vitamers for each of the treatment. If feeding raised thiamine concentrations, the difference between these groups was expected to be greater than zero; then the analysis would have been performed on samples from week 3 to determine when feeding began to impact thiamine concentrations. A paired t-test was also used to test if survivorship between unfed and fed fry for both the untreated and treated groups was significantly different ($\alpha = 0.05$); 20 families had sufficient fry in both groups for analysis. The original study design used 20 pairs of fed and unfed in each treatment; an a priori power analysis showed that an effect size >0.66 was needed to have sufficient statistical power ($\alpha= 0.05$, $\beta =0.80$).

To determine if removing the yolk sac prior to thiamine analysis significantly changed the total thiamine (i.e. removing yolk sac did not cause an increase in thiamine concentration by decreasing the weight of the sample) a paired t-test ($\alpha = 0.05$) was used. Thiamine analysis was complete on 4 families of fry from the same family and treatment group at week 0 with and without yolk-sacs.

Results

2014

The mean (± standard deviation) egg thiamine level from lake trout eggs collected from Lake Champlain in 2014 was 4.64 ± 1.80 nmol/g. The low thiamine group comprised 12 families with a mean egg thiamine of 2.96 ± 0.92 nmol/g. The high thiamine group included 25 egg families with a mean egg thiamine of 5.42 ± 1.58 nmol/g.

Thiamine concentrations of fry from the high initial thiamine, untreated group were significantly different between unfed and fed fry, however, contrary to our hypothesis, thiamine was higher in the unfed fry ($\bar{X}^{\text{Untreated/Unfed}}$ = 6.44 nmol/g ± 0.68, $\bar{X}^{\text{Untreated/Fed}}$ = 5.97 nmol/g ± 0.93, N =14 paired within-family comparisons; P = 0.01, β =0.38). There were no significant differences (P > 0.05) between unfed and fed fry in both the untreated and treated low initial thiamine group, or the high initial thiamine, treated group. Concentrations of the vitamer thiamine pyrophosphate (TPP) were significantly different for unfed and fed fry from the high initial thiamine, untreated group, however, TPP was higher in unfed fry ($\bar{X}^{\text{Untreated/Unfed}}$ = 6.25 nmol/g ± 0.62, $\bar{X}^{\text{Untreated/Fed}}$ = 5.48 nmol/g ± 1.69, N =14 paired within-family comparisons; P = 0.03). Concentrations of thiamine monophosphate were significantly different between unfed and fed fry of the untreated group with high initial thiamine ($\bar{X}^{\text{Untreated/Unfed}}$ = .19 nmol/g ± 0.13, $\bar{X}^{\text{Untreated/Fed}}$ = 0.13 nmol/g ± 0.12, N =16 paired within-family comparisons; P = 0.01). There were no significant differences for the free thiamine vitamer of the high initial thiamine groups. There were no significant differences for each of the different

thiamine vitamers, thiamine pyrophosphate, thiamine monophosphate, and free thiamine, among unfed and fed fry of the low initial thiamine, untreated group or of the unfed and fed fry of the high initial thiamine, treated group. In 2014, mean thiamine concentrations for all treatments increased from egg stage to hatching and then decreased to week 6 (Table 1, Figure 3). This change was not due to differential distribution of thiamine in the fry body versus the yolk sac, which we removed before analysis, as there were no significant differences in thiamine content of fry with their yolk-sac in comparison to fry with yolk-sac removed prior to thiamine extraction. No visible signs of TDC occurred throughout the experiment in any treatment groups. Survival could not be calculated for the 2014 samples due to air system failures resulting in loss of all or part of each family, leaving insufficient fry for survival analysis.

2015

The mean egg thiamine from eggs collected from Cayuga Lake in 2015 was 10.65 ± 7.02 nmol/g with a range of 1.94 to 28.14 nmol/g. Only three of the twenty families at the egg stage were below the 4 nmol/g threshold that would be expected to show thiamine deficiency signs. There were no significant differences between thiamine concentrations of fry analyzed with and without yolk sacs. Thiamine concentrations of the untreated group in 2015 were not significantly different ($P > 0.05$) between unfed and fed fry. There were no significant differences between the unfed and fed fry of the treated group. There were no significant differences among treatment

groups for each of the different thiamine vitamers, thiamine pyrophosphate, thiamine monophosphate, and free thiamine. Overall, mean thiamine concentrations for all treatments in 2015 increased from egg stage to hatching, then decreased to week 6 (Table 2, Figure 4).

In 2015, the mean survival of the unfed and fed fry in the untreated group was 86.5 ± 11.2% and 88.3 ± 8.7% (Figure 5). Mean survival of the fed and unfed fry in the treated group was 84.2 ± 13.6% and 85.5 ± 11.57%. There were no significant differences in survival between unfed and fed fry for either treatment ($P > 0.05$). Several families displayed signs of TDC, specifically lethargy, however, no families in 2015 had high mortality throughout the experiment.

Discussion

The results of this study suggest either that lake trout fry are unable to increase thiamine through feeding on zooplankton or thiamine metabolism is greater than uptake of thiamine through feeding. We hypothesized early feeding by lake trout fry could sufficiently restore thiamine levels to ameliorate thiamine deficiency. We expected low-thiamine, fed fry would show an increase in thiamine concentrations relative to the unfed group of the corresponding families. Thiamine treated families were expected to have sufficient thiamine to fulfill metabolic needs, therefore the effect of feeding on thiamine concentrations would be minimal. In fact, there were no significant differences between any of our comparisons except in 2014, when feeding slightly but significantly *decreased* thiamine levels. A greater difference between mean thiamine was expected for the fed and unfed treatment groups. The differences between means in our

experiments were reflected in the TPP vitamer, however, these differences were not biologically significant enough to make a difference in the occurrence of TDC in fry. Feeding was expected to have a greater impact on fry with low thiamine concentrations (< 4.0 nmol/g); however, the fry in our experiments had thiamine concentrations above 4.0 nmol/g at hatching. Thiamine concentrations of fry decreased throughout the post-hatch period but never fell below 4.0 nmol/g. Thiamine is water soluble and excess is not stored in the body, therefore when thiamine concentrations in fry were above the metabolic requirements, thiamine concentrations would not be expected to change from feeding. Consequently, the fry we used may not have had sufficiently low thiamine to detect the effect of feeding on thiamine levels.

Our results do not support our second hypothesis that survivorship would be higher in families fed zooplankton. We expected that survival would be higher in untreated, fed families relative to untreated, unfed families and we expected survival to be higher in thiamine-treated families relative to the untreated families. Overall, survivorship did not differ among treatments. High survival of all families could be related to high thiamine levels at hatching and throughout the experiment.

Increases in thiamine concentrations from fertilization to hatching in the untreated fry were unanticipated. The treated group was expected to increase thiamine from egg to hatching because they received a thiamine treatment during fertilization, whereas thiamine concentrations were expected to decrease in the untreated group during egg development to hatching. Little information is available on the metabolism of thiamine in lake trout eggs and the few studies that have focused on eggs did not

measure thiamine from egg stage to hatching, usually measuring thiamine later in development when TDC signs and mortality occur. Thiamine is assumed to decrease throughout development as maternal sources of thiamine are used for energy metabolism. The increases in thiamine from egg to hatching in both years suggest that there was an exogenous source of thiamine that lake trout eggs were able to absorb. Possible causes of the thiamine increase from egg stage to hatching include contamination, analytical errors, thiamine production by bacteria, differential mortality of eggs dependent on thiamine levels, and absorption of available thiamine from the water in which eggs were reared in.

Contamination and analytical errors can be eliminated as possible causes for the increase in thiamine. Contamination between untreated and thiamine treated groups in 2014 could have occurred, as the untreated and thiamine treated eggs were raised in separate incubators on a re-circulating water system that mixed the water in a head tank. Excess thiamine from the thiamine-treated group could have leached into the water and been absorbed by the untreated group, resulting in all treatment groups having similar thiamine concentrations at hatching. However, in 2015 treatment groups were completely isolated from each other and kept on separate water sources throughout rearing. Analytical errors could have occurred during thiamine analysis but would have been detected, as replicate samples were run at each time point for each family measured. If replicates had >20% difference in thiamine the samples were re-run and any samples still with >20% difference were eliminated from the analysis.

Bacteria in the intestinal tract of humans are known to synthesize thiamine that can be absorbed by the gut for use (Burkholder and McVeigh 1942; Aruguman et al. 2011; Nabokina et al. 2014). Bacteria could have become established in the lake trout embryos and synthesized thiamine that was available for uptake, though this has not been studied in lake trout. Lake trout eggs were disinfected with a betadine solution during water hardening, but this process may not eliminate all bacteria (USFWS 2004). Water used for rearing was de-chlorinated water from the Burlington Water Treatment Facility; standard water treatment should eliminate any living bacteria in the water. Thus, bacterial contamination does not appear to be a likely source of thiamine in eggs.

If thiamine concentrations vary substantially among eggs from a single female, high mortality during the egg stage could have led to skewed results from the feeding experiment. Egg thiamine measurements were performed on a random subsample from each family and represent an average, but the variance among individual eggs is unknown. If eggs with low thiamine levels died during embryonic development, only those with high thiamine concentrations would survive to hatching and be available for analysis. Similarly, if fry with high thiamine were more likely to survive, sampled fry would represent a biased sample; we could not measure the thiamine of dead fry. However, no groups showed high mortality at either the egg or fry stages, and the families with slightly higher mortality relative to others did not correspond with any of the families with low egg thiamine.

Alternatively, eggs could have obtained thiamine from exogenous sources during development, i.e., from the water in which they were reared. Thiamine is

absorbed through the chorion, as evidenced by the use of thiamine baths to treat lake trout eggs to prevent TDC (Wooster et al. 2000; Brown et al. 2005). Thiamine is naturally present in water in the environment; however, very little is known about differences in thiamine concentrations across water bodies (Hutchinson 1943, Kurata and Kadota 1981). Plants and bacteria in water synthesize thiamine; during decomposition they could leach thiamine into the water column (Bender 1999). Thiamine may also be available from excretion by fishes and other vertebrates (Manzetti et al. 2014). However, whether natural thiamine is retained in water after standard water treatment is not known; analytical methods for measuring low concentrations of thiamine in water have not yet been fully developed. Thus, the source of water for rearing lake trout eggs could be a significant variable that affects exposure to thiamine.

Hypothetically, different water sources could affect thiamine concentrations in lake trout eggs. Eggs raised in different hatcheries with different water sources do show varying occurrences of EMS/TDC (personal comm. Dale Honeyfield/Jacques Rinchard). Many hatcheries rear fry in well water while others draw water from streams or the hypolimnion of nearby lakes. Photosynthetic productivity is absent in well water, but the presence of thiamine-synthesizing bacteria in well water is unknown. In contrast, algae, bacteria, and plants in lake water produce natural thiamine which is available to fry. In our experiments, eggs and fry from Cayuga Lake reared in Burlington, VT had different survival from eggs and fry from the same Cayuga Lake parents reared in Brockport, NY (personal comm. Jacques Rinchard). But, both

laboratories use treated city water that is drawn from nearby lakes, so the cause of the difference in thiamine concentrations and survival is unclear.

Lake trout eggs and fry adsorb sufficient thiamine for metabolic use when exposed to high-concentration thiamine baths, but the concentrations of thiamine in lake water are generally very low (Hutchinson 1943, Kurata and Kadota 1981). However, lake trout eggs incubate for up to six months in the wild, which may allow adsorption of sufficient thiamine even at very low concentrations. Theoretically, the amount of thiamine absorbed by the egg could be calculated if the concentration of thiamine in the water, the rate of diffusion, and the total incubation time are known. Based on laboratory and hatchery practices, we know that incubating eggs in a thiamine bath of a specific concentration for a set amount of time results in an elevated egg thiamine concentration. To calculate the thiamine uptake at a much lower concentration we need to assume 1) there is a constant uptake of thiamine and 2) the relationship between uptake of thiamine and thiamine concentration in the environment is linear. Empirical data on thiamine concentrations in eggs incubated for long periods at very low thiamine concentrations would be highly useful.

Thiamine deficiency in hatchery-reared lake trout has been extrapolated to the wild and has been frequently suggested as a cause for lake trout recruitment failure in the Great Lakes (Fisher et al. 1996; Marcquenski and Brown 1997; Brown et al. 2005; Muir et al. 2012). In Lake Champlain, similar to the Great Lakes, lake trout egg thiamine concentrations decreased following the invasion of alewife in the early 2000s, from a pre-alewife concentration of 11.1 nmol/g to a low of 2.9 nmol/g in 2009 (Ladago

et al. in review). However, in 2015 we began sampling for juvenile lake trout and found over 30% of ages 0 to 2 years were wild recruits; recruitment appears to be higher in 2016 (unpublished data). The occurrence of thiamine deficiency in the wild is still unknown and cannot be ruled out. However, the beginning of recruitment *after* the establishment of alewife populations and despite low egg thiamine concentrations supports our initial hypothesis that thiamine is in some way mitigated during development in the wild. Our experiments do not rule out a role of feeding in thiamine acquisition, but add the possibility that thiamine may be adsorbed from water.

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Table 1. Mean (± SD) total thiamine concentration (nmol/g) of Lake Champlain lake trout alevins hatched from low- and high-thiamine treatment groups in 2014. Eggs were either treated with thiamine after fertilization or untreated, and hatched alevins from each group were either fed zooplankton or starved. Thiamine was measured at week 1 post-hatch (587 degree days) and week 6 (981 dd).

Treatment Group	Sample Date (degree Days)			
Low Egg Thiamine (Mean = 2.96, SD = 0.92)	Week 1 (587)		Week 6 (981)	
Untreated/Unfed	8.50	(1.60)	5.91	(0.96)
Untreated/Fed	8.09	(1.00)	5.81	(1.20)
Treated/Unfed	10.95	(1.78)	7.47	(0.43)
Treated/Fed	10.80	(1.37)	7.86	(1.77)
High Egg Thiamine (Mean = 5.42, SD = 1.58)				
Untreated/Unfed	8.91	(1.57)	6.44	(0.68)
Untreated/Fed	8.53	(1.42)	5.97	(0.93)
Treated/Unfed	9.56	(1.71)	8.31	(1.01)
Treated/Fed	10.16	(2.37)	8.19	(1.98)

Table 2. Mean (± SD) total thiamine concentrations (nmol/g) at hatching (478 dd), week 3 (855 dd), and week 6 (1088 dd), and mean (± SD) percent survival at week 6 of lake trout alevins from Cayuga Lake in 2015. Eggs were either treated with thiamine after fertilization or untreated, and hatched alevins from each group were either fed zooplankton or starved. Thiamine was measured at hatching (478 dd) before groups were split into fed and unfed treatments, week 3 (855 dd), and week 6 (1088 dd).

Treatment Group	Sample Date (degree Days)							
Mean = 10.65, SD =7.02	Hatching (478)		Week 3 (855)		Week 6 (1088)		Mean Survival (%)	
Untreated/Unfed	14.59	(3.32)	10.16	(2.23)	7.85	(2.02)	86.51	(11.23)
Untreated/Fed			10.04	(2.50)	8.27	(1.85)	88.34	(8.65)
Treated/Unfed	15.99	(5.06)	12.11	(1.15)	8.76	(1.05)	84.23	(13.84)
Treated/Fed			11.42	(1.33)	8.89	(0.69)	85.5	(11.57)

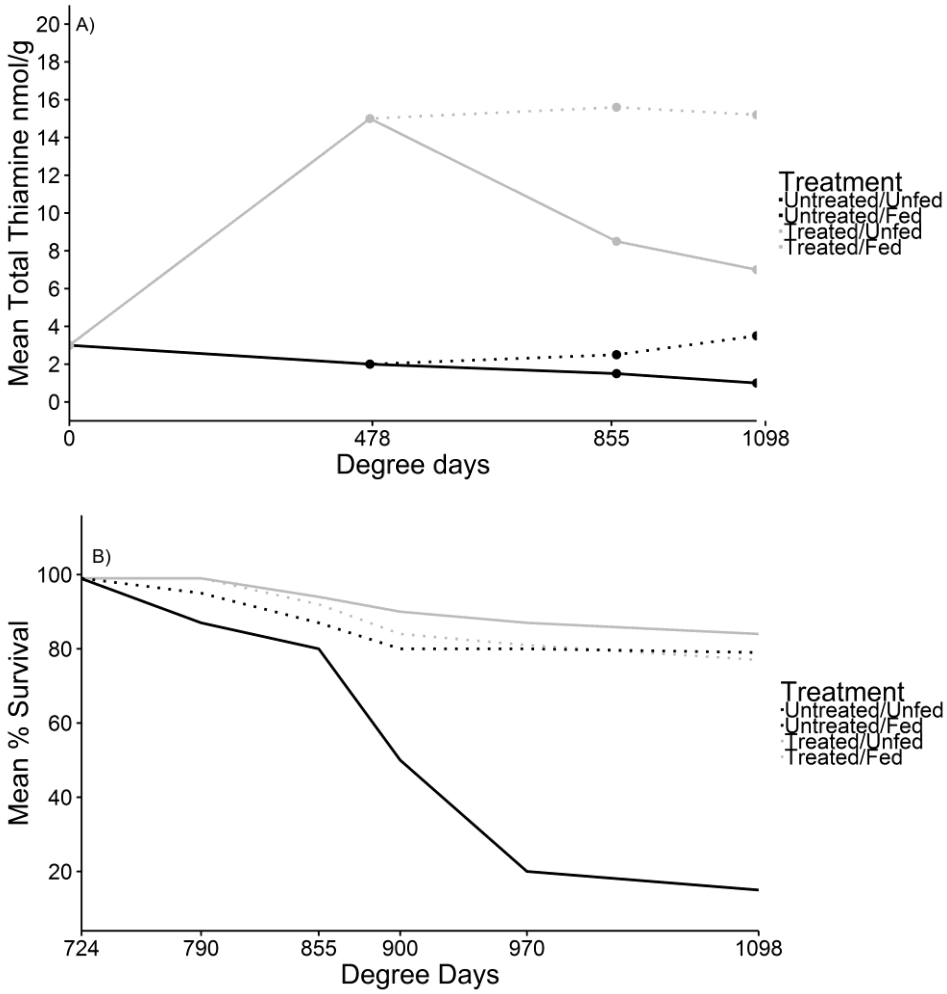

Figure 1. Expected mean thiamine concentrations (A) and expected mean percent survival (B) over time. Each group of eggs was either treated with thiamine during fertilization (grey lines) or left untreated (black lines); alevins from each group were split into two and either fed or not fed.

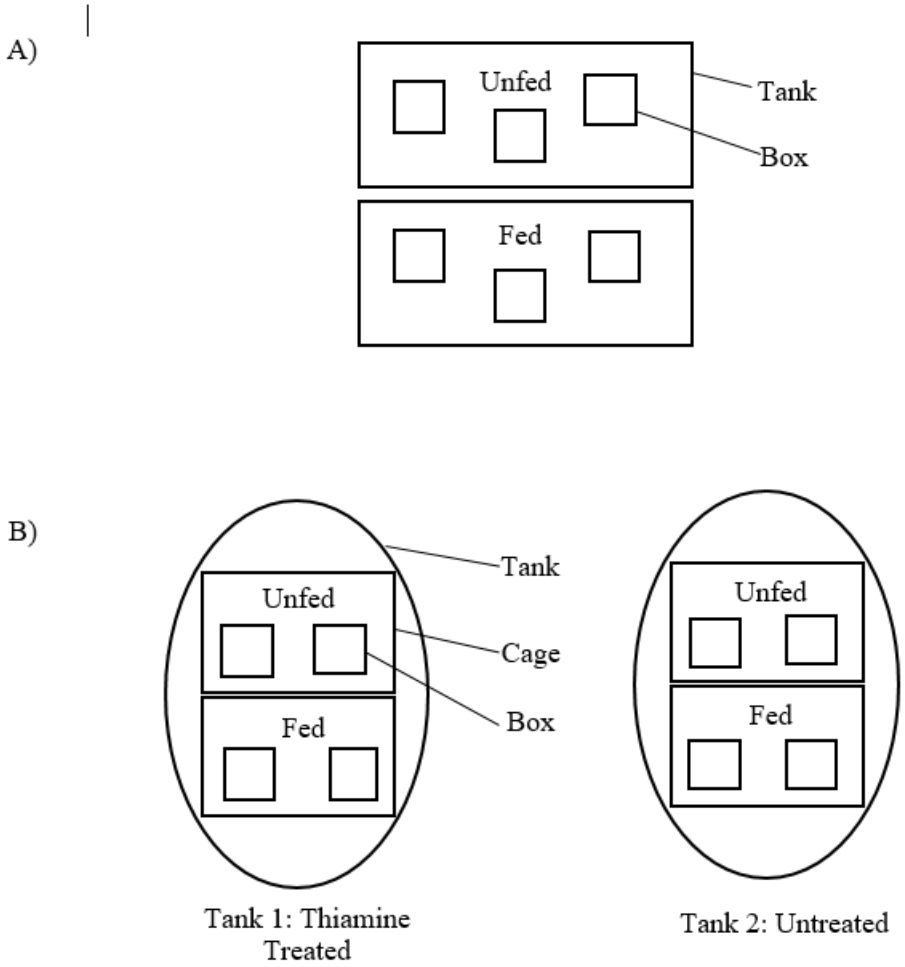

Figure 2. Tank set-up for feeding experiments in 2014 (A) and 2015 (B). In 2014 (A), boxes inside the tank represent the boxes constructed of plastic canvas that contained alevins. Boxes for each family were placed in tanks either receiving zooplankton (fed) or unfed. Thiamine treated and untreated families were kept in the same tanks. In 2015 (B), tanks were connected to a re-circulating water system. Boxes inside the tank represent the fed and unfed treatment cages constructed from Nitex mesh to prevent zooplankton from entering the unfed treatment group. Alevins were once again held in plastic canvas boxes. Not all families (boxes) are pictured.

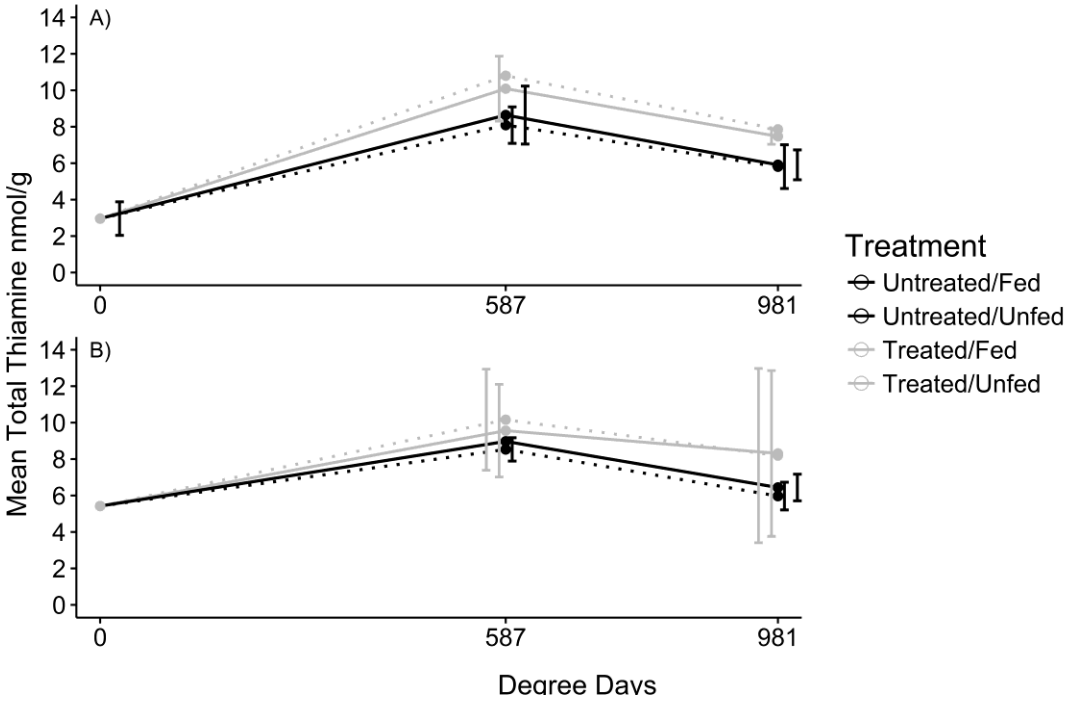

Figure 3. Mean total thiamine of lake trout eggs and fry in 2014 experiments. The low egg thiamine group (upper panel) had an initial egg thiamine <4.0 nmol/g; the high egg thiamine group (lower panel) had initial egg thiamine >4.0 nmol/g. Each group of eggs was either treated with thiamine during fertilization (grey lines) or left untreated (black lines); fry from each group were split into two and either fed or not fed.

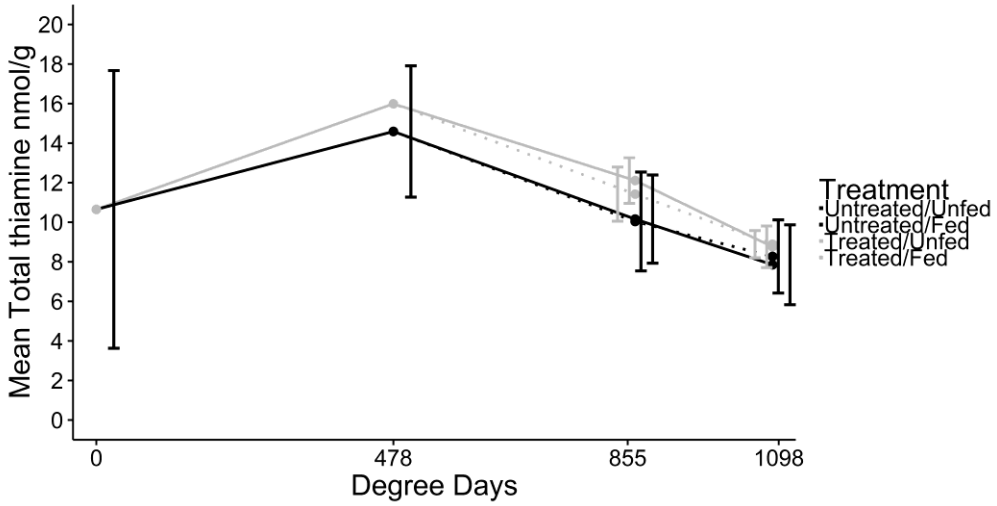

Figure 4. Mean total thiamine of lake trout from 2015 experiments. Each group of eggs was either treated with thiamine during fertilization (grey lines) or left untreated (black lines); fry from each group were split into two and either fed or not fed. Thiamine treated families were expected to increase thiamine from fertilization to hatching while

thiamine concentrations in untreated families were expected to decrease. Feeding was expected to increase thiamine concentrations with a larger effect in untreated fry.

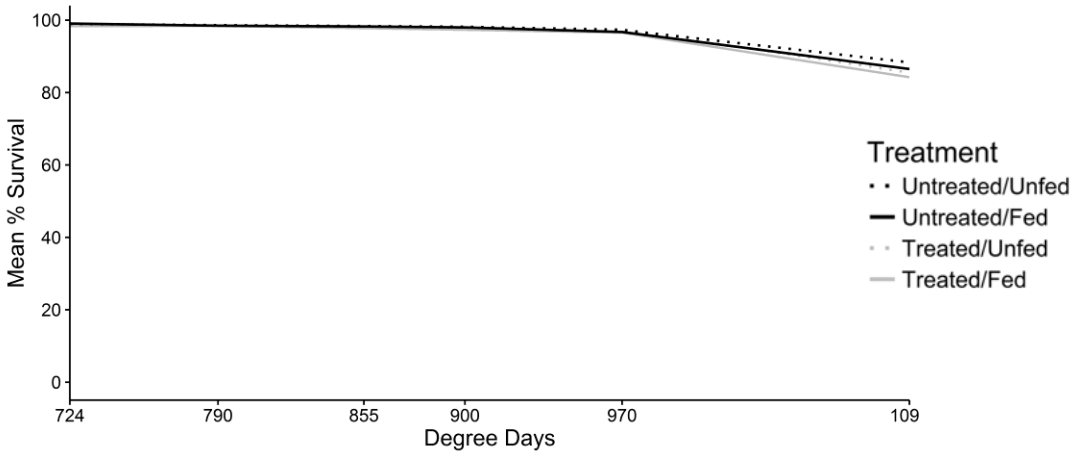

Figure 5. Mean percent survival of lake trout fry from 2015 experiments. Each group of eggs was either treated with thiamine during fertilization (grey lines) or left untreated (black lines); fry from each group were split into two and either fed or not fed. Feeding was expected to increase survival with a larger effect in untreated fry.

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Appendices

List of Abbreviations

EMS – Early mortality syndrome

DD – Degree days

HPLC – High performance liquid chromatography

SD – Standard deviation

TDC – Thiamine deficiency complex

TH – Free thiamine

TMP – Thiamine monophosphate

TPP – Thiamine pyrophosphate

TTP – Thiamine triphosphate