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Assessing limitations on reintroduction success: the influence of spawning habitat and thiamine deficiency on Atlantic salmon in Lake Champlain

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General Abstract

Assessing limitations on reintroduction success: the influence of spawning habitat and thiamine deficiency on Atlantic salmon in Lake Champlain

With the decline of freshwater fish populations, reintroductions are becoming more prevalent. In this study, we evaluated two limiting factors on the reintroduction success of Atlantic salmon (*Salmo salar*) within the Lake Champlain Basin: 1) the effects of dam removal on spawning habitat availability; and 2) the influence of thiamine deficiency, caused by the consumption of non-native alewife, on the survival and behavior of salmon embryos. The removal of the Willsboro dam increased availability of spawning habitat for Atlantic salmon by allowing them to access large areas of good quality habitat upstream of the former dam site and improving the quality of the small area of habitat available downstream. However, reproductive success may still be limited by thiamine deficiency. High young-of-the-year mortality occurred in families with egg thiamine levels up to 8.5 nmol/g, indicating a larger influence on recruitment then previously thought based on the 1.1nmol/g threshold for Atlantic salmon given in the literature. Though we did not find any behavioral evidence to suggest low thiamine embryos could not partake in early feeding, which may help to naturally mitigate the effects of the deficiency, recruitment remains low within the basin, suggesting the potential need for more controlled management. While this study helps to fill a gap in the literature on reintroduction monitoring and demonstrates the complex nature of population reestablishment, the results also contribute to the overall knowledge on dam removal and thiamine deficiency, which can be applied to research and management outside of the framework of reintroductions.

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Study concept and design: Hill, Grant, Fraser, and Ardren

Acquisition of data: Hill, Trueman, and Prevost

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Acquisition of data: Hill and Prevost

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Drafting of manuscript: Hill

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General Introduction

While freshwater ecosystems are of significant value economically, culturally, and biologically they are also extremely vulnerable to anthropogenic influences (Vörösmarty et al., 2010). Urban and agricultural run off pollutes local river and lakes, dams modify flows and fragment habitats, and commercial and recreational harvests deplete fish communities (Dudgeon et al., 2006). Consequently, many species that inhabit freshwater systems are at risk (Dudgeon et al., 2006). Two-hundred and eighty taxa of freshwater fishes are in decline in North America alone while another 61 have been extirpated (Jelks et al., 2008). Species reintroductions have been increasingly employed as a conservation tool to combat this biodiversity loss (Cochran-Biederman et al., 2015). A reintroduction is the intentional movement and release of an organism within its indigenous range in an effort to re-establish a viable population (IUCN, 2013). Many reintroduction programs have been successful in re-establishing extirpated populations, however, approximately the same number have been unsuccessful (George et al., 2009; Cochran-Biederman et al., 2015).

While population reintroductions are conceptually simple, there are many biotic and abiotic factors that influence reintroduction success that need to be considered (Cochran-Biederman et al., 2015; Galloway et al., 2016). Though efforts may be limited by administrative factors such as funding, the biological limitations on reintroduction success can be grouped broadly into two main categories: 1) those pertaining to the ability of a donor population to support the reintroduction and 2) those pertaining to the ability of the recipient ecosystem to support the reintroduction (Dunham et al., 2011). The ability of a donor population to support a reintroduction can be constrained by its genetic and biological similarity to the extirpated population or its' overall genetic diversity (Fischer and Lindenmayer, 2000; Dunham et al.,

2011). These factors can limit the genetic variation within a population and their ability to successfully adapt to the new environment (Fischer and Lindenmayer, 2000). Of equal importance is the ability of the receiving ecosystem to support the reintroduced population, which is influenced by factors such as habitat and food availability, or the presence of invasive species that might outcompete or prey upon the reintroduced native (George et al., 2009; Cochran-Biederman et al., 2015). It is often assumed that a species' presence within a given range historically indicates that the same area will be the best location in which to return it. However, many ecosystems are undergoing accelerated rates of change due to climate change and continual human influence (Seddon et al., 2014) and may no longer be suitable for the population (IUCN, 2013; Seddon et al., 2014).

In this thesis I will be focusing on the second category of limitations, factors within the receiving ecosystem that influence reintroduction success, and how they have effected the recolonization success of reintroduced Atlantic salmon in the Lake Champlain Basin. Atlantic salmon were extirpated from Lake Champlain in the 1800s due to overfishing and the damming of many of the tributaries which feed the lake (Marsden and Langdon, 2012). However, they were reintroduced in the 1970s. Since that time roughly 450,000 fry and 240,000 smolts have been stocked into Lake Champlain's major tributaries yearly in hopes of creating a self-sustaining population. While adult salmon return annually to these tributaries to spawn, there has been limited reproductive success and a self-sustaining population has yet to be achieved. Reintroduction success in Lake Champlain may be constrained by several different factors including, but not limited to, the presence of invasive alewife which cause thiamine deficiencies in salmonids when consumed in large quantities, habitat degradation due to land development for commercial and recreational use, and disrupted migration due to the damming of many of the

tributaries in which salmon traditionally spawned. As part of an ongoing adaptive management program employed by the United States Fish and Wildlife Service I have examined two specific factors thought to be of concern. In chapter 1 I explore the effect of dam removal on the availability and quality of spawning habitat for Atlantic salmon on the Boquet River, a major tributary of Lake Champlain. In chapter 2 I examine the influence of thiamine deficiency on juvenile recruitment within the system by assessing differences in survival and behavior of salmon larvae with naturally varying egg thiamine levels.

Chapter 1.

Effect of dam removal on the quality and availability of spawning habitat for a reintroduced Atlantic salmon population

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Abstract

By impeding migration and degrading habitat downstream, dam construction has caused severe population declines in many migratory fish populations. The landlocked Atlantic salmon (*Salmo salar)* reintroduction program in Lake Champlain provides a useful opportunity to study the effects of dam removal on spawning habitat quality and availability. Spawning habitat surveys were conducted downstream of the former Willsboro Dam on the Boquet River, New York, before and after its removal in 2014, 2016, and 2017 and in historical spawning grounds farther upstream in 2016 and 2017. Habitat use was quantified by measuring depth, water velocity, and substrate size at each redd. To ensure a sufficient sample of redds above the former dam site, 81 and 87 adult salmon were translocated upstream in the fall of 2016 and 2017, respectively. Mean habitat use did not differ between the two sites for any habitat variables in 2016 and only differed for depth in 2017. However, variation in used depth and substrate were lower in traditional spawning grounds upstream in 2016, likely due to an abundance of habitat. Downstream, the mean and variance in depth at redds decreased after dam removal as did the variance in substrate size, increasing habitat suitability within the site overall. When compared to primary literature data, habitat used upstream of the former dam was of good quality in both 2016 and 2017 and improved downstream after dam removal. This study illustrates that positive shifts in habitat quality and use can occur rapidly following dam removal through increased access to suitable spawning habitat upstream and improvements in habitat downstream.

Keywords: Dam removal, Reintroduction, Spawning habitat, Atlantic salmon

Introduction

Anthropogenic activities have altered freshwater ecosystems worldwide, putting both species and the ecosystem services they provide at risk (Dudgeon et al., 2006; Vörösmarty et al., 2010). In North America alone, 39% of all described freshwater fish species are considered to be imperiled, and 61 taxa are now presumed to be extinct (Jelks et al., 2008). In some systems where populations have been extirpated, reintroduction programs are ongoing, but these programs are not always successful in creating self-sustaining populations (Cochran-Biederman et al., 2015; Gephard and McMenemy 2004). While reasons for this lack of success vary between systems, often the initial cause of decline has not been remedied and continues to hinder any efforts at restoration (Cochran-Biederman et al., 2015).

Habitat degradation and fragmentation are the greatest threats to freshwater biodiversity (Jelks et al., 2008; Venter et al., 2006), particularly for migratory species that utilize different habitats throughout their life cycle. Anthropogenic barriers such as dams and weirs often impede upstream migration, limiting access to traditional spawning and rearing habitat for migratory species, leading to severe population declines in many species (Baras and Lucan, 2001; Limburg and Waldman, 2009). While some habitat may be available below a dam site, it may be insufficient to support a viable population. Obstructed flow and sediment transport can also lead to the loss of important gravel bars downstream, decreasing habitat quality (Ligon et al., 1995). Installation of fishways and fish lifts improve passage in some areas, but in many cases less than half of the fish that attempt passage succeed (Brown et al., 2013; Noonan et al., 2011). With an increasing awareness of their negative effects, the removal of aging dams is becoming more common in the United States and Canada, as well as parts of Europe and Australia (Harris et al., 2017; Lejon, et al., 2009; O'Connor et al., 2015; Stanley and Doyle, 2003).

The Atlantic salmon reintroduction program in Lake Champlain provides a useful opportunity to study the relationship between spawning habitat availability and recolonization success. Lake Champlain had a thriving landlocked Atlantic salmon population (Marsden and Langdon, 2012), which supported commercial fisheries in both the United States and Canada. The native population was extirpated in the 1800s, due in part to the damming of tributaries flowing into the lake. A stocking program was initiated in the lake and its tributaries in 1962; each year an average of 450,000 fry and 240,000 smolts are stocked into the system (Marsden and Langdon, 2012). However, a self-sustaining population has yet to be established; dams that still obstruct most rivers in the Lake Champlain basin likely contribute to this lack of success.

In August 2015, a run-of-the-river dam was removed from the Boquet River, in Willsboro, New York. Located 3.2 kilometers from the mouth of the river, the dam prevented returning Atlantic salmon from reaching their historical spawning grounds upstream. While a fish ladder was installed in 1982, passage numbers were variable in the years prior to removal (Lake Champlain Fish and Wildlife Management Cooperative, 2009 - 2014) and most reproduction took place in a 400-meter reach below the dam. Habitat within this reach was thought to be of poor quality, as there had been no confirmed fry production (Ashlee Prevost, Concordia University, 2016, personal communication).

Atlantic salmon redd surveys were conducted below the former dam site in 2014, 2016, and 2017 and in traditional spawning grounds further upstream in 2016 and 2017 as part of an ongoing monitoring program exploring the effects of the Willsboro dam removal. Because passage success was unknown after dam removal, we supplemented the breeding population upstream of the previous dam site by trapping adult salmon below the cascades in 2016 and 2017 and moving them upstream to traditional spawning sites. These surveys had two main objectives.

First, we quantified the habitat used by spawning individuals (i.e. redds) both above (upper site) and below (lower site) the former dam site to explore differences in the mean and variance of habitat used between the two sites. Second, we quantified whether habitat quality changed in the lower site after dam removal. To do so we measured depth, substrate size and water velocity at redds, and at randomly selected sites nearby that were not used for spawning. Habitat quality was inferred by assigning habitat suitability scores to our data based on a meta-analysis (Louhi et al. 2008). These data were used to test the following predictions: 1) habitat used for spawning would differ upstream and downstream of the dam/cascades; 2) the variance in used habitat would be higher downstream than upstream, because of the limited habitat below the dam/cascades; and, 3) habitat suitability would be higher upstream than downstream of the dam/cascades.

Materials and Methods

Study Site

The Boquet River originates in the Adirondack Mountains, and flows 125 km before emptying into Lake Champlain just east of Willsboro, New York (Wu and Kalma, 2013). Until the summer of 2015, the upstream migration of salmon was inhibited by the Willsboro dam, a 3 x 61 m (height x length) timber crib structure originally built in the early 1800's and most recently rebuilt in 1983 (American Rivers, 2018; Putman, 2015). The dam was constructed directly upstream of a series of bedrock cascades, hereafter referred to as the Willsboro Cascades, that present the first natural obstacle to salmon migrating upstream (Harbicht et al., 2018; Putman, 2015). Obstruction caused by the dam was partially mitigated by a denil-style fishway, but the number of fish passing upstream in recent years was low and variable, ranging from 0-43 per year (Harbicht et al., 2018; Lake Champlain Fish and Wildlife Management Cooperative, 2009 - 2014). The dam and fishway were removed to allow spawning salmon to migrate through the cascades as they did in the 1800's (Marsden and Langdon, 2012). However, after removal, sediment that had accumulated upstream of the dam was released, and may have filled important plunge pools within the cascades, making the cascades mostly impassable (Lake Champlain Fish and Wildlife Management Cooperative, 2015). Migration monitoring was conducted in the fall of 2015 using a fyke net both above and below the former dam site. While these data could not confirm the passage of any fish, a genetic analysis of young-of-the-year captured in traditional spawning grounds upstream, during the summer of 2016, indicated that some adults were able to bypass the cascades successfully (Ashlee Prevost, Concordia University, 2018, unpublished).

Translocation

To facilitate salmon migration, returning adults were intercepted during the fall of 2016 and 2017 in the two large pools directly below the Willsboro Cascades. Pools were fished twice a week from 13 September to 11 November, 2016, and 18 September to 17 November, 2017. A 15 m Duluth gill net, with a stretched mesh size of 8 cm, was deployed either by canoe or on foot within each pool for a maximum of three minutes, up to 15 times per day. Once a salmon was caught, we removed it immediately from the net and placed it in a flow-through bin at the side of the pool to recover. Each fish was identified with a Floy tag, and measured for length and weight. Eighty-one adult salmon (49 males and 32 females) were translocated in 2016, and 87 (58 males and 29 females) in 2017. Fish were out of water for less than 30 seconds, so we did not use anesthetic for this procedure. Up to ten salmon at a time were placed in a 946 L oxygenated tank and transported via truck to a release site in North Branch of the river, 13 km upstream. To protect spawning Atlantic salmon, fishing is prohibited in this area by the New York Department of Environmental Conservation during the spawning run. While most fish were released in the same site, the location was changed to a more remote area 5.3 km farther upstream in late October 2017 due to concerns about poaching.

Habitat Surveys

In the fall of 2014, during a previous study on the Boquet River, GPS coordinates were collected for 80 redds located below the Willsboro Dam using by Andrew Harbicht (Karlstads Universitet, 2015, personal communication). In July 2015, habitat measurements could only be taken for a random sample of 30 of these redds, as time was limited before the removal of the dam.

In late November 2016, redd surveys were conducted on foot below and above the old

dam site. We found 69 redds in a 400 m reach below the Willsboro Cascades, and 83 redds in a 15-km reach of the North Branch, beginning 4 km downstream of the release site (Figure 1.1). Due to early ice formation on the river in fall 2016, redd habitat measurements could not be taken until 2017. River discharge rates were too high during the spring to be representative of spawning conditions, so measurements were taken between 30 July and 16 August, when the average river discharge was within 2.8 m^3/s of fall conditions (Table 1.1).

In 2017, we found and measured 32 redds during the spawning run, from 29 September to 4 December, in a 20 km reach in the North Branch, beginning 9 km downstream of the original release site and continuing 11 km upstream from the release site (Figure 1.1). We found only 9 redds in the same 400 m reach below the Willsboro Cascades (Table 1.1). Habitat measurements and GPS coordinates were taken at each redd on the day it was located.

Three habitat variables were measured at each redd: water depth, mean water column velocity (40% of the water column) and dominant surface substrate size. Substrate was quantified using the modified Wentworth Scale: 1, 0.07–2 mm; 2, 2.1–8 mm; 3, 8.1– 16 mm; 4, 16.1–32 mm; 5, 32.1–64 mm ; 6, 64.1–128 mm; 7, 128.1–256 mm; 8, 256.1–512 mm; 9, 512.1–1024 mm; 10, bedrock (Heggenes and Dokk, 2001; Mäki-Petäys, 2004). Water velocity was measured using either a Marsh McBirney Flo-Mate 2000 velocity meter or a Swoffer 2100 flow meter, depending on availability. These habitat variables were chosen because they are considered to be important for reproductive success and are routinely measured when quantifying redd habitat (Louhi et al., 2008).

For each redd, the same three habitat variables were measured at a random point that was not used for spawning. In 2014, unused points were extracted randomly from transects of the

area taken on the same day. These unused points were on average 19 m away from a nearby redd. In 2016 and 2017, unused points were measured 3 to 4 m away from each redd.

Statistical Analysis

Welch's t-tests were used for each habitat variable to compare differences in habitat use above and below the previous dam site in 2016 and 2017. The Welch's adjustment was necessary to account for unequal variances between samples. Variance in habitat use was compared between the upper and lower sites using F tests. Variance in habitat not used was also compared between sites to control for differences in the overall variability of habitat between sites.

To compare changes in habitat use between years for data collected in the lower site, one-way ANOVAs were used. Levene's tests were used to compare variance between years for each habitat variable, for both habitat used and habitat not used. Depth and substrate were particularly heteroscedastic between years, even after a square root transformation, so a Welch's correction for heteroscedasticity was applied. When ANOVAs were significant, post-hoc Welch's t-tests were used with Bonferroni-corrected p-values; standard posthoc tests, such as Tukey's test, are not compatible with Welch's ANOVA.

We evaluated the quality of the habitat used for spawning in each site and each year by comparing our data to suitability curves from a meta-analysis (see Figure 2 in Louhi et al., 2008). We interpolated suitability values for each habitat measurement from these curves and generated mean suitability scores for each habitat variable in a site. Suitability scores were generated for both used and not used habitat. We considered the habitat used in a site to be of good quality if it had an average suitability score > 0.5 for all habitat variables (Brown et al., 2000).

Results

Contrary to our first prediction, there were few indications that the habitat used for spawning differed above and below the former dam (Figure 1.2). In 2016, there were no differences in mean depth (Welch's $t_{103.7} = -0.87$, $p = 0.39$), velocity ($t_{129.55} = 0.76$, $p = 0.45$), and substrate ($t_{100.69} = -0.06$, $p = 0.96$) of redds in the upper and lower sites (Figure 1.2). Similarly, in 2017, velocity ($t_{9.78} = 2.00$, $p = 0.073$) and substrate ($t_{14.09} = 0.75$, $p = 0.48$) did not differ, but the mean depth of redds was greater in the upper than the lower site (Welch's t-test, $t_{13.4} = 2.45$, $p = 0.029$).

Consistent with our second prediction, the variance in habitat used in 2016 was typically higher below the former dam site than upstream (Figure 1.2). The variance in depth (F test, $F_{65,77} = 2.69$, $p < 0.001$) and substrate ($F_{65,77} = 2.96$, $p < 0.001$) were higher in the lower than in the upper site, but not for current velocity ($F_{65,77} = 1.34$, $p = 0.21$). For substrate, these differences were not seemingly due to a difference in the variability of available habitat within each site, as the variance in unused habitat did not differ significantly above and below the cascades ($F_{65,77} = 1.08$, $p = 0.38$). However, the greater variance in depths used below the cascade may have been related to the greater variance in available depths in the lower than in the upper site (F test, $F_{65,77} = 1.90$, $p = 0.004$). In 2017, when few fish spawned below the cascades, the variance in habitat used did not differ between the lower and upper sites for any habitat variables (all P-values > 0.28).

There were few changes in habitat used for spawning above the cascades between 2016 and 2017 (Figure 1.2). Substrate ($t_{41.3} = 0.07$, p-value = 0.95) and current velocity ($t_{82} = -0.51$, p-value = 0.62) used did not differ between years, but salmon used greater depths in 2016 than in 2017 (Welch's t-test, $t_{67} = 1.76$ $p = 0.041$). Below the cascades, depth over redds decreased after

the dam removal (Welch's ANOVA, $F_{2, 28.8} = 12.77$ p-value < 0.001). However, substrate size (Welch's ANOVA, $F_{2,22.2} = 2.04$, $p = 0.15$) and current velocity (ANOVA, $F_{2, 102} = 3.00$, $p = 0.055$) did not change significantly after dam removal. Variance in use of substrate (Levene's test, $F_{2,102} = 6.31$, $p = 0.0026$) and depth (Levene's test, $F_{2,102} = 6.62$, $p = 0.0020$) in the lower site decreased significantly after dam removal. Variance in substrate also decreased in areas not used for spawning (Levene's test, $F_{2,102} = 3.64$, $p = 0.030$).

If a suitability value of 0.5 is an indicator of good quality habitat (Brown et al., 2000), then the habitat used above the cascades was of excellent quality. Based on the confidence intervals for all three habitat variables, the average suitability scores above the cascades were significantly higher than 0.5 (Figure 1.3). As predicted, the suitability scores were higher above rather than below the cascades before the dam was removed. After dam removal, the suitability values improved markedly below the cascades for current velocity, slightly for substrate size, but did not improve for depth (Figure 1.3).

Discussion

Though dam removals are becoming more prominent globally, our knowledge of their ecological impacts is far from complete (Harris et al., 2017; Lejon, et al., 2009; O'Connor et al., 2015; Stanley and Doyle, 2003). Post removal monitoring is critical to adaptive management, but few studies have monitored or reported whether restoration goals were achieved following dam removal (Babbitt, 2002; Brewitt, 2016). Every removal differs, due to the particularities of each dam and watershed, but the data and insight collected from each case contributes valuable knowledge to the growing science of dam removal. Our study helps to fill the literature gap on post removal monitoring and will hopefully lend support to similar policy decisions regarding aging dams in other systems.

At the time of the study, no young-of-the-year (YOY) salmon had been observed below the Willsboro Cascades, though they had been documented in traditional spawning grounds upstream. Because of this, the habitat in this lower site was thought to be of poor quality. However, mean habitat used did not differ upstream and downstream of the Willsboro Cascades in 2016 and 2017. While suitability scores were consistently higher upstream than downstream of the cascades in both years, the differences were small. These data suggest that the habitat at both sites was of good quality after removal of the dam. The amount of habitat available below the cascades, rather than its quality, is likely the limiting factor on overall spawning success in this site.

Variance in habitat use was greater downstream than upstream of the Willsboro Cascades for both depth and substrate. This difference was not due to differences in the overall variability of habitat available within each site, as the variance in depths and substrates not used for spawning did not differ between the two sites. Rather, the greater variance in the lower site was

likely due to the small area of habitat available for spawning downstream of the cascades. These observations are consistent with the theory of density dependent habitat selection (Fretwell and Lucas, 1970): the high density of spawners in the lower site in 2016 likely forced some salmon to spawn in less than ideal micro-habitats. While Atlantic salmon are not territorial after spawning, they will defend their redd site for multiple days during construction of the redd and oviposition (Fleming, 1996). In 2017, the variance in habitat use was similar for all habitat variables above and below the Willsboro Cascades, presumably due to the low density of spawners below the cascades and the abundance of habitat above. While suitability scores for depth and substrate size were above 0.5 below the cascades, column velocity had a low suitability score, suggesting that velocities both used and available for salmon were not ideal.

Differences in habitat quality between upstream and downstream sites appeared to be decreasing over time, perhaps due to the removal of the dam. Below the cascades, mean depth used for spawning decreased progressively after dam removal. Though discharge on days sampled was highest in 2014, it was similar between 2016 and 2017, suggesting that this change was not merely a reflection of changes in discharge (Table 1.1). The variance in habitat used and not used also decreased for substrate after dam removal, potentially indicating that the habitat has become more uniform. Suitability scores were in fact higher in 2016 and 2017 compared to 2014 for both used and not used habitat (Table S1.1), suggesting the change in mean habitat use and variability may reflect better habitat quality overall. These trends are consistent with other research on the downstream effects of dam removal. For example, Hatten et al. (2016) found a 50% decrease in pools and an overall increase in salmon spawning habitat following dam removal.

Given the apparent increase in spawning habitat quality, it is tempting to speculate whether the habitat below the cascades can become a productive habitat for Atlantic salmon in the future. However, regardless of quality, the habitat directly below the cascades is limited and would likely not be sufficient to support the large number of YOY emerging from any successful redds. Additionally, any emerging fry that dispersed downstream would encounter poor habitat, characterized by sand and slow current velocities. However, the small amount of habitat below the cascades does not explain the complete absence of YOY found in the previous surveys. Other habitat variables that were not measured in this study could also be influencing reproductive success. Qualitative observations suggest that fine sediment (< 2 mm) infiltration, which can lead to the suffocation of gametes during the overwintering stage, may also be a limiting factor.

Fewer than 100 YOY were observed in traditional spawning sites upstream (Ashlee Prevost, Concordia University, 2017, personal communication), suggesting factors other than habitat may be inhibiting reproductive success. Early Mortality Syndrome, a condition caused by maternally transferred thiamine deficiency, causes up to 100% mortality in juvenile salmon (Fisher et al. 1995, Ketola et al. 2000, Werner et al. 2006) and is known to occur in the Lake Champlain watershed. Currently there are no measures in place to mitigate its effects for naturally spawning individuals (Harbicht et al., 2018).

Though unavoidable, measuring used habitat for the 2014 and 2016 redds the following summer, rather than during the fall spawning run, raised two important caveats with our data: 1) that habitat conditions may have changed at redd sites between seasons and 2) that habitat measurements may have been taken at incorrect locations due to the accuracy of the Garmin eTrex 20x gps (±3 meters). While it is important to acknowledge these caveats, there is evidence to support the validity of our data in representing spawning habitat choice. First, the redds

located below the Willsboro Cascades in 2014 and 2016 were densely packed within a small area, meaning that any location within 3 m of a given redd would have been "suitable" habitat for spawning. Additionally, habitat used for redds in the downstream site did not differ significantly from random sites that were within 3-19 m for 7 of 9 (3 years x 3 variables) comparisons (Table S1.1). These data suggest that microhabitat was relatively consistent at a 3 m spatial scale. The locations of redds at the downstream site were also very consistent over years, indicating that the river did not change markedly over time. Above the cascades, neither the suitability scores for habitat used nor mean substrate or water velocity used deferred between 2016, when habitat was measured eight months after spawning, and 2017, when habitat was measured during the spawning season. These data suggest that the river did not change markedly over time and that the timing of the measurements had little effect. While our data have some limitations, these analyses suggest that they provide a valid description of habitat quality in the Boquet River.

Overall, the removal of the Willsboro dam has been a positive step towards the re-establishment of a naturally reproducing Atlantic salmon population in Lake Champlain. With our help, salmon were able to bypass the Willsboro Cascades and rapidly recolonize the newly accessible habitat upstream of the former dam site. They are also using habitat within the suitable range for the species (Louhi et al., 2008) despite the potential novelty of the area. Our study suggests that salmon will benefit from an increase in spawning habitat quantity and quality after dam removal and that improvements in spawning habitat below a former dam site can occur within a few years.

Tables

Table 1.1 The number of Atlantic salmon redds located each year in the Boquet River and the average river discharge, from a United States Geological Survey water gauge located just upstream of the Willsboro Cascades, during the time they were located and the time during which habitat measurements were recorded.

Year	Site	Redds Located	Redds Measured	Average Discharge During Spawning (m^3/s)	Average Discharge During Habitat Surveys (m^3/s)
2014	Lower	67	30	5.90 (2.71 – 39.64)	7.33
2016	Lower	69	66*	2.61 (1.03 – 8.64)	4.08 (3.06 - 5.13)
2016	Upper	85	78*	2.61 (1.03 – 8.64)	4.47 (3.14 – 5.10)
2017	Lower	9	9	5.21 (4.70 – 5.58)	5.21 (4.70 – 5.58)
2017	Upper	32	32	5.61 (2.92 – 12.37)	5.61 (2.92 – 12.37)

*redds in water deeper than 1 m were not measured

Figures

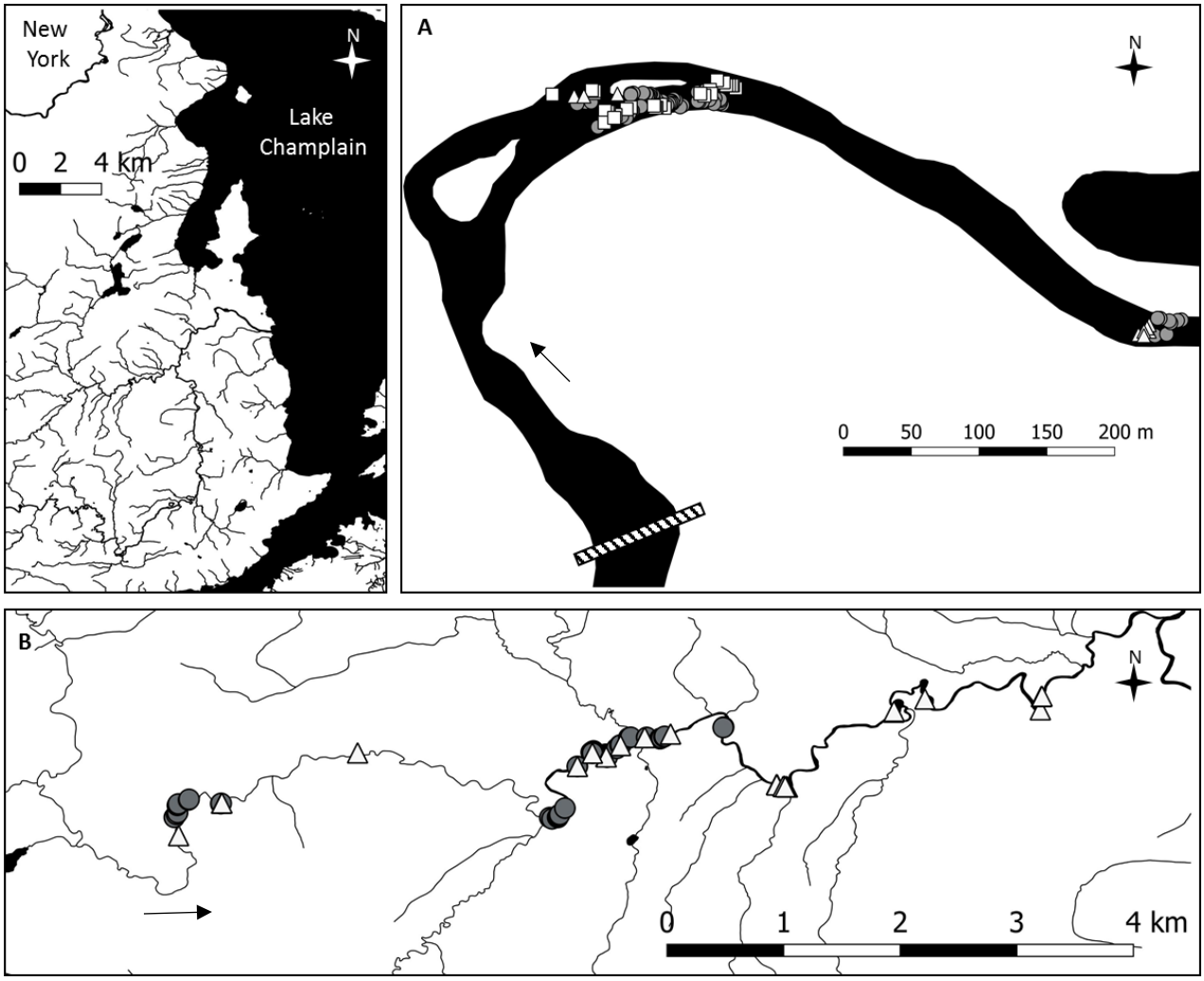

Figure 1.1 The Boquet River in New York, U.S.A., showing the lower site, below the Willsboro Cascades (Box A) and the upper site in the North Branch (Box B). Atlantic salmon redd locations are indicated by squares (2014), circles (2016), and triangles (2017). The former dam is indicated by the striped box and the downstream flow of the river is indicated with an arrow.

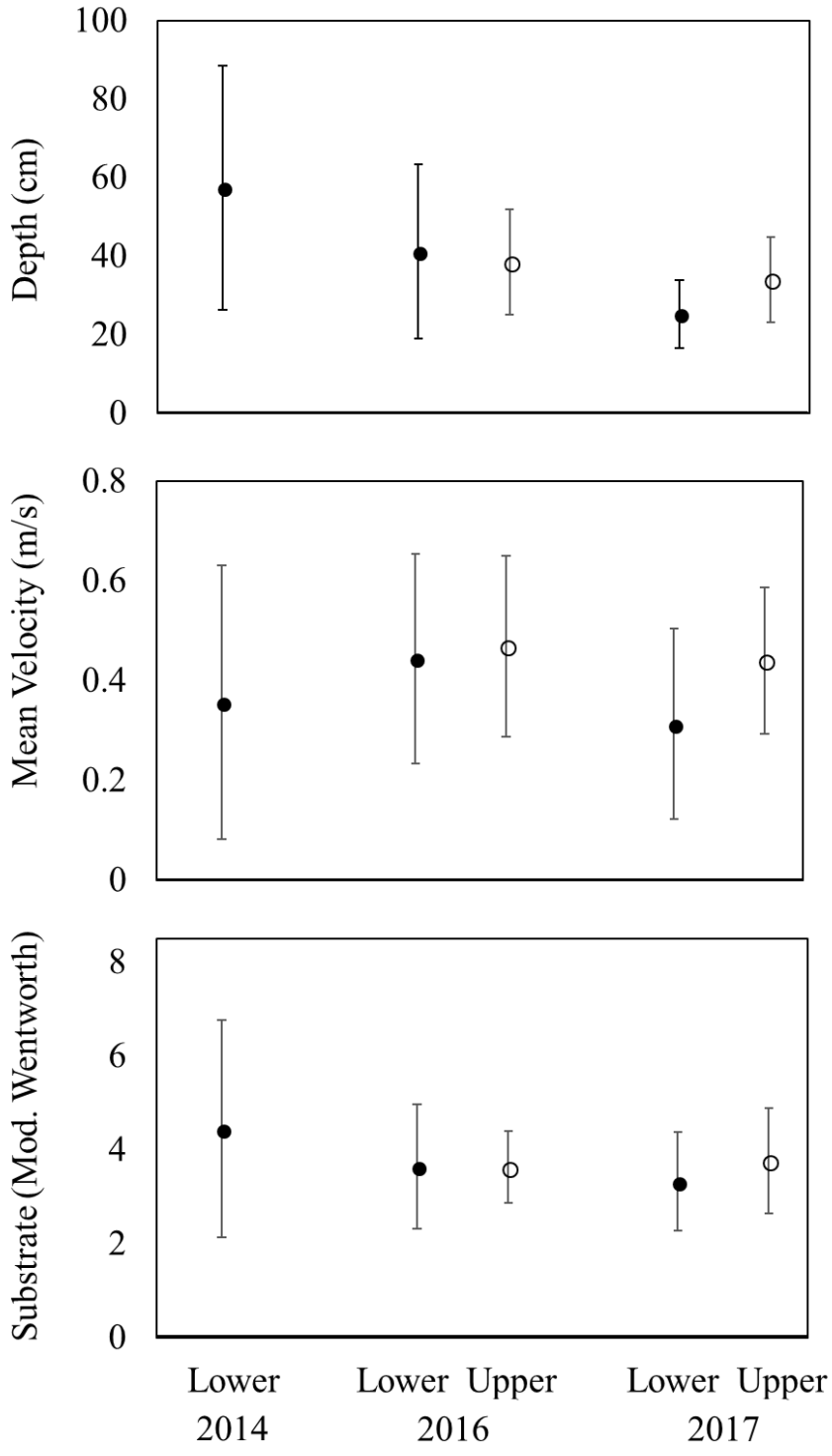

Figure 1.2 Mean and standard deviation of Atlantic salmon spawning habitat on the Boquet River for depth, column velocity, and substrate size downstream (lower) and upstream (upper) of the former dam site, respectively.

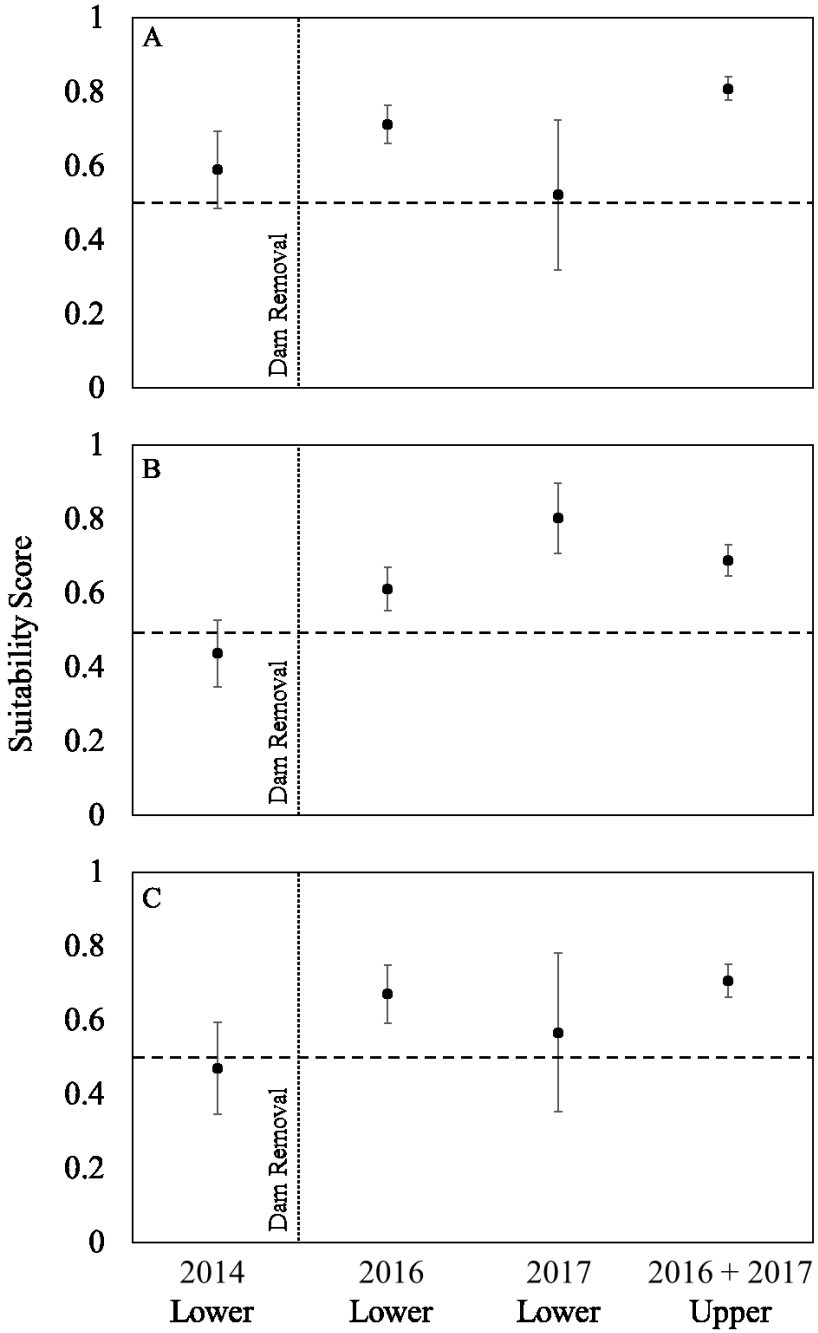

Figure 1.3 Mean (±95% CI) suitability scores for depth (A), column velocity (B), and substrate size (C) of used Atlantic salmon spawning habitat on the Boquet River downstream (lower) and upstream (upper) of a former dam site. Scores for the upper site were combined as there were no significant differences between years. The dashed line denotes the 0.5 threshold for good quality habitat while the dotted line indicates the removal of the Willsboro dam in 2015.

Supplementary Material

Table S1.1 Average suitability scores (± 95% CI) for Atlantic salmon spawning habitat on the Boquet River calculated from the suitability curves in Louhi et al., (2008). Scores range from 0 to 1 with 1 being the highest. Scores for the upper site were combined as there were no significant differences between years.

Habitat Variable	Suitability Score			
	Lower 2014	Lower 2016	Lower 2017	Upper 2016 + 2017
Water Depth (Used)	0.59 ± 0.10	0.71 ± 0.05	0.80 ± 0.20	0.80 ± 0.03
Water Column Velocity (Used)	0.44 ± 0.09	0.61 ± 0.06	0.52 ± 0.10	0.69 ± 0.04
Substrate (Used)	0.47 ± 0.12	0.67 ± 0.08	0.57 ± 0.21	0.71 ± 0.05
Water Depth (Not used)	0.59 ± 0.09	0.72 ± 0.05	0.67 ± 0.12	0.75 ± 0.04
Water Column Velocity (Not used)	0.38 ± 0.12	0.57 ± 0.06	0.39 ± 0.17	0.61 ± 0.06
Substrate (Not used)	0.31 ± 0.12	0.65 ± 0.15	0.56 ± 0.27	0.52 ± 0.06

Chapter 2.

Variation in egg thiamine levels amongst reintroduced Atlantic salmon and its effects on embryo behavior and survival

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Abstract

The introduction of alewife to freshwater ecosystems in the eastern United States and Canada has resulted in the development of thiamine deficiencies in many local salmonids. These deficiencies often lead to a condition known as Early Mortality Syndrome (EMS) in which high mortality is experienced in salmonid embryos between hatching and button up (i.e. when yolk is fully absorbed). We explored the influence of egg thiamine level (ETL) on the behavior and mortality of embryos from 17 families of landlocked Atlantic salmon (*Salmo salar*) from Lake Champlain with naturally varying egg thiamine levels. Embryos from each family were divided into two groups, one treated with thiamine and one untreated, allowing for treatment comparisons within families. Behavioral responses to light and physical stimulus were evaluated at the free embryo and button up life stages. Survival was consistently lower in untreated fish than thiamine treated fish, but increased to > 80 % as ETL increased to 8.5nmol/g. EMS occurred in untreated individuals in all families with ETL below 2.7 nmol/g, and varied in families between 2.7 and 8.5 nmol/g; ETLs much higher than the previously suggested threshold of 1.1 nmol/g for Atlantic salmon. However, no significant behavioral differences were detected between treated and untreated salmon at the free embryo life stage, prior to the occurrence of high rates of mortality. Our results suggest that the occurrence of EMS may be influenced by factors other than ETL at intermediate thiamine levels and that free embryos may be capable of foraging on thiamine rich food sources, which could help to mitigate the deficiency before the onset of EMS.

Keywords: Thiamine deficiency, Early Mortality Syndrome, Atlantic Salmon, Behavior

Introduction

Invasive species pose a major threat to native populations in freshwater ecosystems worldwide (Ricciardi and MacIsaac, 2011). Though the impact of an invasive is often associated with their ability to out-compete or prey upon the native species, they may also have more subtle effects on local biota (Simon and Townsend, 2003). For instance, the introduced zebra mussel has outcompeted native bivalves in the Laurentian Great Lakes, but it has also lead to an unanticipated increase in outbreaks of avian botulism (Ricciardi and MacIsaac, 2011) by creating environments conducive to the growth of botulism bacteria which they then concentrate within their tissues, and pass up the food chain.

The impact of introduced alewife (*Alosa pseudoharengus*) on freshwater salmonid populations in the eastern United States and Canada is another example in which an invasive species has affected the survival or performance of a native species through unforeseen interactions. These small forage fish contain gut bacteria that are high in thiaminase, an enzyme that breaks down thiamine (Werner et al., 2006). When alewife make up a large portion of the salmonid diet, thiamine deficiencies often result, leading to reduced energy levels in adult fish, which limit their ability to successfully complete spawning migrations (Fitzsimons et al., 2005; Harbicht et al., in press; Ketola et al., 2005). While these effects on adults are serious, the effects of the deficiency on the embryos of successful spawners can be even more detrimental. Thiamine deficiency is maternally transferred to developing embryos, causing yolk coagulation and improper absorption, vascular congestion, and neurobehavioral abnormalities (Fisher et al., 1995). Such deficiencies often result in high mortality rates (up to 100%) by the button up life stage, defined as the stage at which healthy embryos would have depleted their yolk and begun exogenous feeding (Fisher et al., 1998; Fisher et al., 1995; Ketola et al., 2000; Werner et al.,

2006). The condition has been termed Early Mortality Syndrome (EMS) in the Laurentian Great Lakes, Cayuga Syndrome in the Finger Lakes of the eastern U.S., and M74 in the Baltic Sea of Europe (Brown et al., 2005c; Fisher et al., 1995; Madenjian et al., 2008).

Research on EMS has been ongoing since the early 1990s. Brown et al. (2005a), Fisher et al. (1998), and Werner et al. (2006) all found species-specific threshold egg thiamine levels above which high rates of mortality were not experienced. However, few studies have explored sublethal effects of thiamine deficiency in individuals that survive with thiamine levels near these thresholds. Anything that reduces the ability to capture prey, avoid predation, or hold position within the current may inhibit survival beyond the button up life stage (Carvalho et al., 2009; Fitzsimons et al., 2014). The negative effects on survival can be mitigated by immersion of EMS fry in a thiamine solution (Fitzsimons, 1995). Further research has shown that thiamine baths can be successfully administered to eggs during water hardening (Brown et al., 2005b), a technique which has been adopted by many hatcheries to prevent EMS in their stocks. While encouraging for hatchery production, these baths do not solve the problem for naturally reproducing populations.

Limited work on EMS has investigated potential mitigating factors within the natural environment that may not be present in a hatchery setting. Ladago et al. (2016) suggested that foraging on thiamine rich zooplankton during the free embryo stage, before yolk sac absorption, could allow individuals to counteract the deficiency. They showed that lake trout fry begin to feed as early as two weeks post hatching, when the yolk sac is still present, and could consume enough zooplankton to provide the thiamine needed to offset mortality. Many salmonids are thought to begin feeding while yolk is present (Heming et al., 1982; Koss and Bromage, 1990; Skoglund and Barlaup, 2006), making this a viable possibility for multiple species. However, if

free embryos are weakened by thiamine deficiency early in their development they may not be able to forage successfully.

In this study, we explored the effects of egg thiamine level on behavior and mortality of landlocked Atlantic salmon embryos in Lake Champlain. Alewife are thought to have been introduced to Lake Champlain in 2003 through an unauthorized stocking event (Marsden and Hauser, 2009). Though the native Atlantic salmon population was extirpated in the 1800s, a reintroduction program has been present since the 1970s in an effort to re-establish a self-sustaining population. While this goal has yet to be reached, some natural reproduction was documented within the lake's tributaries in 2016 and 2017 (Ashlee Prevost, Concordia University, 2018, unpublished data), suggesting either variability in thiamine levels within the population or the presence of a mitigating factor within the river environment.

The eggs from each of 17 females, that were collected during the 2016 spawning run, were fertilized by a separate male, and divided into two groups; one group received a thiamine treatment, the other did not. Total egg thiamine levels (ETL) were determined for each family and mortality was recorded from hatching to the button up stage. Behavioral assays were performed at the free embryo and button-up life stages to explore difference between treatment groups, and families with varying ranges of ETL. We predicted that 1) the threshold ETL above which EMS would not occur would be around 1.1 nmol/g, based on previous studies by Fisher et al. (1998) and Ketola et al. (2000); 2) there would be behavioral differences between thiamine treated and untreated individuals, as well as high thiamine and low thiamine individuals at the free embryo stage that might prohibit early feeding success or survival; and 3) there would be behavioral differences between surviving thiamine treated and untreated individuals at the button up life stage indicating potential sublethal effects of thiamine deficiency.

Materials and Methods

Egg Collection and Rearing

Spawning salmon were collected on the 15 and 17 November 2016 as they migrated from Lake Champlain to Hatchery Brook, a small stream originating at the Ed Weed Fish Culture Station in Grand Isle, Vermont, U.S.A. Eggs were collected from 17 females over the two days (11 and 6, respectively). Each female's eggs were placed into a 3.8 L bucket for transportation. Milt was collected from 14 males on the two days (11 and 3, respectively) and stored in 50 mL conical tubes. After collection, eggs and milt were stored on ice and transported to the White River Fish Hatchery in Bethel Vermont, 150 km away.

Eggs were fertilized upon arrival at the hatchery. On 15 November, each female's eggs were fertilized with sperm from a single, unique male. However, on 17 November, due to the small number of males collected, each male's milt was used to fertilize the eggs of 1 to 3 females (Table S1.1). After fertilization a sample of eggs from each family was collected for thiamine analysis. The remaining embryos were then disinfected in a 50ppm iodophor bath for 30 minutes (Kevin Kelsey, Ed Weed Fish Culture Station, 2016, personal communication). Following disinfection embryos from each family were split into two groups, one which received a 10,000ppm thiamine bath for 30 minutes, using PureBulk food grade thiamine mononitrate, to treat for thiamine deficiency (Kelsey, Ed Weed Fish Culture Station, 2016, personal communication) and one which was bathed in water for 30 minutes. This design allowed for the evaluation of both thiamine treated and untreated individuals within each family. Each group of fertilized embryos was randomly placed into a separate incubation tray for rearing. Embryos were reared on well water (5.7 - 10.9^{0}C) and dead individuals were removed from each tray once every two weeks between January and February, and once daily from March to April. Total

mortality was calculated on 2 April, prior to moving larvae to open tanks to begin exogenous feeding. Degree days (dd) were used as a standard unit for monitoring developmental rate amongst groups throughout the study. One dd was equal to a 24-hour period in which the mean water temperature was 1^{0}C.

Thiamine Analysis

Egg thiamine was analysed for each family, prior to thiamine treatment, following Futia et al. (2017). Free thiamine, thiamine monophosphate, and thiamine pyrophosphate were extracted from a 1g sample of fertilized egg tissue and summed to give total thiamine. Once extracted, high performance liquid chromatography was used to determine thiamine levels within each sample. Each sample was run twice, and the two values were averaged. These analyses were completed by Dr. Jacques Rinchard at the College at Brockport, New York.

Behavioral Study

Behavioral assays were performed between 662 and 674 dd for the free embryo life stage, and 857 and 863 dd for the button up life stage. Previous work has shown that behavioral manifestations of EMS arise between 641 to 700 dd (Fisher et al., 1995; Ketola et al., 2000), prior to the onset of mortality. Behavior was measured (see below) as a response to light and a physical stimulus. During early development, larval salmon are photonegative (Dill, 1977), responding to light by hiding within the gravel, protecting them from potential predators. A lack of photonegative response would suggest a deviation from normal larval behavior that may increase mortality in a natural setting (Fast and Stober, 1984). A physical stimulus mimics contact by a predator, which should elicit an even stronger flight response than light (Hale, 1999).

To control for light exposure, assays were performed within a $20m^2$, portable tent, which kept the environment dark throughout the experiment. Three-liter rectangular plastic tanks were set up in a two by two pattern on a table within the shelter. Each tank held a single individual per trial and was filled with well water (5.7 - 10.9^0C) to a depth of 2.5 cm to encourage horizontal rather than vertical movement. Water was replaced after each trial. Behavior was filmed using Canon GL1 and GL2 cameras mounted on tripods above the tanks. Portable clamp lights with 2700k bulbs were secured to the side of the table and used to provide the light stimulus. The physical stimulus was applied as a gentle tap on the caudal fin using a 4.5 mm wide plastic rod.

Individuals were given 5 minutes to acclimate to the experimental tanks before the light stimulus was provided. Response to the light stimulus was recorded for 1 minute, followed by a 10 minute acclimation period to the lit conditions. After this period the physical stimulus was given, and responses were recorded for another minute. Multiple trials were run over a 2-3 day period to obtain 8 replicates for each of the two treatments within each family (Table 2.1). To account for potential differences caused by the time of day during which a trial was conducted, a single fish from each family group was tested before moving on to succeeding individuals, thereby dispersing the effect of time of day evenly amongst families. To ensure that no fish was assayed twice, individuals were put in a separate hatchery tray after their trial and were euthanized using MS-222 upon the completion of the experiment. Due to mortality and equipment malfunctions, usable data was not always obtained for all 8 replicates (Table 2.1). Only families with at least 1 representative individual from both thiamine treated and non-treated groups were included in the behavioral analysis.

Atlantic salmon embryos from a Merrimack River domestic brood stock were assayed as a “control” for this experiment. These individuals, which were also housed at White River Fish

Hatchery, were second generation hatchery reared-fish from a line of sea-run salmon that had no previous exposure to thiamine deficiency. These fish provided baseline data of how "normal" fish should behave. Because Merrimack and Lake Champlain stocks were on different developmental schedules, Merrimack fish were tested during alternative sessions on 25 March and 21 April, which corresponded to the appropriate developmental stages. The same experimental procedures were followed for 20 individuals per life stage.

Video Analysis

Videos were analysed using KINOVEA 0.8.15, an open source software used to track and study movement. A two by two grid was superimposed upon each tank and the number of times a gridline was crossed throughout the 1 minute response period was recorded. To ensure this was a good metric for movement, a subsample of individuals from each family was analysed for time spent moving (s) and distance traveled (cm). These measurements were taken using the motion tracking and stop watch functions in KINOVEA. Results were compared to the gridline data for the same individuals. A strong correlation was found between the number of grids crossed and distance travelled ($r = 0.92$, $df = 281$, $p < 0.001$) and time spent moving ($r = 0.71$, $df = 281$, $p < 0.001$).

Statistical Analysis

To explore the influence of ETL and thiamine treatment on survival and behavior, generalized linear mixed models were constructed using packages lme4 and glmmTMB in R 3.4.3 (Table 2.2). ETL was $\log_{10}$ transformed prior to testing to reduce the influence of outliers from two families that had markedly higher thiamine levels than the rest of the group (Table S2.2). ETL, treatment, and their interaction term were analysed as fixed effects in all models. The interaction term was only reported when significant. To evaluate the effect of ETL and

treatment on survival, a model was constructed using a binomial probability distribution. Treatment and family were included as crossed random effects to account for variation amongst individuals in different treatment groups within each family.

When evaluating the effects of ETL and treatment on behavioral response to stimuli, each life stage and stimulus was tested separately. Family was incorporated as a random effect in each model to account for within family variability. Treatment was not incorporated as a crossed random effect as it was for analysing mortality, as it explained very little variance and was highly correlated with family in all cases ($r > 0.9$). Zero inflated negative binomial models were constructed for behavioral responses to physical stimulus at both life stages. The negative binomial distribution was used to account for higher variance within the data than normally observed in a Poisson distribution (over-dispersion) (http://qcbs.ca/wiki/r_workshop7). Zero-inflated models fit two different distributions to the data, one which modeled count data (number of times a gridline is crossed, Poisson/negative binomial distribution) and one which modeled the excess of zeros through presence/absence (Bernouilli distribution; whether a fish will cross a gridline or not) (Hu et al. 2011). This procedure allowed the models to deal with the large number of individuals that did not exhibit a movement response. Akaike information criterion (AIC) were used to compare the zero inflated models to their non-zero inflated counterparts, and models with the lowest AIC value were selected (Akaike 1987). A zero inflated Poisson model was used to analyse the effects of ETL and treatment on light stimulus response at the free embryo life stage as over dispersion was minimal ($\Phi = 1.37$). For analysing behavioral response to light at the button up stage, a non-zero inflated model with a negative binomial distribution was constructed as the data were over dispersed and the non-zero inflated model had a better fit (lower AIC) than its zero-inflated counterpart.

To compare differences in behavioral responses between our experimental groups from Lake Champlain and from the Merrimack River, generalized linear models were constructed using MASS and pscl in R 3.4.3. Behavioral responses were tested for each stimulus at each life stage separately. Treatment group (thiamine treated, untreated, or Merrimack) was the only fixed effect in each model, as ETL was not known for the Merrimack fish. Family was not included as a random effect because the family origins for the Merrimack fish were unknown. Zero inflated negative binomial models were constructed for response to a physical stimulus at both life stages and response to light stimulus at the free embryo life stage. A negative binomial model was constructed for response to light stimulus at the button up stage. As above, models were selected based on AIC (Akaike 1987).

Results

Survival

As expected, there was a significant interaction between the effects of ETL and treatment on salmon embryo survival (Figure 2.1; Wald's test, $Z = 3.252$, $df = 27$, $p = 0.001$; all other test statistics reported are for Wald's tests). Survival increased with ETL in untreated fish ($Z = 2.896$, $df = 27$, $p = 0.004$), but not in the treated fish ($Z = -1.217$, $df = 27$, $p = 0.224$). Despite this interaction, and consistent with previous research, survival was higher in the thiamine treated fish than the untreated (Figure 2.1; $Z = -4.804$, $df = 27$, $p < 0.001$), but this difference diminished as ETL approached 9.3 nmol/g.

Survival was low (< 20%) for untreated individuals in 11 of the 17 families tested (Table S2.2). Out of the remaining 6 families, 3 had moderate survival (20-80%), and 3 had high survival (> 80%) amongst untreated individuals. Within the thiamine treated group, 3 and 14 families had moderate and high survival rates, respectively. Defining EMS as the occurrence of 20% or greater mortality within a family (Brown et al., 2005b; Fisher et al., 2007; Fitzsimons et al., 1998), untreated fish had a higher incidence of EMS than treated fish (Fisher's exact test: $P<0.001$). Amongst our sample group the threshold level above which survival was at least 80% in untreated fish was 8.5 nmol/g. Survival below this threshold was variable. While families 1, 3, and 8 all had ETLs of 3.1 nmol/g survival varied between 7.4% and 97.3%. In all families with an ETL less than or equal to 2.7 nmol/g, survival in untreated individuals was low, varying between 1.1% and 12.4% (Table S2.2).

Behavior – free embryo stage

Untreated fish were significantly more likely to move in response to the light stimulus than treated individuals (Table 2.2; Z = -2.038, df = 215, p = 0.042). Overall, only 23% of 106 untreated individuals moved compared to 11% of 119 of treated. However, amongst individuals that did respond to light, there was no significant difference in the amount of movement between treated and untreated groups (Figure 2.2A; Z = -1.513, df = 215, p = 0.130), nor was there a significant effect of ETL (Z = 0.074, df = 215, p = 0.941). There were no significant differences between the control fish from the Merrimack River and the thiamine treated (Z = 1.185, df = 238, p = 0.236) and untreated (Z = -0.056, p = 0.955) fish from Lake Champlain in whether an individual would respond to light stimulus (Figure 2.3A); only 30% of the 20 control fish responded to the light stimulus. Similarly, there were no significant differences between groups in individuals that did respond (Figure 2.3A; treated Z = 0.242, df = 238, p = 0.809; untreated Z = -1.687, p = 0.0922)

There was no significant effect of treatment (Z = 0.170, df =182, p = 0.864) or ETL (Z = -0.827, df = 182, p = 0.408) on whether a fish moved in response to the physical stimulus at the free embryo stage. Amongst fish exhibiting a response, untreated fish tended to move less than treated fish (Z = -1.862, df = 182, p = 0.063), but there was no significant effect of ETL (Figure 2.2B; Z = -0.100, df = 182, p = 0.920). There were no significant differences between treatment groups and the Merrimack control group in whether a fish would respond to physical stimulus (treated Z = 1.295, df = 206, p = 0.195; untreated Z = 1.435, p = 0.151): 48% of 104 treated fish, 54% of 89 untreated fish, and 35% of 20 control fish did not move. Amongst individuals that did respond to the stimulus, thiamine treated fish moved more than the control fish (Figure 2.3B; Z =

3.466, df = 206, p = 0.001), but untreated and control fish did not differ significantly (Z = 1.796, df = 206, p = 0.963).

Behavior – button up stage

Neither treatment (Z = 1.680, df = 122, p = 0.093) nor ETL (Z = 0.522, df = 122, p = 0.602) was a significant predictor of a fish's response to the light stimulus (Figure 2.2C). The response of control fish did not differ significantly from either thiamine treated (Figure 2.3C; Z = -0.898, df = 145, p = 0.369) or untreated groups (Z = -0.491, df = 145, p = 0.624). The percentage of individuals within each group that did not respond was similar to that for the light stimulus at the free embryo stage; 71% of 71 treated; 68% of 57 untreated; and 65% of 20 control fish.

There were no significant predictors of whether a fish moved in response to the physical stimulus at the button up life stage (ETL Z = -0.343, df = 105, p = 0.731; treatment Z = 0.560, p = 0.576). However, amongst individuals that did move there was a significant interaction between ETL and treatment (Figure 2.2D; Z = 3.173, df = 105, p = 0.002). Movement was not affected by ETL in treated individuals (Z = -0.343, df = 105, p = 0.731), but increased significantly with increasing ETL in untreated individuals (Z = 3.521, df = 105, p < 0.001) until it equaled and even surpassed that of treated individuals at 5.9 nmol/g (Figure 2.2D). When compared to the control group, untreated individuals were significantly less likely to exhibit a movement response (Z = 2.148, df = 129, p = 0.032) relative to control fish; 53% of 52 untreated fish, 27% of 64 treated fish, and 20% of 20 control fish did not move. Amongst moving individuals, there were no significant differences between groups (Figure 2.3D; treated Z = -0.692, df = 129, p = 0.489; untreated Z = -0.769, p = 0.442).

Discussion

Prior to this study, the threshold ETL above which Atlantic salmon experienced normal survival was 1.1 nmol/g (Fisher et al., 1998; Ketola et al., 2000). Despite differences in populations, rearing conditions, or analysis this threshold has remained relatively consistent across studies (Werner et al., 2006). Accordingly, all Lake Champlain embryos sampled should have had sufficient ETL to prevent thiamine deficiency related mortality. However, high mortality rates symptomatic of EMS (> 20%) (Brown et al., 2005b; Fisher et al., 2007; Fitzsimons et al., 1998) occurred in untreated embryos for 14 out of 17 families (Table S2.2). Families with ETLs as high as 4.9nmol/g experienced mortality over 80%. Though survival significantly increased with increasing ETL in untreated embryos, the distinction between families that did and did not experience EMS was not clearly defined. The 8.5 nmol/g threshold correctly classified the 1 family with an ETL above the threshold, but incorrectly assigned 2 out of the 16 families below the threshold, overestimating the number of families effected by EMS (Figure 2.1). This variability suggests that factors other than ETL determine whether a family will be affected by EMS. While mortality rates were lower in the thiamine treated group than the untreated group, there was one notable exception: in family 4, which had an ETL of 7.7 nmol/g, treated individuals had lower survival (59%) than their untreated counterparts (74%). This family may have suffered from other developmental issues rather than just EMS.

The survival rates observed in this study indicate that EMS may be influencing reproductive success in a larger proportion of the Atlantic salmon population within Lake Champlain than previously suspected. Eggs sampled in both 2014 and 2015 were at or above the 1.1 nmol/g threshold, implying the potential for strong survival and recruitment (William Ardren, United States Fish and Wildlife Service, 2017, personal communication). However, in

2014 only 3% of families had thiamine levels above 8.5 nmol/g, with 60% of families within the variable area between 2.7 and 8.5 nmol/g, and 37% below 2.7 nmol/g. While thiamine levels were higher in 2015, only 17% of the sample had total ETLs at or above 8.5 nmol/g (William Ardren, United States Fish and Wildlife Service, 2017, personal communication).

Despite these discouraging results, successful natural reproduction has been documented within two of Lake Champlain's major tributaries (Ashlee Prevost, Concordia University, 2018, unpublished data); though fewer fry have been found than would be expected given the number of redds observed each year. The low number of fry may indicate that success is limited to high thiamine individuals, as they likely make up only a small portion of the population. However, the observed fry were clustered in a few small areas and produced by several females (Ashlee Prevost, Concordia University, 2018, unpublished data). Because it is unlikely that all high thiamine females spawned in the same location, these data suggest that local habitat characteristics may be influencing embryo survival. These could include factors such as sediment input or over wintering conditions but may also include the availability of prey for early feeding individuals.

Behavioral manifestations of thiamine deficiency at the free embryo life stage are frequently described in studies on EMS (Fisher et al., 1995; Ketola et al., 2000; Wooster et al., 2000). In our data, however, we detected few, if any, behavioral differences that might indicate decreased survival in a natural environment or an inability to partake in early feeding. Overall, untreated individuals differed little across all thiamine levels and were more likely to show a behavioral response to a light stimulus than thiamine treated individuals; while there were no significant differences between the two groups in those that did respond. Though there was a trend for treated fish to move more than untreated when responding to physical stimulus, the

difference was not statistically significant and may not represent a biologically relevant distinction. These results suggest that thiamine deficiency did not adversely affect photonegativity or the reaction to a physical stimulus, both of which are important antipredator responses at the free embryo life stage. Furthermore, untreated fish did not differ from the Merrimack River control fish in their response to light or physical stimulus. While neither a photo-stimulus nor physical stimulus are direct predictors of foraging capacity, they provide a baseline for behavioral comparison that could be expanded to encompass more complex responses in future research. Overall the lack of significant behavioral differences is encouraging and suggests early feeding may be a viable mitigating factor within a natural environment.

Out of 17 families with egg thiamine levels ranging from 2.3 to 12.7 nmol/g, 9 had surviving, untreated individuals at the button up life stage. While there were no significant differences between groups in response to light stimulus, salmon tend to be less photonegative by the button up stage (Carey and Noakes, 1981; Dill, 1977); therefore, light may not have been a strong enough stimulus at this life stage to detect sublethal effects. Significant differences in behavior were detected between treatments and across egg thiamine levels in response to the physical stimulus. However, 6 of the 9 families that survived to this life stage experienced heavy mortality in untreated individuals prior to completion of the experiment, suggesting the observed differences were precursors of EMS rather than sublethal effects. While there may be a slight trend within the remaining 3 families for untreated individuals to move more at higher thiamine levels (Figure 2.4), the sample size was too small to make any general conclusions about the occurrence of sublethal effects.

Though our knowledge of the Early Mortality Syndrome and the effects of thiamine deficiency on salmonid populations has increased in the past twenty years there is still much to

be learned about the intricacies of the condition. The results of this study suggest that EMS may be having a larger impact on wild Atlantic salmon recruitment than previously thought, not only within Lake Champlain but in other systems where thiamine deficiency is prevalent. However, they also demonstrate variability in mortality in families with moderate thiamine levels, suggesting that not all genotypes are equally susceptible to EMS. This may allow hatcheries to develop broodstocks that are more resistant to thiamine deficiency, which could then be used to supplement threatened populations. A lack of significant behavioral differences detected between low thiamine and high thiamine fish at the free embryo stage suggests that fish may be able to partake in early feeding to offset the deficiency. This behaviour may be a crucial mitigating factor within the natural environment that is not present in a hatchery setting. To date most research on EMS has been done in closed, experimental environments, not allowing for the presence of natural complexities which may lessen the severity of this deficiency in the wild. To truly understand the impact of thiamine deficiency on natural populations more work is needed within a natural environment. Such research will be imperative to the proper management of salmonid fisheries in freshwater systems influenced by EMS.

Tables

Table 2.1 The expected and observed number of experimental replicates for thiamine treated and untreated Lake Champlain Atlantic salmon embryos from 17 families at two different life stages, free embryo and button up.

		Light Stimulus				Physical Stimulus			
		Free Embryo		Button Up		Free Embryo		Button Up	
Family	Expected	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	8	7 [2]	7 [2]	8	8	6 [2]	6 [2]	8	8
2	8	8	8	8	1	7 [2]	7 [2]	7 [2]	0 [1]
3	8	8	8	8	8	7 [2]	7 [2]	7 [2]	7 [2]
4	8	8	7 [1]	8	8	7 [2]	6 [12]	7 [2]	7 [2]
5	8	8	8	8	0 [1]	8	8	8	0 [1]
6	8	8	1 [1]	8	0 [1]	8	1 [1]	7 [2]	0 [1]
7	8	7 [2]	5 [12]	7 [2]	0 [1]	6 [2]	4 [12]	6 [2]	0 [1]
8	8	7 [2]	7 [2]	7 [2]	0 [1]	7 [2]	7 [2]	8	0 [1]
9	8	8	0 [1]	8	0 [1]	8	0 [1]	7 [2]	0 [1]
10	8	8	8	8	0 [1]	6 [2]	6 [2]	8 [2]	0 [1]
11	8	8	8	8	0 [1]	8	8	7 [2]	0 [1]
12	8	8	8	6 [2]	6 [2]	6 [2]	6 [2]	7 [2]	7 [2]
13	8	8	8	8	8	8	8	7 [2]	7 [2]
14	8	8	8	8	8	6 [2]	6 [2]	8	8
15	8	8	4 [1]	8	3 [1]	8	4 [1]	8	3 [1]
16	8	8	8	7 [2]	7 [2]	7 [2]	7 [2]	6 [2]	6 [2]
18	8	8	8	8	1 [1]	6 [2]	6 [2]	6 [2]	1 [1]

[1] losses due to mortality

[2] losses due to video malfunction

Table 2.2 The results of generalized linear models and generalized linear mixed models for survival and behavioral response to stimuli of thiamine treated and untreated Atlantic salmon embryos from 17 families of varying egg thiamine levels in Lake Champlain; in addition to a control group from the Merrimack River that had no previous exposure to thiamine deficiency.

	Model	Residual df	AIC	Fixed Effects	Count Model				Zero Inflated Model			
					Estimate	SE	Z-value	Pr(>|z|)	Estimate	SE	Z-value	Pr(>|z|)
Survival	GLMM	27.00	396.50	(intercept)	3.329	1.036	3.213	0.001	-	-	-	-
				Treatment	-10.718	2.231	-4.804	0.000	-	-	-	-
				ETL : Treatment	10.886	3.348	3.252	0.001	-	-	-	-
				ETL: Treated	-1.890	1.554	-1.217	0.224	-	-	-	-
				ETL : Untreated	8.996	3.107	2.896	0.004	-	-	-	-
Free Embryo: Light	GLMM	215.00	314.00	(intercept)	0.411	1.596	0.257	0.797	1.048	1.353	0.774	0.439
				Treatment	-4.860	3.212	-1.513	0.130	-7.826	3.840	-2.038	0.042
				ETL	0.197	2.664	0.074	0.941	1.131	2.110	0.536	0.592
				ETL : Treatment	6.903	5.645	1.223	0.221	10.278	6.034	1.703	0.885
	GLM	238.00	371.48	Control (intercept)	0.313	0.722	0.433	0.665	-1.003	1.946	-0.515	0.607
				Treated	0.190	0.785	0.242	0.809	2.106	1.777	1.185	0.236
				Untreated	-1.236	0.736	-1.684	0.092	-4.940	87.526	-0.056	0.955
Free Embryo: Physical	GLMM	182.00	755.50	(intercept)	1.812	0.401	4.514	< 0.001	0.553	0.856	0.646	0.519
				Treatment	-1.088	0.585	-1.862	0.063	0.219	1.280	0.171	0.864
				ETL	-0.058	0.582	-0.100	0.920	-1.068	1.292	-0.827	0.408
				ETL : Treatment	0.966	0.805	1.200	0.230	-0.076	1.893	-0.040	0.968
	GLM	206.00	827.79	Control (intercept)	0.808	0.268	3.016	0.003	-1.403	0.944	-1.487	0.137
				Treated	0.984	0.284	3.466	0.001	1.242	0.959	1.295	0.195
				Untreated	0.528	0.294	1.796	0.725	1.382	0.963	1.435	0.151

Button Up: Light	GLMM	122.00	355.75	(intercept)	-0.917	1.665	-0.551	0.582	-	-	-	-
				Treatment	3.124	1.860	1.680	0.093	-	-	-	-
				ETL	1.181	2.262	0.522	0.602	-	-	-	-
				ETL : Treatment	-4.205	2.569	-1.637	0.102	-	-	-	-
	GLM	145.00	423.93	Control (intercept)	0.854	0.582	1.467	0.142	-	-	-	-
				Treated	-0.595	0.663	-0.898	0.369	-	-	-	-
				Untreated	-0.333	0.679	-0.491	0.624	-	-	-	-
Button Up: Physical	GLMM	105.00	567.10	(intercept)	2.240	0.448	5.003	< 0.001	-0.754	1.278	-0.590	0.555
				Treatment	-2.602	0.785	-3.314	0.001	0.964	1.723	0.560	0.576
				ETL : Treatment	3.267	1.030	3.173	0.002	0.338	2.401	0.141	0.888
				ETL : Treated	-0.411	0.633	-0.650	0.516	-0.639	1.863	-0.343	0.731
				ETL : Untreated	2.856	0.811	3.521	< 0.001	-0.301	1.508	-0.200	0.842
	GLM	129.00	690.53	Control (intercept)	2.107	0.219	9.646	< 0.001	-1.695	0.747	-2.269	0.023
				Treated	-0.175	0.252	-0.692	0.489	0.387	0.813	0.476	0.634
				Untreated	-0.217	0.283	-0.769	0.442	1.708	0.795	2.148	0.032

Figures

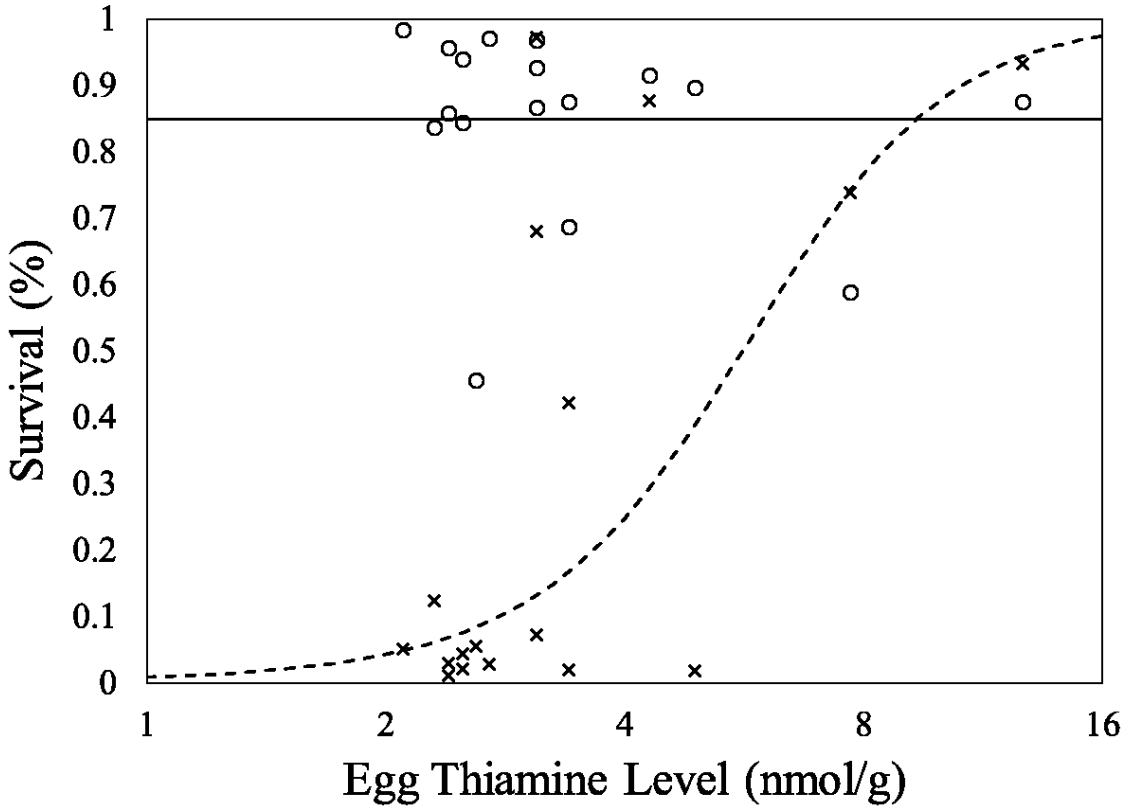

Figure 2.1 The survival of thiamine treated (open circles) and untreated (x's) Lake Champlain Atlantic salmon from 17 families of varying egg thiamine levels from hatch to button up. The dashed line indicates the predicted relationship for untreated individuals based on the results of a generalized linear mixed model while the solid line indicates the non-significant trend for treated individuals with a slope of 0.

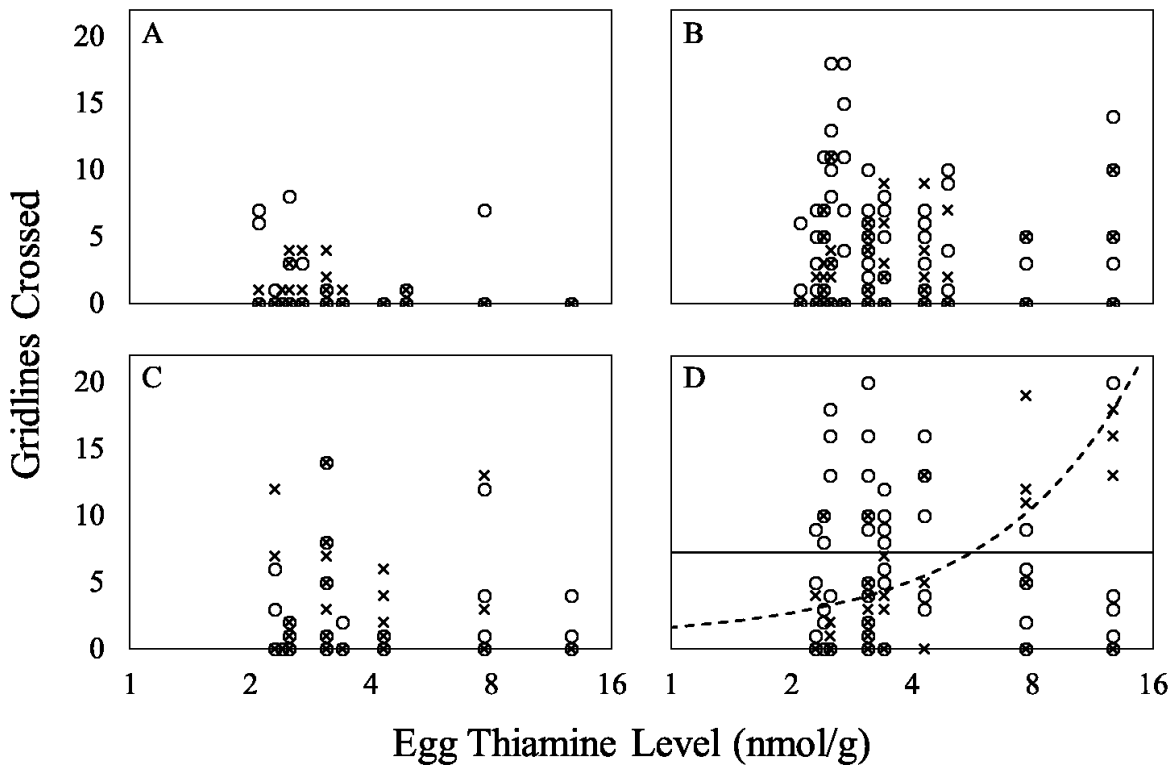

Figure 2.2 The behavioral response of thiamine treated (open circles) and untreated (x's) Lake Champlain Atlantic salmon to light stimulus at the free embryo stage (A), physical stimulus at the free embryo stage (B), light stimulus at the button up stage (C), and physical stimulus at the button up stage (D). The dashed line indicates the predicted relationship for untreated individuals based on the results of a generalized linear mixed model while the solid line indicates the non-significant trend for treated individuals with a slope of 0. When neither relationship was significant no trendlines are depicted.

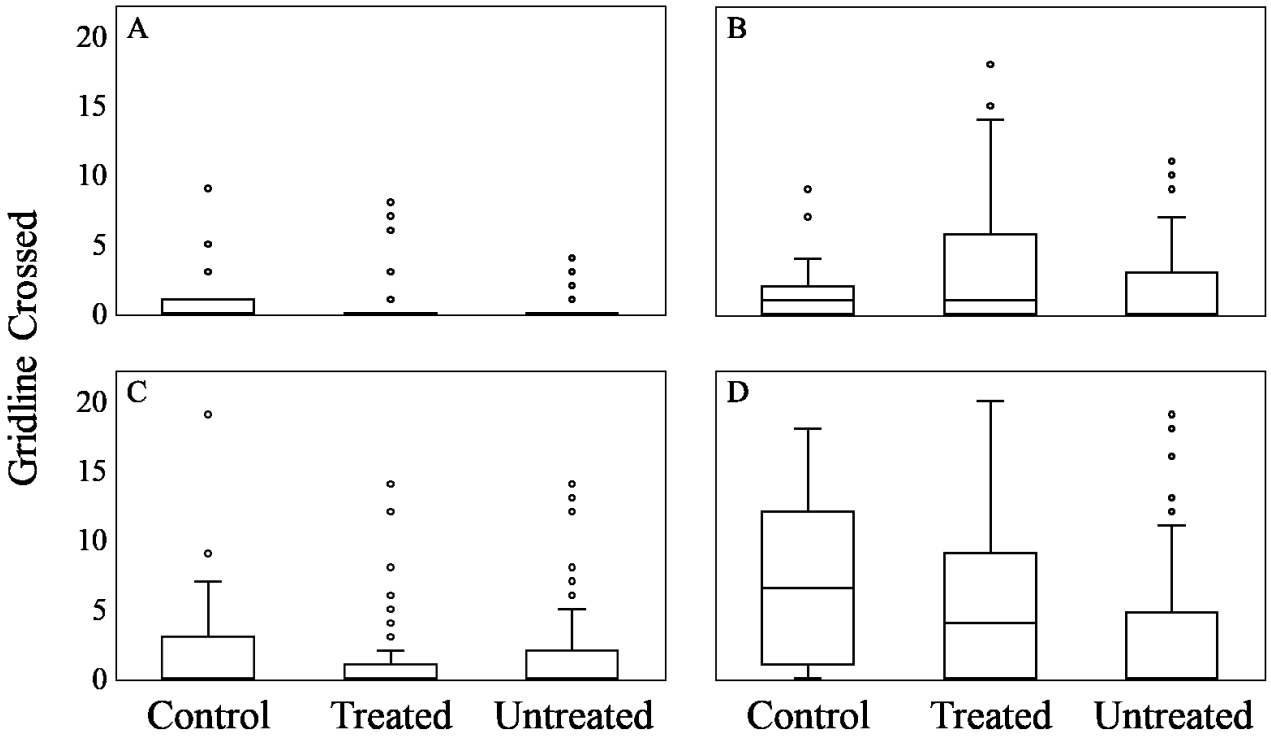

Figure 2.3 Box and whisker plot of the median behavioral response of thiamine treated and untreated Atlantic salmon larvae from Lake Champlain, and a control group from the Merrimack River to light stimulus at the free embryo stage (A), physical stimulus at the free embryo stage (B), light stimulus at the button up stage (C), and physical stimulus at the button up stage (D). The median is indicated by the horizontal line within the box while the box itself depicts the 25th to 75th percentile. The whiskers indicate the inter quartile range, while outliers are depicted as open circles.

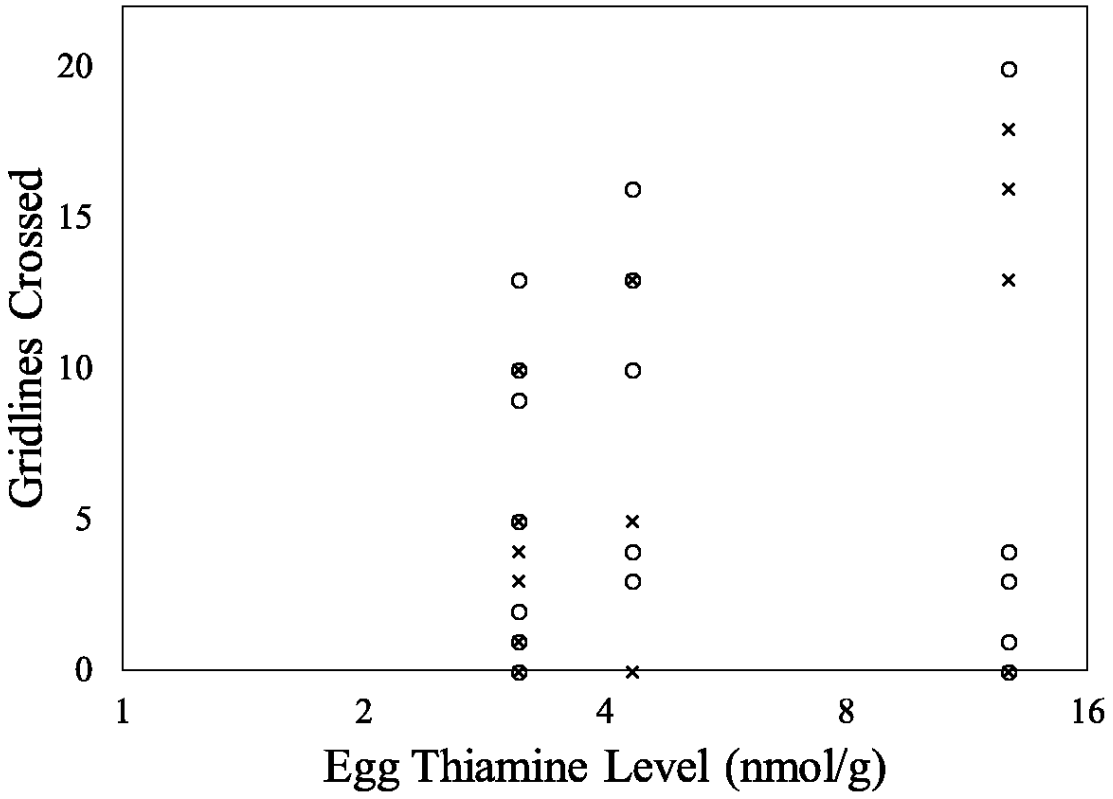

Figure 2.4 The behavioral response to physical stimulus at the button up stage of thiamine treated (open circles) and untreated (x's) Lake Champlain Atlantic salmon in the three remaining families which did not experience Early Mortality Syndrome.

Supplementary Material

Table S2.1 Mating crosses for 17 families of Atlantic salmon collected from Lake Champlain. Each family was assigned a number based on the ID number of the Female.

Date Crossed (dd/mm/yy)	Female ID	Male ID
15/11/16	1	1
15/11/16	2	2
15/11/16	3	3
15/11/16	4	4
15/11/16	5	5
15/11/16	6	6
15/11/16	7	7
15/11/16	8	8
15/11/16	9	9
15/11/16	10	10
15/11/16	11	11
17/11/16	12	12
17/11/16	13	12
17/11/16	14	13
17/11/16	15	14
17/11/16	16	13
17/11/16	17	12

Table S2.2 The average total egg thiamine level for 17 families of Lake Champlain Atlantic salmon and percent mortalities amongst thiamine treated and untreated groups within each family.

Family	Egg Thiamine Level (nmol/g)	Survival (%)	
		Thiamine Treated	Untreated
1	3.1	96.9	97.3
2	2.4	95.7	1.1
3	3.1	86.7	68.1
4	7.7	58.9	73.9
5	2.7	97.1	2.9
6	2.5	84.4	2.1
7	2.1	98.4	5.2
8	3.1	92.8	7.4
9	2.6	45.6	5.6
10	3.4	87.5	2.0
11	4.9	89.7	1.9
12	3.4	68.7	42.3
13	4.3	91.6	87.8
14	2.5	94.0	4.5
15	2.3	83.8	12.4
16	12.7	87.6	93.3
17	2.4	85.9	3.0

General Conclusions

The re-establishment of a native population often takes many years and requires persistence on behalf of those undertaking the endeavour (George et al., 2009). An adaptive management approach is fundamental in monitoring and evaluating progress throughout the duration of the program and identifying any limitations on recolonization success. (Cochran-Biederman et al., 2015; IUCN, 2013; Seddon et al., 2014).

The continued presence of factors which contributed to the original extirpation of the population is one of the most consistent predictors of reintroduction failure (Cochran-Biederman et al., 2015; Fischer and Lindenmayer, 2000). Theoretically, these factors should be addressed prior to reintroduction; though this can be difficult when the limiting factors provides benefits to society. In the case of Lake Champlain, the damming of many tributaries is thought to have contributed to the extirpation of Atlantic salmon within the system. Despite this, most have not been removed, as they provide services to the surrounding communities such as hydroelectric power. However, the timber crib dam on the Boquet River, Willsboro was no longer in use and beginning to fail, making it a good candidate for removal (Lake Champlin Fish and Wildlife Conservation Office, 2016). The removal of the dam has potentially increased spawning habitat availability for Atlantic salmon on the Boquet, by allowing access to high quality spawning grounds upstream and improving habitat quality below the former dam site. While the Willsboro Cascades may still represent a significant migration barrier, some untagged fish were observed in spawning grounds upstream. Further monitoring will be needed to determine the percentage of the population that can successfully traverse the cascades on their own. Continued translocation would require extensive external intervention and is not a viable, long term solution.

Spawning habitat availability, however, is not the only factor limiting reintroduction success in Lake Champlain. The invasion of non-native alewife and their interaction with the local Atlantic salmon population has resulted in a more complex constraint on recruitment in the form of Early Mortality Syndrome (EMS). Until an effective management plan is in place to mitigate the occurrence of EMS in naturally spawned embryos, spawning habitat restoration will likely not be sufficient to support a viable population. Our results suggest that the egg thiamine level needed to circumvent deficiency induced mortality is significantly higher than described in the previous literature. Out of the 17 families we sampled, 14 (or 82%) experienced EMS in untreated embryos. A lack of behavioral differences at the free embryo stage suggests that some fish may be able to off set the deficiency in the wild by early feeding. However, this does not seem to be occurring in a large proportion of the population as less than 200 naturally spawned fry have been observed in the lake's major tributaries in the past two years. To properly manage EMS, a better understanding of its effects on reproductive success in a natural environment will be needed. It is still likely that there are external factors that influence the occurrence of EMS, and which cannot be accounted for within a hatchery environment. While alewife populations may eventually decline naturally, it is likely that further intervention will be necessary to either mitigate this issue or maintain the Atlantic salmon population. By selecting for embryos that survive without thiamine treatment, hatcheries may be able to develop a deficiency resistant broodstock which could be used to help augment the low population numbers. However, there is currently no definitive research on the heritability of these traits.

There is no single or simple solution to reintroduction success. There are many different factors that influence a populations ability to re-establish within a given environment, not all of which are easily mitigated. However, consistent monitoring and an adaptive approach to

population management may help to increase the likelihood of success (Cochran-Biederman et al., 2015). While a self-sustaining population of Atlantic salmon has not yet been established in Lake Champlain, by learning about and working towards the management of issues such as habitat availability and the effects of invasive species, the program is making slow progress towards its goal.

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