

A molecular-telemetric approach to Atlantic salmon reintroductions: how human intervention
can promote the establishment of new populations

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Abstract

A molecular-telemetric approach to Atlantic salmon reintroductions: how human intervention can promote the establishment of new populations

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With increases to the number of species and populations impacted by human activities, the need for human involvement in their maintenance and/or conservation has grown in turn. This involvement increasingly takes the form of re-introductions, relocations, or supplementation of populations in decline or that have been lost. As a result, reintroduction biology has become a quickly growing field of research that attempts to answer the many questions related to reintroductions and translocations. When and how to release individuals into the wild, at what age should they be released, and in what situations is the introduction of new individuals sufficient to establish new populations.

To advance our understanding of species reintroductions and address some of these questions in situ, adaptive management experiments and new techniques were developed for the Atlantic salmon reintroduction program in the Lake Champlain basin. These experiments assessed the suitability of commonly used methods in salmonid reintroduction and supplementation for re-establishing or strengthening self-sustaining populations and meta-population structure through direct comparisons of long-term survival, spawning returns, and dispersal rates. Concurrently, to address how changes in the species composition of Lake

Champlain have affected the ability of reintroduced salmon to establish themselves, radio telemetry was employed to monitor spawning migrations through a challenging, high velocity section of the Boquet River, a tributary to Lake Champlain. In doing so, new, transferrable radio telemetric techniques for continuous fine scale monitoring were developed.

In comparing alternative rearing/release techniques to the standard method of producing large 1+ parr through the use of elevated rearing temperatures, several significant trends were apparent. First, while fry (age 0+) are commonly held to exhibit reduced survival to adulthood relative to 1+ parr, once natural mortality during the first year was accounted for, fry returns exceeded those of standard production parr while their dispersal rates were more consistent with Atlantic salmon meta-populations. Second, all three alternative parr rearing/release methods reduced straying rates relative to standard methods. Third, while advancing release dates lowered parr-to-adult survival relative to standard procedures, using seasonal water temperatures prior to release significantly improved both survival and spawning returns.

Once released, growing salmon fed on alewife, an invasive prey species containing thiaminase, which lowered thiamine levels among mature adults, potentially impacting energy levels and swimming performance. At a challenging, high velocity section of the Boquet River, we detected a significant effect of this thiamine deficiency on downstream movement rates from both high and low flow sections of the river as well as an effect of thiamine supplementation, particularly among male salmon. To confirm this, however, a new telemetric technique was required. We therefore developed a technique that successfully estimated the location of tagged salmon to within several meters using readily available equipment and statistical models.

Overall, these results will assist the reintroduction efforts in Lake Champlain and their implications are highly transferrable to the reintroduction/supplementation of other at-risk or

extirpated populations. The methods developed below are readily implementable and provide positive returns on investment, both over the short- and long-term, while representing a step forward for the reintroduction of species of high economic, cultural, and ecological importance.

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have been possible. All research was conducted under strict adherence to the regulations stipulated within our sampling permits and was in accordance with the guidelines set by the Concordia Animal Research Ethics Committee.

Contribution of the Authors:

The following chapters were prepared as manuscripts, with each intended for submission to peer-reviewed journals. In each case, Dr. Dylan Fraser and Dr. William Ardren assisted considerably in the conception and implementation of each experiment, both in the planning stages and in the field. I was primarily involved in conception, planning, data collection and analysis, along with the preparation of all manuscripts. Dr. Theodore Castro-Santos was instrumental in the planning, preparation and analysis stages of both chapters related to radio telemetry, while also assisting in the preparation of those manuscripts. Finally, Drs. Dimitry Gorsky and David Hand both provided expert opinions and assistance regarding field techniques for the radio telemetric and thiamine chapters along with contributing to the preparation of the final submitted manuscripts.

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Table of Contents

List of Figures	xi
List of Tables.....	xiv
General Introduction.....	1
Chapter 1: Minor shifts towards more natural conditions in captivity improve long-term survival and reduce dispersal in reintroduced salmon populations.	17
Abstract:.....	19
Introduction:	20
Methods:.....	23
Results:	28
Discussion:	32
Chapter 2: Novel, continuous monitoring of fine-scale movement using fixed-position radio telemetry arrays and random forest location fingerprinting	46
Abstract:.....	47
Introduction:	48
Methods:.....	50
Results:	59
Discussion:	64
Chapter 3: Environmental, anthropogenic, and dietary influences on fine scale movement patterns of Atlantic salmon through challenging waters	74
Abstract:.....	75
Introduction:	76
Methods:.....	79
Results:	87
Discussion:	92
General Discussion	115
General Conclusion	126
References	128
Chapter 1 Appendices	160
<i>Appendix 1A, Offspring production and release conditions</i>	<i>160</i>
<i>Appendix 1B, Sample collection and supplemental analysis of potential sampling bias</i>	<i>166</i>
<i>Appendix 1C, Genotyping and parentage methods and results</i>	<i>173</i>
<i>Appendix 1D: Parr-to-adult survival, spawning returns, and straying at the cohort level</i>	<i>184</i>
Chapter 3 Appendix	196

List of Figures

Figure 1.1: The temperature regime and stocking dates experienced by parr experimental Atlantic salmon released into the Lake Champlain Basin in two experimental tributaries (Boquet River, NY, and Winooski River, VT) from 2012 to 2014 as part of an adaptive management experiment to improve river returns and restore wild populations and meta-population structure.

Figure 1.2: The location of two experimental tributaries to Lake Champlain where fry (empty circles) and parr (filled circles) were released over the course of 4 years (2011 – 2014). Dams are indicated by triangles and the sampling site for returning adult salmon is indicated by a filled triangle.

Figure 1.3: Recapture proportions in Lake Champlain and two experimental tributaries along with straying proportions according to the average parr size-at-release including trend lines based on linear models.

Figure 1.4: The lake recapture frequencies, river returns, and straying rates for multiple growth conditions and release dates for Atlantic salmon stocked in two tributaries of Lake Champlain, the Boquet (a) and the Winooski (b) rivers. Lake recaptures and river returns are expressed as a proportion of the numbers originally released while straying rates represents the frequency of salmon that were recaptured in a non-origin tributary as a proportion of total number of each release group intercepted during a spawning migration.

Figure 2.1: The study reach within the Boquet River, NY, USA, along with locations of four fixed-position aerial radio antennas, one submerged radio antenna, and landmark locations at which the received signal strength from mobile VHF radio transmitters were characterized for each antenna.

Figure 2.2. The mean received signal strengths from a radio transmitter tag at varying distances (bottom) along the migratory route of Atlantic salmon climbing a section of the Boquet River, NY, USA. Mean values were modeled using loess curves fit to 671 received signal strength values at 49 separate distances throughout the study reach.

Figure 2.3. Panel a) the prediction error (in meters) between the true distances from the river mouth of landmarks throughout the study reach and the distances estimated by the random forest location fingerprinting method. The dotted line represents an ideal fit (a slope of 0) while the solid line is the fitted slope from a linear model (slope = 0.017, $p > 0.05$). Panel b) the prediction errors as a function of the random forest model's confidence (the 95% prediction interval width).

Figure 2.4. The mean received signal strengths at known locations as modeled using loess curves (colored lines), and the RSS values averaged over 24 tagged Atlantic salmon at distances estimated by random forest localization model (points, panel 4a). The remaining two panels display the mean RSS values for those same 24 salmon after application of a Kalman smoother to (4b) the raw distance estimates, and (4c) only those distance estimates with confidence intervals $< 100\text{m}$.

Figure 2.5: The mean received signal strength at estimated distances from the river mouth for two radio tagged Atlantic salmon captured and subsequently released into the lower pool of the study reach on September 29th and monitored until November 19th 2014. Histograms show the number of tag transmissions (1 every 5 seconds) received from each fish at each 1 m section of the study reach.

Figure 3.1: Map of the Willsboro rapids used to assess the impact of various biotic and abiotic factors on upstream migrations through challenging sections of river, along with the effect of thiamine supplementation among the thiamine deficient landlocked Atlantic salmon in the Lake

Champlain basin. Antennas remained fixed and recorded transmission signal strength from tagged salmon from September 26th to November 19th, 2014.

Figure 3.2: Two of the ancillary time varying covariates; discharge (a) and temperature (b) included in Cox proportional hazards models to explain migratory behaviour of mature Atlantic salmon migrating through a difficult section of the Boquet River, along with the number of salmon (c) present in the Willsboro rapids during the observation period of September 26th to November 19th, 2014.

Figure 3.3: The predicted survival and cumulative incidence curves by treatment from Cox Proportional Hazards models of salmon, respectively, advancing through or retreating from pools within the Willsboro Rapids, Essex County, NY. While the analysis was stratified over three pools, only the lower pool's curves are displayed. Grey lines represent all tagged salmon regardless of thiamine treatment status.

Figure 3.4: The predicted survival and cumulative incidence curves from Cox Proportional Hazards models of salmon, respectively, advancing through or retreating from sets of rapids located in the Willsboro Rapids, Essex County, NY. While the analysis was stratified over two sets of cascades, only the first cascade's curves are displayed. Grey lines represent all tagged salmon regardless of thiamine treatment status or sex.

Figure 3.5: The predicted cumulative incidence curve from a Cox Proportional Hazard model of salmon retreating from the Willsboro Rapids, Essex County, NY.

List of Tables

Table 1.1: Adult recapture frequencies, pooled across replicate cohorts within a tributary, for Atlantic salmon produced using alternative rearing/release conditions and released into two experimental tributaries to Lake Champlain as fry or parr. Lake sampling occurred throughout the year, while river returns were assessed during fall spawning migrations. Strays represent adults observed migrating into tributaries other than their tributary of origin. Values represent the number of salmon/number of families/mean (st.dev.) number of offspring per family. Subscripts indicate significant similarities or differences based on Fisher's Exact tests.

Table 1.2: Adults recapture frequencies, pooled across replicate cohorts across tributaries, for Atlantic salmon produced using alternative rearing/release conditions and released into two experimental tributaries to Lake Champlain as fry or parr. Lake sampling occurred throughout the year, while river returns were assessed during fall spawning migrations. Strays represent adults observed migrating into tributaries other than their tributary of origin. Values represent the number of salmon/number of families/mean (st.dev.) number of offspring per family. Subscripts indicate significant similarities or differences based on Fisher's Exact tests.

Table 3.1: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in pools within the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

Table 3.2: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in cascades within the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

Table 3.3: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in the whole of the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

General Introduction

Reintroduction Biology

As we continue to advance through the Anthropocene, the number of species extinctions and populations in decline will continue to rise (Seddon et al. 2007, Frankham 2008, Pimm et al. 2014), impacting natural and human environments alike in unpredictable ways (Potts et al. 2010, Dirzo et al. 2014). This is perhaps particularly true for freshwater ecosystems as they support a disproportionately high proportion of fish species and are highly vulnerable to human activities and global climate change (Meybeck 2003, Dudgeon et al. 2006, Vörösmarty et al. 2010). If we are to avoid or at least minimize such losses, direct human intervention will be required (Novacek and Cleland 2001, Ceballos et al. 2015). To this end, species reintroductions - the movement of individuals into vacant historic ranges from captive or wild sources (IUCN/SSC Reintroduction Specialist Group 1987) - have become a popular form of intervention due to their direct positive impact on local biodiversity (Armstrong and Seddon 2008, Seddon et al. 2014).

Though reintroductions are an attractive means of counteracting population declines, their success is not guaranteed and in many situations they fail to establish viable populations (Griffith et al. 1989, Fischer and Lindenmayer 2000, Cochran-Biederman et al. 2015). Reintroductions may fail for a myriad of reasons, though the most common predictor of unsuccessful reintroductions is the failure to identify and remedy the initial cause of population decline (Fischer and Lindenmayer 2000). This is therefore included as an initial step in virtually all reintroduction guidelines (Williams et al. 1988, Meffe 1995, Dunham et al. 2011, IUCN/SSC Reintroduction Specialist Group 2013). When the initial cause of decline has been remedied, reintroductions can still fail due to various reasons that can be broadly classified into three

general categories: issues with the receiving environment, issues with release techniques, or issues with the reintroduced individuals themselves.

Among plants (Godefroid et al. 2011), mammals and birds (Wolf et al. 1998), and freshwater fish (Cochran-Biederman et al. 2015) the most common causes of failure in reintroduction programs are issues with the receiving environment. This includes reintroductions into environments without suitable spawning or juvenile habitat (Harig et al. 2000), environments with an excess of competitors, both native and non-native (Al-Chokhachy et al. 2009, Godefroid et al. 2011), or environments with an excess of predators (Wolf et al. 1996, Sinclair et al. 1998). Yet, even when great care is taken to insure receiving habitats meet the requirements of a reintroduced species, increases in temperature, drought, or fires resulting from climate change can put small, newly reestablished populations at risk, particularly along the retreating edges of their range (Aitken et al. 2008). Invasive species are also impacted by climate change, though not always negatively (Rahel and Olden 2008) which can exacerbate problems for reintroduction programs. Finally, human land use and resource requirements, particularly in freshwater environments (Vörösmarty et al. 2010) can reduce the suitability of environments within the historic ranges of a species. How such issues will affect reintroduction efforts in the future is often unpredictable, emphasizing the need for reintroduction programs to employ active adaptive management strategies (Walters and Hilborn 1978).

The methods by which individuals are released into the wild can greatly benefit from adaptive management techniques to improve the likelihood of reintroduction success. For example, among prey species, survival rates tend to increase with age, and through experimental releases and subsequent monitoring, biologists can identify which age classes maximize the frequency of mature adults in a receiving environment. Doing so has improved natural

recruitment from reintroduced species in a number of situations (Sarrazin and Legendre 2000, Ostermann et al. 2001, Cochran-Biederman et al. 2015), though this strategy carries with it numerous risks associated with extended exposure to the captive environment (see below). Where, how, and when individuals are released can also significantly affect survival over the short- and long-term. Releasing individuals into predator-free enclosures before releasing into the wild, for example, has been found to significantly improve short- and long-term survival (Jonsson et al. 1999, Hamilton et al. 2010, Mitchell et al. 2011). Such soft-release methods have also been shown to reduce dispersal from the release site which reduces the Allee effect, improving recruitment (Armstrong and Wittmer 2011). Synchronizing releases to correspond with seasonally abundant food sources or seasonally low predator abundances can also improve post-release survival (Chittenden et al. 2010). Finally, increasing the number of individuals released greatly improves the likelihood of successful establishment in a target environment (Wolf et al. 1996, Armstrong and Wittmer 2011, Cochran-Biederman et al. 2015).

Reintroductions can also fail when the released individuals are from a captive maladapted population (Fischer and Lindenmayer 2000, Frankham 2008, Fraser 2008). When losses to wild populations have been considerable, however, the use of captive populations may be unavoidable (Frankham 2008, Fraser 2008). When captive populations are used, care must be taken to insure such populations remain viable in the wild by avoiding cumulative maladaptive traits from unintentional selection or relaxed selective pressures in the captive environment (Frankham 2008, Araki et al. 2008, Fraser 2008, Williams and Hoffman 2009), inbreeding and/or outbreeding depression (Marshall and Spalton 2000), or the loss/reduction of local adaptations (Araki et al. 2008). Finally, simple exposure to the captive environment, which typically differs greatly from the wild environment, can disrupt crucial developmental processes during early life-

stages, resulting in carry-over effects that decrease the fitness of released individuals at a later date (Araki et al. 2009, Milot et al. 2013, Clarke et al. 2016).

It was through studies addressing the question of why some efforts succeed while others fail that the science of reintroduction biology developed into the recognized branch of biology it is today (Seddon et al. 2007, Ewen et al. 2012). Since its creation, the study of reintroduction biology has resulted in the creation of numerous guidelines for the development and improvement of new and current reintroduction programs (e.g. IUCN/SSC 2013). Based on the results of mathematical models, past reintroduction efforts, and adaptive management experimentation, these guidelines, generalized over all plant or animal species, cover the entire reintroduction process including when/how wild species should be relocated (Richardson et al. 2009, McDonald-Madden et al. 2011), and how best to conduct captive breeding in order to maintain fitness in the wild (Lynch and O’Hely 2001, Frankham 2008, Fraser 2008). When the use captive populations is required, following guidelines for optimal rearing and captive husbandry is key to the successful release of viable individuals (IUCN/SSC Re-introduction Specialist Group 2016), while in all cases, long-term monitoring is required to determine the ultimate outcome of various reintroduction methods (Sarrazin and Barbault 1996, Ewen and Armstrong 2007).

Despite the widespread use of these generalized guidelines, and the development of less general guidelines for specific species and habitat types (e.g. guidelines for freshwater fish reintroductions; Williams et al. 1988, Meffe 1995, Dunham et al. 2011, Cochran-Biederman et al. 2015), failure is still possible and reintroduction programs should critically assess each step of the process to identify areas where improvements can be implemented. In this thesis, I will look at two such areas of potential improvement for the reintroduction of a culturally and

economically important fish species, Atlantic salmon (*Salmo salar*). This includes how salmon rearing conditions and the timing of release impact development at a crucial early life stage and the carry-over effects this can have on lifetime survival and homing abilities of captive-bred salmon. I also address how active management at later stages of a reintroduction program may help the establishment of self-sustaining populations following the appearance of an invasive species. In doing so, I address a shortcoming of current fish tracking methods in high energy fluvial environments and develop a new radio telemetric technique for monitoring fine scale movement.

Captive rearing and releasing techniques

The dramatic differences between captive and wild environments have long been known to produce behavioral, morphological, physiological and genetic differences between captive and wild individuals from a multitude of species (Lyles and May 1987, Frankham and Loebel 1992, McPhee 2004, Håkansson and Jensen 2005, Bowlby and Gibson 2011, Reading et al. 2013). These differences almost always reduce the fitness of captive-bred individuals in the wild, as has been particularly well documented among fish species (Reisenbichler and McIntyre 1977, Fleming and Gross 1993, Ford 2002, Araki et al. 2008, Christie et al. 2014). Regardless, captive breeding programs still represent our primary means of augmenting the densities of declining wild populations through supplementation as well as for reintroducing populations that have been lost (reviewed in Brown and Day 2002). As a result, a wealth of studies have investigated how captive breeding programs and release methods can be altered to reduce these fitness losses experienced by captive-bred fish.

The two primary means by which the fitness of captive-bred fish is reduced in the wild are through genetic or environmental influences. Genetic influences on post-release fitness often take the form of inbreeding depression (Kincaid 1976, Ryman and Ståhl 1980), the loss of adaptive genetic variation (Meffe 1990), or the fixation of maladaptive traits (Hedrick 1994). A number of reviews of captive breeding programs (Lynch and O’Hely 2001, Frankham 2008, Fraser 2008) have outlined techniques for minimizing the risks of such genetic effects which include, but are not limited to, the use of large broodstocks, equalizing family sizes in each generation, minimizing kinship within controlled crosses, and reducing exposure to selection in the captive environments (by releasing earlier or by reducing differences between the captive and wild environments). Regardless of how closely hatchery managers follow such recommendations, however, the fitness of captive-bred fish, salmonids in particular, remains lower than those of individuals born in the wild (Araki et al. 2007a, Milot et al. 2013, Christie et al. 2014, Clarke et al. 2016).

Captive-bred individuals can also be genetically maladapted to specific receiving environment if they initially lack locally adapted traits (Krueger et al. 1981), or if they lose such traits over successive generations in captivity through relaxed or unintentional domestication selection (Allendorf and Luikart 2007). Reducing the geographic distance between the source population, and the receiving environment, along with the regular use of feral gametes, can reduce this risk (Ford 2002, Araki et al. 2008), though the genetic effects of captivity can arise quickly, even within a single generation (Christie et al. 2012, 2014). Apart from following genetic guidelines and releasing individuals from early generations of local broodstocks, carry-over effects from the captive environment will still reduce fitness in the wild and fisheries

managers should carefully consider which rearing and release methods are most appropriate, using adaptive management experimentation to address areas where knowledge is still unclear.

An example of how release conditions can produce carry-over effects is with age-at-release (exposure to the captive environment) and its effect on fitness post-release. Salmon from identical genetic backgrounds can display significant behavioral, developmental, and fitness differences over their lifetimes that negatively affect fitness as a result of exposure to the captive environment as juveniles (Fleming et al. 1996). Even the extent of exposure can significantly and negatively affect fitness related traits (Thériault et al. 2011, Clarke et al. 2016), sometimes after only a few months (Metcalf et al. 2003). These effects result from differences in selective regimes or differences in learnt behavior and can be difficult to avoid as the use of rearing facilities is usually necessary. Commonly suggested means of mitigating the effects of a captive environment on behavior and development include release at earlier life stages (Metcalf et al. 2003, Clarke et al. 2016), enriched captive environments (Berejikian et al. 2001, Brown et al. 2003), or reduced rearing densities (Barnes et al. 2013).

Similarly, differences in both short- and long-term post-release survival among release groups have prompted fisheries biologists to recommend various release methods in order to improve survival. For example, anadromous salmonids released at the smolt stage can experience higher post-release survival when transported to the release site in water containing salt and a mild anesthetic to reduce stress (Finstad et al. 2003). Also, local variations in prey availability should be considered when selecting a release date as synchronizing juvenile presence with optimal prey abundances can significantly improve salmonid survival following outmigration to the sea or lakes (Duffy and Beauchamp 2011). Care must be taken to avoid releasing pre-smolts too late, however, as spring releases produced significantly fewer strays than fall releases

(Hansen and Jonsson 1991). When earlier life stages are released (fry or fingerlings), releasing individuals over a dispersed area rather than mass releases into a single location can reduce density dependent effects on growth and survival (Crisp 1995, Brunsdon et al. 2017). Finally, providing released individuals with an acclimation period of several days in a predator-free enclosure, where they can experience local conditions, can significantly improve post-release survival (Johnson et al. 1990, Jonsson et al. 1999) though this does not seem to be the case with all salmonid species and populations (e.g. Savitz et al. 1993, Kenaston et al. 2001).

While the use of such experimentally derived recommendations can improve survival, they do not necessarily improve overall fitness and for many fisheries managers, questions such as how to maximize survival without sacrificing overall fitness (such as reproductive success) remain unanswered. This is of particular concern for salmon reintroduction and supplementation efforts as most make use of salmon production operations that are geared to maximize juvenile-to-adult survival, not to maximize fitness traits such as reproductive success. While the methods employed by such operations do tend to increase the number of adult fish recruiting into a fishery or returning to a stocked tributary, their contribution to a population's reproductive output is nearly always lower than that of wild salmonids (Araki et al. 2007b, Thériault et al. 2011, Christie et al. 2014). For this reason, fisheries managers must seek out means of adjusting their methods to improve fitness in order to increase the chances of such releases resulting in the establishment of new self-sustaining populations.

Salmon production facilities commonly employ the method of accelerating the growth rates of juveniles to produce larger individuals that will transform from parr (the freshwater life stage) to smolt (the stage at which they begin their downward journey to feeding grounds in lakes or marine environments) in their first year (Farmer 1994, Duffy and Beauchamp 2011).

These larger 1+ parr possess a survival advantage over smaller conspecifics as their larger size permits access to a wider range of food, allowing them to maintain elevated growth rates and escape gape limited predators earlier (Beamish and Mahnken 2001). These survival advantages do not necessarily translate into greater fitness, however, as a common carry-over effect of such husbandry methods among migratory species, such as salmonids, is a tendency to stray more than wild individuals (Quinn 1993). Though multiple possible explanations likely exist for these elevated straying rates, a highly probable contributing factor is that the high growth rates allow juveniles to begin their parr-smolt transformation earlier, prior to release into the wild. Doing so can result in incorrect or interrupted olfactory imprinting during the parr-smolt transformation period (Dittman and Quinn 1996). Insuring that salmonid juveniles are released prior to olfactory imprinting is crucial, though not always an easy task as salmonids exhibit species- and population-specific imprinting times/sizes (Keefer and Caudill 2014). Adaptive management is therefore an appropriate means of counteracting this negative effect. In addition to elevated straying rates, the faster growth rates of captive-bred salmonids can allow them to mature earlier (Kallio-Nyberg and Koljonen 1997, Thériault et al. 2011). This earlier maturation, in conjunction with behavioral differences, can result in females releasing fewer eggs in less well constructed redds while males compete less successfully for females (Fleming and Gross 1993, Jonsson and Jonsson 2006).

In order for salmonid reintroduction efforts to successfully establish self-sustaining populations and reestablish sustainable metapopulation structure within an area, rearing and release methods that maximize survival while promoting natural dispersal rate are required. By releasing salmon with abnormally high dispersal rates, fisheries managers undermine their own efforts in several influential ways. First, each stray represents a potentially critical loss from the

donor population, particularly when the population size is small. Second, when nearby wild populations still exist, but at low levels, straying of captive-bred fish into these native population can result in hybridization and outbreeding depression (Waples 1991). Third, as local adaptations can develop quickly among salmonids (Fraser et al. 2011), each salmon that homes back and spawns in its tributary of release represents a step towards the establishment of a locally adapted self-sustaining population.

Despite the importance of maintaining natural dispersal rates for population and metapopulation restoration, few studies have attempted to address this issue by combining methods known to produce high survival with methods believed to reduce dispersal by promoting olfactory imprinting post-release. While some attempts have been made, such as rearing eggs using water from the reintroduction environment (Dittman et al. 2015), past efforts have shown limited success and a definitive answer has yet to be found. Currently, the most effective way to insure salmon imprint properly is to sacrifice high survival by releasing juveniles at earlier life stages. A desirable method would maintain high parr-to-adult survival by allowing for accelerated growth rates pre-release, while permitting rearing conditions or releasing methods to be altered once a critical size is reached without any significant negative effect on parr-to-adult survival.

Impact of Invasive Alewife

Species reintroductions are often hindered by the presence of exotic invasive species in the receiving environment, particularly when the exotic species is a predator (Short et al. 1992, Sinclair et al. 1998, Moseby et al. 2011, Hardman et al. 2016). Other types of interactions do occur, however, and competition with exotics (Lindsay and Cunningham 2011), or parasitism by

exotics (Swinnerton et al. 2005) can be equally detrimental to reintroduction efforts. Given the great financial and ecological costs associated with the removal of established invasive species, especially if they have assumed the role of displaced native species (Zavaleta et al. 2001), reintroduction biologists often have no choice but to incorporate interactions with exotics in their reintroduction plans. This includes an often-overlooked interaction that can develop when a reintroduced predator species preys on an established invasive. An example of such an interaction is between the invasive prey species alewife (*Alosa pseudoharengus*) and native salmonid predators.

Though alewife occur naturally within the native ranges of salmonids throughout coastal waters in northeastern North America, under normal conditions they only make up a small portion of salmonid diets and impart no negative effects on fitness. When alewife invade lakes however, they outcompete native pelagic species and cause considerable changes to the trophic system and forage base (Wells 1970, Harman et al. 2002, Mihuc et al. 2012). Following such invasions, alewife quickly become a primary prey species for landlocked salmonids, as they have done in lakes Erie, Huron, Ontario, and Michigan (Brown et al. 2005, Riley et al. 2011), many of the Finger Lakes (Fisher et al. 1996), and Lake Champlain (Ladago et al. 2016). This poses a problem to the nutritional state of local salmonid populations as alewife contain elevated levels of thiaminase, an enzyme that breaks down thiamine (vitamin B₁) and produces thiamine deficiencies among salmonids which negatively impacts survival and recruitment (Werner et al. 2006). A similar dietary thiamine deficiency exists among salmonid populations around the Baltic sea (Amcoff et al. 1999). In this case, however, the deficiency stems from the decline and overabundance of sprat (*Sprattus sprattus*), a species with a naturally low ratio of thiamine to

fatty acids, resulting in a similar thiamine deficiency among Baltic salmonids (Keinänen et al. 2012, 2017).

Thiamine deficiencies produces a suite of symptoms that are often referred to in North America as a thiamine deficiency complex (TDC) or in Northern Europe as M74 syndrome. Passed from mother to offspring via thiamine deficient eggs, low thiamine levels among sac fry produce a range of symptoms that eventually lead to death upon yolk sac absorption (Fisher et al. 1995, Fitzsimons et al. 1999). Mortality rates among thiamine deficient juveniles can be as high as 100% in captivity and is often referred to as early mortality syndrome (EMS). Among adults, mortality resulting from low thiamine levels occurs much less often (Morito et al. 1986), and under controlled settings, even diets containing 100% alewife do not always cause mortality (Honeyfield et al. 2005). For this reason, initial research on thiamine deficiencies focused on their impacts on recruitment due to juvenile mortality.

Low thiamine levels do affect adult fitness, though in less direct ways, such as by reducing energy levels, causing equilibrium and coordination issues, and reduced swimming performance (Morito et al. 1986, Brown et al. 2005, Houde et al. 2015). As a consequence, adults and subadults may be more susceptible to disease, predation, or starvation (Ketola et al. 2000, Ottinger et al. 2012). Additionally, recruitment may be inhibited if physically challenging sections of river occur along the migratory path, prevent access to suitable spawning habitat further upriver. Such partial migratory barriers can therefore cause a bottleneck effect on reproduction by forcing thiamine deficient adults to spawning in sub-optimal habitat or abandoning their spawning attempt altogether (reviewed in Aas et al. 2010). While complete abandonment of a spawning attempt as a result of low thiamine levels has not been observed in

the wild, studies have demonstrated a link between thiamine levels and salmonid migratory capabilities (Fitzsimons et al. 2005, Ketola et al. 2005, 2009).

The presence of invasive alewife and other prey fishes high in thiaminase therefore represents a serious risk to the maintenance or reestablishment of self-sustaining landlocked salmonid populations. While a recent study indicated that TDC related juvenile mortality in the wild may not be as extensive as observed in hatcheries (Ladago et al. 2016), the degree to which thiamine deficiencies, along with other biotic and abiotic factors, impact natural recruitment by deminishing the ability of salmonids to pass challenging, high velocity sections along migratory routes, is still unknown. So too is the effectiveness of mitigation options such as thiamine supplementation for returning spawners. For this reason, it is important to investigate such phenomena more closely to fully understand the impact thiamine deficiencies have on salmonid migration and therefore restoration, and whether mitigation actions on the part of reintroduction biologists can be successful.

Thesis aims and study system

In this thesis, I intend to address several of the issues facing the reintroduction of landlocked Atlantic salmon in Lake Champlain, a population currently supported entirely by the release of captive-bred individuals. Landlocked Atlantic salmon were extirpated from Lake Champlain in the mid 1800s, primarily as a result of the damming of spawning tributaries and unregulated fishing pressure. Since their disappearance, periodic reintroduction attempts, along with the removal or alteration of artificial migratory barriers, has been unsuccessful in reestablishing natural populations. A more concerted effort by the U.S. Fish and Wildlife Service, along with the Vermont Department of Fish and Wildlife and the New York Department

of Environmental Conservation began in the 1970s involving annual releases of fry and parr into tributaries along with the release of smolt directly into the lake. Thus far these efforts have succeeded in producing a successful lake fishery that attracts anglers from a wide area, creating funds that are channeled back into the reintroduction program. Despite this success, however, salmon have yet to recolonize tributaries and establish self-sustaining populations. Multiple possible explanations exist for why this may be, and in this thesis, I will address two in particular.

First, as part of the “lake first” approach used for salmon restoration in Lake Champlain, hatcheries use accelerated growth rates in the first year to allow for the release of large parr (age 1+) that transform into smolt and migrate downstream shortly after their release into the wild. While this method has proven successful at producing high recruitment into the recreational lake fishery, it can increase the likelihood of salmon beginning the parr-smolt transformation prior to release and greatly increasing the likelihood of interrupted or incorrect olfactory imprinting. This undermines the overarching goal of re-establishing self-sustaining populations as juvenile salmon that fail to imprint on the tributaries into which they are released are considerably more likely to disperse as mature adults (Quinn 1993). These strays then enter new tributaries during their spawning migrations that may lack mature conspecifics, or suitable spawning habitat (Poethke et al. 2003, Keefer and Caudill 2014). For small or newly reestablished populations, this Allee effect negatively impacts their chances of establishing a self-sustaining population (Armstrong and Wittmer 2011) as those individuals that stray represent losses to the population. Conversely, when small at-risk populations of conspecifics persist in the vicinity of reintroductions, excessive dispersal (particularly following large scale releases) can genetically swamp smaller populations, eroding local adaptations and lowering fitness through outbreeding

depression (Waples 1991, Quinn 1993, Hendry et al. 2007). I will therefore investigate whether alternative rearing conditions and release methods can reduce dispersal behavior among stocked salmon without reducing parr-to-adult survival.

Second, I will assess the possibility that low thiamine levels among mature adult salmon is reducing migratory capacity and preventing successful spawning migrations in a tributary to Lake Champlain containing a high velocity section that serves as a migratory barrier to many adult salmon. As the suitable spawning habitat for Atlantic salmon and many other salmonids tends to occur in lower order tributaries (Louhi et al. 2008), the potential for migrating salmon to encounter high energy environment (riffles, runs, cascades, and rapids) along their journey is high. When salmon are deficient in thiamine, such challenging environments may become wholly or partially impassible, resulting in delayed spawning or a failure to spawn at all. As Lake Champlain salmon are known to be exhibiting a thiamine deficiency as a result of dietary alewife (early mortality syndrome is common in Lake Champlain hatcheries that use feral gamete sources), there is reason to believe that a high-energy environment in the Boquet River (the Willsboro Rapids) is presently acting as a barrier to thiamine deficient migrating salmon. I will experimentally test this possibility and quantify the extent to which various other biotic and abiotic factors influence movement through such partial migratory barriers. By doing do so I will assess the feasibility of thiamine supplementation as a means of mitigating the effects of a thiamine deficiency under a natural setting along with potential confounding variables.

Finally, in order to answer the question of whether a thiamine deficiency is hindering migration through high-energy environments, it is necessary to continuously monitor the fine scale movement patterns of adult salmon during their upstream migration. Currently available methods such as acoustic telemetry are not appropriate in such settings due to the irregular

morphology of the rapids (alternating shallow and deep sections) along with the high degree of turbulence (Bergé et al. 2012) while the short detection range of alternative methods such as PIT tag arrays would require the use of a dense array of antennas (Castro-Santos et al. 1996, Zydlewski et al. 2006). For this reason, I develop a technique based on the fingerprint localization models employed in the telecommunications industries (Bahl and Padmanabhan 2000, Li et al. 2015) to allow for the recording of fine scale movements of radio tagged individuals while requiring a limited infrastructure (Harbicht et al. 2017). Location Fingerprinting is a general term used to describe any localization techniques that compares measured system characteristics to a pre-recorded database of system characteristics at known locations (Kjærgaard 2007). By reversing the roles of the transmitters and receivers relative to previous methods that used fixed position broadcasters (i.e. wifi routers) and mobile receivers (i.e. cellular phones), I describe a new telemetric tool that allows for continuous monitoring of location and movement using relatively inexpensive equipment. I then discuss the applicability of this method of localizing radio tagged individuals beyond the one dimensional migratory situation in the Boquet River, to multiple dimensions and terrestrial systems. The applicability of these methods extends beyond the monitoring of aquatic species in linear environments such as rivers and can be applied to any species that can be tagged and any areas where overlapping antenna coverage is possible.

Chapter 1: Minor shifts towards more natural conditions in captivity improve long-term survival and reduce dispersal in reintroduced salmon populations.

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Abstract:

Common actions when rearing salmonids, like elevating water temperatures to increase post-release survival, can disrupt important developmental processes (olfactory imprinting), representing a genetic risk to nearby conspecifics. To test whether more natural developmental conditions (seasonal temperatures, earlier release dates) can reduced dispersal without reducing survival, we released three cohorts of fry (age 0+) and parr (age 1+) landlocked Atlantic salmon, produced under two thermal conditions (seasonal brook water and above-seasonal groundwater) and release times (early or normal), into two tributaries. Among the resulting adults, parr releases strayed half as often when released earlier and 6.3 times less often when exposed to brook water prior to release. Though not consistently higher than fry survival, parr releases exposed to brook water had adult recaptures and spawning returns 4.4- and 5.1-fold higher than standard release methods while earlier release dates had no effect on adult recaptures, but halved spawning returns. By taking ecology and developmental requirements into account, these cost-effective changes reduced dispersal while improving lifetime survival and should prove useful for releasing species with complex life histories.

Introduction:

To achieve the ultimate goal of establishing self-sustaining populations (Montalvo et al. 1997), many species reintroductions (programs that release extirpated species, from wild or captive sources, into parts of their historic ranges) strive to maximize the number of mature adults in a target environment. As survival increases with age in many species (Sarrazin and Legendre 2000, Aaltonen et al. 2009, Campbell-Thompson et al. 2012), older individuals are often chosen for release despite the additional financial expenses and costs to wild fitness. However, prolonged captivity may not be possible or advisable when dealing with migratory species with complex life-histories, as various life-stages often have unique dietary, habitat, and/or developmental requirements that are difficult to replicate. Neglecting such requirements can produce carry-over effects on fitness in subsequent life-stages or generations (Araki et al. 2009, Roberts et al. 2011, Ogilvy et al. 2012).

Migratory species inherit many of the necessary morphological, sensory and physiological traits for migration (Liedvogel et al. 2011) but others must be acquired, such as olfactory imprinting in salmonids (Quinn 1993) or knowledge of migratory routes among birds (Mueller et al. 2013). Ensuring such traits are acquired is important for the reintroduction or conservation of migratory species, not only to minimize losses from the donor population (Poethke et al. 2003) but also to avoid increasing dispersal rates among populations, particularly for species possessing metapopulation structure (Keefer and Caudill 2014), as this can break down local adaptations (Kisdi 2002, Kawecki and Ebert 2004).

Salmonid fish populations exhibit high levels of local adaptation (Fraser et al. 2011) yet often display metapopulation characteristics by maintaining low-level dispersal rates among populations (Cooper and Mangel 1999, Rieman and Dunham 2000, Consuegra et al. 2005,

Schtickzelle and Quinn 2007, Fraser et al. 2007). This dispersal occurs during spawning migrations when returning adults enter non-natal tributaries to spawn and is crucial for the maintenance of genetic diversity among small populations and for colonization or recolonization of habitats that have suffered from population loss in the past (Pess et al. 2014). However, the likelihood of dispersal occurring depends heavily on juvenile developmental conditions (Morin et al. 1989, Quinn 1993, McCormick 2013), particularly during the parr-smolt transformation period, when salmon undergo physiological, behavioral, and morphological changes in preparation for their downstream migrations to the oceans and lakes where they will grow and mature. It is during this period that olfactory imprinting occurs (Hasler and Scholz 1983, Dittman and Quinn 1996, McCormick 2013) and salmonids bred in captivity, experiencing developmental conditions that deviate from the wild, often exhibit greater and more variable dispersal rates than salmonids in the wild (Quinn 1993). This occurs especially when there is selection for production traits (Jonsson et al. 2003a), or when release dates interrupt the imprinting process (Pascual et al. 1995).

Salmonid supplementation and conservation programs that employ elevated rearing temperatures to accelerate individual growth rates during winter months (e.g. Atlantic salmon (Farmer 1994), chinook salmon (Beckman et al. 2017) and steelhead trout (Berejikian et al. 2012)) are particularly at risk of interrupting olfactory imprinting. Such departures from normal developmental conditions increase the likelihood of improper or incomplete olfactory imprinting (Schtickzelle and Quinn 2007), and can therefore increase dispersal rates. Despite this risk, accelerated growth rates are often used by conservation programs as they permit earlier smoltification and downstream migration which subsequently increases juvenile-to-adult survival. Concurrently, such captive breeding programs can often exert near complete control

over the rearing environment and release conditions, making them well suited to adaptive management experimentation (Waples 1999, Molony et al. 2003). Experimental releases can therefore be used to identify captive rearing methods that reduce straying without reducing survival, thereby limiting risks to neighboring populations (Waples 1991, Anderson et al. 2014, Bett et al. 2017).

Landlocked salmonids with feeding migrations to lakes are particularly well-suited for reintroduction experimentation that investigates captive rearing practices in relation to migration and life history. These fishes are relatively easier to monitor over their lifespan than their anadromous conspecifics, whilst having analogous ecological, genetic and life history characteristics (Hindar et al. 1991, Nilsen et al. 2008). By quantifying the effects of alternative captive rearing methods on lifetime survival (adult recapture rates in the lake), spawning returns (return rates in tributaries during spawning migrations), and straying (the rate at which migrating individuals enter non-natal tributaries), experimental releases can identify new methods to assist reintroduction/supplementation efforts in focal or other systems.

In the present study, annual releases of landlocked Atlantic salmon parr (age 1+) into tributaries of Lake Champlain, USA, produced using standard (accelerated) growth methods were paired with four alternative methods. These alternatives shifted the environmental conditions experienced by juveniles towards more natural states by adjusting rearing temperatures or release life-stages/dates. These adjustments included reducing exposure to the captive environment through the release juveniles at an earlier life stage (fry, age 0+), as well as three minor alterations to the accelerated parr production methods. In an effort to maintain high parr-to-adult survival, each of the alternative parr production methods began with accelerated growth, allowing juveniles to reach a size sufficient to permit smoltification and downstream

migration within the first year (a fork length of at least 75 mm by February, Thorpe et al. 1980), before deviating from the standard procedure. In this way, none of the alterations to parr production methods were hypothesized to reduce parr-to-adult survival. Alterations involved switching some juvenile salmon from groundwater (with above-seasonal temperatures) to brook water (with seasonal surface water temperatures) during their first winter to delay the parr-smolt transformation and promote natural levels of seasonally influenced juvenile hormones (McCormick 2013), and/or by releasing juveniles earlier to improve the likelihood of olfactory imprinting post release (Unwin and Quinn 1993). Relative to standard production methods, each alternative rearing or release condition was hypothesized to reduce dispersal of adult salmon during spawning runs, resulting in reduced straying rates among tributaries.

Experimental releases were repeated over three consecutive cohorts and the resulting adults were monitored over four years, both in the lake and in experimental tributaries. This experimental design provided a robust basis by which to identify combinations of alternative captive rearing practices that reduce straying (dispersal) without reducing lifetime survival in a natural setting, thereby assisting reintroduction programs for migratory species with comparable life-histories.

Methods:

Juvenile production

In the fall of 2010, 2011, and 2012, one-to-one crosses were performed using a total of 620, 586, and 480 adults, respectively, of the Sebago Lake strain of landlocked Atlantic salmon at the Vermont Fish & Wildlife Department's Bald Hill fish culture station (Vermont). Fertilized embryos were then pooled according to their treatment group and immediately transported to the

US Fish and Wildlife Service's (USFWS) D.D. Eisenhower National Fish Hatchery (Vermont, Table S1.1). Embryos were incubated on groundwater ($\sim 10^{\circ}\text{C}$, Figure 1.1) in vertically stacked egg trays until they reached the first feeding stage when they were transferred to circular tanks (2.4 m diameter) and reared on groundwater heated to $\sim 15^{\circ}\text{C}$. In May, when seasonal water temperatures roughly matched rearing temperatures, families selected for release as fry were planted into upper reaches of the experimental tributaries (details below). Remaining families were moved to outdoor raceways and switched to water from nearby Furnace Brook, whose warmer seasonal temperatures maintained rapid growth rates. As water temperatures dropped in the fall, all juveniles were switched back to groundwater to maintain high growth rates through winter. In February, families selected to experience delayed smoltification and seasonal shifts in environmentally regulated hormones (McCormick 2013) were gradually (over several days) transferred back to brook water on which they remained until their release in the spring. Families selected for earlier release into the wild, thereby enhancing their likelihood of correctly imprinting to their stocking location (Unwin and Quinn 1993), remained on groundwater until their release date, 4-7 weeks before the standard release dates in late April/early May (Table S1.1). Aside from rearing temperature and release timing, environmental conditions within the hatchery (i.e. lighting, tank densities, and feeding schedules) were identical across groups. While experimental alterations to captive procedures resulted in the release of parr at significantly smaller sizes (Table S1.1, S1.2), all parr were sufficiently large to receive a left ventral fin clip (LV) distinguishing them from smolts released elsewhere within the Lake Champlain basin. Fry were too small at their release date to be marked and hence had no identifying clip (NC). Further information is available in Appendix 1A.

Experimental tributaries: stocking and sampling

The Winooski River basin (Figure 1.2) covers 2797 km² and contains three hydroelectric dams between the river mouth and its fry stocking locations in the Huntington River, 54 km upriver. Though each dam permits downstream smolt passage, parr were released below the lowest dam. Only standard release dates were used for parr in this tributary and similar stocking densities were used for both the standard growth and seasonal growth release groups all over three years (Table S1.1). Spawning returns were sampled each fall by USFWS staff as they entered a permanent fish trap situated at the lowest dam (16 km upriver from the mouth). In total, biological data and tissue samples were collected from 284 returning adults (215 LV and 69 NC salmon, Table S1.3).

The Boquet River basin covers 725 km² (Figure 1.2). As with the Winooski, unfed fry were released into smaller tributaries of the upper reaches of its North Branch, which separates from the main stem 9 km from the river mouth. Parr were released at multiple sites throughout the upper main stem. All juvenile salmon were required to fall over a shallow crib dam in the town of Willsboro (~ 4 km upriver from the mouth) as they migrated down to Lake Champlain. Similar densities of parr were released at early or standard dates in all three years, and both groups experienced standard growth conditions in 2010 and 2011, and seasonal growth conditions in 2012. Spawning returns were sampled in an identical manner to Winooski salmon by N.Y Department of Environmental Conservation staff upon entering a fish ladder at the Willsboro dam. From 2014 – 2016, trap nets and gillnets were used below the dam to boost sample numbers. Overall, 282 salmon were sampled over four years (184 LV and 98 NC salmon, Table S1.3). The collection of biodata and tissue samples from adults conformed with the

guidelines of the Canadian Council on Animal Care in science and was in accordance with the guidelines set by the Concordia University Animal Research Ethics Committee.

Lake sampling:

To assess parr-to-adult survival prior to their spawning migration, tissue samples were collected from adult salmon captured in Lake Champlain via boat electrofishing and angling. As concurrent salmon reintroduction efforts in the basin were releasing unclipped fish during our sample collection window, only LV clipped fish (parr releases) were sampled in the lake. Over four years of sampling, electrofishing resulted in the collection of 130 samples while angling produced another 161 samples (Table S1.3). A supplemental analysis insured that recapture proportions were independent of sampling method prior to combining the two sampling groups into a single ‘lake recapture’ group. More information on sample collection in Lake Champlain and the supplemental analysis is available Appendix 1B.

Genetic parental based tagging

Details regarding the extraction, amplification and scoring of microsatellite loci as well as the estimation of genotyping error rates, exclusionary power, and parentage assignments are available in the supporting information, Appendix 1C. Briefly, parents and putative offspring (totaling 2618 individuals) were genotyped at 7 - 10 microsatellite loci using polymerase chain reaction (PCR) and allele sizes were determined using an ABI 3500 sequencer and manually scored using Genemapper software (*Life Technologies Inc.*, Burlington, Ontario, Canada). Genotyping error rates were estimated by repeating the above process (DNA extraction, PCR amplification, and scoring) on a subsample of individuals (14%) from each of the primary groups

(broodstock and putative offspring). These results were directly compared to previous scoring results and the number of full and partial matches as well as full-mismatches was calculated. The per-locus and overall genotyping error rates were then calculated for the total number of alleles observed.

The use of known parental pairs to produce experimental releases permitted a high level of confidence in parentage assignments using exclusionary methods (Jones and Ardren 2003). Nevertheless, the exclusionary power of the suit of loci used was assessed using the program P-Loci (Matson et al. 2008) and based on the results of this analysis, offspring assigning to parental pairs at 5 or fewer loci were omitted from further analysis. Parental assignments were made using the SOLOMON package in R (Christie et al. 2013). Once offspring were assigned to parents, their cohort, release location, release date and rearing conditions were then identified by cross-referencing the parental database.

Data analysis:

As experimental treatments were expected to impact straying rates, and therefore spawning returns, survival was assessed both before and during the spawning migration via adult recapture rates in the lake, for parr release groups, and spawner returns in both of the stocked tributaries respectively for all release groups. As NC salmon (fry release groups) were not sampled in Lake Champlain, they were not included in the assessment of adult recapture rates in the lake. In both cases, recapture frequencies were compared to release frequencies for each group via Fisher's exact tests. Bonferroni corrections were applied to the critical p-values to account for multiple comparisons. In the case of fry releases, natural mortality was expected to reduce the number of offspring in the experimental tributaries prior to the release of experimental

1+ parr. This was accounted for by adjusting the fry stocking densities to account for natural mortality post release. Among wild Atlantic salmon, survival rates for the 0+ fry to 1+ parr period can range from 14.0 to 74.4% (Cunjak and Therrien 1998), though for hatchery fry these rates tend to be considerably lower, ranging from 3% to 38% (Bley and Moring 1988). Assuming a constant survival rate over the first two years in the wild, a fry-to-parr survival rate of 17.4% was capable of producing the observed fry-to-smolt survival estimates of 3.4% in the Winooski watershed (VTFWS pers. comm.). A conservative fry-to-parr survival estimate of 10% was therefore applied to the fry stocking densities in both tributaries prior to calculating the parr-to-adult river return rate for fry stocked groups.

Straying rates were assessed in a similar way to survival estimates, but by contrasting spawner returns to non-origin tributaries with the overall spawning returns for each group. Analyses were conducted at the tributary level (across replicate cohorts within a tributary), and the basin level (across cohorts and tributaries). An analysis of the data at the cohort level (within cohort and tributaries) is available in the supplemental information, Appendix 1D. To assess the potential influence of sampling bias in the lake on the survival results, a supplemental sensitivity analysis was conducted at the tributary level which indicated that recapture ratios in the lake among release groups were highly robust against sampling bias. Further details are available in the supplemental information, Appendix 1B.

Results:

Genetic parental based tagging

While exclusionary power simulations, using estimated null-allele frequencies and genotyping error rates, indicated >98% of assignments were correct with four matching loci, a

threshold of six matching loci was chosen to ensure confidence in parentage assignments (Table S1.8). Overall, 77% of the putative offspring sampled in the lake and experimental tributaries assigned back to known parental pairs. This included 25% and 89% of the samples collected from NC and LV clipped salmon respectively. Unassigned salmon represented a combination of fin clip misidentification, failure to meet parentage assignment requirements, an inability to assign to a single parental pair (exclusion failure), or (for NC salmon only) salmon from external stocking efforts straying into experimental tributaries. Further details on parentage assignments are available in the supplemental information, Appendix 1C.

Survival to maturity in the lake

Tributary level – Seasonal rearing conditions and standard release dates produced the greatest recapture rate in the Boquet (0.237%), slightly exceeding those of seasonal rearing conditions with early release times (0.225%), but significantly improving upon standard rearing temperatures with standard (0.067%) or early (0.010%) release times (Table 1.1). In the Winooski, salmon that experienced seasonal rearing conditions had a 4.6-fold greater recapture rate (0.091% vs. 0.020%) than salmon reared on standard, accelerated conditions (Table 1.1). Among all release groups, linear models indicated a non-significant negative trend between size (length) at release and lake recapture rates ($R^2 = 0.14$, $p\text{-value} = 0.124$, Figure 1.3).

Basin level – The recapture rate of salmon exposed to seasonal rearing conditions and released early (0.225%) was approximately 1.6-fold to 22.5-fold higher than salmon exposed to seasonal rearing conditions and standard release times (0.138%) or salmon exposed to standard rearing conditions and released at standard (0.051%) or early (0.010%) dates: a significant improvement in each case. When experimental variables were considered separately, recapture

rates in the lake significantly increased 4.4-fold when seasonal rearing conditions were used (0.162%) relative to standard rearing conditions (0.037%), but decreased slightly when early release dates were used (0.082%) relative to standard release dates (0.086%).

Spawning returns

Tributary level – In the Boquet, yearlings exposed to seasonal rearing conditions and released at standard times had the greatest spawning return rates (0.310%), 36% higher than yearlings exposed to seasonal rearing conditions and released early (see above) and 3-fold, 3.6-fold, and 26.2-fold greater than fry (0.102%), standard production parr (0.085%), and parr reared in standard conditions but released early (0.012%) times, respectively (Table 1.1). In the Winooski, spawning return rates for parr exposed to seasonal rearing conditions (0.368%) were 3.4-fold and 4.1-fold greater than those of fry releases (0.109%) or parr reared under standard conditions (0.091%) respectively (Table 1.1). A linear model of river recapture proportions (Figure 1.3) indicated a non-significant negative relationship between the average size-at-release for parr, and the proportion that returned to the experimental tributaries during spawning runs ($R^2 = 0.19$, p -value = 0.088).

Basin level – Yearlings exposed to seasonal rearing conditions and released at standard times had the greatest spawning return rates (0.350%), 53% higher than seasonal rearing conditions and early release times (0.229%), 3.3-fold greater than returns from fry releases (0.105%), and 4-fold or 29.5-fold higher rates than standard (0.087%) and early (0.012%) release times under standard rearing conditions: a significant improvement in all contrasts (Table 1.2). When experimental variables were considered separately, spawning return rates for yearlings exposed to seasonal rearing conditions (0.317%) were 5.1-fold greater than those reared under

standard conditions (0.062%), while the rates for standard release dates (0.193%) were 2.3-fold greater than those of early release dates (0.085%): a significant difference in each case.

Straying

Tributary level – In the Boquet River, under standard rearing conditions (2010, 2011 cohorts), standard release times produced a straying rate of 14.3% (8 in 56) (Figure 1.4a). This exceeded that of early release times, which did not produce any detectable strays, as well as stray rates of early and standard release dates under seasonal rearing conditions (1 in 59 and 0 in 68 respectively); the differences were only significant with the seasonal rearing and standard release time. Fry in the Boquet River had a variable straying rate (Figure 1.4a) which, across replicate cohorts, exceeded all other groups (21%, 4 in 19), but only significantly so when compared with seasonal rearing conditions with standard released dates (Table 1.1). In the Winooski, straying rates remained relatively constant across replicate cohorts and were low for salmon exposed to seasonal rearing conditions 1.8% (3 in 170) but which exceeded those of salmon reared under standard conditions and fry as neither group produced detectable strays (Figure 1.4b). Overall straying rates were higher and more variable among the Boquet cohorts (Figure 1.4a); males tended to stray more than females (9 males, 7 females), though not significantly more (binomial exact test, $p = 0.80$). A linear model of straying proportions indicated a non-significant positive relationship between the proportion of salmon straying into non-natal tributaries and the average yearling size at release ($R^2 = 0.05$, $p\text{-value} = 0.228$, Figure 1.3).

Basin level – The straying rate for yearlings reared under standard conditions and released early (9.1%) was between 5-fold and 7-fold greater than early and standard release dates under seasonal rearing conditions (1.7 and 1.3% respectively, Table 1.2). It also exceeded that of

yearlings reared under standard temperatures and released early (who did not produce detectable strays), though the difference was only significant with juveniles that experienced seasonal rearing conditions and standard release dates. Fry produced a greater straying rate (10.3%) than all yearling groups, though not significantly so. When experimental variables were considered separately, straying rates for salmon reared under standard conditions (8.5%) were significantly higher than for salmon reared under seasonal conditions, while standard release dates (3.37%) were 2.2-fold greater than those of salmon released early (1.54%): a non-significant difference.

Discussion:

Using DNA parentage based tagging, we assessed the efficacy of multiple captive rearing and release conditions to reduce straying rates to more natural levels without negatively affecting survival in a model migratory fish species. Overall, once the additional year of mortality in the wild was accounted for with fry releases, parr produced using elevated rearing temperatures produced similar returns to fry releases. Parr exposed to seasonal rearing temperatures, however, exhibited significantly higher survival rates than all other rearing/release methods. Additionally, the overall straying rate among release groups was low and significantly associated with rearing conditions experienced prior to release. Altering standard yearling production methods via earlier release dates or with seasonal rearing temperatures shortly before release reduced the overall straying rate. Releasing salmon as fry, while producing generally lower straying rates (no detected strays for five of six experimental releases), produced the greatest range in straying rates, resulting in a higher overall rate comparable to standard parr production methods. These results suggest that occasionally high dispersal may be unavoidable, even when exposure to the captive environment is minimized, but on average, dispersal behavior can be reduced through

minor changes to the captive experience. Doing so may even improve survival, a requirement for the restoration of distinct populations and the development/maintenance of metapopulation structure.

Survival

Relative to the range or smolt-to-adult return rates observed for anadromous Atlantic salmon populations, 1% to 12% (Saunders and Allen 1967, Friedland et al. 1993, Jonsson et al. 2003b), the parr-to-adult returns observed in Lake Champlain were quite low. Despite this, adult returns to stocked tributaries and recapture rates in the lake were both significantly improved by exposing 1+ parr to a water source with seasonal temperature for 2-4 months before release. As brook water salmon were smaller upon release, this improved survival contradicts the well supported positive relationship between size at release and survival to maturity in salmonids (e.g. Ward and Slaney 1988a, Koenings et al. 1993), but is consistent with the idea that more natural environmental conditions early in life improves fitness at later stages (Berejikian et al. 2012, Evans et al. 2015). This view, that seasonally warmer summer and cooler winter temperatures improve juvenile-to-adult survival by promoting growth patterns that follow a 'wild fish template' has also recently been documented among chinook salmon (Beckman et al. 2017). In contrast to these findings, however, the improved recapture rates (survival) observed in the present study were achieved by switching to cooler seasonal temperatures for only 2-4 months prior to release, permitting extended use of above seasonal water temperatures and increasing the proportion of offspring sufficiently large to smoltify within their first year.

Conversely, under identical rearing conditions, advancing the release date by two months relative to the typical release date resulted in fewer recaptures in the lake, and fewer salmon

returning to spawn. Advancing the release date by an entire year, however, by releasing fry did not result in a reduction in the parr-to-smolt survival rates, once mortality within the first year was accounted for. Indeed, fry release groups were recaptured in the experimental tributaries at a higher rate than the standard production parr. This suggests that achieving a critical size threshold to allow smoltification and downstream migration within the first year may not be sufficient to impart a survival advantage; other factors, such as the timing of release, play a significant role. Once this critical size is reached, however, our results support that a significant survival advantage can be gained by exposing juveniles to seasonal temperatures/growth conditions prior to release.

As survival was measured among adult salmon in the lake and during spawning migrations, the underlying cause for the differential abundances observed remains unclear. Several potential explanations exist. First, survival differences could develop shortly after release if cold shock from dramatic decreases in water temperature occurs. Cold shock can cause short term spikes in stress hormone levels, and lower blood pressure and respiratory rates among salmonids (Barton and Peter 1982, Seth et al. 2013). Under such conditions, the effect of additional stressors such as handling or transport (Hyvärinen et al. 2004) can result in long lasting effects on survival (Donaldson et al. 2008). In our study, despite all yearling release groups experiencing a pre-release acclimation period in captivity, during which temperatures were gradually lowered to seasonal levels, there was a negative correlation between survival and the temperature differential experienced at release (Figure 1.1). Previous studies have confirmed that preconditioning salmonids in ponds (Näslund 1992), in-stream acclimation pens (Finstad et al. 2003), or within captive environments (Beckman et al. 2017) can significantly improve survival. If the same mechanism is acting here, our study supports the evidence for

preconditioning significantly improving lifetime survival while also representing the first study, to our knowledge, to have controlled for many of the confounding variables associated with staging ponds or in-stream holding pens (e.g. natural food, habitat complexity, luminosity). A second potential mechanism could be the effect of seasonal growth rates on energy reserves. Salmonids exhibit energy consumption and growth rate optima that shift as a function of seasonal temperature (Finstad et al. 2004). Owing to being reared on brook water for several months prior to release, salmon exposed to seasonal rearing conditions may have been closer to their seasonal phenotypic optimum at the time of release. With metabolic and energy consumption rates at or near their optimum, such salmon would experience an advantage over more maladapted salmon reared on groundwater in terms of predator avoidance, dispersal, microhabitat selection, or feeding.

Dispersal rates

Along with improved survival, seasonal rearing conditions also produced salmon with lower dispersal rates (1.7 and 1.3% for early and standard release dates respectively, at the basin level) that more closely resembled those of wild anadromous Atlantic salmon (5%, Jonsson et al. 2003). Conversely, standard production salmon reared on elevated temperatures to facilitate rapid growth rates through winter and released at larger sizes, exhibited higher straying rates (9.1%) more akin to those of farmed salmon under artificial selection for production traits (15%, Jonsson et al. 2003), unless they were released earlier. These results suggest that, while standard production methods may improve parr-to-adult survival relative to fry releases, it also greatly increases the proportion of juveniles likely to experience interrupted or incorrect olfactory imprinting. As a result, these salmon are significantly more likely to stray from their release

tributary during spawning migrations, representing losses from the donor population and a potential outbreeding risk to neighboring locally adapted populations.

Salmon released as fry underwent their parr-smolt transformation in the receiving environment, and should therefore have demonstrated the most natural straying behavior of all release groups. Despite this, their overall straying rate was the highest (10.25%) with 4 strays among 39 returning spawners. This unusually high overall straying rate in part could be due to small sample size, though a more natural explanation does exist. Straying rates were generally more variable among salmon stocked into the Boquet River, which is the only location where a fry cohort (1 of 6) exhibited any sort of straying (33% or 4 of 12 returning spawners). Such temporally variable dispersal rates are not uncommon for Atlantic salmon or other salmonids (Quinn 1993, Keefer and Caudill 2014) and may have been a natural response to some unmeasured variable in the river triggering colonization behavior.

With natural straying rates for Atlantic salmon being anywhere from 5% (Jonsson et al. 2003a), 7% (Pess 2009), or potentially as high as 10% (Keefer and Caudill 2014), our inability to detect strays from 5 of 6 fry cohorts - our proxy for natural fish - suggests that our observed straying rates may be underestimates of true rates. Given that we measured straying in only two of Lake Champlain's eleven tributaries that historically supported salmon populations (Fisheries Technical Committee 2009), five of which are currently stocked annually, and that three quarters of the NC salmon recaptured in the experimental tributaries likely originated from those external stocking efforts, the straying rate for standard production yearlings is likely much higher than 9%. This further supports the use of alternative captive rearing methods, particularly seasonal temperature variations, for the reintroduction of migratory salmonid populations and, potentially, the reestablishment of sustainable metapopulation structure.

Caveats

While both water sources used in captivity were filtered and treated prior to entering the raceways, other characteristics, besides temperature, may have varied among them.

Subsequently, it could be argued that yearlings exposed to seasonal rearing conditions imprinted in captivity to the same extent as those reared on groundwater, but strayed less because the brook water more closely resembled their receiving environment. This is unlikely, however, as both receiving tributaries were a considerable distance from the hatchery (74 and 82 km) and were in different drainage basins. A more likely alternative explanation may be that yearlings reared on groundwater were deprived of olfactory stimulation prior to olfactory imprinting, resulting in reduced olfactory activity and brain development (Marchetti and Nevitt 2003). As this could have occurred in conjunction with delayed imprinting due to cooler rearing temperatures, further experimentation would be required to fully understand the underlying mechanisms. Given the significant improvements in both survival and straying rates as a result of exposure to seasonal rearing conditions prior to release, confirming such hypotheses may have wide ranging applications to the restoration of multiple migratory populations and species.

We also acknowledge that the low overall returns during spawning migrations resulted in few strays being detected and a potential lack of statistical power. Despite this, some significant differences resulting from rearing conditions were detected. It is therefore possible that such differences would be reinforced had sampling effort and survival to maturity been higher in Lake Champlain.

Conclusion

By challenging the idea that releasing larger individuals always improves adult returns, the present study has emphasized the importance of adaptive management experimentation in species reintroductions and the potential advantages of considering how production methods can affect multiple life stages of a species with a complex life history. While our results support the idea that releasing salmonids at later life stages improves survival to maturity, the same cannot be said for straying rates. However, through the use of simple alterations to the captive environment, lifetime survival can be improved and dispersal rates reduced to more natural levels. By exposing juveniles to water sources with seasonal temperature variations that reduce growth rates and promote natural hormonal cycles prior to release, biologists may increase the likelihood of reintroduced populations establishing themselves while promoting more natural dispersal rates necessary to reestablish or maintain metapopulation structure. Such eco-evolutionary approaches to species reintroduction and restoration, taking metapopulation structure into account, are increasingly becoming the norm for similar species with complex life histories, including Pacific salmon and trout species (Anderson et al. 2014, Fullerton et al. 2016), oysters (Schulte et al. 2009), butterflies (Kuussaari et al. 2015) and mammals (McGeachy et al. 2017) and their frequency is likely to continue growing as habitat fragmentation and population declines continue. To this effect, the results and implications of the present study extend beyond Lake Champlain, to the reintroduction of locally extirpated populations in a multitude of environments.

Table 1.1: Adult recapture frequencies, pooled across replicate cohorts within a tributary, for Atlantic salmon produced using alternative rearing/release conditions and released into two experimental tributaries to Lake Champlain as fry or parr. Lake sampling occurred throughout the year, while river returns were assessed during fall spawning migrations. Strays represent adults observed migrating into tributaries other than their tributary of origin. Values represent the number of salmon/number of families/mean (st.dev.) number of offspring per family. Subscripts indicate significant similarities or differences based on Fisher’s Exact tests.

Release Location	Age Class	Rearing Temperatures†	Release Date‡	Cohort	Stocked*	Lake Recaptures	River Returns	Strays
Boquet	Fry			2010-2012	18597	NA	19/18/1.06(0.24)a	4/4/1(0)a
	Parr	Standard	Standard	2010-2011	65660	44/19/2.32(1.25)b	56/35/1.6(1.24)a	8/7/1.14(0.38)a
			Early	2010-2011	50639	5/5/1(0)a	6/5/1.2(0.45)b	0/0/0(0)a, b
		Seasonal	Standard	2012	21905	52/32/1.62(1.04)c	68/35/1.94(1.39)c	0/0/0(0)b
			Early	2012	25775	58/30/1.93(1.62)c	59/29/2.03(1.21)c	1/1/1(0)a, b
Winooski	Fry			2010-2012	18376	NA	20/19/1.05(0.23)a	0/0/0(0)a
	Parr	Standard	Standard	2010-2012	35274	7/5/1.4(0.55)b	32/27/1.19(0.4)a	0/0/0(0)a
			Seasonal	2010-2012	46158	42/25/1.68(0.95)a	170/77/2.21(1.8)b	3/3/1(0)a

* numbers of stocked fry are corrected for a 10% survival rate between the 0+ fry and 1+ parr stages

† Parr rearing temperatures were either standard (groundwater through the winter) or seasonal (surface water for 2-4 months prior to release).

‡ Release dates were either standard for a particular tributary, or 4-7 weeks earlier.

Table 1.2: Adults recapture frequencies, pooled across replicate cohorts across tributaries, for Atlantic salmon produced using alternative rearing/release conditions and released into two experimental tributaries to Lake Champlain as fry or parr. Lake sampling occurred throughout the year, while river returns were assessed during fall spawning migrations. Strays represent adults observed migrating into tributaries other than their tributary of origin. Values represent the number of salmon/number of families/mean (st.dev.) number of offspring per family. Subscripts indicate significant similarities or differences based on Fisher’s Exact tests.

Age	Rearing	Release		Survival	Migrating	Straying
Class	Temperatures†	Date‡	Stocked*			
Fry			369730	NA	39/37/1.05(0.23)a	4/4/1(0)a,b
Parr	Standard	Early	50639	5/5/1(0)a	6/5/1.2(0.45)b	0/0/0(0)a,b
		Standard	100934	51/24/2.12(1.19)b	88/62/1.42(0.98)a	8/7/1.14(0.38)a
	Seasonal	Early	25775	58/30/1.93(1.62)c	59/29/2.03(1.21)c	1/1/1(0)a,b
		Standard	68063	94/57/1.65(0.99)d	238/112/2.12(1.68)d	3/3/1(0)b

* numbers of stocked fry are corrected for a 10% survival rate between the 0+ fry and 1+ parr stages

† Parr rearing temperatures were either standard (groundwater through the winter) or seasonal (surface water for 2-4 months prior to release).

‡ Release dates were either standard for a particular tributary, or 4-7 weeks earlier.

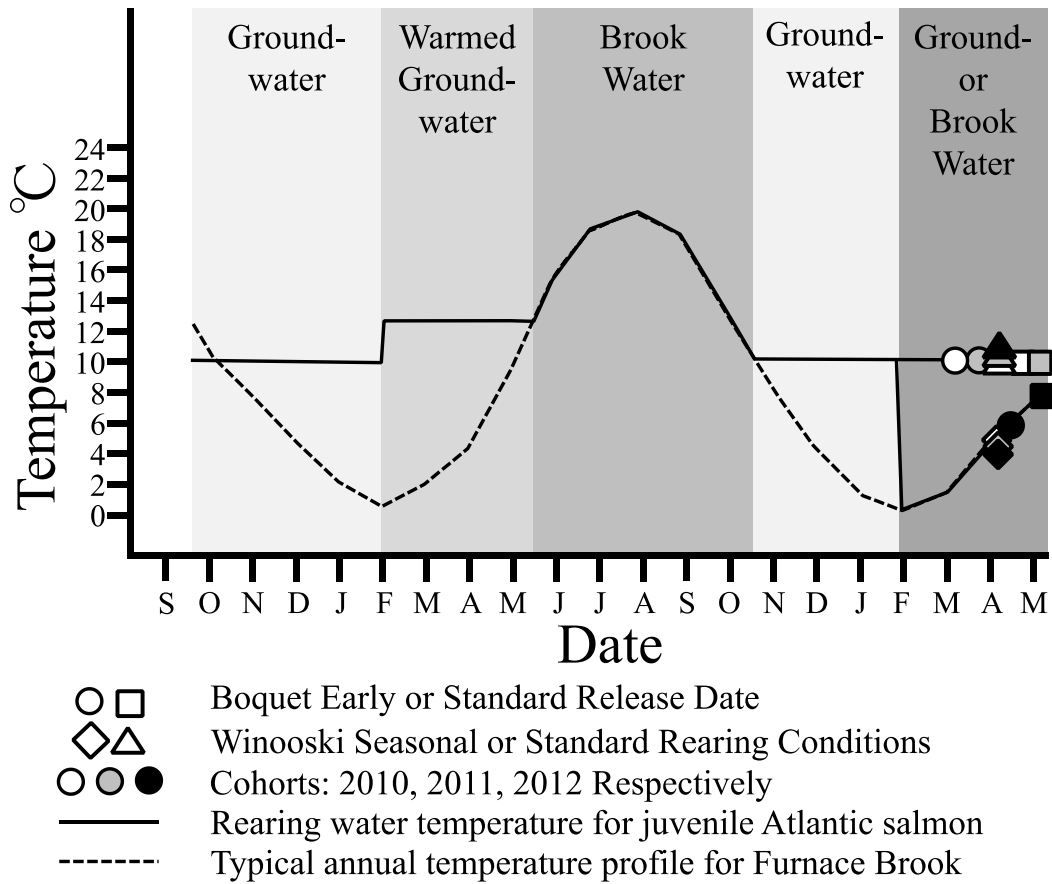


Figure 1.1: The temperature regime and stocking dates experienced by parr experimental Atlantic salmon released into the Lake Champlain Basin in two experimental tributaries (Boquet River, NY, and Winooski River, VT) from 2012 to 2014 as part of an adaptive management experiment to improve river returns and restore wild populations and meta-population structure.

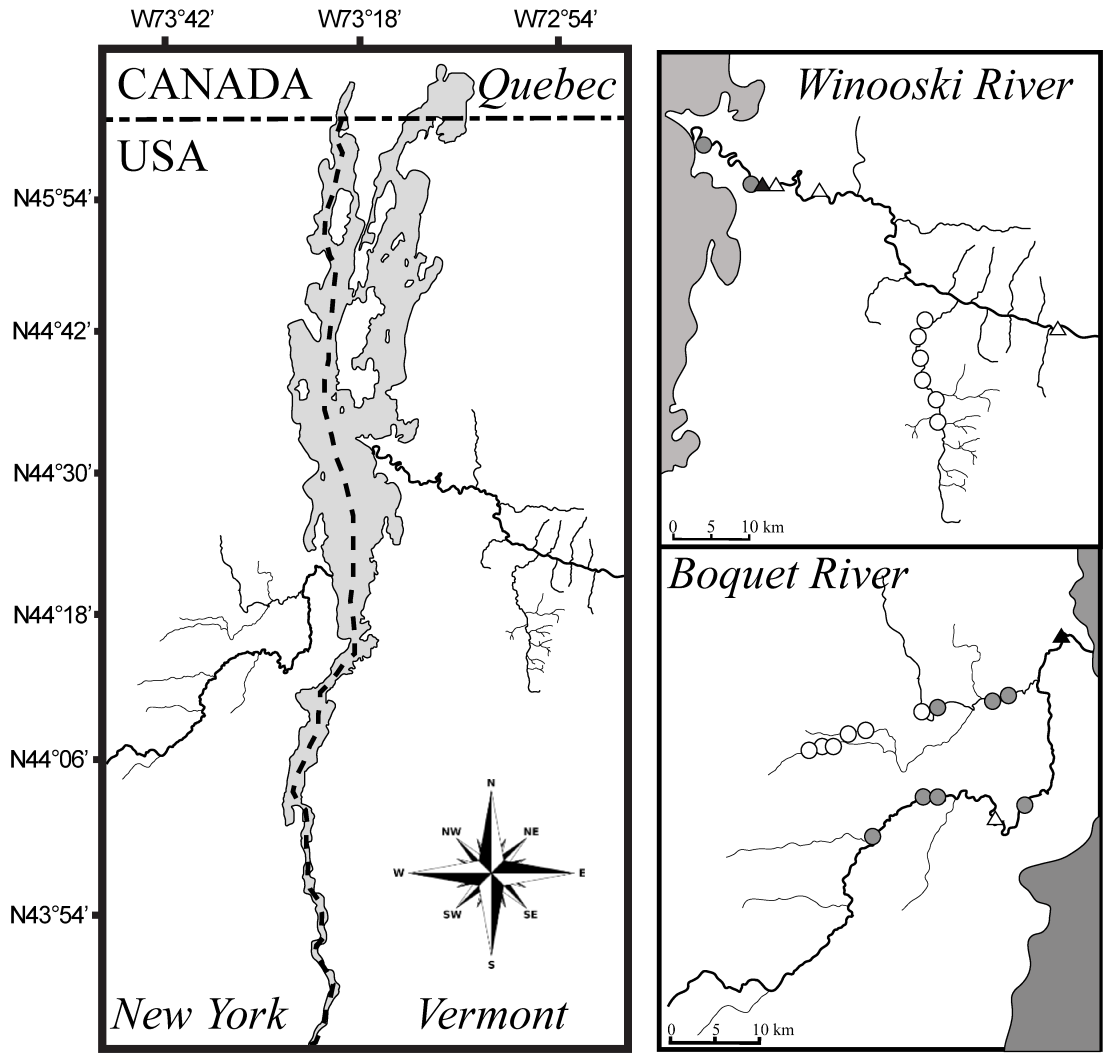


Figure 1.2: The location of two experimental tributaries to Lake Champlain where fry (empty circles) and parr (filled circles) were released over the course of 4 years (2011 – 2014). Dams are indicated by triangles and the sampling site for returning adult salmon is indicated by a filled triangle.

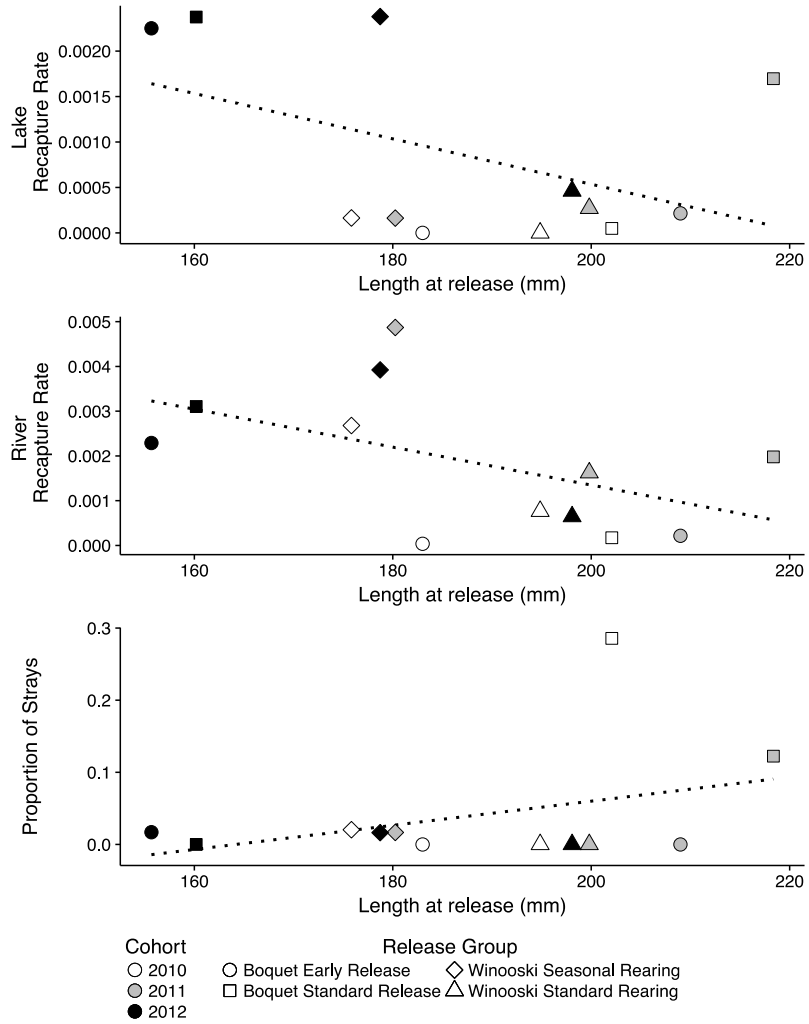


Figure 1.3: Recapture proportions in Lake Champlain and two experimental tributaries along with straying proportions according to the average parr size-at-release including trend lines based on linear models.

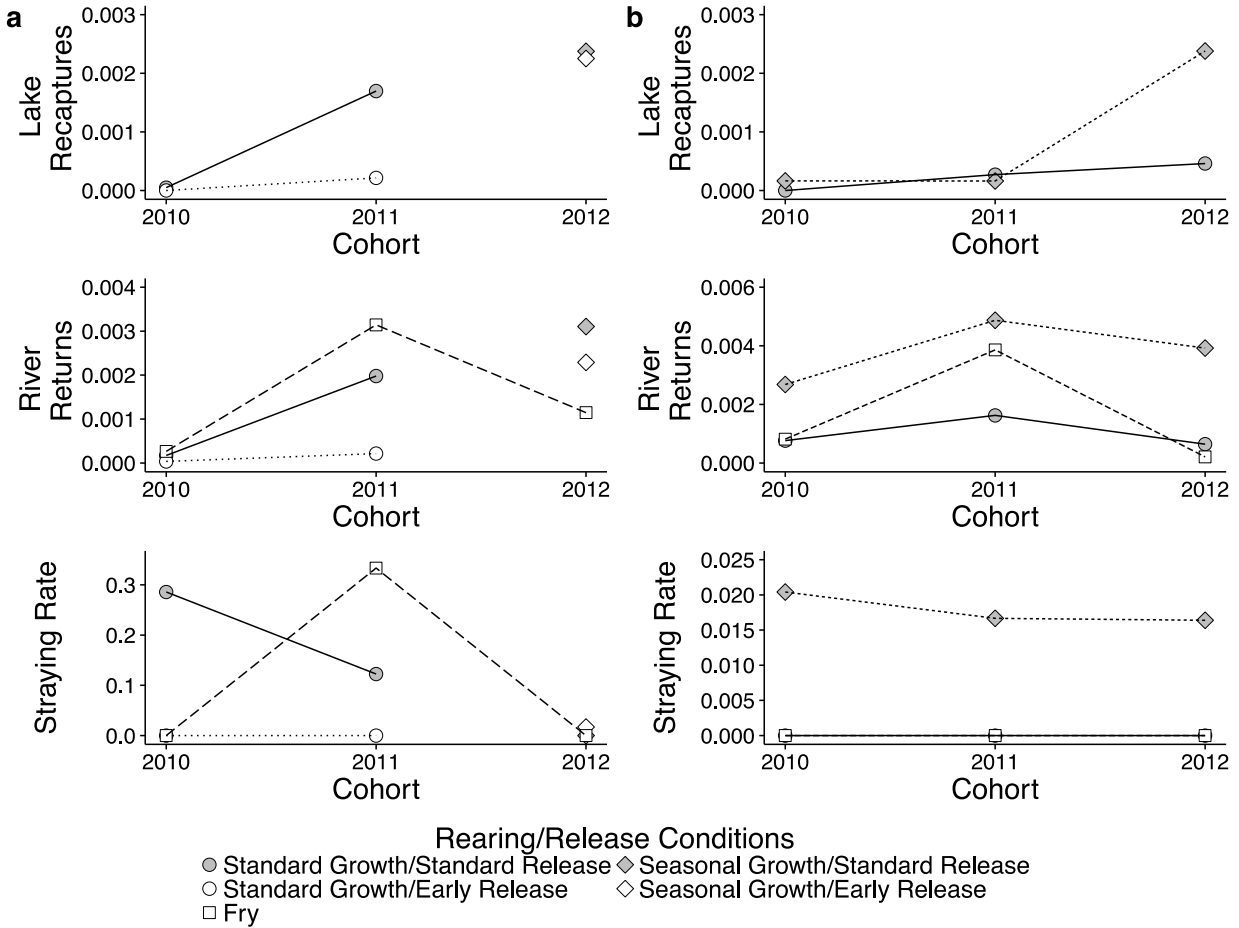


Figure 1.4: The lake recapture frequencies, river returns, and straying rates for multiple growth conditions and release dates for Atlantic salmon stocked in two tributaries of Lake Champlain, the Boquet (a) and the Winooski (b) rivers. Lake recaptures and river returns are expressed as a proportion of the numbers originally released while straying rates represents the frequency of salmon that were recaptured in a non-origin tributary as a proportion of total number of each release group intercepted during a spawning migration.

Chapter 2: Novel, continuous monitoring of fine-scale movement using fixed-position radio telemetry arrays and random forest location fingerprinting

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Migration? Oral presentation to the International Association for Great Lakes Research in

Burlington, VT - International.

Abstract:

1. Radio-tag signals from fixed-position antennas are most often used to indicate presence/absence of individuals, or to estimate individual activity levels from signal strength variation within an antenna's detection zone. The potential of such systems to provide more precise information on tag location and movement has not been explored in great detail in an ecological setting.

2. By reversing the roles that transmitters and receivers play in localization methods common to the telecommunications industry, we present a new telemetric tool for accurately estimating the location of tagged individuals from received signal strength values. The methods used to characterize the study area in terms of received signal strength are described, as is the random forest model used for localization. The resulting method is then validated using test data before being applied to true data collected from tagged individuals in the study site.

3. Application of the localization method to test data withheld from the learning dataset indicated a low average error over the entire study area ($< 1\text{m}$) while application of the localization method to real data produced highly probable results consistent with field observations.

4. This telemetric approach provided detailed movement data for tagged fish along a single axis (a migratory path) and is particularly useful for monitoring passage along migratory routes. The new methods applied in this study can also be expanded to include multiple axes (x, y, z) and multiple environments (aquatic and terrestrial) for remotely monitoring wildlife movement.

Introduction:

Numerous aspects of a species' life history, habitat requirements, dispersal, migratory routes, foraging and home-range characteristics can be studied by monitoring individual movement over time (White & Garrott 1990; Cooke *et al.* 2013). In many environments, however, this presents a challenge due to difficulties associated with visibility limitations (e.g. aquatic environments) or manpower (e.g. around the clock observations). Hence, several methods have evolved to monitoring movement, the most fruitful of which make use of electronic tags; particularly passive integrated transponder (PIT) tags, acoustic transmitters (in aquatic environments), GPS tags, and radio (VHF) transmitters.

Fixed-position PIT tag detection arrays (Armstrong *et al.* 1996; Lucas *et al.* 1999) provide location information via proximity detection (within 2m of the detector) and therefore provide accurate estimates of location at a specific time. A drawback of PIT arrays is their limited detection range which requires a fairly dense array of detectors to provide fine-scale movement data (Castro-Santos *et al.* 1996; Zydlewski *et al.* 2006). In contrast, acoustic telemetry arrays have much larger detection areas and, depending on the array deployment and tag type, can provide highly accurate estimates of location in two or three dimensions (Heupel *et al.* 2006; Hanson *et al.* 2007). Yet the susceptibility of acoustic arrays to interference from turbulent waters and heterogeneous substrates (Bergé *et al.* 2012) limits their applicability in fast-flowing or shallow aquatic environments. GPS tags can conversely provide fairly accurate continuous movement data in many environments, but their high cost and size place limitations on study designs (Wikelski *et al.* 2007; Hebblewhite & Haydon 2010). In many situations, radio telemetry is often the preferred choice. Fixed-position radio antenna arrays detect and record the presence

of tagged individuals over a much larger detection area than PIT tags, while functioning well in habitats where acoustic systems cannot. Modern VHF tags are also smaller and cheaper than GPS tags. In large environments, radio antennas can act as checkpoints, monitoring movement along a migratory path such as a river, while in smaller environments, where a single antenna's detection area may include most of the area of interest, fluctuations in signal strength can be used to estimate activity levels (David & Closs 2001). Currently, the use of radio telemetry to estimate location on a fine scale is an active area of research (Kays *et al.* 2011; Ward & Raim 2011; Ward *et al.* 2013).

Herein, we describe a new method of continuously estimating fine-scale location of tagged individuals using VHF radio transmitter tags and fixed-position antenna arrays. The method provides a means of remotely monitoring individual movement through an environment with overlapping detection areas that differs considerably from previous proximity detection and angulation methods. Location Fingerprinting (LF) describes localization techniques that compare measured system characteristics to a pre-recorded database of system characteristics at known locations (Kjaergaard 2007). They are commonly employed in non-wildlife fields for providing location-aware services where standard localization techniques (i.e. GPS) are ineffective (i.e. indoors). LF methods are typically applied to estimate the location of mobile computing devices (e.g. cellular phones) based on the signal strength they receive from signal emitting base-stations with known, fixed locations, often IEEE 802.11b based WLAN signals (Bahl & Padmanabhan 2000; Li 2015). The recorded system characteristics (e.g. received signal strength) are then compared, using various estimation methods, to a pre-recorded database of system characteristics and locations, often referred to as a radio map. By reversing the roles of the transmitters and

receivers (mobile transmitters and stationary receivers), we provide researchers with a new telemetric tool to allow continuous monitoring of location and movement using relatively inexpensive equipment.

Methods:

Study site and species

The Boquet River is a tributary of Lake Champlain, New York, USA. Each fall, landlocked Atlantic salmon (*Salmo salar*) migrate up the river towards spawning habitat located in the river's upper reaches. Four kilometers upstream from the river mouth, migrating salmon encounter their first barrier, a series of rapids followed by a dam equipped with a fish ladder. These rapids were chosen as our study site because they represent a typical environment in which information on the fine-scale movement patterns of a migratory species would be valuable to researchers. In the present setting, such information would previously only have been possible using an extensive short-ranged PIT tag array due to the irregular substrate and high degree of turbulence. A total of 24 adult salmon were intercepted along their upstream migration and fitted with radio transmitters (Pisces model, Sigma Eight Inc.; Newmarket, Ontario, Canada) placed inside their abdominal cavity. Each radio transmitter broadcasts a unique code at regular intervals. To minimize signal cancellation at the receivers due to simultaneous signal reception, transmission rates were staggered over four to six seconds per transmission and over four separate frequencies (164.31, 164.48, 164.34 and 164.33 MHz).

Tagged fish were then allowed to continue upstream into the ~295 m long study reach containing three resting/staging pools and two sections of rapids (Figure 2.1). The lower pool (90

m × 45 m, 3 m max. depth) often contains many salmon in the fall that appear to use it as a resting/staging area before attempting to climb the first section of rapids. These first rapids span roughly 100 m in length and have an average width of 24 m, with a depth varying from 20 cm to 70 cm. Immediately above the first set of rapids fish encounter the middle pool, measuring 47 m x 28 m with a maximum depth of 2.5 m and is divided into two equally sized sub-basins. Above the middle pool is a second set of rapids that contains a natural ridge that cross-cuts the river's flow and provides migrating salmon with the only suitable means of achieving the third and final pool (10 m x 4 m, 1.5 m max. depth), which contains three small sub-basins. Once in the final pool, salmon must enter and pass a fish ladder to continue upstream.

Antenna array

Six receiver stations with fixed-position antennas were used to monitor the study reach from September 26th through November 19th, 2014. Each station consisted of a receiver/data logger (Orion model, Sigma Eight Inc.; Newmarket, Ontario, Canada) coupled with an antenna by a section of 4 m long coaxial cable. Five of the antennas were six-element Yagi-Uda antennas positioned three to four meters high, and angled towards the center of their respective zone (the lower pool, first rapids, middle pool, the second rapids/upper pool, and the river mouth). A sixth, whip style antenna consisting solely of a coaxial cable with 10 cm of shielding stripped from the distal end, hereafter referred to as a 'dropper', was used to provide a more localized detection range immediately in front of the entrance to the fish ladder. All stations continuously scanned the four radio frequencies employed, recording the frequency, id number, timestamp and strength of each transmission detected. The station at the river mouth was intended to detect fish

potentially leaving the river. All receivers measured received signal strength (RSS) on a decibel scale and a noise floor was set at -115 dB (-120 dB for the dropper). Once data were downloaded, transmissions received by the dropper with RSS values below -115 dB were bumped up to the overall noise floor and all RSS values were increased by 116 to create a positive noise floor of one. Data collected by the receivers were stored on MMC memory cards and downloaded every 2-3 days.

Data collection and preparation

Noise was removed from downloaded data by cross-referencing each transmission with the id number/frequency combinations programmed into our tags. Timestamps from tag transmissions received by multiple receivers were then synchronized. Data were arranged to resemble a separate multivariate time-series for each tag/fish, with regular time intervals beginning the moment each fish was tagged (September 28th – October 7th, 2014) and finishing upon study completion (November 19th, 2014). Any tag transmissions received by both the river mouth station and upriver stations were considered erroneous due to the distances involved and were removed from the data set. Presence thresholds were then identified as per Castro-Santos and Perry (2012) for each antenna. Gaps in a fish's time series for a detection zone in excess of that zone's presence threshold (720 seconds for each of the four aerial antennas in the study reach, 320 seconds for the dropper, and 200 seconds for the antenna at mouth of the river) signified that fish leaving the detection zone, thereby ending an exposure period. Any exposure periods for a particular fish containing only one or two transmissions were considered erroneous and were removed. Next, within exposure periods, gaps in the multivariate time series produced

by incomplete transmissions (transmissions not detected by all antennas in the study reach) were imputed using one of two methods. If the gap's duration did not exceed the presence threshold for the antenna, linear interpolation was used to estimate the RSS values. Gaps in excess of an antenna's presence threshold, but which occurred while that fish was within the study reach (i.e. detected by other antennas), were imputed with RSS values of zero. No data were imputed between exposure periods to avoid estimating a fish's location when it might not be present.

Radio map

Estimating LF-based tag location first required constructing a radio map characterizing RSS patterns for the 5 antennas over the entire the study reach. In traditional LF applications, this would involve moving a mobile receiver through the target environment in a grid-like manner while recording its location and system characteristics, such as RSS, received from multiple fixed position base-stations (Kjaergaard 2007). However, our primary interest was upstream/downstream movement rather than side-to-side movement within the river. For this reason, RSS values were recorded at locations (i.e. landmarks, Figure 2.1) along the most likely migratory route for salmon (the fish path, Figure 2.1). The fish path assumed migrating salmon enter the lower pool from the western side of the island, and follow the most direct route through the first two pools and first set of rapids. Salmon then deviate from the most direct passage as they climb the second rapids diagonally along a natural ridge, before passing through the center of the final pool in the direction of the fish ladder. To account for the effect depth has on RSS (David & Closs 2001; Kuechle & Kuechle 2012), tag depth was varied at each landmark while RSS values were being recorded. Landmarks were assigned a distance from the river mouth

based on their nearest point along the fish path. This method produced 785 partial and complete RSS patterns at 49 distances along the fish path. Incomplete transmissions were imputed using either the average RSS value for that receiver and distance, or with a value just below the noise floor (zero) where no transmissions were received by an antenna at a given distance. This latter case occurred when landmarks were beyond the detection range of the receiver's antenna.

Next, landmark data were split into two groups: learning data, used to construct the radio map, and test data, representing a random selection of 15% of the data for model testing. Learning data were plotted against distance from the river mouth and loess models were constructed for each aerial antenna using the 'stats' package in R (version 3.2.4, R Core Team 2016). These models allowed mean RSS values of each antenna to be extrapolated into unsampled distances. Initial loess models used α values of 40, 45, 40, and 40 respectively for the first four antennas (Figure 2.1) and the resulting curves appeared reasonable through the shallower sections of the study reach. A sparseness of landmarks through the deeper sections of the pools, however, prevented these initial curves from representing biologically realistic mean RSS patterns in these sections. To compensate for this, the four loess curves were adjusted by providing the models with 'observations' created using: i) knowledge of the system and ii) observations from data of tagged fish. For i, tagged fish entering the study reach from downstream were expected, and observed, to initially express RSS values at the pre-determined noise floor for each of the antennas/receivers in the study reach. The loess models were therefore provided with RSS values at the noise floor occurring at the downstream limit of the array's detection range (3720 m). For ii, data from the 24 tagged fish were examined and RSS patterns maintained for long periods of consecutive transmissions were identified. These prolonged RSS

patterns largely corresponded with RSS patterns expected at distances that fell within holding pools and were therefore provided to the loess models in a manner identical to that above, but at distances corresponding to the 3 holding pools. Additionally, within the upper pool, RSS values received by the dropper antenna were observed to vary more among sub-basins of the pool rather than within sub-basins. For this reason, a stepped ‘curve’ was incorporated into the radio map for the dropper rather than a smooth loess curve.

While the five curves above provided estimates of mean RSS values for each antenna throughout the study reach, they did not include information about the error associated with these means. To incorporate this information into the radio map, the standard deviation (SD) around the mean RSS values recorded at each of the original 49 landmarks was calculated for each antenna. Average SD values were then used to construct a normal distribution around the mean RSS values produced by each curve, by repeating each mean RSS value (\pm random error with an antenna specific SD) ten times for every 1 m section of the study reach. Finally, the RSS patterns from the original learning data were added to the modeled RSS values \pm their variance to form the final extrapolated radio map.

Estimating tag location

Along with the extrapolated radio map created above, a random forest model was used to estimate tag location. Random forests are an ensemble machine learning method that combine multiple models based on ‘weak’ subsets of the learning data to create a single model with high accuracy (Breiman 2001). When applied to LF techniques, random forests outperform other machine learning methods such as artificial neural nets and support vector machines while

requiring less training time complexity (Mo et al. 2014). Random forest models can also provide the user with the distribution of conditional predictions produced by the trees, rather than only a single conditional mean for the forest. These conditional prediction distributions were used to assess confidence in each prediction by calculating the 0.025 and 0.975 quantiles for each estimate (the prediction interval, Meinshausen 2006). The random forest model used to estimate tag distance was created using the randomForest package in R (Liaw & Wiener 2002) and used RSS values from the five antennas as explanatory variables, along with a variable indicating which antenna had the maximum RSS value per tag transmission. The tendency of RSS values to decrease proportionally when tags were lower in the water column necessitated the inclusion of relative RSS values (RSS values divided by the sum of RSS values for each transmission) as explanatory variables as well. A total of 1000 trees ($n_{tree} = 1000$ in R) were created within the model and three explanatory variables were assessed at each branching event ($m_{try} = 3$). The model was allowed to potentially over-fit its predictions by allowing a minimum terminal node size of one ($nodesize = 1$) as this assisted in the estimation of the prediction intervals. To reduce computation time, predictions were made on a subset of the original data containing only one copy of each unique RSS pattern observed from tagged fish. The resulting distance estimates were then joined to the original data by matching the RSS patterns.

Localization trend

Since RSS values received by the stationary antennas represented the true signal strength from tags at a given location plus some error, the resulting raw distance estimates also reflected this error. Thus, a Kalman smoother was applied to each fish's time series of raw distance

estimates to approximate true distance trends. Such smoothers are used with LF methods to increase overall accuracy of location estimates (Guvenc 2003; Chan et al. 2009) by iteratively estimating the true location at a given time based on the previous location, Bayesian inference, and an estimation of its joint probability distribution for each time step. The iterative application of prediction and measurement update steps are what allow Kalman smoothers to quickly move through a time series of noisy location data and identify the most likely position at each time step. When dealing with complete time series (i.e. all location estimates have been made), this filtering process can be taken one step further by incorporating information from both past and future observations when estimating the true location at any given time. This is the basis of the Kalman smoother implemented in R (tsSmooth command, 'stats' package; R Core Team 2016). Smoother parameters were estimated separately for each fish using local level state space models constructed with the StructTS command (also in the R 'stats' package). A new variable, Estimate Difference, was calculated for each tag transmission as the difference (in meters) between raw and smoothed distance estimates.

Model Calibration

Once the distance trend was identified for each tagged fish, certain irregularities presented themselves as periods of a fish's time series where Estimate difference was above average. These irregularities were handled one of two ways according to their duration. For short irregular periods (< 10 transmissions or 50 seconds) we omitted raw distance estimates with prediction intervals exceeding 100 m prior to applying the Kalman smoother. Long irregularities (> 10 transmissions) were thought to arise from tagged fish deviating from the expected

migratory path and were anticipated as our extrapolated radio map is a one-dimensional representation of a three-dimensional environment. These deviations produced sequential raw distance estimates with larger Estimate differences and wider prediction intervals. To account for such deviations, the extrapolated radio map was expanded to include additional RSS patterns at various distances from the river mouth. These additional RSS patterns were calculated from the mean RSS values recorded during longer irregular period, while the associated distances corresponded to a distance halfway between the preceding and following distance estimates.

Model performance

Model performance was assessed in three different ways by: i) using the test data removed from the learning data prior to construction of the extrapolated radio map, ii) comparing the extrapolated radio map constructed above to ‘estimated’ radio maps constructed by calculating the average RSS patterns produced by the 24 tagged fish at estimated distances from the river mouth, and iii) examining the realism and reliability of the approach using two case studies.

Assessment i) Cross-validation of the random forest model was conducted using the hold-out method by estimating distances for the 114 RSS patterns recorded at known locations and withheld from the learning data. These 114 randomly selected RSS patterns originated from 41 of 49 landmarks and spanned 87.6% of the study reach’s length. Once distance estimates were produced, method accuracy was assessed by calculating the differences between the predicted and actual distances, the prediction error, and comparing this to zero using a t-test. Trends in estimation accuracy and precision were also evaluated by regressing prediction error against the

known distances using a linear model; a slope of zero indicated consistent error throughout the study reach.

Assessment ii) Here the extrapolated radio map was compared to three ‘estimated’ radio maps. Estimated radio maps were created by averaging antenna-specific RSS values across the 24 tagged fish for each 1 m section of the fish path using a) raw distance estimates produced by the random forest model, b) Kalman-smoothed raw distance estimates, and c) a subset of Kalman-smoothed distance estimates with prediction intervals below a threshold of 100 m. Our aim was to identify distances along the fish path where measured RSS values differed consistently from extrapolated RSS values due to mischaracterization of the fish path (irregular differences) or the effect of depth (proportional differences), and to demonstrate the effect of applying Kalman smoothers to raw and filtered distance estimates.

Assessment iii) We assessed model plausibility by evaluating movements of two tagged individuals. We selected one salmon known to have successfully climbed the fish ladder situated at the top of the study reach and another, tagged on the same day, which displayed considerably different behavior.

Results:

Radio Map

The loess models used to extrapolate mean RSS values across the study reach produced curves that decreased with distance in front of each antenna in a manner similar to the standard negative power attenuation curves typically observed with radio signals (Whitehouse et al. 2007), but with additional undulations. These undulations likely reflect changes in signal

attenuation produced by differences in depth and elevation along the irregular terrain of the migration path (Figure 2.2). A radio tag moving in an upstream direction (left to right in Figure 2.2) along the expected migratory path, moved sequentially into, then out of each antenna's primary detection zone. At the downstream limit of antenna coverage, RSS values received by each of the 5 antennas are at the noise floor. As the tag enters the lower pool however, 3720 – 3800 m, mean RSS values detected by antenna 1 (situated at 3720 m, Figure 2.2) increase rapidly until they reach their maximum of 29. They then gradually decrease over the entire study reach, with the exception of a slight increase at the downstream limit of the two sets of rapids, 3800 m and 3950 m, as the tag re-enters shallower water. Mean RSS values at antenna 2 (situated at 3829 m, Figure 2.2) remain near the noise floor throughout most of the lower pool but begin to climb near its upstream limit. Antenna 2's RSS values plateau at around 23 until the tag passes under the antenna, when they climb rapidly to a maximum of 45 at 3857 m. Beyond this maximum, RSS values received by antenna 2 decrease until the tag reaches the downstream limit of the second set of rapids where they begin to increase again before falling once more as the tag exits the second rapids 3985 m and enters the final pool. Antenna 3 (situated at 3921 m, Figure 2.2) was positioned to overlook the middle pool and exhibited its maximum RSS values directly in front of the antenna, with RSS values tapering off rapidly in both the upstream and downstream directions. An exception to this is a leveling off of RSS values as the upstream limit of the first rapids. Antenna 4 (situated at 4018 m, Figure 2.2) expressed RSS values that began rising above the noise floor at the downstream limit of lower pool and which increased throughout the entire study reach until the tag passes directly under the antenna. Noticeable periods of steep increases in RSS for antenna 4 occur at the lower and upper limits of the first rapids, and the lower limit of

the second rapids, all coinciding with shallow water depth. The dropper antenna (antenna 5, 4010 m, Figure 2.2) in the upper pool only began receiving tag transmissions at the upper limit of the second rapids. RSS values at this antenna climbed in a stepwise manner until reaching a maximum immediately in front of the entry way to the fish ladder, 4015 m, before dropping again as the tag enters the concrete fish ladder at 4020 m.

Model Assessment

Test data: To test the accuracy of our localization method over the entire study reach, we estimated distances for withheld RSS data and calculated the prediction error for these estimates (the difference between predicted and true distances). Predictions tended to be slightly upstream from the true distances and had a mean error of 0.6 m and a 95% confidence interval of -1.48 to 2.70, which did not differ significantly from zero ($p = 0.56$, $t = 0.57$, $df = 113$). When regressed against the known distances, prediction errors produced a non-significant positive slope of 0.017 ± 0.015 (\pm SE, Figure 2.3a), with the greatest errors occurring primarily at either end of the first rapids (3800 – 3885 m). There was no apparent trend between prediction errors and prediction intervals (confidence) for the withheld data, though there was a tendency for the dispersal of the prediction errors around its mean to be greater at greater prediction intervals (Figure 2.3b). Interestingly however, even the most ‘uncertain’ estimates differed from the true distance by less than 10 m, as the greatest errors were associated with predictions with intermediate prediction intervals spanning 30 – 60 m.

Extrapolated vs. estimated radio maps

Several interesting results were produced by comparing estimated and extrapolated radio maps (Figure 2.4a). The distribution of mean RSS values at estimated distances were generally below those modeled from learning data throughout the entire study reach and at each of the antennas; two exceptions were antenna 1 (purple, Figure 2.4) at the upstream limit of the lower pool (3780 – 3800 m) and antenna 4 (red, Figure 2.4) in the upper pool (3995 – 4018 m), where RSS values from tagged fish at estimated locations were higher. These trends were even more apparent for Kalman-smoothed estimates (Figure 2.4b). Interestingly, despite the Kalman filter being applied to the distance estimates and not the RSS values, the overall effect was a reduction in variation in RSS trends throughout the study reach. Unexpected fluctuations in mean, Kalman-smoothed RSS values were apparent, such as the local maximum exhibited by the first (purple), third (blue) and fourth (red) antennas at around 3845 m. This overall increase in signal strength across three of the aerial antennas might reflect tagged fish preferring to pass through a particularly shallow section in the first rapids situated at ~3845 m. Additionally, antennas two (orange), three (blue) and four (red) produced a local minimum at 3900 m. This drop coincided closely with the downstream limit of the second pool, a particularly deep sub-basin that would have been the first suitable resting area for fish that successfully climbed the first rapids. Filtering out uncertain observations prior to applying the Kalman smoother did not result in a noticeable difference with the raw Kalman smoothed data (Figure 2.4c).

Case example

The following two case studies demonstrate the ability of LF methods to produce continuous fine-scale movement data for tagged individuals over extended time periods. Both

individuals, named 'Bill and Ted' for simplicity, were captured, tagged, and released into the lower pool on September 29th 2014. Bill was released at 15:30, and remained in the lower pool for 71.2 hours before attempting to climb the first set of rapids. This attempt lasted only 1.7 minutes and was ultimately unsuccessful, though it was followed by two other attempts over a span of four hours. The third attempt was successful and Bill arrived in the middle pool on October 2nd, 19:55, where he remained for the next 48 hours. Bill's first attempt at the second rapids occurred after 15.7 hours and lasted 3.5 minutes, but ultimately resulted in a further 47.9 hours being spent in the middle pool. During this time, Bill shifted towards the downstream edge of the middle pool and moved into and out of the first rapids a total of 14 times, averaging 7 minutes each. On October 5th, at 11:41:35, Bill reentered the second rapids and successfully climbed to the third pool in 2.8 minutes. Once in the upper pool, Bill remained relatively immobile for another 10 days, dropping back into the upper section of the second rapids several times throughout. Finally, on October 16th at 3:28:23 the signal dropped out as Bill moved through the fish ladder and into the fish trap operated by the NY Department of Environmental Conservation (NYDEC). Bill remained in the trap until the next day when, at 11:00 the trap was tended by NYDEC staff and he was passed above the dam and out of the study reach. In general, the mean RSS values received from Bill (Figure 2.5, upper two panels) at Kalman smoothed distance estimates match closely with the estimated RSS patterns produced by all 24 tagged fish (Figure 2.4) with the exception of a spike in RSS values for antenna one at the upstream limit of the bottom pool as well as generally higher RSS values received by antenna two in the lower sections of the first set of rapids. Bill spent relatively little time in these two sections, preferring to spend most time in the middle and upper pools.

In contrast to Bill's successful climb up the rapids, Ted did not reach the final pool. Tagged and released into the lower pool only 30 minutes after Bill (16:00), Ted remained in the lower pool for 76.5 hours before exiting the study reach in a downward direction on October 2nd at 20:31:10. Ted then returned to the study reach after 12.3 hours, always remaining near the downstream limit of the lower pool and exiting the range a total of 15 times in the first 10.4 days. Ted then attempted to climb the first set of rapids 4 times before again exiting the study reach in a downstream direction. Each of these attempts to climb the first rapids lasted less than 3 minutes. Ted then spent the remainder of the study period either in the lower pool or below the study reach, in the lower section of the river, though he was never detected at the river mouth. Most of Ted's time was in the downstream half of the lower pool (Figure 2.5, lower two panels), or at the downstream limit of the study reach, likely at greater depths than originally modelled in the extrapolated radio map based on relatively lower RSS values within the lower pool.

Discussion:

We provide a new telemetric tool with which to remotely study animal movement in their natural environment. Our results support that location fingerprinting (LF) techniques employing random forest models are a practical and accurate means of observing and recording the movement patterns of individuals over long periods, while requiring a relatively limited amount of equipment and time. The successful application of LF methods to ecological telemetry greatly expands the uses of fixed-position antenna arrays from simple estimates of activity levels over time (David & Closs 2001; Tucker *et al.* 2014) and opens the door to many different possibilities. The ability of LF methods to adjust and expand the level of detail required allows

such methods to be applied equally well in a variety of aquatic and terrestrial environments wherever overlapping antenna coverage is possible.

When tested using withheld data, the LF method produced distance estimates with very little error on average (within 2 m). This was despite our random forest model using an extrapolated radio map which modeled RSS patterns between landmarks in order to provide continuous RSS estimates. This result highlights the robustness and flexibility of random forests as an estimation algorithm for LF methods. Additionally, use of a radio map allows for flexibility when choosing the desired level of precision by adjusting the number of landmarks, artificially binning RSS patterns, or extrapolating RSS patterns into unsampled sections of the study reach (Krumm & Platt 2003; Lee & Han 2012).

Differences between the extrapolated and estimated radio maps provides insights into various features of the study environment and the behavior of tagged individuals. For instance, areas of the study reach where RSS values differed proportionally relative to the modelled RSS values suggest that tagged individuals spent more time deeper in the water column than expected. Alternatively, such proportional differences may have resulted from the positioning of the VHF tag antennas. Tag antennas exited the fish on their ventral surface, placing them in close proximity to the rocky substrate in shallower sections of the study reach, potentially causing signal interference that would not have been detected when the landmarks were characterized using a tag attached to a long wooden dowel. Finally, non-proportional differences between observed and extrapolated radio maps could arise when individuals depart from the modelled migratory path, though these departures were fairly evident upon inspection of the estimate differences, and prediction intervals and could be incorporated into the radio map before re-

estimating locations. In fact, such differences may even provide more information about the study range. For example, unnoticed landscape features may have caused the increase in mean RSS values, relative to the expected values, at ~3845 m by the third and fourth antennas or the decrease in RSS values at ~3890 m by antenna 2. Rerunning the random forest model using this estimated radio map may therefore increase prediction consistency or reduce prediction intervals in these areas. This should be done with care, however, and the resulting model should be cross-validated using more withheld data.

From our case examples, it is clear that LF methods can provide detailed estimates of movement patterns for tagged individuals beyond the standard presence/absence data typically collected with VHF tags, while providing greater detail about fine-scale habitat use (Figure 2.5). This level of accuracy, combined with the flexibility of programmable VHF tags, makes LF techniques well suited to the study of movement patterns for a wide variety of species. For example, LF methods are highly applicable to studies of fish passage where it is important to understand how individuals approach a particular passage structure and how long they remain in each stage of passage (the rates of passage, Castro-Santos & Perry 2012).

Such path selection and passage rates can also be of value in terrestrial environments at highway crossings or when studying movement patterns among meta-populations. In such situations, LF methods may be expanded to collect two- or three-dimensional movement data by altering three aspects of the above methods: first, by constructing a multi-dimensional radio map; second, by using a categorical dummy response variable in the random forest model; and third, by using multiple or omni-directional antennas with the receivers. With any LF exercise, accurate location estimation requires the study environment to be thoroughly characterized in

terms of RSS patterns. As with the present study, however, environments may contain inaccessible areas in which RSS values may need to be modeled as a function of their x and y (and z) coordinates. The resulting radio map, in table form, would require an additional categorical variable summarizing the location (e.g. “B1” corresponds to $x = 2, y = 1$) as random forest models only predict single response variables. The resulting predictions would then be back-transformed to provide location estimates. A limitation of LF methods, however, is that each tag transmission must be received by multiple antennas in order for location to be estimated. Larger study environments will therefore require a greater number of antennas, while receivers may need to be paired with a single omni-directional, or multiple directional antennas in order to avoid ‘blind spots’ within the environment.

Such multi-antenna stations have already proven useful when combined with automated radio telemetry (ART) methods for studying small mammals, reptiles and birds (Kays *et al.* 2011; Ward & Raim 2011; Ward *et al.* 2013). For the moment, however, the accuracy of the LF methods developed in our study (within 2 m) exceeds the accuracy of such methods (typically within 50 m), though whether this will hold when LF methods are applied to multi-dimensional environments has yet to be seen. One advantage of LF methods over ART methods is that they do not rely on theoretical models of radio signal attenuation curves as do ART systems, so accuracy is less affected by obstacles in non-ideal (i.e. no clear line-of-sight) environments. LF methods can simply account for the effect of such obstacles in their radio maps either by characterizing the RSS values surrounding the obstacle or by applying a correction factor to modelled RSS values (Lee & Han 2012). Methods also exist within the LF literature to account

for temporal changes in radio mapped environments (Yin *et al.* 2008) allowing for accuracy to be maintained despite temporal variation in signal attenuation.

In summary, the fingerprint localization methods employed in this study, using a random forest estimation method, offer a novel approach to continuous automated wildlife telemetry. The level of precision that fingerprint localization can offer in fluvial environments, from tens of meters to within a meter, was formally available only through the use of dense arrays of PIT antennas in shallow waters (e.g. Sullivan 2004) or acoustic telemetry arrays in deep, open water (e.g. Daniel Deng *et al.* 2011). Additionally, the ease with which received signal strength throughout an environment can be characterized (both for aquatic and/or terrestrial environments), and the ability of such systems to be expanded to include 2d or 3d environments should make them an ideal choice in many research situations.

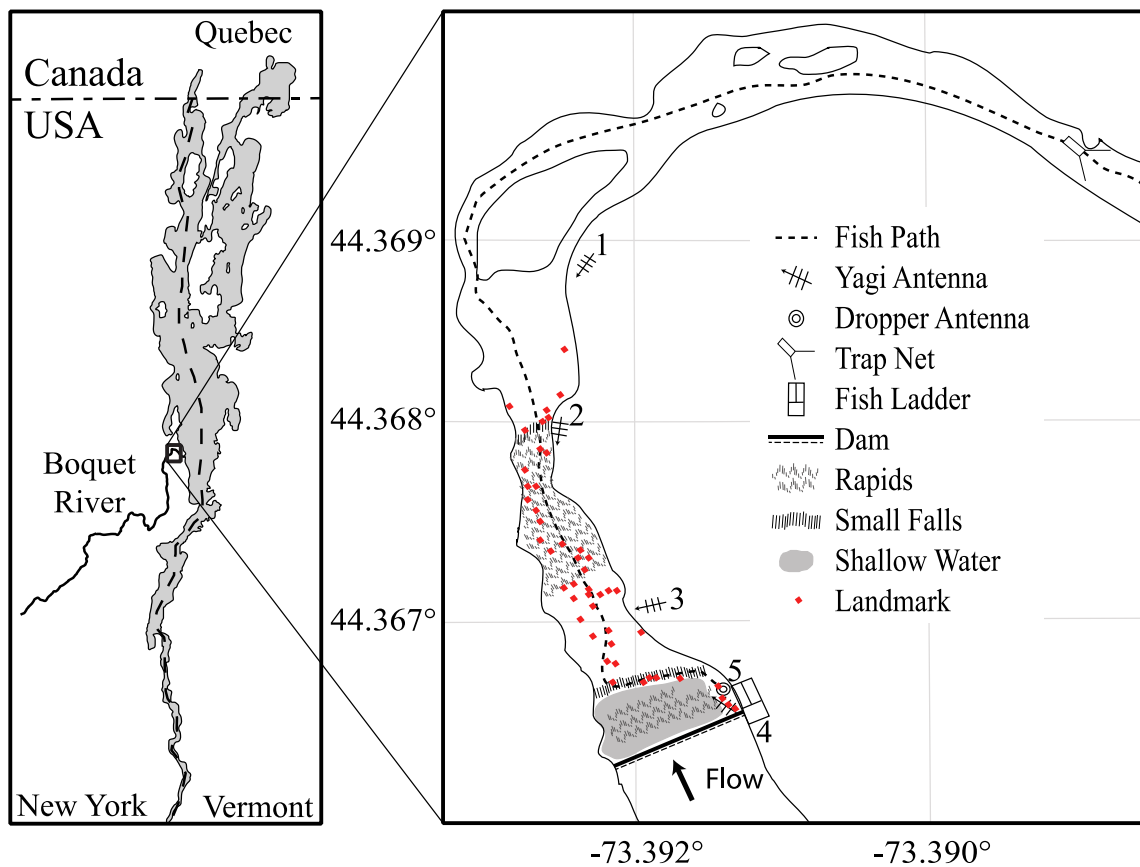


Figure 2.1: The study reach within the Boquet River, NY, USA, along with locations of four fixed-position aerial radio antennas, one submerged radio antenna, and landmark locations at which the received signal strength from mobile VHF radio transmitters were characterized for each antenna.

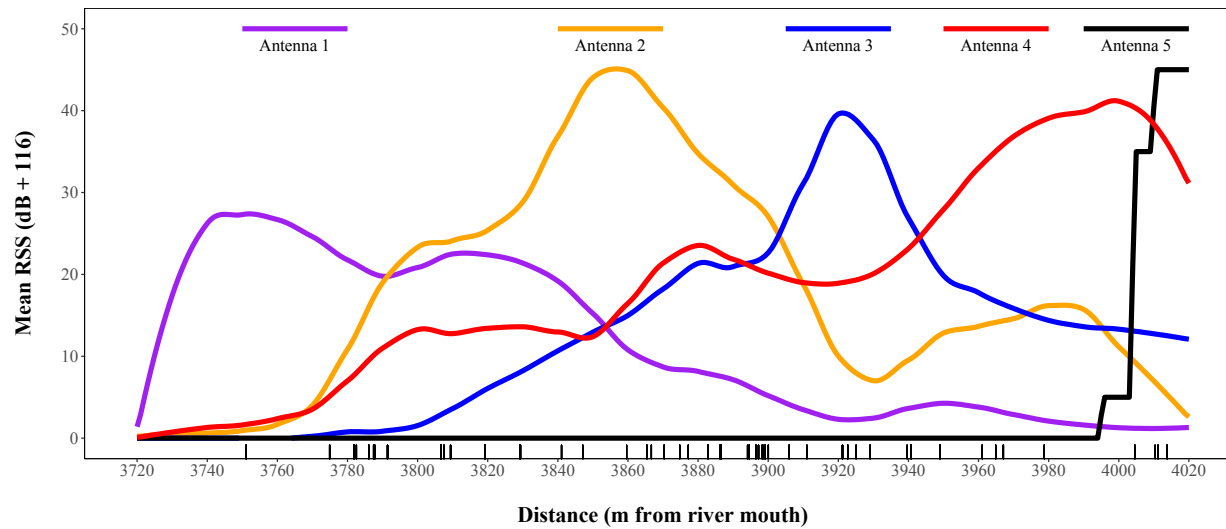


Figure 2.2: The mean received signal strengths from a radio transmitter tag at varying distances (bottom) along the migratory route of Atlantic salmon climbing a section of the Boquet River, NY, USA. Mean values were modeled using loess curves fit to 671 received signal strength values at 49 separate distances throughout the study reach.

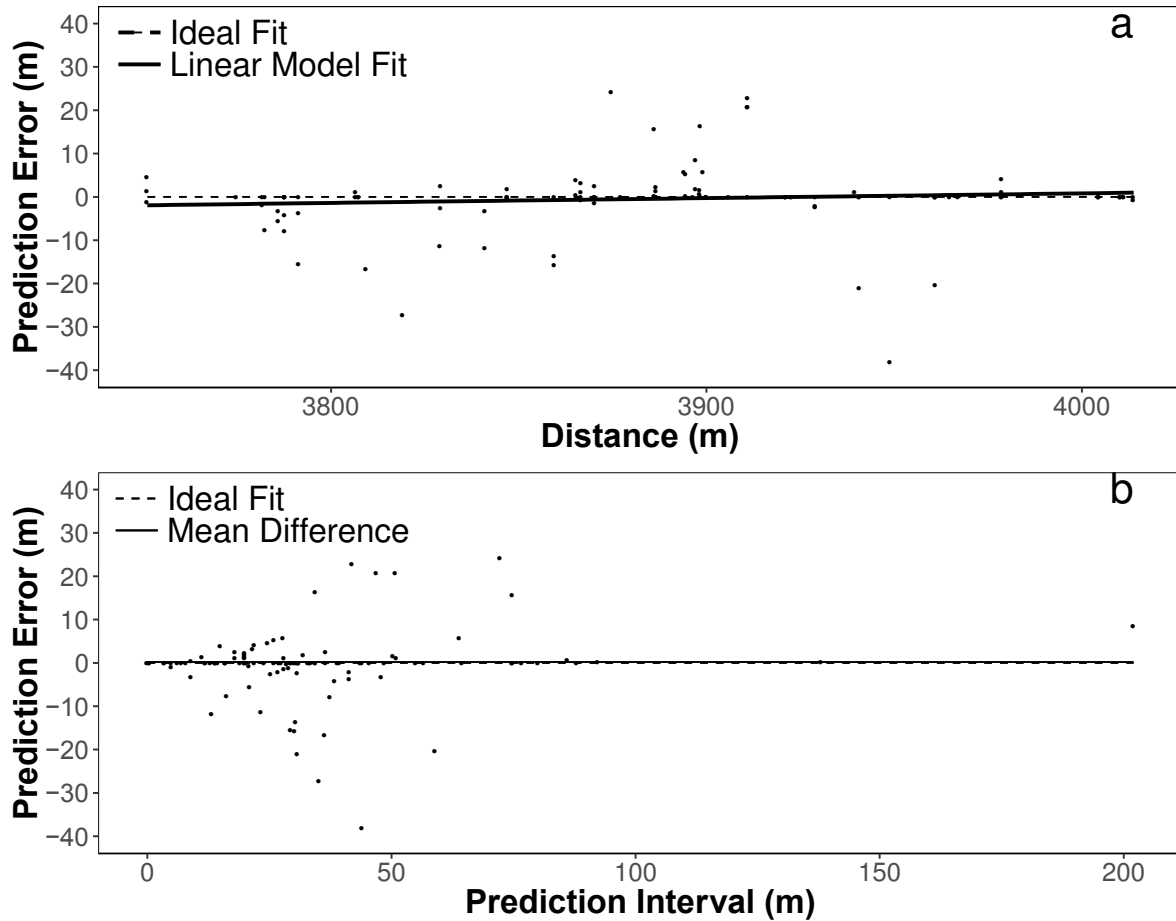


Figure 2.3: Panel a) the prediction error (in meters) between the true distances from the river mouth of landmarks throughout the study reach and the distances estimated by the random forest location fingerprinting method. The dotted line represents an ideal fit (a slope of 0) while the solid line is the fitted slope from a linear model (slope = 0.017, $p > 0.05$). Panel b) the prediction errors as a function of the random forest model's confidence (the 95% prediction interval width).

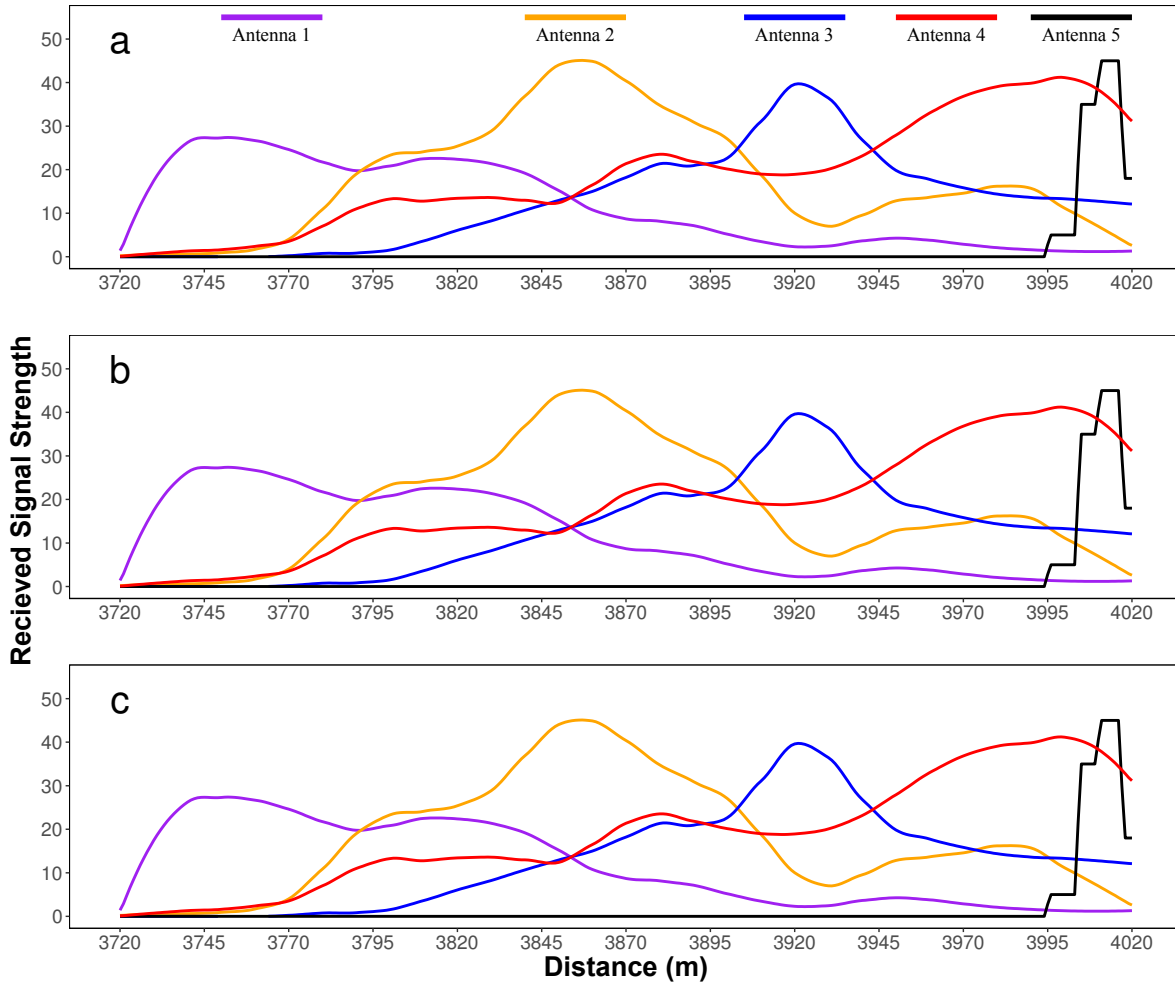


Figure 2.4: The mean received signal strengths at known locations as modeled using loess curves (colored lines), and the RSS values averaged over 24 tagged Atlantic salmon at distances estimated by random forest localization model (points, panel 4a). The remaining two panels display the mean RSS values for those same 24 salmon after application of a Kalman smoother to (4b) the raw distance estimates, and (4c) only those distance estimates with confidence intervals $< 100\text{m}$.

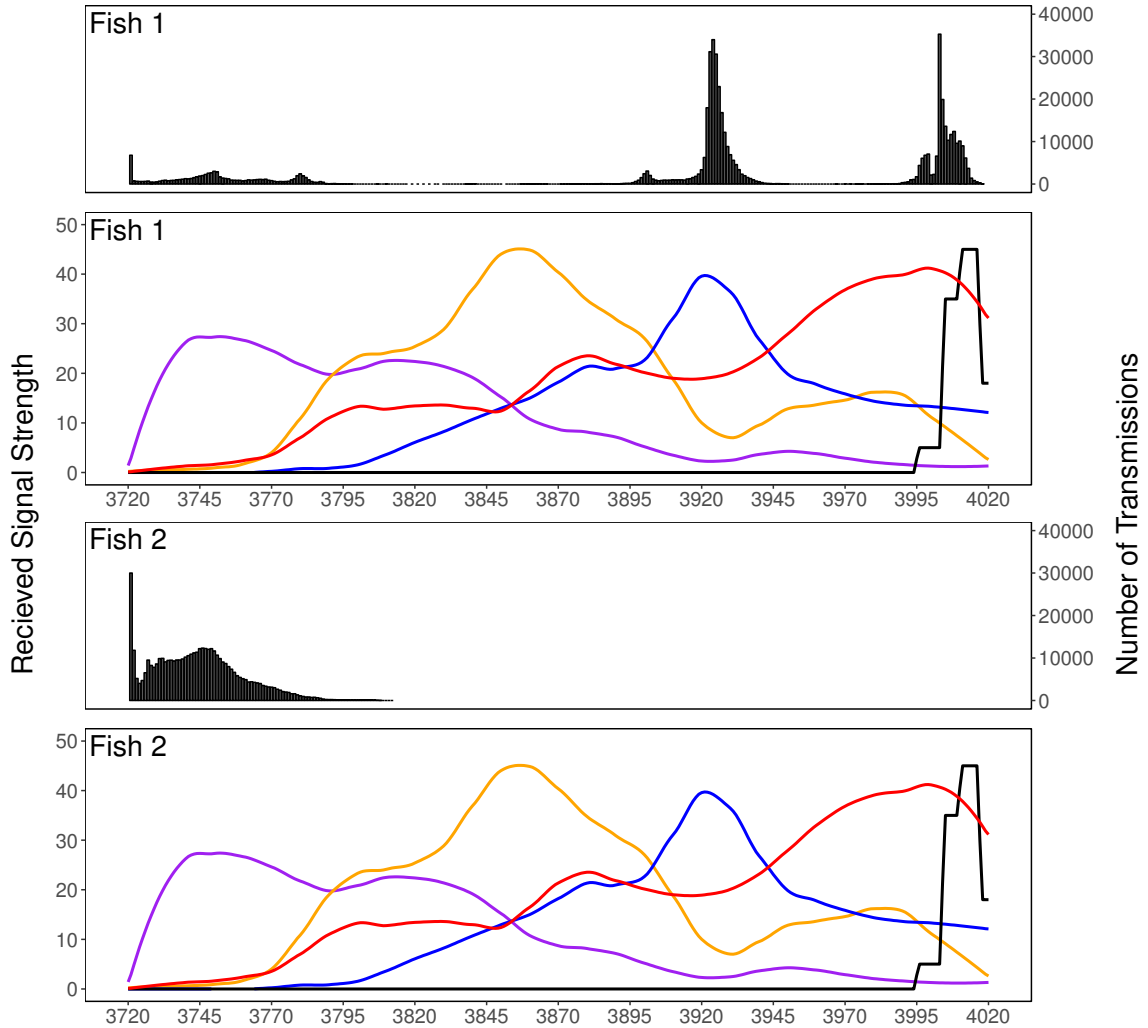


Figure 2.5: The mean received signal strength at estimated distances from the river mouth for two radio tagged Atlantic salmon captured and subsequently released into the lower pool of the study reach on September 29th and monitored until November 19th 2014. Histograms show the number of tag transmissions (1 every 5 seconds) received from each fish at each 1 m section of the study reach.

Chapter 3: Environmental, anthropogenic, and dietary influences on fine scale movement patterns of Atlantic salmon through challenging waters

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Abstract:

Partial barriers to migration can have significant effects on migratory fish population dynamics and evolution. The extent to which passage is limited depends on many biotic, abiotic, and anthropogenic factors. Additionally, malnutrition (e.g. thiamine deficiencies) can decrease swimming performance and further limit passage through natural and human-altered partial barriers. We investigated how such factors impact fine-scale movement patterns of landlocked Atlantic salmon in situ within high velocity, human-altered sections of river. Salmon were monitored as they migrated upriver using a novel radio telemetric technique, location fingerprinting. Time-to-event analyses identified a strong effect of multiple exposures to a section of the river along with water temperature on movement rates. High or increasing discharge encouraged downstream movement and males displayed greater upstream movement rates than females, but abandoned migratory attempts more quickly after 24 days in the study reach. Thiamine supplementation influenced movement in high and low energy sections. These results emphasize the importance of biotic variables, along with timing and seasonal shifts in abiotic variables for migratory success while indicating a potential benefit from thiamine supplementation.

Introduction:

River environments are becoming increasingly fragmented by infrastructure that alters natural hydrological flows (Nilsson et al. 2005, Kemp and O'Hanley 2010), impeding the movement of fish between areas used for feeding and growth and those necessary for spawning and reproduction (Neraas and Spruell 2001, Hall et al. 2011). Among obligate migrators, such blockages often result in the extinction of local breeding populations (Rieman and Dunham 2000, Gehrke et al. 2002). To prevent this, passage features, such as fish ladders or culverts, are often added to such infrastructure, but their passage efficiency can vary greatly (Bunt et al. 2012, Noonan et al. 2012) and may allow some, but not all fish to pass. Such partial barriers often impose migratory delays which negatively impact survival and reproductive success (Castro-Santos and Haro 2003, Burnett et al. 2014) while impacting upstream population densities, sex ratios, gene flow, and applying new selective pressures to migratory populations (Agostinho et al. 2007, Deiner et al. 2007, Keefer et al. 2009, Burnett et al. 2014). For these reasons, passage structures that function as partial barriers have received considerable attention in the literature, while natural sections of rivers with high velocity, low water levels, or rapid changes in gradient/elevation can be equally challenging to migrating fish and act as partial barriers, but receive much less attention.

Challenging sections of riverine habitat can represent partial or complete barriers to migration under several conditions. Anthropogenic or natural alterations to the hydrologic flow upriver can result in excess velocity (Castro-Santos 2005), or insufficient depth (Reinfelds et al. 2009), to permit fish passage. Ecological forces such as parasitism, pathogens, or disease may impact a migrating individual's swimming performance, thereby limiting their ability to overcome normally passable but challenging sections of river (Wagner et al. 2005, Palstra et al.

2007). Temperature can also influence metabolic functions within a migratory species by altering swimming performance or energy use (Lee et al. 2003, Burnett et al. 2014) such that challenging sections become too difficult when temperatures are above or below the energetic optimum. Finally, an often-overlooked mechanism is the nutritional state of migratory species. As many migratory species exhibit reduced feeding behavior during migrations, they must rely on stored energy reserves, as well as behavioral and bioenergetic traits to maximize efficiency during migration if they are to be successful (Bernatchez and Dodson 1987, Hinch and Bratty 2000). When prey species are introduced or lost from feeding grounds, the quantity and quality of food available may become insufficient, restricting energy or nutrient reserves required for passage through difficult sections of river.

An example of nutritional state affecting migratory success can be seen among anadromous and landlocked salmonid populations afflicted with thiamine (vitamin B₁) deficiencies. In freshwater environments where alewife (*Alosa pseudoharengus*) have established themselves through natural or human-mediated colonization events, this thiaminase containing prey species becomes a major part of the salmonid diet (Madenjian et al. 2008) resulting in the enzymatic breakdown of thiamine within the digestive systems of salmonid predators (Fitzsimons et al. 1999). Thiamine deficiencies can also arise in other ways, such as through the dramatic decline of a native predatory species, Atlantic cod (*Gadus morhua*), from the Baltic sea (Jonzén et al. 2002). The loss of cod has resulted in a shift in the forage base available to anadromous salmonids towards sprat (*Sprattus sprattus*), a species with a naturally low ratio of thiamine to fatty acids, resulting in a similar thiamine deficiency among Baltic salmonids (Keinänen et al. 2012, 2017). Ultimately, all thiamine within aquatic systems originates from primary producers, phytoplankton, whose thiamine levels can fluctuate as a response to

environmental stresses (Sylvander et al. 2013), influencing thiamine availability at all higher levels within the food web. When such thiamine deficiencies arise, afflicted salmonids can exhibit whirling behavior, weakness, difficulty maintaining equilibrium, and reduced swimming performance (Morito et al. 1986, Brown et al. 2005, Houde et al. 2015). These side effects have been found to negatively impact the capacity of salmonids to move upstream during spawning migrations (Fitzsimons et al. 2005, Ketola et al. 2005, 2009). Affected populations may therefore be forced to spawn in sub-optimal habitat or abandon spawning attempts altogether (reviewed in Aas et al. 2010).

Understanding how the above-mentioned conditions, or relevant biotic and abiotic variables impact movement rates through partial migratory barriers in natural settings is important for the proper management of migratory species, many of which are of ecological, cultural, and economic importance. To this end, landlocked Atlantic salmon from the Lake Champlain basin, which are known to possess reduced thiamine levels due to dietary alewife, were tagged and observed during their spawning migration through a challenging, high velocity section of the Boquet River, NY, USA. Their progress through the study reach was followed continuously over 54 days using a novel method of monitoring fine-scale movement employing radio transmitter tags and fixed-position radio antennas with overlapping coverage.

By modelling such fine scale movement patterns within a challenging section of river known to act as a partial migratory barrier, it is possible to quantify the effect of relevant biotic, abiotic, and ecological factors on movement rates. As alewife have become fully established in Lake Champlain since their arrival in 2003 (Marsden and Hauser 2009), it is also important to assess exactly how thiamine deficiencies impede upstream migration. Specifically, we assessed rates of advance and/or retreat in three specific environment types within a partial migratory

barrier (low energy environments, i.e. pools; high energy environments, i.e. cascades, and overall challenging environments, i.e. the study reach as a whole). In doing so, we further the understanding of how a thiamine deficiency hinders spawning migrations and clarify under what circumstances treatment is effective, providing biologists with a means of targeting their management efforts for at-risk thiamine deficient salmonid populations. Additionally, by identifying how biotic and abiotic factors impact migration through challenging river sections, we advance our understanding of how migratory bottlenecks function as a selective force on culturally and economically important migratory fish species.

Methods:

Study site

The Boquet River is a tributary to Lake Champlain with a drainage basin of 725 km². Each year, fry are released into suitable juvenile habitat within the upper reaches of this tributary, while fall fingerling (age 0+) and parr (age 1+) are released into the mainstem of the river. Following smoltification, these juvenile salmon migrate downstream to Lake Champlain where they remain until they mature. For these downstream migrating juveniles, a low (~1 m) crib dam in the town of Willsboro may result in a minor delay as juveniles must fall over the dam to complete their migration. To mature adults returning to the Boquet River to spawn in its upper reaches, however, this dam represents a significant barrier to migration, despite being equipped with a Denil style fish ladder. This is due, in part, to its location. By significantly widening the river's width, this dam alters the hydrologic flows over exposed bedrock which drops considerably in elevation over the 300 m of river immediately below the dam (Figure 3.1).

This results in a technically challenging, high velocity section of river that not all returning spawners are capable of passing.

Sampling, injections, and tagging

Gravid female and mature male salmon were intercepted in the Boquet river during their fall spawning run, which typically begins mid-September, ends by early November, and peaks in early October. A total of 20 salmon were captured with the use of a passive trap net approximately 0.4 km below the study reach from September 26th to October 7th, 2014, while supplemental short-set gill netting on September 29th in the lower pool of the study reach (Figure 3.1) added another 4 salmon. The length and mass of each captured salmon were recorded before salmon were anesthetized via electronarcosis as per Hudson et al. (2011). Once anesthetized, salmon were internally implanted with a uniquely coded VHF radio transmitter (Pisces-5, *Sigma Eight Inc.*, Aurora, Ontario) via a small incision (~1 cm) on the ventral surface of the fish, anterior to the pelvic fins. The transmitter antenna exited the abdominal cavity through the fish's side, below the lateral line. Once a transmitter was implanted, the incision was closed with 1-2 closely placed sutures (PDS-II 1-0 absorbable sutures, *Ethicon Inc.*, Somerville, New Jersey). Additionally, experimental salmon were given an intraperitoneal injection (1 ml/kg body weight) of either physiological saline (0.9% NaCl; control) or food grade thiamine mononitrate dissolved in physiological saline (150 nmol/g of body weight; treatment) posterior to their pelvic fins. No leakage through the closed incision was observed during a test injection of physiological saline with food grade colorant. The minimum concentration of thiamine required in muscle tissue to avoid impaired swimming performance in Atlantic salmon is estimated to be ~5 nmol/g (Houde et al. 2015). Our injections of 150 nmol/g body weight therefore contained a sufficient

thiamine to increase muscle thiamine concentration to levels exceeding this threshold while accounting for absorption into organs and eggs. Identical injection concentrations of thiamine hydrochloride, an analogue of thiamine mononitrate with similar bioavailability (Ketola et al. 2008), were found to be absorbed into the bloodstream of wild or farmed Atlantic salmon within hours (Koski et al. 2005), suggesting the effects of thiamine treatment would be felt among treated salmon shortly after release. Thiamine mononitrate was used instead of thiamine hydrochloride, however, as it can be dissolved directly into saline solution at the concentrations required more easily than thiamine hydrochloride, which often requires the addition of NaOH to facilitate dissolution. All experimental salmon were also given a unique T-bar anchor tag next to their dorsal fin before being placed into a recovery container of fresh river water. Once fully recovered (1 - 5 minutes), salmon were released above the trap net, or back into the lower pool in the event they were captured via gill netting. All manipulations of adult salmon conformed with the guidelines of the Canadian Council on Animal Care in science and was in accordance with the guidelines set by the Concordia University Animal Research Ethics Committee.

The thiamine status of the experimental salmon was not tested directly as collecting sufficient tissue from each salmon would have negatively impacted their ability to climb this challenging section of river. Instead, muscle samples were taken from Atlantic salmon collected within the lake as feral broodstock for a nearby hatchery (32 km away). White muscle tissue samples were collected from all male salmon euthanized for disease testing while egg samples were collected from gravid females. All samples were immediately frozen and stored at -80 until being analyzed using the methods of Futia et al. (2017).

Radio antenna array

An array of six fixed-position antennas was used to monitor the progress of tagged salmon as they migrated through the study reach. A total of four aerial six-element Yagi antennas monitored movement through the study reach while a fifth antenna, with a more localized detection range (termed a dropper), was positioned immediately before the entrance to the fish ladder (Figure 3.1). The dropper consisted of a 5 m coaxial cable with the shielding removed from approximately 10 cm of the distal end (Figure 3.1). Detection ranges of these 5 antennas overlapped, and provided the data required for continuous tracking of salmon movements throughout the reach (see below). A sixth Yagi antenna was positioned at the mouth of the river to detect fish that returned to the lake. Each antenna was paired with its own broadband receiver/data logger (Orion, *Sigma Eight Inc.*, Aurora, Ontario), powered by a deep-cycle marine battery. Batteries were changed and data recorded by the loggers were downloaded every 2 – 3 days. Three radio beacons (TX-V4-B, *Sigma Eight Inc.*, Aurora, Ontario), programmed to transmit every 5 minutes (or less) were used within the study reach, along with a fourth at the mouth of the river, to ensure antenna monitoring was continuous. Additionally, a handheld antenna was used in conjunction with regular weekly floats between the study reach and the mouth of the river to identify salmon in the lower stretch of the river. For more information regarding the antenna array, refer to Harbicht et al. (2017).

Salmon localization

A random forest model in conjunction with a one-dimensional radio map of the study reach was used to estimate the location of tagged fish at 5 second intervals (Harbicht et al. 2017). This process involves constructing a radio map that models the relationship between an active

tag's location (distance along the migratory path) and the transmission signal strength received by each of the five antennas within the study reach. Using this radio map as learning data, a random forest model in R (Liaw and Wiener 2002) was used to estimate each tagged salmon's location for each recorded tag transmission. Kalman smoothers were then applied to the time series of position estimates to reduce noise and improve accuracy (Guvenc et al. 2003) while data from an activated tag at known locations were used to assess the accuracy of position estimates (Harbicht et al. 2017).

Environment-specific passage zones

To investigate the three environments of interest (high and low energy sections along with the study reach as a whole), locations within the study reach were first assigned to mutually exclusive zones (states) based on geophysical features of the river, i.e. pools or cascades. These initial zones (lower pool, first cascade, middle pool, second cascade, and upper pool, Figure 3.1) were then grouped together to create habitat-specific passage zones to be used in subsequent time-to-event analyses. Within these analyses, downstream movement from the lower pool and upstream movement from the upper pool resulted in salmon exiting the study reach and being censored from further analysis until re-entered the study reach. All other possible movement along the migratory route resulted in a transition between two passage zones. To account for potential inaccuracies in the location estimates for tagged fish, detection thresholds for each passage zone were designated via interval analyses (Berdoy 1993, Castro-Santos and Perry 2012) and movement into or out of passage zones shorter than the threshold duration was deemed to constitute potential artifacts of the monitoring methods and were not considered.

To investigate which biotic and abiotic variables were associated with rates of advance through, or downstream retreat from low energy environments within the study reach, the lower, middle, and upper pools collectively formed the passage zone for the first time-to-event analysis. Variables impacting the advance and retreat rates in high-energy environments were assessed by combining both the first and second sets of cascades into a passage zone for the second analysis. Finally, to determine which variables influenced the rejection rate from the study reach as a whole, all of the initial zones within the study reach collectively formed a single passage zone for the third analysis.

To avoid temporal autocorrelation among observed behaviors within passage zones (e.g. multiple exits and re-entries in quick succession during an attempt to climb a set of cascades), only salmon entering a habitat-specific passage zone from downriver were considered. Similarly, repetitive series of exits and re-entries at the downstream limit of a passage zone were accounted for by requiring salmon to descend more than one zone downriver (i.e. from the middle pool, through the first cascades and into the lower pool) or remain downriver for a minimum of 5 minutes before a reentry into the passage zone was considered for analysis.

Time-to-Event analysis

As salmon entered a passage zone, they entered the risk-set for a given time-to-event analysis (the population of individuals at risk of experiencing the event of interest at a given point in time) and could only exit one of two ways: continued upstream migration into the next zone (advancing) or by returning downstream (retreating). Thus, competing risk analyses were conducted for the first two habitat types. These consisted of two separate time-to-event analyses wherein one possible outcome (e.g. advancing upstream) was treated as the event of interest,

while the alternative outcome (retreating) was treated as a censored observation. Until they retreat downstream, censored individuals contribute to the denominator when calculating fraction of the population that advance, but not the numerator. For the study reach as a whole, as our primary concern was to characterize rejection rates, a single time-to-rejection analysis was performed and successful climbs to the top were treated as censored observations. Within each analysis, inherent differences between sections of a passage zone (e.g. the lower, middle, and upper pools of the passage zone for low energy environments) were accounted for by stratifying each analysis by zone. Each stratum therefore had its own underlying baseline hazard, with each being similarly influenced by covariates, i.e. no covariate-by-stratum interactions. Additionally, given our requirement for a downstream sojourn prior to reentry into a passage zone, analyses were conducted on a gap-time scale (Therneau et al. 2003). Cox proportional hazards models were constructed using the ‘survival’ package in R (Therneau 2015) and were fit using the Anderson-Gill framework to account for internal correlations among observations by the same individual when multiple re-entries by an individual occurred (Andersen and Gill 1982). As this framework assumes a single underlying hazard for recurrent events that is unaffected by prior history (Andersen and Gill 1982) a time-dependent covariate distinguishing between each salmon’s first, second, and all subsequent attempts within a zone was incorporated as a time-dependent covariate.

Model selection:

Model selection consisted of constructing a global model for each analysis, containing all of the covariates and interactions considered biologically relevant (Figure 3.2). Along with treatment (thiamine/control) and the reentry identifier mentioned above (1, 2, or 3+), this global

model included the biotic covariates sex (male/female), length (mm) and origin (native/stray) based on a genetic pedigree analysis (Harbicht et al. submitted manuscript). It also contained the time-dependent abiotic covariates: temperature ($^{\circ}\text{C}$), luminosity (i.e. day/night), discharge ($\text{m}^3 \text{s}^{-1}$), and Δ -discharge (the change in discharge rate over each time interval). Hourly temperature measurements (to the nearest $^{\circ}\text{C}$) were recorded within the study reach using a submerged temperature logger, while discharge was recorded at 15-minute intervals at a USGS monitoring station located ~ 1 km upriver from the study reach. Interactions between treatment and sex were considered along with interactions between temperature and daylight, to account for the seasonal shifts, and between discharge and Δ -discharge.

A complete set of models nested within the global model was then constructed using the *MuMIn* package in R (Bartoń 2016) and ranked according to AICc values (Anderson 2008). Models were required to have a minimum of five events per covariate as suggested by Vittinghoff and McCulloch (2007); only models within 2 AICc units of the best fit model (Δ_{\min}) were considered. Models within 2 AICc units of the best model, but containing uninformative covariates (i.e. additional covariates that fail to improve a model's overall AIC score, Burnham and Anderson 2004; Arnold 2010) were also not considered further. Exceptions to this second rule were allowed when the uninformative covariate was treatment, as quantifying the effect of thiamine injections was one of our primary objectives. Once a set of best models was selected for each habitat, the proportionality of each model's covariates (an assumption of proportional hazard models) was confirmed using the *cox.zph* command in the survival package in R (Therneau 2015).

Results:

Among the 24 tagged salmon, 8 were mature males and 16 were mature females. Males had longer fork lengths (mean = 586.62 mm, SD = 62.72) and weighed more (mean = 2335 g, SD = 769.1) than females (mean = 557.25 mm, SD = 22.96 and mean = 2060 g, SD = 400 respectively). In neither case, however, were these differences significant (Wilcoxon rank sum test, $W = 82.5$ and 78 , p -value = 0.27 and 0.40 respectively). Thiamine levels in white muscle tissue from feral male broodstock indicated a mean concentration among Lake Champlain salmon of 7.92 nmol/g (SD = 2.95). This suggests that at a minimum, ~15% of the salmon in the Lake Champlain basin were experiencing reduced swimming performance as a result of a thiamine deficiency during the experimental period. This may also have been biased towards larger fish as a multivariate linear regression indicated a significant negative relationship between salmon length and thiamine levels in both muscle and egg tissue (Figure S3.1). According to the model, with each 1 mm increase in total length, muscle thiamine concentrations reduced by 0.01 nmol/g, while eggs contained an average of 4.7 nmol/g less thiamine than white muscle tissue ($F_{(2, 56)} = 28.18$, p -value < 0.01, $R^2 = 0.5$).

Of the 24 tagged salmon, nineteen were identified by a genetic pedigree analysis (Harbicht et al. 2017 submitted manuscript) as having originated as juveniles stocked into the Boquet River, 18 as yearlings and one as a fry. The remaining five salmon represented strays that were originally released into other tributaries. Among the 19 salmon with known pedigrees, all but one (the fry release) were returning to the Boquet River as 2+ year olds, after only one summer in the lake. During the tagging period, alternating between thiamine and control injections among returning salmon produced no significant association between the treatment and sex (Fisher Exact test, $p = 0.67$), length (two-sample t-test, $t = -0.11$, d.f. = 18.69, p -value =

0.91) or origin (Fisher Exact test, $p = 0.32$). All tagged salmon released from the trap net downstream of the study reach (20 out of 24 salmon) eventually migrated into the study reach, though how quickly they did so depended on whether they initially descended to the river mouth beforehand. This delayed entry into the study reach ranged from 8 hours to 17 days, but was not significantly associated with treatment (two-sample t-test, $t = 1.29$, d.f. = 11.72, p -value = 0.23).

Low energy pools

Rates of advance through low energy environments (pools) were best explained by additive relationships between prior exposure to the specific pool, whether it was day or night, the sex of salmon, and the water temperature (Table 3.1). In this model, males advanced out of pools at a rate over three times that of females, while all salmon exhibited rates of advance over 4 times higher during the day and after having entered the pool once before. This increase in advancement rates as a result of prior exposure only became significant, however, beyond the second exposure. Additionally, each 1°C increase in temperature increased the rate of advance by 53%. An identical model containing treatment was within $2 \Delta_{\text{min}}$ of the best model (Table 3.1) though in this model, treatment had a non-significant impact on rates of advance through low energy environments (Figure 3.3).

Rates of retreat from low energy environments were best explained by prior exposure, temperature and treatment. Salmon entering either of the two pools for the second time retreated at a rate more than double that of salmon entering for the first time, while those that entered more than twice retreated at a rate over five times that of salmon in their first entry. The overall rate of retreat decreased by 6% with each 1°C increase in temperature, but increased by 37% when salmon were treated with thiamine (Table 3.1, Figure 3.3).

Overall, 100% of experimental salmon entered at least one of the pools one time, 87.5% entered at least twice, and 50% entered a pool at least 6 times. The maximum number of times that a tagged salmon entered either of the pools within the study reach was 43 while the average number of exposures per salmon was 10.9. The average duration of each exposure was 22 hours. Once inside a pool, tagged salmon advanced upstream and out of the pool 1.5 times on average, which produced 36 advances in total. An abundance of salmon entering the lower pool and never attempting to climb the first cascade resulted in a much higher average number of retreats 10.1 and a total of 242 retreats from low energy environments over the observation period (Table 3.1, Figure S3.1).

High-energy environments

The rate of advance through high-energy environments (cascades) was best described by a null model where the common underlying hazard rate, which determines the rate of advance for all salmon, was unaffected by any of the measured covariates. Several models containing a single covariate were within $2 \Delta_{\min}$ of the null model but their support was limited, potentially due to the low number of events observed ($n = 10$). Among these was a model containing treatment which suggested a slightly positive, albeit non-significant, impact of treatment on the rate of advance (Table 3.2).

Rates of retreat from high-energy environments (Table 3.2) were best described by a model containing an indicator of prior exposure and an interaction between treatment and the sex of a salmon. Contrary to rates of advance in low-energy environments, all candidate models for the rates of retreat in high-energy environments (Table 3.2) indicated a negative effect of past exposure to cascades. This resulted in subsequent re-entries into cascades lasting longer overall.

The best fit model indicated a significant interaction between treatment and sex wherein treatment did not have a significant effect among females, nor did sex have an effect among untreated salmon, however treated males had a rate of retreat less than one third that of untreated females (Figure 3.4). This interaction was not present in other competitive models, though, as both indicated a significant effect of sex, but not treatment (rates of retreat decreased by around a half for males). Both of the competitive models indicated that salmon not originally stocked into the Boquet river as juveniles (i.e. strays) had significantly higher rates of retreat from high energy environments (79 and 64% higher). One competitive model indicated a slight, non-significant negative impact of temperature on the rate of retreat (Table 3.2).

Overall, 75% (18) of the tagged salmon experienced 1 or 2 exposures to a high-energy environment (i.e. a cascade), while the remaining 25% did not advance beyond the lower pool. Fewer than 50% of salmon entered a set of cascades more than 5 times, while the maximum number of re-entries was 38. On average, tagged salmon entered cascades 10.6 times throughout the observation period while the mean duration of these exposure periods was 1.7 hours. Tagged salmon advanced through high-energy environments into upstream sections 0.6 times, and retreated downstream from them 11.2 times on average resulting in a total of 10 advances and 202 retreats (Table 3.2, Figure S3.2).

The study reach as a whole

Rejection rates from the entire study reach produced a best fit model and a single competitive model (Table 3.3). Within the best fit model, both discharge and Δ -discharge, along with an indicator of prior exposure, had significant positive effects on the rejection rate while length, treatment, and sex (initially) had non-significant effects on the rejection rate. In both the

best fit model and the competitive model, each increase in the discharge rate of $1 \text{ m}^3\text{s}^{-1}$ resulted in an increase to the rejection rate of 9%, while an increase in Δ -discharge (the change in discharge over a 15 minute interval) of 1 increased the rejection rate by ~ 3.5 -fold. Additionally, salmon that had previously entered the study reach once had rejection rates nearly 4 times higher than those entering for the first time, while subsequent re-entries exhibited rejection rates over 30-fold higher than the initial entry. Both length and treatment had non-significant impacts on the rejection rates, with greater length slightly increasing the rejection rate and thiamine treatment decreasing the rejection rate by 25%. Neither of these covariates were present in the competitive model, but both models indicated that sex had a non-significant effect initially, which became a strong, significant effect after the 24 days within the study reach, such that males became over four times more likely to retreat from the study reach than females.

Overall, 100% of tagged salmon entered the study reach at least once, while 87% entered at least twice. Half of the tagged salmon re-entered at least six times, and the greatest number of observed re-entries was 43. On average, salmon re-entered the study reach 10.9 times and the mean exposure duration was 1.5 days. Once in the study reach, salmon retreated an average of 10.75 times, totaling 258 rejections from this challenging section of river (Table 3.3, Figure S3.3). While three salmon reached the upper pool, only two (one treated and one untreated) successfully advanced into the fish ladder. Due to the low number of successful advances through the entire study reach prevented any meaningful models from being fit to the rates of advance, only the rates of retreat from the study reach were considered and both of these successful advances were treated as censored observations. Both of the salmon that successfully climbed the entire study reach eventually re-entered and were again considered as part of the experimental risk set. All salmon retreated from the study reach multiple times though, with one

exception, a salmon which entered the study reach and was subsequently removed by a predator/angler. This individual's follow-up period therefore terminated with a censored observation. In addition, while three tagged salmon reached the upper pool, only two entered the fish ladder, resulting in censored observations. Both of these salmon subsequently descended the study reach at a later date and re-entered again (Figure S3.3).

Discussion:

The combined use of time-to-event analyses with fine-scale movement data on Atlantic salmon through a challenging section of river (where a dam alters flow, creating a partial barrier), has provided new insights into how biotic, abiotic, and human intervention influences upstream migration. Among the abiotic covariates considered, past exposure to sections of the study reach impacted the greatest number of movement rates. Entering a section of the river for the second or third time significantly increased the rates of advance and retreat from low energy sections and the rate of retreat from the study reach as a whole. However, in high energy sections, past exposure significantly decreasing the rate of retreat. Water temperature had an overall positive effect on upstream movement while discharge and changes in the rate of discharge encouraged downstream movement. Among biotic variables, sex and origin were both associated with increased rates of movement, though not consistently in one direction. Thiamine supplementation significantly increased the rate of retreat from low energy environments, but decreased the rate of retreat from high-energy environments. Furthermore, when non-significant effects within the best or competitive models were considered, thiamine supplementation had an overall positive effect on upstream movement.

Past Exposure:

The overall effect of past exposure to sections of the study reach was inconsistent in terms of the overall impact in a particular direction. In low energy environments, past exposure positively affected the rate of advance and retreat, resulting in a net reduction in the amount of time spent in pools for each subsequent re-entry. As salmon initially entered the study reach near the beginning of the spawning migration, subsequent re-entries were, by necessity, later in the migratory period. This reduced willingness to hold in low energy environments could, therefore, represent a greater sense of urgency felt by salmon as their window of opportunity to locate suitable spawning habitat diminished. In support of this idea, the rate of retreat from the study reach as a whole increased over subsequent re-entries, such that salmon re-entering the study reach were less willing to remain in the study reach, attempting to climb the challenging cascades, than those entering for the first time. Such retreating salmon may have been seeking alternative spawning areas, represent an example of forced dispersal within their 'natal' tributary. Conversely, in high-energy sections (cascades), repeated entries had the effect of reducing the rate of retreat, such that sequential attempts to climb through cascades increased in duration. This result would be expected if salmon were expressing an increasing desire to climb the cascades over time and/or were learning from past unsuccessful attempts.

Temperature:

For migrating salmon within the study reach, temperature played a role in determining movement patterns in both pools and cascades. In pools, the rate of advance increased with increasing temperature while the rate of retreat decreased, suggesting that over the range of temperatures observed during our observation period, recovery/resting time in pools was reduced

by warmer temperatures. This result suggests that the tendency for upstream movement among migrating adult salmon is positively associated with temperature within the range of temperatures we observed (0 - 21°C). Such relationships between activity levels, recovery time, or speed, with temperature were not unexpected (see Videler 1993). Indeed, most ectothermic species, including Atlantic salmon (Booth et al. 1997), sockeye salmon (Brett and Glass 1973), chinook salmon and steelhead (Salinger and Anderson 2006), experience increased metabolism with increasing temperatures, up to a certain critical temperature above which the inverse relationship holds. Despite this, very few studies have quantified this increased activity among fish in the wild under natural or semi-natural conditions (but see Castro-Santos, 2004; Goerig & Castro-Santos, 2017; Nyqvist et al., 2017). The positive influence of warmer temperatures on upstream movement from holding pools observed during our study, combined with the overall trend for temperature to decrease throughout the observation period, might explain why salmon reentering the study reach after their first exposure exhibited higher abandonment rates than their earlier attempts.

Discharge:

Greater and increasing discharge rates did not influence movement patterns within high- or low-energy sections of the study reach individually, but did have an overall positive effect on rejection rates from the study reach as a whole. Higher flows are known to be associated with a greater incidence of Atlantic salmon beginning their upstream migrations (Smith et al. 1994, Thorstad and Heggberget 1998), but how flow affects instream migration behavior is less well understood (Thorstad et al. 2008). The increased rejection rate from the study reach as a whole at higher and increasing discharge rates contradicts past work on multiple fish species, including

salmonids, wherein discharge was found to positively influence attempt rate and upstream movement (Weaver 1963, Castro-Santos 2004, Goerig and Castro-Santos 2017). It is, however, consistent with past work on landlocked Atlantic salmon by Trepanier et al. (1996) who found that landlocked salmon were less likely to move upstream from Lac Saint-Jean during high flow periods (freshets) and preferred, rather, to move upstream during periods of descending flows. While we did not observe an increase in the rate of upstream movement during descending flows as Trepanier found, we did see an increased tendency to abandon a migratory attempt (i.e. to move downstream from the study reach) when discharge was rising. As the water level within the lower Boquet River is influenced by the lake level only a short distance downriver from the study reach, tagged salmon may have been retreating back to lake influenced section of the river to wait for more favorable, presumably decreasing, flows before attempting another climb.

Luminosity:

Luminosity (day/night) was associated with upstream movement from low energy sections (pools) into high energy sections (cascades) within the study reach. The rate of advance from pools was greater during the day than at night. This increased preference for tagged salmon to move upstream during daylight hours contradicts the observed tendency of Atlantic salmon to move more at night during spawning migrations (reviewed in Thorstad et al. 2008) particularly when water turbidity is low (Hellawell et al. 1974), as it commonly is in the Boquet River. It is, however, consistent with other past work that Pacific and Atlantic salmon prefer to move through obstructions or technically challenging sections of river during the day (Neave 1943, Banks 1969, Gowans et al. 1999, Nyqvist et al. 2017), perhaps relying more heavily on eyesight in such technical sections. Similar patterns of elevated movement through challenging sections

of river have been observed in other species, such as American shad (Castro-Santos and Letcher 2010). Another possibility is that salmon were more likely to advance from pools during dawn or dusk periods, which were classified as daylight in the present study. This would also be consistent with past observations of upstream migratory patterns for salmonids (Banks 1969, Goerig and Castro-Santos 2017).

Sex:

Rates of movement differed between the sexes both in high- and low-energy sections of the study reach. In pools, males exhibited upstream attempt rates over three times higher than those of females, perhaps suggesting a greater drive to reach upstream spawning sites during our observation period. Males also remained in high-energy environments longer than females before retreating, indicating potentially greater motivation levels or a greater ability to hold their position in fast flowing water. Alternatively, females may have been more susceptible to the stresses associated with our tagging protocol, similar to female Pacific salmon that are more susceptible to environmental stresses during spawning migrations than males (Burnett et al. 2014). Despite the evidence to suggest that males were more motivated to migrate upstream, once in the study reach for 24 days, males showed a 4-fold greater tendency than females to abandon migratory attempts within the study reach. While sex is seldom listed among the variables associated with migratory behavior for Atlantic salmon (Thorstad et al. 2008), the increased tendency for upstream movement observed among males is consistent with the observation that females express slower, or less direct migratory patterns than males (Karppinen et al. 2004, Nyqvist et al. 2017). The increased tendency for males to leave after unsuccessfully attempting to climb the study reach may be the result of sex biased movement behavior, a

phenomenon previously observed among other salmonids (Hutchings and Gerber 2002, Fraser et al. 2004, Neville et al. 2006).

Thiamine Injections and Biotic Variables:

When all modelled effects of thiamine injections were considered, thiamine supplementation had an overall positive effect on upstream movement rates among tagged salmon. In low-energy environments, supplementation significantly increased the rates of retreat, while a competitive model for upstream movement indicated a positive, but non-significant effect on rates of advance. This resulted in an overall reduction in time spent by treated fish resting in holding pools. In high-energy environments, treatment significantly decreased the rate of retreat, but only for males. As thiamine is important for the production of ATP, allowing pyruvate to enter the citric acid cycle (Morito et al. 1986, Koski et al. 2005), these results (though not all were significant) are consistent with salmon in the Boquet River exhibiting the effects of low thiamine levels. This view is supported by the low thiamine levels observed among salmon sampled from within Lake Champlain, with mean white muscle concentrations (7.92 nmol/g) very near the concentration at which swimming performance is reduced (~5 nmol/g, Houde et al. 2015). Indeed, 17% of the salmon sampled within Lake Champlain had muscle thiamine levels below 5 nmol/g and would therefore likely have exhibited reduced swimming performance as a result of a thiamine deficiency during the study period.

The differential effectiveness of thiamine injections among the sexes in terms of rates of retreat from high-energy environments may result from size differences among male and female salmon and its relation to alewife consumption in Lake Champlain. Male salmon were both longer and heavier than females (albeit not significantly so). Among the salmon sampled from

Lake Champlain, there was a significant negative relationship between total thiamine concentrations and size. Male salmon, being generally larger and heavier than females, may therefore consume a larger proportion of alewife (thiaminase) in the lake, producing a more substantial loss of thiamine. This relationship with size may therefore indicate that larger salmonids may require (1) a stronger concentration of thiamine or (2) more time for thiamine injections to become effective. Curiously, however, no significant direct impacts of size on movement rates were detected in the present study, possibly due to the small sample size.

Conclusions:

The above study demonstrates that even in a system where dietary thiaminase from invasive alewife is likely impeding spawning migrations among salmonids, the strongest impacts may remain biotic and abiotic in nature. Among these, past exposure to the environment had the strongest impact, followed by water temperature, both emphasizing the importance of synchronizing migration attempts with seasonal windows of opportunity. Despite the secondary role that thiamine played in explaining movement rates, thiamine supplementation did impact downstream movement rates, increasing them in low energy, and decreasing them in high-energy environments (particularly among salmon at the greatest risk of a thiamine deficiency: large males). The presence of multiple competitive models containing thiamine as an explanatory variable suggests that thiamine supplementation may impact migratory success among thiamine deficient salmonid populations, but that the human-altered hydraulic flow through our study's cascades represent an insurmountable barrier to most returning salmon regardless of thiamine status which limited sample sizes. Indeed, many more salmon were observed below the study reach than were counted at the fish ladder, while a considerable number of salmon redds (60+)

were observed being constructed throughout the latter half of our observation period in a gravel bar below the study reach despite a complete lack of suitable juvenile habitat nearby. Together, these two observations imply a desire by Atlantic salmon to spawn in the Boquet River, but an inability of most salmon to overcome this challenging stretch of river. This places further emphasis on the importance of understanding the underlying mechanisms affecting fish passage and how these impact the population dynamics and evolution of migratory species. Promisingly, the novel methods employed in this study (Harbicht et al. 2017) did provide unique insights into such mechanisms. Repeating such experiments on less challenging sections of river with higher overall passage rates would add further to this promising area of research. This does not preclude the Boquet River, however, as the crib dam situated above the study reach has been removed since the completion of this study, providing a potentially useful “before” dataset against which comparisons could be drawn. In the meantime, further studies employing similar methods in new environments that present different challenges will undoubtedly produce new information about when, where, and how biotic and abiotic influences at migratory barriers impact migratory species.

Table 3.1: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in pools within the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

K	Log Likelihood	AICc	Δ_{\min}	w	Covariate	Hazard Ratio	LCL	UCL	P-Value	
Advance - 36 events										
					Exposure + Luminosity + Sex + Temperature	Exposure(2)†	4.06	0.88	18.69	0.07
5	-75.60	161.20	0.00	0.73		Exposure(3+)†	4.98	1.20	20.59	0.03
						Luminosity(Light)*	4.08	1.86	8.96	0.00
						Sex(Male)	3.31	1.45	7.55	0.01
						Temperature	1.53	1.31	1.78	0.00

Table 3.1 continued

K	Log				<i>w</i>	Covariate	Hazard			
	Likelihood	AICc	Δ_{\min}				Ratio	LCL	UCL	P-Value
	Exposure + Luminosity + Sex + Temperature + Treatment					Exposure(2)†	4.02	0.83	19.47	0.08
6	-75.59	163.19	1.99	0.27		Exposure(3+)†	4.93	1.17	20.78	0.03
						Luminosity(Light)*	4.08	1.85	8.97	0.00
						Sex(Male)	3.30	1.46	7.48	0.00
						Temperature	1.52	1.31	1.78	0.00
						Treatment(Thiamine)‡	1.05	0.48	2.33	0.90
Null										
0	-48.95	97.90	12.69	0.00						

Table 3.1 continued

Log					Hazard				
K	Likelihood	AICc	Δ_{\min}	w	Covariate	Ratio	LCL	UCL	P-Value
Retreat - 242 events									
Exposure + Temperature + Treatment					Exposure(2) [†]	2.69	1.18	6.14	0.02
4	-1051.75	2111.50	0.00	1.00	Exposure(3+) [†]	5.42	2.64	11.11	0.00
					Temperature	0.94	0.91	0.97	0.00
					Treatment(Thiamine) [‡]	1.37	1.08	1.72	0.01
Null									
0	-1092.72	2185.44	73.94	0.00					

[†] Exposure corresponds to the particular entry (re-entry) into a passage zone (first, second, or more than second)

* Luminosity corresponds to diurnal cycles (day/night).

[‡] Treatment describes whether salmon were supplemented with thiamine or not.

Table 3.2: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in cascades within the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

K	Log				Covariate	Hazard			
	Likelihood	AICc	Δ_{\min}	w		Ratio	LCL	UCL	P-Value
Advance - 10 events									
Null									
0	-22.06	44.11	0.00	0.43					
Luminosity									
1	-21.23	44.46	0.35	0.36	Luminosity(Light)*	3.00	0.86	10.49	0.09
Treatment									
1	-21.80	45.60	1.49	0.21	Treatment(Thiamine)‡	1.80	0.52	6.22	0.36

Table 3.2 continued

Log					Hazard				
K	Likelihood	AICc	Δ_{\min}	<i>w</i>	Covariate	Ratio	LCL	UCL	P-Value
Retreat - 202 events									
Exposure + Sex * Treatment					Exposure(2)†	0.39	0.24	0.62	0.00
5	-707.33	1424.76	0.00	0.44	Exposure(3+)†	0.41	0.27	0.64	0.00
					Sex(Male)	1.19	0.87	1.62	0.29
					Treatment(Thiamine)‡	1.25	0.81	1.95	0.31
					Sex:Treatment	0.30	0.17	0.55	0.00
Exposure + Origin + Sex + Temperature					Exposure(2)†	0.39	0.24	0.63	0.00
5	-707.48	1425.07	0.30	0.38	Exposure(3+)†	0.36	0.24	0.55	0.00
					Origin(Stray)§	1.79	1.23	2.62	0.00
					Sex(Male)	0.48	0.37	0.63	0.00
					Temperature	0.93	0.82	1.04	0.21

Table 3.2 continued

K	Log Likelihood	AICc	Δ_{\min}	<i>w</i>	Covariate	Hazard Ratio	LCL	UCL	P-Value
					Exposure + Origin + Sex				
					Exposure(2)†	0.38	0.24	0.60	0.00
4	-709.21	1426.48	1.72	0.19	Exposure(3+)†	0.35	0.23	0.54	0.00
					Origin(Stray)§	1.64	1.14	2.37	0.01
					Sex(Male)	0.50	0.38	0.67	0.00
<hr/>									
Null									
0	-726.26	1452.53	27.76	0.00					

† Exposure corresponds to the particular entry (re-entry) into a passage zone (first, second, or more than second).

* Luminosity corresponds to diurnal cycles (day/night).

‡ Treatment describes whether salmon were supplemented with thiamine or not.

§ Whether a salmon was originally stocked as a fry or parr into the Boquet River, or an alternative stocking effort within the Lake Champlain Basin as determined by a genetic parentage analysis.

Table 3.3: Results of AICc-based model selection for Time-to-Event analyses on rates of advance and retreat of salmon in the whole of the Willsboro Rapids, Essex County, NY. The columns: Model, K, LL, AICc, Δ_{\min} , and w correspond to the fitted model, number of covariates, log likelihood, corrected Akaike value, difference between each model's AICc and that of the best model, and the model weight respectively. The hazard ratios, upper and lower 95% confidence intervals (UCL and LCL respectively) and p-values correspond to the covariates included within each fitted Cox proportional hazards model. A hazard ratio of 1 represents no effect on movement rates.

Table 3.3

K	Log Likelihood	AICc	Δ_{\min}	w	Covariate	Hazard			
						Ratio	LCL	UCL	P-Value
Downriver - 258 events									
Discharge + Delta-Discharge + Exposure +									
					Discharge	1.09	1.03	1.16	0.00
Length + Sex + Treatment									
8	-308.32	632.65	0.00	0.58	Delta-Discharge	3.47	1.95	6.19	0.00
					Exposure(2)†	3.92	1.45	10.57	0.01
					Exposure(3+)†	31.21	13.65	71.33	0.00
					Length	1.00	1.00	1.01	0.14
					Treatment(Thiamine)‡	0.75	0.49	1.15	0.19
					Sex(Male) < 24 days§	0.93	0.45	1.93	0.84
					Sex(Male) > 24 days§	4.71	1.63	13.63	0.00

† Exposure corresponds to the particular entry (re-entry) into a passage zone (first, second, or more than second).

‡ Treatment describes whether salmon were supplemented with thiamine (Thiamine) or not.

§ The impact of Sex (male/female) was found to differ over time and was therefore estimated for two time periods: the first 24 days within the Willsboro rapids, or later than 24 days

Table 3.3 continued

K	Log Likelihood	AICc	Δ_{\min}	w	Covariate	Hazard Ratio	LCL	UCL	P-Value
S ~ Discharge + Delta-Discharge + Exposure + Sex					Discharge	1.09	1.03	1.15	0.00
6	-310.66	633.33	0.68	0.42	Delta-Discharge	3.30	1.88	5.80	0.00
					Exposure(2)†	3.98	1.51	10.46	0.01
					Exposure(3+)†	31.00	13.64	70.46	0.00
					Sex(Male) < 24 days§	1.01	0.49	2.10	0.98
					Sex(Male) > 24 days§	4.67	1.59	13.73	0.01
Null									
0	-480.72	961.43	328.78	0.00					

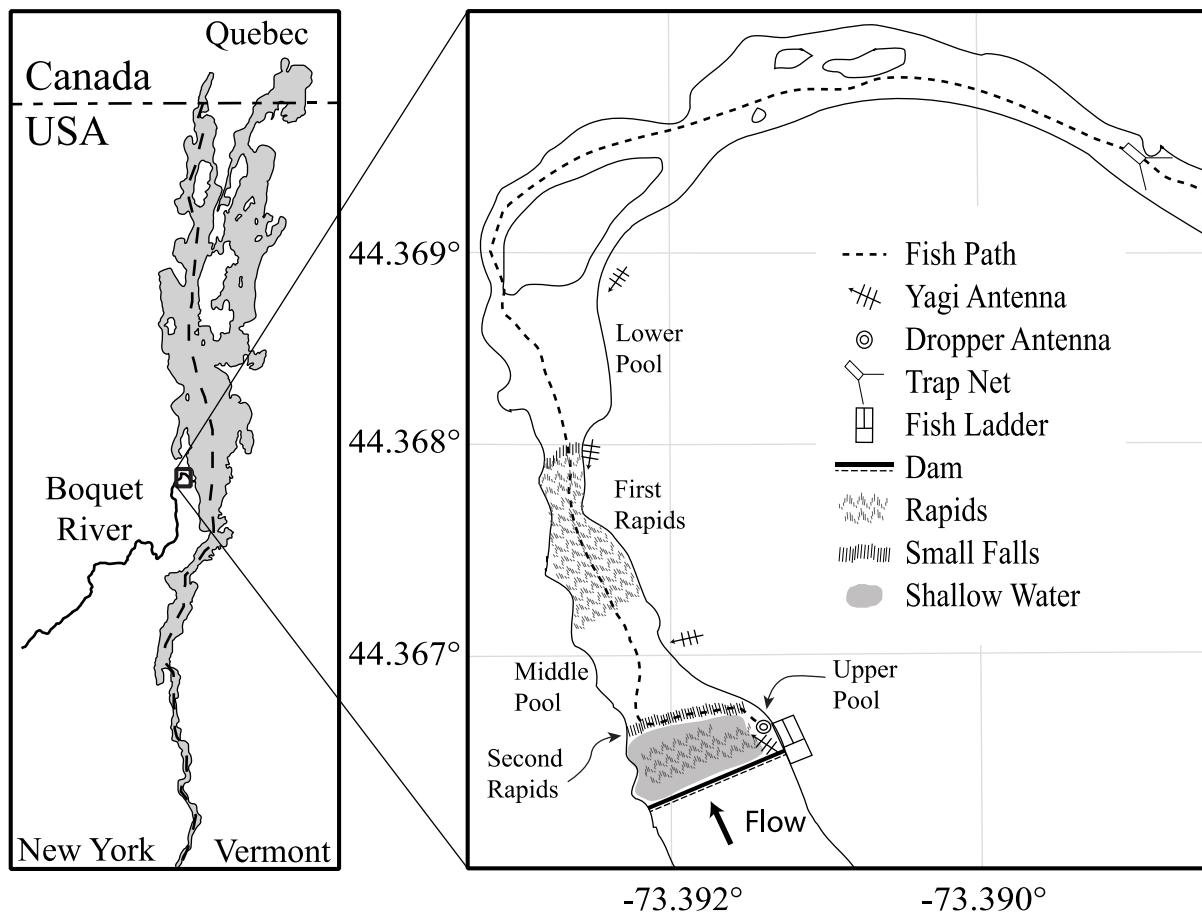


Figure 3.1: Map of the Willsboro rapids used to assess the impact of various biotic and abiotic factors on upstream migrations through challenging sections of river, along with the effect of thiamine supplementation among the thiamine deficient landlocked Atlantic salmon in the Lake Champlain basin. Antennas remained fixed and recorded transmission signal strength from tagged salmon from September 26th to November 19th, 2014.

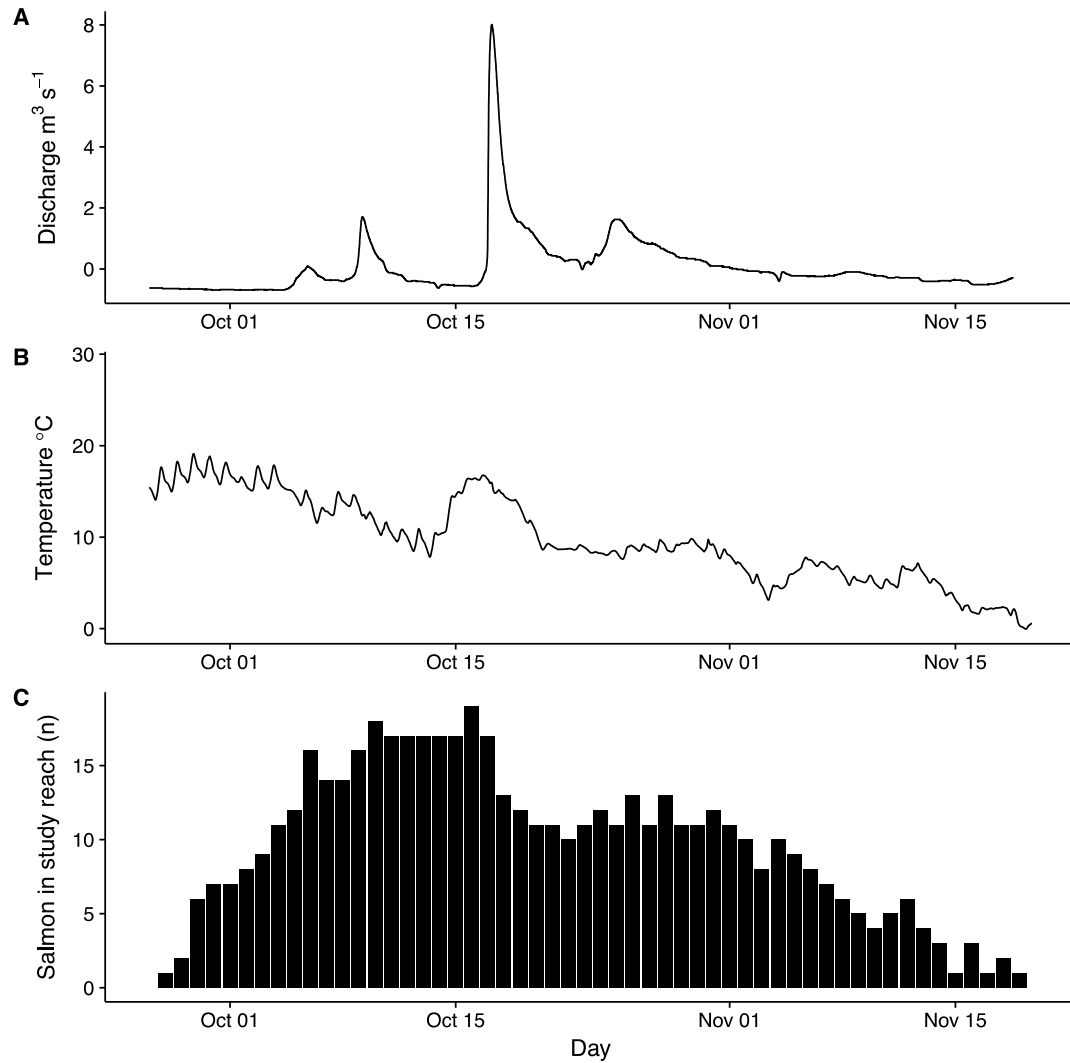


Figure 3.2: Two of the ancillary time varying covariates; discharge (a) and temperature (b) included in Cox proportional hazards models to explain migratory behaviour of mature Atlantic salmon migrating through a difficult section of the Boquet River, along with the number of salmon (c) present in the Willsboro rapids during the observation period of September 26th to November 19th, 2014.

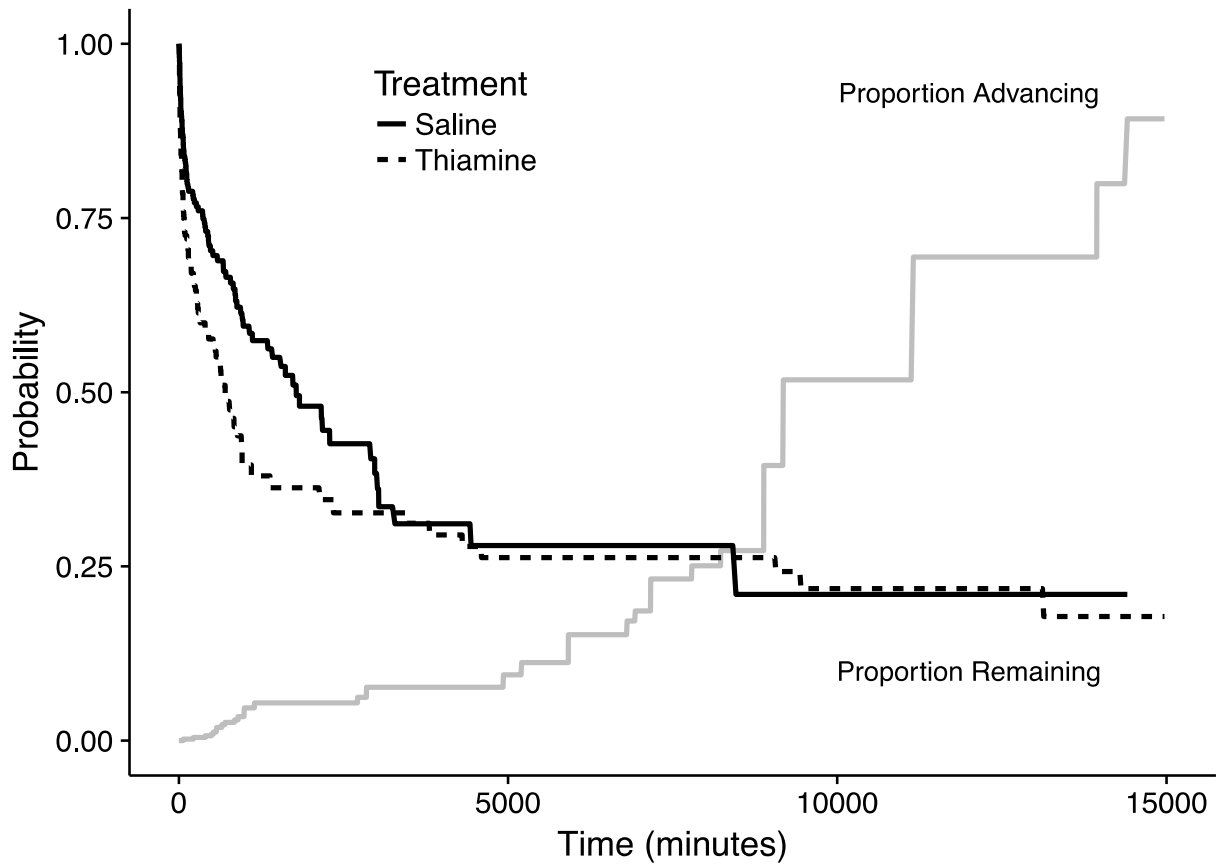


Figure 3.3: The predicted survival and cumulative incidence curves by treatment from Cox Proportional Hazards models of salmon, respectively, advancing through or retreating from pools within the Willsboro Rapids, Essex County, NY. While the analysis was stratified over three pools, only the lower pool's curves are displayed. Grey lines represent all tagged salmon regardless of thiamine treatment status.

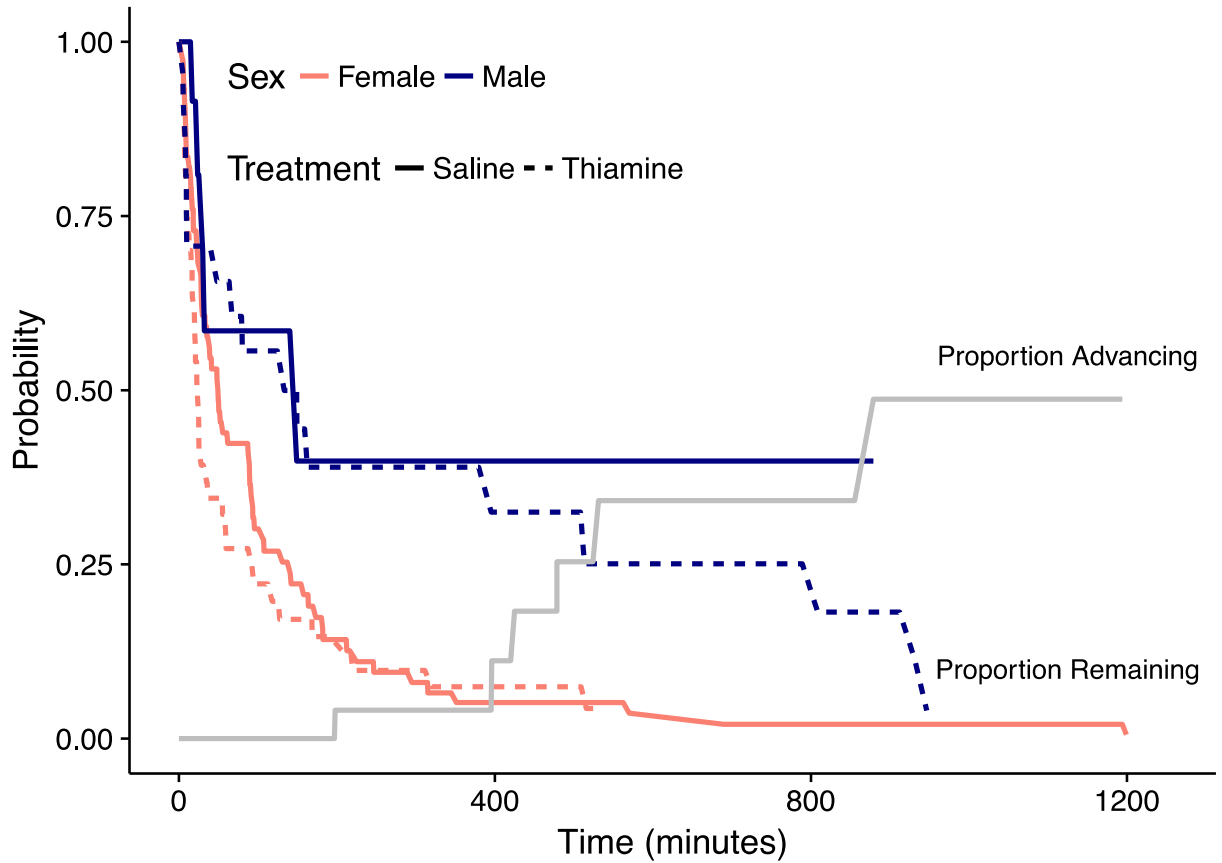


Figure 3.4: The predicted survival and cumulative incidence curves from Cox Proportional Hazards models of salmon, respectively, advancing through or retreating from sets of rapids located in the Willsboro Rapids, Essex County, NY. While the analysis was stratified over two sets of rapids, only the first rapids' curves are displayed. Grey lines represent all tagged salmon regardless of thiamine treatment status or sex.

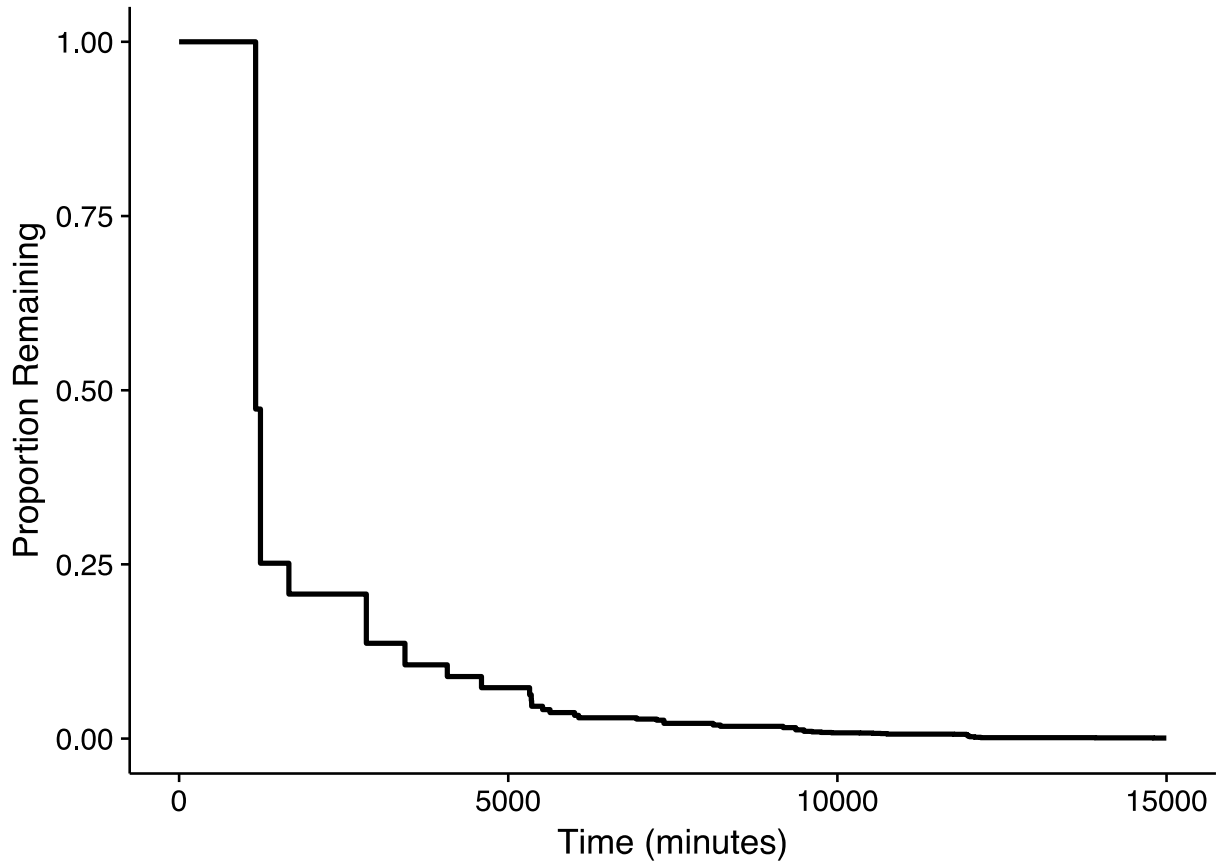


Figure 3.5: The predicted cumulative incidence curve from a Cox Proportional Hazard model of salmon retreating from the Willsboro Rapids, Essex County, NY

General Discussion

Though reintroductions face many challenges in the modern world, their use as a means of overcoming the continued loss of plant and animal populations in nature is projected to increase (Sarrazin and Barbault 1996, Novacek and Cleland 2001, Frankham 2008, Ewen et al. 2012, Seddon et al. 2014). With most reintroduced individuals beginning life in the wild at a disadvantage as a result of exposure to captive environments (Frankham and Loebel 1992, Ford 2002, Frankham 2008, Fraser 2008, Araki et al. 2009), strict adherence to guidelines intended to minimize such effects is necessary. So too is the need for continuous, long-term monitoring and adaptive responses to unexpected results or changing environmental and ecological conditions (Fischer and Lindenmayer 2000, Ewen and Armstrong 2007, Armstrong and Seddon 2008, Rout et al. 2009, Godefroid et al. 2011). In the face of present and future climate change and the continued human-mediated movement of species into new habitats, reintroduction programs may need to expand their current scopes and continue developing new techniques and testing new theories (Seddon 2010, Williams and Brown 2016) such as experimental comparisons of release methods (Jonsson et al. 1999, Moseby et al. 2014). New and unforeseen interactions with invasive predators, parasites, and prey means the number and extent of challenges faced by reintroduction biologists will likely increase. For reintroductions to succeed over the long term, biologists must continuously monitor and assess the results of their actions while attempting new approaches when risks are minimal (Rout et al. 2009). By reassessing each step of a reintroduction program using controlled experiments, such as in this PhD thesis on Atlantic salmon, new techniques and relationships can be identified that improve both present and future reintroduction efforts (Armstrong and Seddon 2008, Ewen et al. 2012, IUCN/SSC Reintroduction Specialist Group 2013, Westgate et al. 2013, Canessa et al. 2016).

Captive rearing and releasing methods

For salmonid reintroductions to successfully reestablish populations and metapopulation dynamics, a balance must be struck between maximizing adult production (juvenile-to-adult survival), and promoting natural dispersal rates. Focusing solely on survival can lead to excessively high dispersal rates that reduce overall reproductive output via the Allee effect (Armstrong and Wittmer 2011) and prevent the formation of locally adapted populations. Within the Lake Champlain basin for example, tributaries with and without native brook trout populations are routinely stocked with captive-bred Atlantic salmon fry. In tributaries where inter-specific interactions exist, juvenile fry must avoid predation and/or compete with brook trout for food and overwintering habitat. Consequently, populations may adapt as juvenile that adjust their feeding schedules and growth rates in response to the selective pressures (Kennedy and Strange 1986) exhibit higher survival. When dispersal rates from other tributaries are high, however, gene flow from systems without brook trout can prevent the development of such adaptations.

In an attempt to find such a balance, my colleagues and I released four competing genetically tagged parr release groups along with fry releases into two Lake Champlain tributaries. The resulting adults frequencies were then compared for the lake and in tributaries during spawning migrations. Using a comprehensive DNA pedigree analysis, recaptured salmon were reassigned to their rearing/release method and locations, permitting comparisons of survival, migration, and straying frequencies.

Overall, chapter 1 results indicate that once juveniles reached a critical size threshold that allowed them to transform into smolt in their first year, rearing temperatures (and therefore

individual growth rates), can be adjusted with no negative effect on parr-to-adult survival. In fact, rearing parr at cooler, seasonal temperatures prior to release significantly improved recapture rates in the lake and tributaries despite decreasing size-at-release. The same could not be said for advancing release dates, however, as under standard parr rearing conditions, earlier release dates resulted in fewer salmon recaptured in the lake and tributaries. In fact, when standard temperatures were used, recapture rates of earlier releases were significantly lower than those of juvenile release as fry, once the additional year of natural mortality in the wild had been accounted for. In terms of their effects on straying rates, at the basin level, all three alternative parr methods reduced straying relative to standard production methods. These results suggest that straying rates for captive-reared salmon can be reduced without sacrificing parr-to-adult survival. As the juveniles reared on seasonal brook water experienced reduced growth rates in the hatchery, these results also contradict the well-supported positive relationship between size-at-release and survival to maturity (Ward and Slaney 1988, Henderson and Cass 1991, Koenings et al. 1993).

Generally, the straying rate among hatchery release groups in the Lake Champlain basin tended to be on the low end of the spectrum for wild populations (Jonsson et al. 2003a, Pess 2009) and were variable among years. This variation is exemplified well by the 2011 cohort of fry-stocked salmon in the Boquet River that, despite experiencing natural conditions and growth rates from a very early age, exhibited the highest straying rates of all release groups (33%, 4 of 12). Such occasionally high straying is not uncommon among Atlantic salmon (reviewed in Keefer and Caudill 2014), however, and the underlying trend among fry and parr release groups was for parr to stray more often. Despite this elevated straying among parr releases, many salmonid supplementation programs follow juvenile rearing procedures that employ elevated

temperatures in order to speed up growth in captivity. Such programs can therefore benefit from the minor changes to rearing conditions outlined in this thesis, that promote more natural dispersal rates. In doing so, fisheries managers can reduce dispersal by captive-bred salmon to levels more akin to natural anadromous populations (Jonsson et al. 2003a, Pess 2009, Keefer and Caudill 2014). In doing so, they reduce the losses to supplemented or reintroduced populations that strays represent due to their generally reduced reproductive success (Peterson et al. 2014). When nearby remnant populations of wild conspecifics exist, reducing dispersal also reduces the transfer of genetic material to at-risk populations, preserving local adaptations and minimizing outbreeding depression. At the same time, reducing dispersal promotes the gradual development of local adaptation among the reintroduced population (Waples 1991, Quinn 1993, Hendry et al. 2007). For these reasons, it is advisable for captive breeding programs employing elevated rearing temperatures to conduct experiment with reduced rearing temperatures when the risk is minimal to do so as the potential gains include improved survival and reduced dispersal.

Although the recapture rates from fry release groups were significantly lower than those of the parr groups exposed to seasonal temperatures, our measure of fitness (survival to maturity, spawning migration, and straying rates) omitted a potentially significant difference: relative reproductive success. Salmonids have been shown numerous times to exhibit reduced reproductive success as a result of exposure to the captive environment (Fleming et al. 1996, Araki et al. 2007a, Frankham 2008, Fraser 2008). These reductions in reproductive success stem from carry-over effects of the captive environment that result in males displaying reduced competitive abilities on spawning grounds (Fleming et al. 1996, Jonsson and Jonsson 2014), females producing smaller and less viable eggs (Clarke et al. 2016) and offspring showing lower survival (Evans et al. 2014). Therefore, salmon released as fry may ultimately be the best choice

if they compensate for lower returns by producing a greater number of more fit offspring. This trade-off between maximizing the number of mature adults returning to a stocked tributary and increasing the quality of the adults that return must be carefully considered by reintroduction biologists in each individual case.

While chapter 1 outlined methods for increasing the number of mature adults returning to stocked tributaries without sacrificing juvenile-to-adult survival, the quality of the returning adults was not assessed, nor were the underlying mechanisms responsible for the improved survival identified. Future studies into this area may address this by assessing not only survival to the adult stage, but also relative reproductive output of alternative rearing/release conditions by sampling out-migrating smolts in the event that natural reproduction occurs. Using a genetic pedigree analysis to identify the grandparents of surviving smolt can identify the optimal release method. Additionally, the precise timing of olfactory imprinting could be narrowed in on by employing a range of release dates and comparing the resulting dispersal rates. By identifying the precise point at which salmon juveniles imprint under controlled hatchery conditions, fisheries managers can minimize the cost to survival that early releases produce. Similarly, experimentally varying the date at which rearing temperatures are reduced to seasonal levels may identify an optimal date which maximizes exposure to warmer rearing temperatures (thereby maximizing the proportion of juveniles that will smoltify in their first year) while minimizing dispersal rates.

Finally, while the reduced straying rates observed for all three alternative parr release methods suggest that our hypothesis regarding standard captive rearing practices interrupting olfactory imprinting were likely correct, the overall recapture rates remained fairly low.

Determining the ultimate cause for this survival difference: cold stock; unmeasured influences of

cold temperature preconditioning; or the adoption of seasonal metabolic rates prior to release, could assist in further improving survival and spawning returns and represent a promising avenue for future research. The future experiments outlined above may go a long way to addressing this question. In the meantime, in the absence of precise knowledge of when various populations imprint, reintroduction programs should invoke a portfolio effect by employing both of the strategies outlined above. Such an action will increase the likelihood of successful imprinting post-release without jeopardizing survival.

The improvements to survival and reductions in straying demonstrated above are a step in the right direction but much work is still needed if landlocked Atlantic salmon are ever to exist in Lake Champlain at their historic densities. Full restoration of this species will require more than simple adjustments to hatchery rearing conditions as limited natural reproduction, limited juvenile survival, interactions with invasive species (sea lamprey, alewife), and continued angling pressures are among the various forces currently hindering reintroduction efforts.

Location Fingerprinting

Efforts to reintroduce Atlantic salmon into the Lake Champlain basin, like many reintroduction programs, have encountered new and unexpected questions that required novel approaches and techniques to answer (e.g. improving sampling methods for cryptic species, Bell (2009); or new training techniques for reintroductions of predator-naïve species, McLean et al. (1996)). In order to assess the impact of a thiamine deficiency on the migratory ability of salmon in challenging river sections, it was necessary to monitor the fine-scale movement patterns of thiamine supplemented and non-supplemented salmon as they climbed an extensive series of rapids. Doing so using conventional methods was not possible due to the conditions present in

the study system or the equipment needed to achieve the level of precision required. I therefore adapted methods in use by alternative areas of research to develop a new technique that permits the continuous monitoring of movement patterns for multiple tagged individuals simultaneously over long periods of time (Harbicht et al. 2017).

The location fingerprinting (LF) techniques developed in chapter 2, employing random forest models, are a practical and accurate means of observing and recording the movement patterns of individuals over long periods, while requiring a relatively limited amount of equipment and time (Harbicht et al. 2017). This technique also represents a next step for continuous radio telemetry based monitoring, following in the footsteps of David and Closs (2001) and Tucker et al. (2014). The adaptability of LF methods means that researchers can use this method with similar materials to address a variety of questions in a wide range of situations accurately.

Use of a random forest model as an estimation method for the localization technique was justified by the high precision observed when locations were estimated for withheld data. Furthermore, the successful use of modelled, rather than directly measured, system characteristics between landmark locations demonstrates the high degree of accuracy that can be achieved from minimal front-end system characterization efforts, saving time and man-power.

An unexpected advantage of the LF techniques developed was its ability to provide new information on the radio landscape following the creation of the radio map. Differences between the extrapolated and estimated radio maps provide insights into landscape features that affect signal propagation but that may not have been apparent during the initial characterization of the study area. Care should be taken when interpreting this new information however and further

cross-validation would be required before such information could be incorporated into a new radio map of the study area.

The case examples provided in chapter 2 demonstrate the level of detail that can be achieved using LF methods, which extends well beyond the simple presence/absence data typically collected with VHF tags. Combined with the flexibility of modern VHF radio tags, LF techniques are well suited to the study of movement patterns for a wide variety of species beyond the situation for which it was developed. Examples range from similar studies of fish passage at a variety of barriers, to path selection by terrestrial mammals at manmade highway crossings. A limitation LF techniques, however, is its dependence on overlapping detection areas by multiple antennas. Larger study environments will therefore require a greater number of antennas, while receivers may need to be paired with a single omni-directional, or multiple directional antennas in order to avoid 'blind spots'.

In summary, the fingerprint localization methods employed in this study are a novel approach to continuous automated wildlife telemetry. Their precision and flexibility make them ideally suited to the variety of situations, both on land or in aquatic environments, and they represent an improvement in our ability to track fine scale movements in aquatic species in environments where dense arrays of PIT antennas or acoustic telemetry arrays are not feasible.

Thiamine impacts on Atlantic salmon migration

In situations where the historic range of a locally extinct species has experienced irreversible changes as a result of invasive species (Short et al. 1992, Swinnerton et al. 2005, Moseby et al. 2011), reintroduction programs are faced with additional challenges. The presence of an invasive species may preclude the achievement of certain original goals and require others

be altered in some way, e.g. the presence of an invasive predator may result in biologists choosing to relocate threatened individuals into new habitat, rather than its historic ranges, i.e. assisted colonization (Hunter 2007, Seddon 2010). The establishment of alewife in Lake Champlain and the Great Lakes, or the overabundance of sprat (*Sprattus sprattus*) in the Baltic sea are problems that are likely to persist into the future due to the difficulties associated with removing the former and the loss of the ecological role of predators on the latter (Atlantic Cod, *Gadus morhua*). This means that thiamine deficiencies among local salmonid populations are likely to remain a problem into the future. Under such circumstances, restoration and reintroduction programs must find feasible, long term solutions to work around such problems, but until such solutions are found, short term mitigation efforts should be employed. In chapter 3, I investigated how a thiamine deficiency can transform a high-energy section of river into a migratory barrier for spawning salmon, impacting their ability to reach spawning grounds and naturally reproduce. In doing so, the influence of several other influential biotic and abiotic variables on passage rates and the suitability of thiamine supplementation as a short-term mitigation option were also assessed.

The results of chapter 3 indicate that the low thiamine levels among Lake Champlain salmon are likely causing migration delays, but that the strongest effects were those of abiotic environmental conditions. Additionally, human intervention was found to improve migratory returns by reducing delays that thiamine deficient salmon experience at low energy environments. As migrating salmon are strongly influenced by a number of environmental cues during their spawning migration, such as seasonal temperatures, discharge rates, and day length (review in Thorstad et al. 2008), migratory delays can result in salmon missing their ‘window of

opportunity'. As a result, salmon are forced to either abandon their spawning attempt or spawn in sub-optimal habitat.

Time-to-event analyses identified temperature as being one of the abiotic factors most strongly associated with movement within difficult sections of river. At warmer temperatures, salmon advanced from pools more quickly and while abandoning an attempt more slowly. This suggests that minor delays stemming from a thiamine deficiency could increase considerably if they occur early in a migration process and increase the chances of salmon migrating as season temperatures begin to fall. Similarly, daylight was positively associated with advancement rates through technically challenging sections of the study reach. In situations where low thiamine levels cause sufficient migratory delays, seasonal shifts in the photoperiod could render such stretches more difficult as the time available to salmon for passage decreases.

It is this association between salmon migration and the progression of seasonal environmental cues that make migratory delays such a risk (Caudill et al. 2007). The supplementation methods employed in chapter 3 showed potential as a means of reducing the impact of low thiamine levels on migration delay, though its effectiveness was not consistent across all salmon. If such human intervention is to provide restoration programs with a better chance of establishing self-sustaining populations of Atlantic salmon, additional research into how such treatments can be most effective will be required.

Finally, despite the positive effect of thiamine supplementation on movement rates in challenging sections of river, the effect of thiamine supplementation is only temporary. Once adult salmon return to the lake and recommence feeding on alewife, their thiamine levels will once again drop (Ketola et al. 2008). This produces a situation where each subsequent spawning migration requires human intervention to insure its success as long as alewife are present in

the lake. For this reason, fisheries managers will need to consider more long-term solutions. Possibilities include installing fish ladders capable of assisting salmon passage without permitting invasive sea lamprey passage; artificially creating suitable spawning and rearing habitat below rapids acting as barriers to migration; or shifting the reintroduction focus to tributaries that lack high energy barriers. With the possible exception of shifting reintroduction efforts to new tributaries, these options all involve a considerable financial investment and do not directly address the core problem. As thiamine deficient salmon populations tends to display a range of deficiency levels, a potential long-term solution may be to identify what allows some individuals to maintain high thiamine levels and, if possible, select for such qualities in breeding programs.

General Conclusion

The problems encountered by the Atlantic salmon reintroduction program in Lake Champlain are not unique to this system nor this species as similar problems are faced by reintroduction biologists around the world. It is for this reason that conducting controlled experiments to improve reintroduction efforts and disseminating the results is so crucial to the success of reintroduction programs (Bearlin et al. 2002, Armstrong et al. 2007, Rout et al. 2009, Mitchell et al. 2011, Hyvärinen et al. 2013, Sard et al. 2015). By assessing each stage of a reintroduction program, asking questions, and developing new techniques, future projects can benefit from the mistakes, lessons, and discoveries of others. In the present study, significant reductions in straying rates and improved returns of mature adults to stocked rivers were achieved by challenging current practices and attempting new methods. In doing so we improved the likelihood of migratory salmon returning to Lake Champlain tributaries in the thousands as they did historically. Similarly, by addressing a problem that is present in Lake Champlain, but not unique to it (i.e. thiamine deficiency), mitigation actions were identified that can reduce migratory delays, thereby helping to re-establish natural reproduction in an altered historic range. In the process, we furthered our understanding of where such interventions are needed and under what situations they can permit the successful reintroduction of a species into a permanently altered sections of their historic home range. Such discoveries are important as a growing number of species conservation biologists are presented with the difficult task of deciding whether to reintroduce species into historic, but permanently altered, habitats or introducing at-risk species into new, but pristine, habitats with the risks this entails (Hunter 2007, Hoegh-Guldberg et al. 2008, Seddon 2010).

By considering a whole life-cycle approach to reintroducing populations and metapopulation dynamics, my colleagues and I arrived at a cost-effective means of improving survival while reducing straying rates, and identified situations in which the restoration of thiamine deficient salmonids can benefit from further human intervention, developing new techniques along the way. The methods employed here are easily implemented and have potential to greatly improve the success of future reintroductions, though some questions remain. For example, the observed results reflect Atlantic salmon in Lake Champlain and may differ somewhat in other situations. Additionally, our assessments, both of the effects of rearing conditions and release timing as well as for thiamine supplementation, were limited by their duration and did not include the most important variable in reintroduction settings, lifetime reproductive success. Fisheries managers should therefore test these methods in their own systems and report on their findings, including lifetime reproductive success whenever possible. By assessing these methods in new settings, using structured experimental techniques, we can further our understanding of the underlying mechanisms while adding to the expanding list of reintroduction guidelines. Conducting similar experiments under different conditions and over longer time spans will further contribute to our understanding of how human actions can influence the lifetime fitness of migratory species at risk; improving their chances of successful reintroduction.

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Chapter 1 Appendices

Appendix 1A, Offspring production and release conditions

To promote seasonal hormone production and retard olfactory imprinting prior to release, or increase the likelihood of imprinting post release, three cohorts of juvenile Atlantic salmon were either released as fry (0+) or as parr (1+) reared on multiple water sources in the final months prior to release or released earlier than usual (Table S1.1). Both the alternate water sources (groundwater with above-seasonal winter temperatures, or brook water with seasonal surface water temperatures) and early or late release dates had a noticeable effect on the size of juvenile salmon at the time of release. To assess the effect of rearing conditions and release dates on the size at release, length and weights for 100 randomly selected juvenile salmon were recorded from each rearing raceway shortly before their scheduled release date. These lengths and weights were then pooled according to release group within a cohort and an ANOVA was used to determine whether lengths differed among release groups. In the event that result indicated a significant difference, a Tuckey honest significant difference analysis was conducted.

Results confirmed that lengths differed significantly within cohorts as a function of rearing conditions and of release dates (Table S1.2). Among juveniles released into the same tributary (Boquet or Winooski), the experimental release groups (Early or Seasonal respectively) were consistently smaller at the time of release than the standard release dates or standard growth conditions respectively.

Table S1.1: Stocking information for fry (0+) and parr (1+) introduced into the Boquet and Winooski rivers between 2011 and 2014 as part of an experimental assessment of captive rearing and release. Rearing temperatures refers to whether salmon reared on groundwater (standard) or surface water (seasonal) for 2-4 months prior to release in the spring. Release date refers to whether parr were released at standard date in spring for each tributary, or a date 4-7 weeks earlier.

Stocking Cohort	Stocking Location	Age Class	Rearing Temperatures†	Release Date‡	Fin Clip	Stocking Date	Number Stocked*	Number of Families	Average Size (mm)
2010	Winooski	Fry			NC	2011-05-25	110360	90	25
		Parr	Seasonal	Standard	LV	2012-04-03	18284	30	174
			Standard			2012-04-03	17024	20	194
	Boquet	Fry			NC	2011-05-10	112890	90	24.5
		Parr	Standard	Early	LV	2012-03-07	27364	39	183
				Standard			2012-04-25	40898	41

* numbers of stocked fry are corrected for a 10% survival rate between the 0+ fry and 1+ parr stages

† Parr rearing temperatures were either standard (groundwater through the winter) or seasonal (surface water for 2-4 months prior to release).

‡ Release dates were either standard for a particular tributary, or 4-7 weeks earlier.

Table S1.1 continued

Cohort	Stocking Location	Age Class	Rearing Temperatures†	Release Date‡	Fin Clip	Stocking Date	Number Stocked*	Number of Families	Average Size (mm)
2011	Winooski	Fry			NC	2012-05-02	25900	51	27
		Parr	Seasonal	Standard	LV	2013-04-04	12320	18	180
			Standard			2013-04-04	7387	18	199
	Boquet	Fry			NC	2012-05-01	38187	146	27
		Parr	Standard	Early	LV	2013-03-21	23275	30	209
				Standard		2013-05-02	24762	30	217

* numbers of stocked fry are corrected for a 10% survival rate between the 0+ fry and 1+ parr stages

† Parr rearing temperatures were either standard (groundwater through the winter) or seasonal (surface water for 2-4 months prior to release).

‡ Release dates were either standard for a particular tributary, or 4-7 weeks earlier.

Table S1.1 continued

Stocking Cohort	Stocking Location	Age Class	Rearing Temperatures†	Release Date‡	Fin Clip	Stocking Date	Number Stocked*	Number of Families	Average Size (mm)
2012	Winooski	Fry			NC	2013-05-08	47500	71	25
		Parr	Seasonal	Standard	LV	2014-04-02	15554	16	178
			Standard			2014-04-02	10863	16	298
	Boquet	Fry			NC	2013-05-07	34983	81	25
		Parr	Seasonal	Early	LV	2014-04-09	25775	28	155
			Standard			2014-05-07	21905	28	159

* numbers of stocked fry are corrected for a 10% survival rate between the 0+ fry and 1+ parr stages

† Parr rearing temperatures were either standard (groundwater through the winter) or seasonal (surface water for 2-4 months prior to release).

‡ Release dates were either standard for a particular tributary, or 4-7 weeks earlier.

Table S1.2: The total lengths and standard deviations of Atlantic salmon parr (1+) released from the D.D. Eisenhower National Fish Hatchery into two experimental tributaries to Lake Champlain, the Boquet and Winooski rivers. Rearing temperature refers to whether parr were reared on groundwater (standard) or brook water (seasonal) for 4-2 months prior to release. Release date refers to whether parr were released 4-7 weeks earlier than the standard date of late April/early May depending on the tributary.

	Release	Rearing	Release	Total
Cohort	Location	Temperature	Date	Length (mm)
2010	Boquet	Standard	Early	183(18.55)a
			Standard	202.07(22.14)b
	Winooski	Seasonal	Standard	175.82(15.08)c
		Standard		194.85(18.29)d

Table S1.2 continued

	Release	Rearing	Release	Total
Cohort	Location	Temperature	Date	Length (mm)
2011	Boquet	Standard	Early	208.99(18.59)a
			Standard	218.36(18.5)b
	Winooski	Seasonal	Standard	180.24(16.72)c
		Standard		199.8(15.03)d
2012	Boquet	Seasonal	Early	155.67(16.12)a
			Standard	160.19(16.71)b
	Winooski		Standard	178.72(16.04)c
		Standard		198.07(21.16)d

Appendix 1B, Sample collection and supplemental analysis of potential sampling bias

Broodstock sample collection in the Hatchery:

Male and female Atlantic salmon were randomly selected from among the available Sebago Lake strain of broodstock maintained at the Bald Hill Fish Culture Station in Newark, Vermont, to conduct one-to-one crosses. Immediately following gamete collection, small fin clips (roughly 5mm²) were excised from the caudal fin and either placed into individually labeled 2.5 ml tubes containing non-denatured 95% ethanol, or pressed onto labelled sheets of filter paper. Samples preserved on filter paper were allowed to dry before being inserted into transparent page protectors for storage (Table S1.3).

Offspring sample collection in Lake Champlain:

To assess survival to maturity prior to the potential influence of differential homing/straying rates among experimental releases of juvenile Atlantic salmon in the Lake Champlain basin, adult salmon were sampled throughout the lake via two methods: boat electrofishing and angling. The boat electrofishing samples were collected during annual lake salmonid surveys conducted by trained U.S. Fish and Wildlife Service biologists while angling samples were collected by volunteers participating in a citizen science outreach program. Volunteers attended annual meetings throughout the 4 years of sampling, during which demonstrations were given of how to properly identify, measure, and sample Atlantic salmon with the appropriate left ventral fin clip. In each sampling method, tissue samples were collected as scales or fin clips that were dehydrated and stored in coin envelopes or filter paper respectively (Table S1.3).

Offspring samples collected in experimental tributaries:

During fall spawning runs (early September to early November), fish traps situated at dams on both the Boquet and Winooski rivers were actively maintained by NY Department of Environmental Conservation and US Fish and Wildlife staff respectively. Traps were inspected on a daily basis during peak run periods, or every two days as runs trailed off. Small 5mm² tissue samples were collected from the caudal fins of captured salmon with left ventral fin clips (LV) or lacking a fin clip entirely (NC). From 2014 to 2016, trap and gill nets were also used within the Boquet river immediately below the dam in order to boost sample sizes. All fin clips were preserved in individually labelled 2.5ml tubes containing non-denatured 95% ethanol. In several cases scale samples were collected rather than fin clips, and were preserved by desiccation in individually labelled coin envelopes (Table S1.3).

Supplemental analysis of potential sampling bias in the lake

Once samples collected in the lake were assigned back to the appropriate parental pair, the cohort, release location, rearing conditions and release date were identified. Samples collected from Lake Champlain were then summed according release location (tributary), release group (groundwater or brook water, early or late release), and sampling method (e-fishing or angling). These recapture frequencies were summed across replicate cohorts and therefore represented recapture frequencies at the tributary level. Independence between the sampling method and recapture frequencies within each release group was then assessed using Fisher's Exact tests in R. Results indicated no significant association among sampling methods and

recapture frequencies for each release group. Subsequent analyses were therefore conducted on samples pooled across sampling methods.

To assess how potential differences in the catchability of release groups could impact the results of our assessment of survival to adulthood in the lake, an informal sensitivity analysis was conducted. This consisted of iteratively repeating adult recapture analyses at the tributary level while varying recapture ratios over a range -10 to +10 individuals per release group. Following each iteration, the significance and result were recorded. The results indicate that in terms of rearing conditions or release date (Figure S1.1 left and middle panels respectively) the observed recapture ratios were highly robust and dramatic deviations from the observed ratios (the center of each plot) were required to affect the outcome. When both the early and standard release date groups were exposed to seasonal rearing conditions in the hatchery prior to release (Figure S1.1 right panel), results switched from a non-significant decrease as a result of earlier release dates, to a non-significant increase, though only a very extreme departure from the observed recapture ratio produced a significant result, suggesting the lack of difference between these two groups is also robust.

Table S1.3: Sample sizes and tissue sample types collected from parental broodstock and putative offspring sampled within the Lake Champlain basin as part of an adaptive management experiment to reduce straying and maintain or improve survival among Atlantic salmon releases.

Source	Sample Type			Total
	Dried Fin Clip	Fin Clip in EtOH*	Scales	
2010 Broodstock	0	620	0	620
2011 Broodstock	586	0	0	586
2012 Broodstock	480	0	0	480
2013 Lake Samples	24	0	22	46
2013 Boquet Spawners	0	14	0	14
2013 Winooski Spawners	0	67	12	79
2013 Straying Spawners	0	0	25	25

* 95% Non-denatured ethanol

Table S1.3 continued

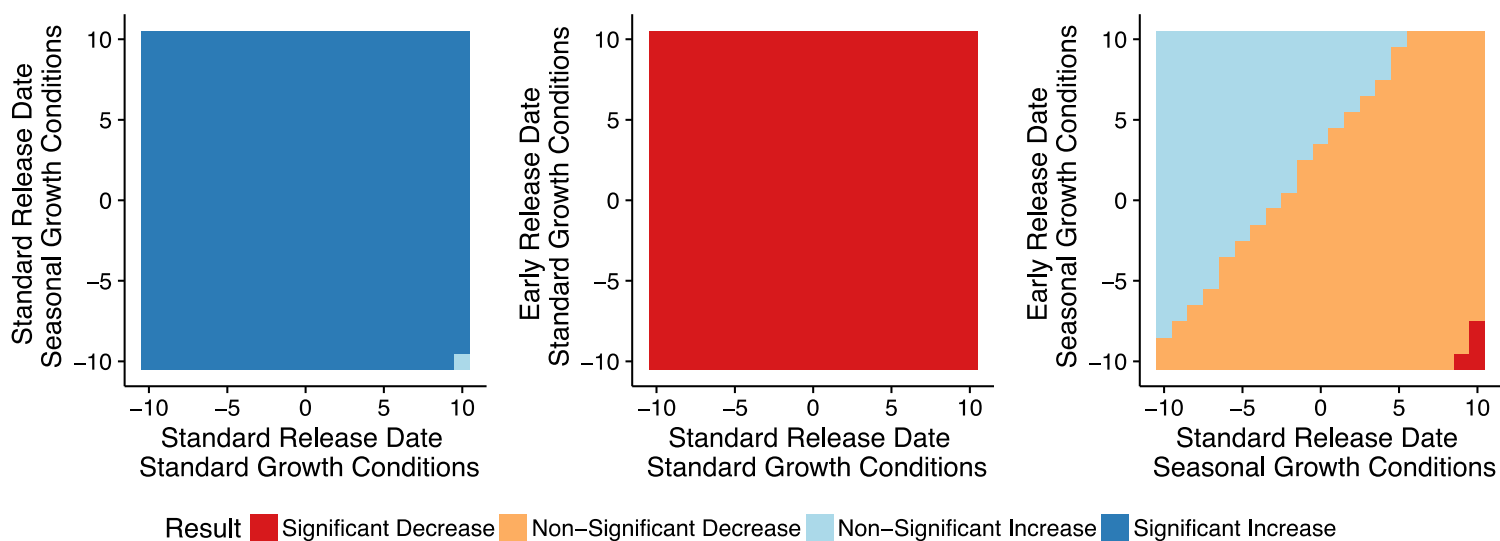
Source	Sample Type			Total
	Dried Fin Clip	Fin Clip in EtOH*	Scales	
2014 Lake Samples	0	0	145	145
2014 Boquet Spawners	0	63	0	63
2014 Winooski Spawners	0	102	0	102
2015 Lake Samples	0	0	97	97
2015 Boquet Spawners	0	134	2	136
2015 Winooski Spawners	0	68	4	72
2015 Straying Spawners	0	0	41	41
2016 Lake Samples	0	0	3	3
2016 Boquet Spawners	0	78	0	78
2016 Winooski Spawners	0	31	0	31
Total	1090	1177	351	2618

* 95% Non-denatured ethanol

Table S1.4: Salmon reared in the hatchery under seasonal or standard temperatures and released into tributaries of Lake Champlain at either standard or early (~2 months earlier) dates, recaptured in the lake via two methods: an angler citizen science program, or during an annual salmonid survey conducted by the USFWS using an electro-fishing boat. P-values are from Fisher’s exact tests for independence between recapture frequencies and sampling method.

	Release	Rearing	Release	Angler	E-Fishing	
Cohort	Location	Temperature	Date	Samples	Samples	P-Value
2010-2012	Winooski	Standard	Standard	5	2	
		Seasonal		26	16	1.00
2010-2011	Boquet	Standard	Standard	34	33	
			Early	4	2	0.68
2012	Boquet	Seasonal	Standard	27	25	
			Early	26	32	0.57

Figure S1.1: Results of a sensitivity analysis on the ratio of recapture frequencies of adult Atlantic salmon released as parr exposed to competing growth conditions/release dates. In each case, the true observed recapture frequencies are represented by the center of the graph (0,0). Ratios were then varied from -10 to +10 individuals for each group and a Fisher’s exact test was conducted on the results. Colors correspond to test results for the y-axis relative to the x-axis.



Appendix 1C, Genotyping and parentage methods and results

Genotyping protocol:

DNA was extracted from tissue samples using a modified chelex extraction method which involved incubating samples in 5% chelex solution (chelex 100, Sigma) containing 125 µg/L of 25 mg/ml proteinase K at 45°C for a minimum of 45 minutes and a maximum of 12 hours. After this incubation period, samples were boiled at 98°C for 5 minutes to insure cell lysis. The resulting cell lysate was vortexed briefly and centrifuged before the supernatant was separated from the chelex beads for use and storage.

Seven polymorphic microsatellite loci (Table S1.5, SSsp2215, SSsp2216, SSspG7, SsaD144, SsaD157, SsaD48, SsaD71) were then amplified in five 10 µL PCR reactions. Each reaction contained 2 µL of a 1:10 dilution of the cell lysate, 0.06 units of Taq, 0.4 µg/µL of BSA, 2.0 mM of MgCl, 0.2 mM of dNTP, 1x Taq buffer, fluorescently labeled forward and unlabeled backward primer (0.18 to 0.35 mM depending on the PCR reaction), and HPLC water. The resulting PCR cocktails are then run through a thermocycler at 95°C for 4 minutes followed by 35 cycles of 95°C for 20 seconds, 60 or 58°C for 20 seconds and 72°C for 20 seconds. The entire process finished with 72°C for 5 minutes before cooling and holding at 4°C. Amplified alleles were then scored using the ABI 3500 sequencer and Genemapper scoring software (ThermoFisher) before being visually proofread. In the event additional loci were require to differentiate between multiple potential parental pairs, three more loci (SSa85, Sssp 2201, and 407) were genotyped for each potential parent-offspring triplet using the methods listed above.

Table S1.5: Microsatellite loci used for genetic analysis and a summary of genetic diversity statistics. N refers to the number of individuals scored at each locus while N_a refers to the total number of observed alleles at each locus; H_{obs} and H_{exp} are the observed and expected heterozygosities, respectively.

Locus	Reference	N	N_a	H_{obs}	H_{exp}
SSsp2215	Paterson et al. 2004	2522	14	0.823	0.835
SSsp2216	Paterson et al. 2004	2518	52	0.912	0.948
SSspG7	Paterson et al. 2004	2522	17	0.896	0.887
SsaD144	King et al. 2005	2519	30	0.901	0.906
SsaD157	King et al. 2005	2509	28	0.943	0.937
SsaD48	King et al. 2005	2518	52	0.946	0.956
SsaD71	King et al. 2005	2517	31	0.947	0.940
Sssp407	Cairney et al. 2000	229	21	0.882	0.886
Ssa85	O'Reilly et al. 1996	192	13	0.823	0.802
Sssp2201	Paterson et al. 2004	225	24	0.907	0.931

Error rate assessment:

In order to assess genotyping error rates among the primary seven loci used to assign offspring back to parental pairs, multiple runs (extraction, amplification, and scoring) were performed on samples from the major sampling groups (broodstock, lake samples, tributary samples) with the exception of 2016 samples (Table S1.6). Samples that failed to amplify at four or more loci (failed PCRs, poor quality DNA) were not included in the analysis of genotyping error rates, while the remaining unscored loci were omitted from further calculations. Direct

comparisons between replicate genotyping results were then made at the per-locus level, and the rate of full matches, partial matches, and complete mismatches was calculated.

Of the 2120 genotypes included in our genotyping error rate assessment (Table S1.6), 53 alleles conflicted with previous scoring results, suggesting an error rate of ~1.3% (Table S1.7). One-third of the conflicts were due to upper allele dropout, while per-locus error rates ranged from 2.8% (SsaD144) to 0.9% (SsaD157).

Table S1.6: Summary of samples collected from Atlantic salmon broodstock and offspring collected at various life stages in the lake, via e-fishing or angling, and in the stocked tributaries via trap netting, gill netting, and permanent fish traps on the Boquet (NY) and Winooski (VT) rivers from 2013 to 2016

Status	Total Samples	Rerun Samples	Rerun Proportions
2010 Broodstock	620	87	14%
2011 Broodstock	586	49	8%
2012 Broodstock	480	68	14%
2013 Boquet Spawner	14	3	21%
2013 Lake Adult	46	20	43%
2013 Winooski Spawner	79	31	39%

Table S1.6 continued

	Total	Rerun	Rerun
Status	Samples	Samples	Proportions
2014 Angler Sample	90	5	6%
2014 Boquet Spawner	63	9	14%
2014 Lake Adult	55	9	16%
2014 Winooski			
Spawner	102	32	31%
2015 Angler Sample	68	17	25%
2015 Boquet Spawner	136	17	13%
2015 Lake Adult	29	3	10%
2015 Winooski			
Spawner	72	11	15%
2016 Angler Sample	3	0	0%
2016 Boquet Spawner	69	0	0%
2016 Winooski			
Spawner	31	0	0%
Grand Total	2543	361	14%

Table S1.7: Per locus error rates for the seven loci used to assign offspring back to parental pairs calculated from direct comparisons between replicate DNA extraction, amplification, and scoring of a subset of samples collected from broodstock and offspring.

Locus	Reruns	Full	Partial	Full	Error
		Matches	Matches	Mismatches	Rate
SSsp2215	352	0.986	0.003	0.011	0.014
SSsp2216	297	0.983	0.003	0.013	0.017
SSspG7	348	0.989	0.006	0.006	0.011
SsaD144	283	0.972	0.018	0.011	0.028
SsaD157	221	0.991	0.005	0.005	0.009
SsaD48	338	0.985	0.003	0.012	0.015
SsaD71	281	0.986	0.007	0.007	0.014

Exclusionary power analysis:

The ability of the microsatellite panel to correctly identify parent-offspring triplets was assessed using simulations in P-Loci (Matson et al. 2008). First, null-allele frequencies were estimated in ML-NullFreq (Kalinowski and Taper 2005) using a genotype database containing parents and offspring. Null-allele frequencies and genotyping error rates from above were then used, along with parental genotyping results, to create 10 simulated offspring per parental pair. Parallel simulations were performed using mutation/genotyping error rates above (2%) and below (1% and 0%) our observed genotyping error rates. In each case, simulated offspring were reassigned back to parental pairs using Mendelian exclusion. The exclusionary power of each locus and combination of loci was calculated based on the proportions of correct and incorrect assignments.

Analysis of broodstock genotypes with ML-NullFreq suggested the presence of null alleles in three of seven loci, but at very low frequencies: 0.015, 0.015 and 0.005 for loci SSsp 2215, SSsp2216, and SsaD48 respectively. Despite this, the suite of seven microsatellite loci maintained high overall exclusionary power. When estimated per-locus scoring error rates were used, the assignment rate and proportion of correct assignments increased quickly with additional loci, with both values exceeding 95% once four or more loci were used. Assignment error reached zero when all seven loci were used, however 2.9% of simulated offspring remaining unassigned due to an inability of P-Loci to exclude all but one parental pair (Table S1.8).

Table S1.8: Results of P-Loci run assessing the exclusionary power of the seven microsatellite loci used to assign putative Atlantic salmon offspring captured in Lake Champlain and its tributaries to known pairs of broodstock as part of a parentage based tagging system. Parallel analyses were conducted using the estimated, locus-specific null allele and scoring error rates, including the error rates estimated for the present genotyping dataset.

	Loci used for Exclusion						
	1	2	3	4	5	6	7
0% mutation/error rate							
Correctly Assigned:	5.96	80.66	98.42	99.69	99.93	99.86	99.85
Incorrectly Assigned:	0.00	0.21	0.02	0.00	0.00	0.00	0.00
Unassigned:	94.05	19.12	1.55	0.31	0.07	0.14	0.15
1% mutation/error rate							
Correctly Assigned:	6.36	76.22	95.48	99.21	99.38	99.21	98.72
Incorrectly Assigned:	0.36	0.53	0.06	0.01	0.00	0.00	0.00
Unassigned:	93.29	23.25	4.46	0.78	0.62	0.80	1.28
2% mutation/error rate							
Correctly Assigned:	5.96	74.35	93.41	98.08	98.36	97.69	96.73
Incorrectly Assigned:	0.56	0.89	0.25	0.04	0.01	0.00	0.00
Unassigned:	93.49	24.76	6.35	1.89	1.63	2.31	3.27

Table S1.8 continued

	Loci used for Exclusion						
	1	2	3	4	5	6	7
Estimated per-locus error rates							
Correctly Assigned:	6.10	75.34	94.47	98.61	98.75	98.06	97.07
Incorrectly Assigned:	0.36	0.87	0.12	0.01	0.01	0.01	0.00
Unassigned:	93.55	23.80	5.41	1.38	1.23	1.93	2.93

Genetic parentage based tagging:

Putative offspring were reassigned to a release group following successful assignment to a known parental pair. The use of one-to-one crosses from known parental pairs allowed for confident assignment using Mendelian exclusionary principles (Jones and Ardren 2003) in the R package SOLOMON (Christie et al. 2013). All seven loci were used to assign offspring to parents, however, based on the results of our P-Loci simulations, parent-offspring triplets were also accepted that matched at 6 loci. In cases where full exclusion to a single parental pair was not possible at seven loci, parent-offspring triplets were genotyped at an additional three loci and rerun.

Overall, 77% of the putative offspring sampled in the lake and experimental tributaries successfully assigned back to known parental pairs, including, respectively, 25% and 89% of the samples collected from NC and LV salmon. The low reassignment rate for NC salmon likely represents the inclusion of samples from salmon stocked elsewhere within the Lake Champlain basin that strayed into the experimental tributaries during spawning runs, emphasizing the potentially high straying rate among the hatchery strains used within the Lake Champlain basin. The proportion of LV salmon that successfully assigned to known parental pairs rose from 89% to 93% when only samples collected by trained biologists were assessed, suggesting some amount of fin clip misidentification among the angler supplied samples. The remaining unassigned LV salmon likely resulted from three potential sources. First, fin-clip misidentification was possible even among trained biologists as many fin clip combinations are in use within the Lake Champlain basin, some of which resemble the LV clip used in this study (e.g. adipose + LV). Second, salmon may have failed to meet our parentage assignment threshold of six or more matching loci due to genotyping error, null alleles, or upper allelic dropout.

Among LV salmon, 14 (2% of the total) assigned to a single parental pair, but were not considered in further analyses as they matched at only 5 loci. Third, genetic similarity among hatchery broodstock parents may have prevented full exclusion to a single parental pair. P-Loci simulations indicated that 2.9% of offspring would remain unassigned due to exclusion failure under our estimated scoring error rate and null allele frequencies. Among the parent-offspring triplets matching at six or more loci, however, only one could not be assigned to a single parental pair.

Appendix 1D: Parr-to-adult survival, spawning returns, and straying at the cohort level

Survival to maturity in the lake

In all three replicate cohorts in the Boquet river (under standard rearing temperatures in 2010 and 2011 and seasonal rearing temperatures in 2012), standard release dates respectively produced greater recapture rates (0.005, 0.017, and 0.237%) than early release dates (0, 0.021, and 0.225%) (Figure 1.4a). This difference was only significant for the 2011 cohort however. In the Winooski River, with release dates held constant, seasonal rearing temperatures produced greater adult recapture rates (0.016 and 0.238%) than standard rearing temperatures (0 and 0.046%) in the 2010 and 2012 cohorts, respectively, but lower recapture rates (0.016 vs 0.027% respectively) for the 2011 cohort (Figure 1.4b). Only the 2012 cohort produced a statistically significant difference. Nearly three quarters of all adults recaptured in the lake were 2-year-olds (Table S1.9) with another 20% captured as 3-year-olds; remaining salmon were comprised of 1-year-olds (6%) and 4-year-olds (<1%).

Spawning returns

In the Boquet (under standard rearing temperatures: 2010 and 2011; and seasonal rearing temperatures: 2012), migration rates for standard release dates (0.017, 0.198, and 0.310% respectively) were between 1.4-fold and 9.2-fold greater than early release times (0.004, 0.021, and 0.229% respectively) (Figure 1.4a; Table S1.9): though this difference was only significant for the 2011 cohort. Relative to fry migration rates (0.027, 0.314, and 0.115% respectively), standard rearing temperatures and release dates produced a 55 – 60% to decrease in spawner returns, while seasonal rearing temperatures and standard release dates produced a 2.7-fold

increase (Figure 1.4a). In the Winooski, with constant release dates, migration rates for the 2010, 2011, and 2012 cohorts under seasonal rearing temperatures (0.268%, 0.162%, and 0.392% respectively) were between 3-fold and 6.9-fold greater than standard rearing temperatures (0.076%, 0.162%, and 0.064%) and between 3.3-fold and 18.6-fold (0.082%, 0.386%, 0.021%) greater than fry releases, a significant improvement in two of three cases (Figure 1.4b; Table S1.9). Overall, 88% of returning spawners were split roughly in half between 2- and 3-year-old salmon, with an additional 12% returning at age 4. No one-year-old salmon were observed returning during spawning migrations.

Straying

Earlier release dates in 2010 or 2011 produced fewer strays (i.e. none) than standard production salmon in the Boquet (2 of 7, 28.6% and 6 of 49, 12.2% respectively); small sample sizes precluded detection of significant differences at this level. The opposite was true when seasonal rearing conditions were used as no strays were observed for the regular release date, while 1 of the 59 early release parr (1.7%) were recaptured in a non-origin tributary. The 2010 and 2012 Boquet fry cohorts did not produce any strays among the 3 and 4 adults recaptured during spawning migrations respectively. The 2011 cohort, however, expressed the highest observed straying rate of any experimental release groups (33.3%, 4 strays among 12 returning spawners). In the Winooski, parr reared at seasonal temperatures strayed more than standard production parr in all three cohorts (1/49 vs. 0/13, 1/60 vs 0/12, and 1/61 vs. 0/7 respectively). Fry releases also did not produce strays in any cohort (from 9, 10, and 1 recaptured spawners respectively). As in the Boquet, however, none of these differences were significant. Overall straying rates were higher and more variable among the Boquet cohorts (Figure 1.4a); males

tended to stray more than females (9 males, 7 females), though not significantly more (binomial exact test, $p = 0.80$).

Table S1.9: Age specific recapture frequencies in Lake Champlain, two experimental tributaries (river returns), and non-origin tributaries (strays) for Atlantic salmon produced using standard methods as well as four alternative rearing/release methods. Total columns represent the number of salmon/the number of families contributing to returns/mean (sd) number of offspring per family. Differing subscripts indicate significance differences according to Fisher’s Exact tests after Bonferroni corrections. Rearing temperatures indicate the use of groundwater throughout the winter (standard) or surface water for 2-4 months prior to release (seasonal). Release date indicates tributary norms (standard) or 4-7 weeks early.

Lake Recapture Frequencies										
Stocking	Age	Rearing	Release							
Cohort	Location	Class	Temperature	Date	Families	Stocked*	1	2	3	Total
2010	Boquet	Fry			90	11289	NA	NA	NA	NA
		Parr	Standard	Early	39	27364	0	0	0	0/0/0(0)a
				Standard	41	40898	0	0	2	2/1/2(0)a
	Winooski	Fry			90	11036	NA	NA	NA	NA
		Parr	Seasonal	Standard	30	18284	0	0	3	3/2/1,5(0,71)a
			Standard		20	17024	0	0	0	0/0/0(0)a

Table S1.9 Continued...(Lake recapture frequencies)

							Lake Recapture Frequencies			
Stocking	Age	Rearing	Release							
Cohort	Location	Class	Temperature	Date	Families	Stocked*	1	2	3	Total
2011	Boquet	Fry			146	3819	NA	NA	NA	NA
		Parr	Standard	Early	30	23275	0	4	1	5/5/1(0)a
				Standard	30	24762	0	40	2	42/17/2.47(1.42)b
	Winooski	Fry			51	2590	NA	NA	NA	NA
		Parr	Seasonal	Standard	18	12320	0	2	0	2/2/1(0)a
			Standard		18	7387	0	1	1	2/2/1(0)a

* Fry stocking numbers have been adjusted to account for 10% survival during the first year in the wild.

Table S1.9 Continued...(Lake recapture frequencies)

Lake Recapture Frequencies										
Stocking		Age	Rearing	Release						
Cohort	Location	Class	Temperature	Date	Families	Stocked*	1	2	3	Total
2012	Boquet	Fry			81	3489	NA	NA	NA	NA
		Parr	Seasonal	Early	28	25775	6	38	14	58/33/1.74(1.25)a
				Standard	28	21905	9	31	12	52/34/1.53(0.96)a
	Winooski	Fry			71	4750	NA	NA	NA	NA
		Parr	Seasonal	Standard	16	15554	6	26	5	37/20/1.76(0.89)a
				Standard	16	10863	0	5	0	5/3/1.67(0.58)b

* Fry stocking numbers have been adjusted to account for 10% survival during the first year in the wild.

Table S1.9 Continued... (River returns)

										River Returns	
Stocking		Rearing		Release							
Cohort	Location	Age Class	Temperature	Time	Families	Stocked	2	3	4	Total	
2010	Boquet	Fry			90	112890	0	3	0	3/3/1(0)a	
		Parr	Standard	Early	39	27364	0	1	0	1/1/1(0)a	
				Standard	41	40898	7	0	0	7/7/1(0)a	
	Winooski	Fry			90	110360	0	9	5	9/9/1(0)a	
		Parr	Seasonal		Standard	30	18284	43	5	2	49/20/2.45(0.39)b
			Standard			20	17024	11	2	1	13/10/1.3(0.48)a

Table S1.9 Continued... (River returns)

										River Returns
Stocking		Rearing		Release						
Cohort	Location	Age Class	Temperature	Time	Families	Stocked	2	3	4	Total
2011	Boquet	Fry			146	38187	4	8	0	12/12/1(0)a
		Parr	Standard	Early	30	23275	4	1	0	5/4/1.25(0.5)b
				Standard	30	24762	47	2	0	49/21/2.33(2.01)a
Winooski	Fry				51	25900	8	2	0	10/9/1.11(0.33)a,b
		Parr	Seasonal	Standard	18	12320	58	2	0	60/17/3.53(2.12)b
				Standard	18	7387	12	0	0	12/8/1.5(0.75)a

Table S1.9 Continued... (River returns)

										River Returns
Stocking		Rearing		Release						
Cohort	Location	Age Class	Temperature	Time	Families	Stocked	2	3	4	Total
2012	Boquet	Fry			81	34893	2	2	0	4/3/1.33(0.58)a
		Parr	Seasonal	Early	28	25775	46	12	1	59/23/2.57(2.88)a
				Standard	28	21905	49	18	1	68/29/2.34(2.06)a
	Winooski	Fry			71	47500	1	0	0	1/1/1(0)a
		Parr	Seasonal	Standard	16	15554	49	12	1	61/21/2.9(2.05)b
			Standard		16	10863	4	3	0	7/6/1.17(0.41)a

Table S1.9 Continued... (Strays)

							Strays
	Stocking	Age	Rearing	Release			
Cohort	Location	Class	Temperature	Time	Families	Stocked	Total
2010	Boquet	Fry			90	112890	0/0/0(0)a
		Parr	Standard	Early	39	27364	0/0/0(0)a
				Standard	41	40898	2/2/1(0)a
Winooski		Fry			90	110360	0/0/0(0)a
		Parr	Seasonal		30	18284	1/1/1(0)a
				Standard	Standard	20	17024

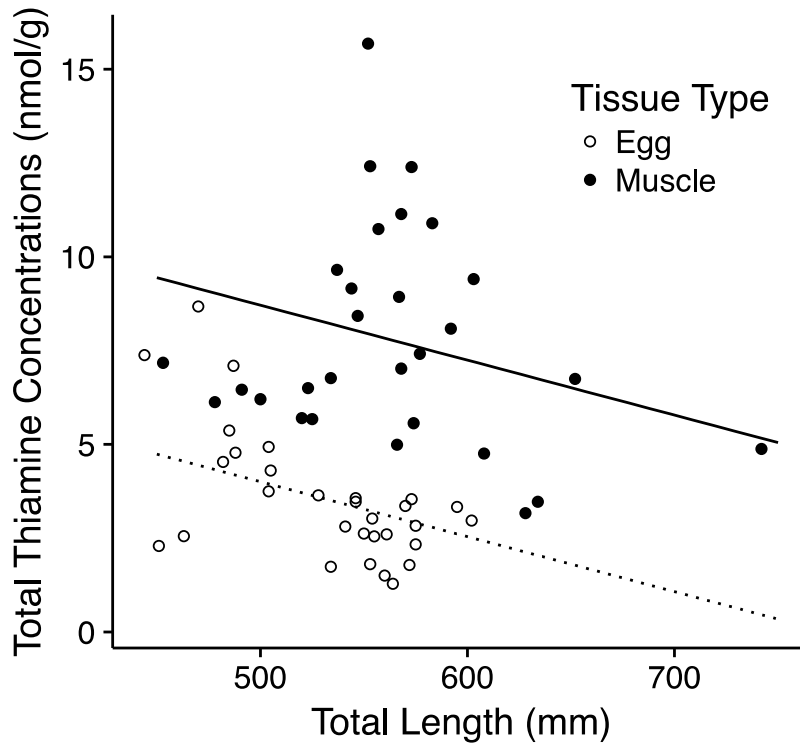
Table S1.9 Continued... (Strays)

							Strays
	Stocking	Age	Rearing	Release			
Cohort	Location	Class	Temperature	Time	Families	Stocked	Total
2011	Boquet	Fry			146	38187	4/4/1(0)a
		Parr	Standard	Early	30	23275	0/0/0(0)a
				Standard	30	24762	6/5/1.2(0.45)a
	Winooski	Fry			51	25900	0/0/0(0)a
		Parr	Seasonal		18	12320	1/1/1(0)a
			Standard	Standard	18	7387	0/0/0(0)a

Table S1.9 Continued... (Strays)

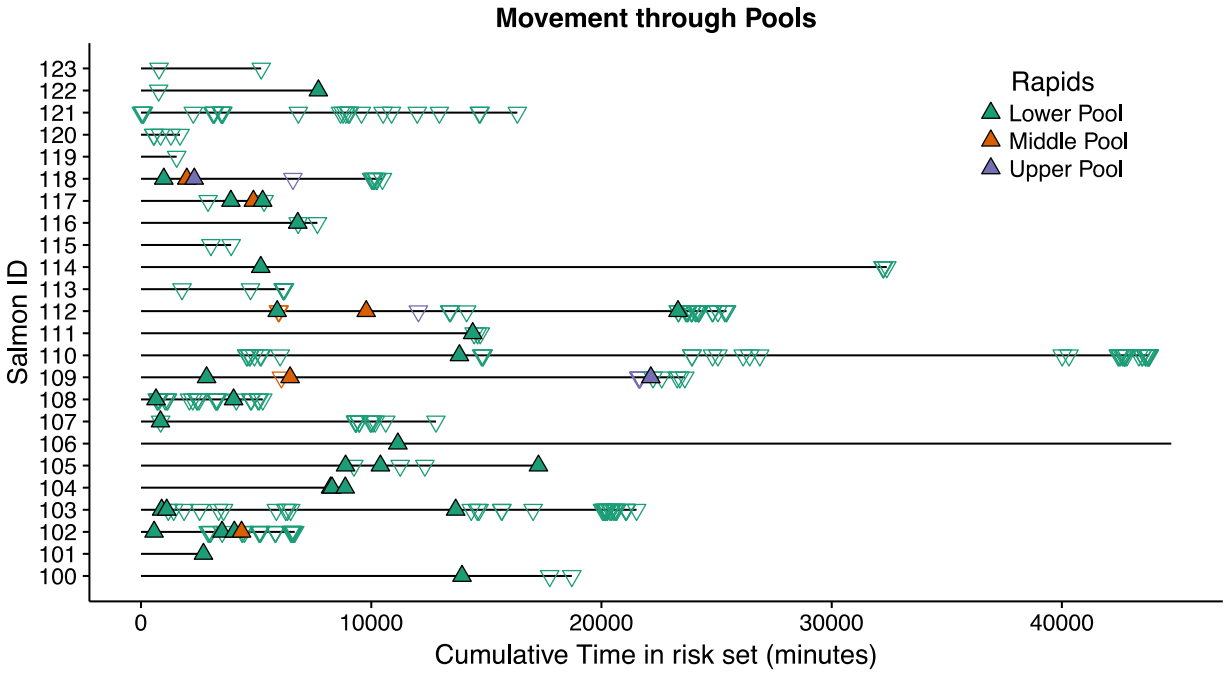
							Strays
	Stocking	Age	Rearing	Release			
Cohort	Location	Class	Temperature	Time	Families	Stocked	Total
2012	Boquet	Fry			81	34893	0/0/0(0)a
		Parr		Early	28	25775	1/1/1(0)a
			Seasonal	Standard	28	21905	0/0/0(0)a
	Winooski	Fry			71	47500	0/0/0(0)a
		Parr	Seasonal	Standard	16	15554	1/1/1(0)a
			Standard		16	10863	0/0/0(0)a

1 Chapter 3 Appendix



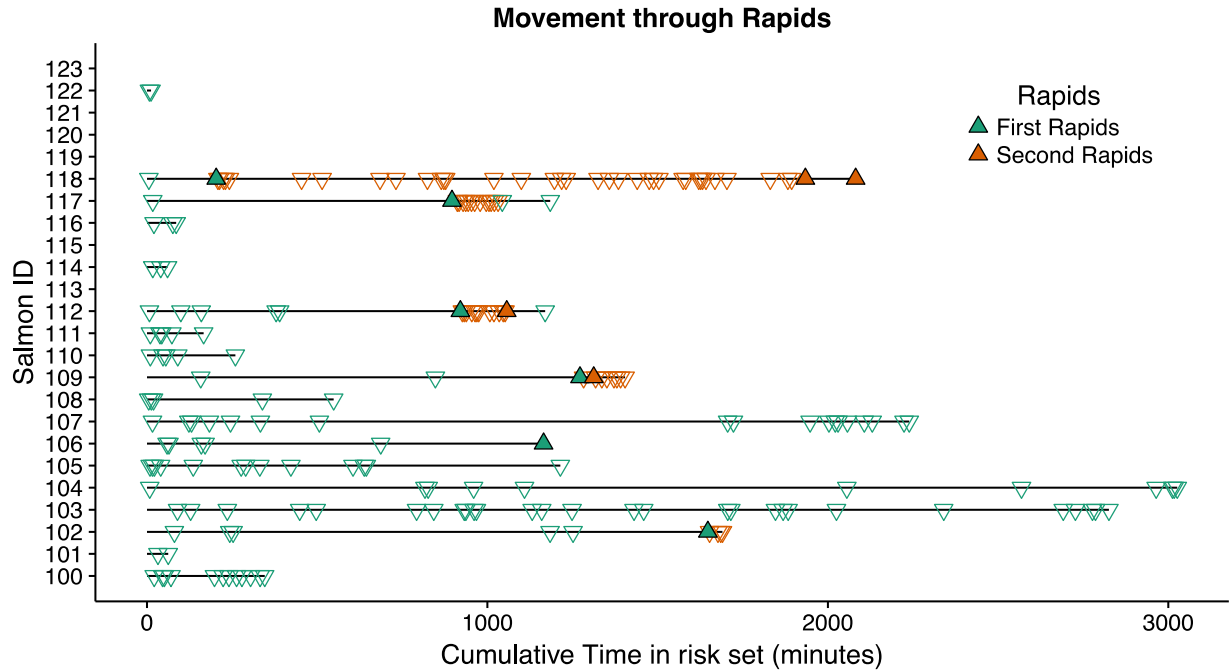
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3 **Figure S3.1:** Total thiamine concentrations in white muscle tissue and egg samples collected from
4 male and eggs collected from female feral broodstock by the Vermont Fish and Wildlife staff at
5 the Ed Weed fish culture station located 30 km from the Boquet River study site.



6

7 **Figure S3.2:** The cumulative time spent in the passage zones comprised of low-energy
 8 environments (pools) within the Willsboro Rapids. Upward and downwards facing arrows
 9 represent advance and retreat movements from rapids while color corresponds to the particular
 10 pool.



11
 12 **Figure S3.3:** The cumulative time spent in the passage zones comprised of high-energy
 13 environments (rapids) within the Willsboro Rapids. Upward and downwards facing arrows
 14 represent advance and retreat movements from rapids while color corresponds to the particular
 15 set of rapids.

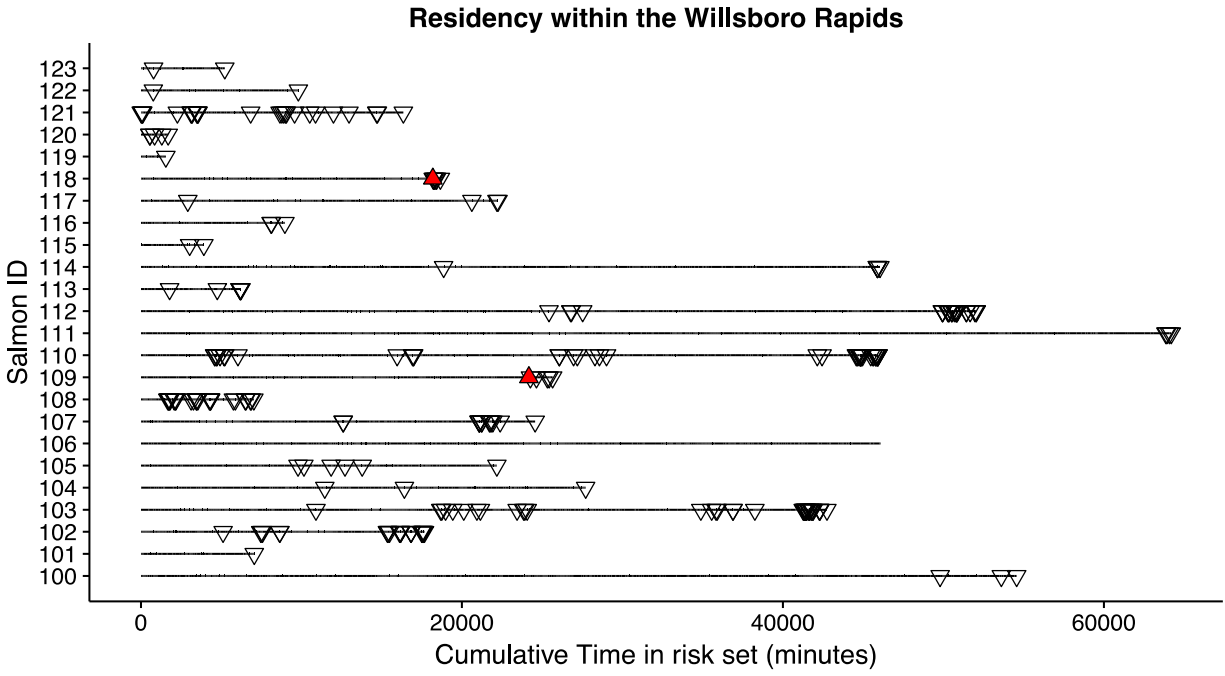


Figure S3.4: The cumulative time spent in the Willsboro Rapids, Willsboro, NY. Upward and downwards facing arrows represent advance and retreat movements from study area respectively.