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Restoration of Native Biodiversity in Altered Environments: Reintroduction of Atlantic salmon into Lake Ontario

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RESTORATION OF NATIVE BIODIVERSITY IN ALTERED ENVIRONMENTS:
REINTRODUCTION OF ATLANTIC SALMON INTO LAKE ONTARIO

(Thesis format: Integrated Article)

by

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Graduate Program in Biology

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Abstract

Less than a quarter of reintroduction programs have succeeded in re-establishing a self-sustaining population of an extirpated species. Optimal source population selection, based on an evolutionary and ecological perspective, could increase the fitness of translocated individuals, thereby improving the success rate of restoring extirpated populations. Here, using three source populations of Atlantic salmon, *Salmo salar* (LaHave River, Sebago Lake, and Lac Saint-Jean), that are being used for reintroduction efforts into Lake Ontario, I examined two optimal source population selection approaches: environment matching and adaptive potential. For environment matching, source populations from locations containing similar key environment features as the reintroduction location should contain adaptations to these features. For adaptive potential, source populations with high heritable genetic variation should have the potential to adapt to new selection pressures, such as the key environment features in the reintroduction location. I tested environment matching using experimental settings by exposing the three source populations to two key environment features that are likely impediments to a successful reintroduction of Atlantic salmon into Lake Ontario: the presence of non-native salmonids and a high thiaminase diet that can lead to a thiamine (vitamin B1) deficiency. I also quantified the amount of within-population heritable (additive) genetic variation for early-life history traits to assess the adaptive potential of the source populations. Although the average amount of heritable genetic variation was the highest for early-life history traits of the Sebago population, the amount was low, suggesting that the traits have a limited potential to adapt to any new selection pressures in Lake Ontario. Overall, the Sebago population (a match to both key environment features) had the highest performance, followed by the Saint-Jean population (match to a

high thiaminase diet but not non-native salmonids), and finally the LaHave population (not a match to either feature). The pattern of overall performance and the low amount of heritable genetic variation of the three source populations generally supports environment matching over adaptive potential; however, further population comparisons are required over the entire life-cycle and in a fully natural setting to make more robust recommendations for large scale reintroduction efforts of Atlantic salmon into Lake Ontario.

Co-Authorship Statement

Chapter 1, 7, and 8

Aimee Lee Houde: Developed the source population selection framework, collected and analyzed the data from a literature review, and drafted the manuscript.

Shawn Garner and Bryan Neff: Provided input on the framework and manuscript.

Chapters 2 and 3

Aimee Lee Houde: Developed the experimental design, collected and analyzed the data on juvenile salmonids in the artificial streams, and drafted the manuscripts.

Chris Wilson: Provided the salmonids and facilities where the experiments were conducted; as well as provided input for the experimental design and manuscripts.

Bryan Neff: Provided input for the experimental design and manuscripts.

Chapter 4

Aimee Lee Houde: Developed the experimental design, collected and analyzed the data on juvenile salmonids in natural streams, and drafted the manuscript.

Andrew Smith: Helped collect the data on juvenile salmonids and microhabitats in natural streams.

Chris Wilson: Provided the Atlantic salmon and facilities for rearing the fish prior to natural stream release; as well as provided input for the experimental design and manuscript.

Pedro Peres-Neto and Bryan Neff: Provided input for the experimental design and manuscript.

Chapter 5

Aimee Lee Houde: Developed the experimental design, collected and analyzed the data on sub-adult Atlantic salmon, and drafted the manuscript.

Patricio Saez and Dominique Bureau: Provided the diets for the experiment.

Chris Wilson: Provided the Atlantic salmon and facilities where the experiment was conducted; as well as provided input for the experimental design and manuscript.

Bryan Neff: Provided input for the experimental design and manuscript.

Chapter 6

Aimee Lee Houde: Developed the experimental design, collected and analyzed the data on Atlantic salmon early-life history traits, and drafted the manuscripts.

Craig Black: Helped collect the data on Atlantic salmon early-life history traits.

Chris Wilson: Provided the Atlantic salmon and facilities where the experiments were conducted; as well as provided input for the experimental design and manuscripts.

Trevor Pitcher and Bryan Neff: Provided input for the experimental design and manuscripts.

Acknowledgments

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List of Abbreviations, Symbols, Nomenclature

ID	Identity
LA	LaHave River population
LaHave	LaHave River population
SD	Standard deviation
Sebago	Sebago Lake population
SE	Sebago Lake population (for population comparisons)
SE	Standard error (for precision around a mean)
Saint-Jean	Lac Saint-Jean population
SJ	Lac Saint-Jean population

Chapter 1

1 General Introduction[*]

1.1 Reintroduction of Extirpated Populations

The extirpation of native populations from historically occupied habitats is a major threat to conserving biodiversity as it is often a precursor to the extinction of the species and the loss of ecosystem services. Reintroduction programs, in which conspecific individuals are translocated into formerly occupied habitats, have emerged as an important conservation tool for reversing extirpations (Armstrong and Seddon 2008; Seddon 2010; IUCN 2013). These programs are intuitively appealing as a means of restoring populations and communities towards a historical baseline, and have been practiced for over a century (Kleiman 1989). In particular, there has been a pronounced increase in the number of reintroduction programs, rising from 124 species in the early 1990s to 424 species in 2005 (Seddon et al. 2014). However, even in the absence of obvious barriers to population reintroduction, less than a quarter of reintroduction programs are successful at restoration (Fischer and Lindenmayer 2000). To increase success, a better understanding of the factors contributing to the outcome of reintroduction programs is needed.

A number of guidelines and best practices for reintroduction programs have emerged, which largely focus on habitat quality and the demographics and logistics of translocation (Montalvo et al. 1997; Armstrong and Seddon 2008). For example, these guidelines indicate that population reintroduction should only be considered if the original causes of

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[*] A part of this chapter (up to Atlantic salmon in Lake Ontario) is in review: Houde ALS, Garner SR, Neff BD. 2015. Restoring biodiversity through reintroductions: strategies for source population selection. *Restor Ecol,* in review.

the extirpation have been addressed and the habitat is again capable of supporting the species; otherwise habitat restoration is advised (Beck et al. 1994; Dobson et al. 1997; Palmer et al. 1997; Cochran-Biederman et al. 2015). Other guidelines suggest avoiding source populations that could suffer from deleterious genetic effects such as inbreeding depression or domestication (Montalvo et al. 1997; Weeks et. al 2011). Inbreeding depression may occur in small source populations when fitness-related traits (e.g. survival and reproductive traits) are reduced by inbreeding, and typically results from either the expression of deleterious recessive alleles or the loss of diversity at loci where heterozygosity is advantageous (Allendorf et al. 2013). Source populations may also be impaired by domestication selection that can result in the accumulation of alleles that are deleterious to individuals released back into the wild (Allendorf et al. 2013). Domestication selection may be especially problematic when a population has had multiple generations of captive breeding (Lynch and O'Hely 2001; Araki et al. 2007). These recommendations on the genetics of source populations have largely been incorporated into reintroduction programs (Armstrong and Seddon 2008; Weeks et al. 2011; IUCN 2013).

Despite potentially major effects on the outcome of reintroduction programs, few clear guidelines exist on how to optimally select source populations for translocation (see Cochran-Biederman et al. 2015). Based on case studies reviewed by the International Union for Conservation of Nature (Soorae 2008, 2010, 2011), reintroduction programs typically select one source population for reintroduction based on: (1) the only remaining source population; (2) a source population of sufficient size that should not have a reduction in viability if individuals were removed for translocation; or (3) the closest

geographic source population to the reintroduction location. However, I propose that source population selection based on an evolutionary and ecological perspective could greatly improve the success of reintroduction programs, and the strategies for identifying these source populations are part of the focus of this introductory chapter. Previous work on source population selection can be broadly categorized into the PRE-EXISTING ADAPTATION STRATEGY, which focuses on populations with a high frequency of genotypes that confer adaptations (i.e. high fitness) in the reintroduction location, or the ADAPTIVE POTENTIAL STRATEGY, which focuses on populations with high heritable genetic variation that confer the potential to adapt (i.e. respond to selection pressures) in the reintroduction location. Here I review the theoretical and empirical support for these two strategies and develop needed recommendations for selecting source populations.

1.2   Pre-Existing Adaptation Strategy

Source populations may differ in their viability in the reintroduction location because of genetically-based differences in individual fitness resulting from local adaptation. Local adaptation is a genotype by environment pattern in which the genotypes of local individuals have higher fitness in their local environment than they do in a foreign environment (Kawecki and Ebert 2004). Local adaptation can occur when local environments differ among populations of a species, resulting in different natural selection pressures. Provided gene flow among populations is restricted, genetically-based differences in individual fitness can accumulate among the populations. Local adaptation can be driven by a wide range of key environment features, including temperature, competitors, predators, prey type, parasites, and pathogens (for reviews in plants see Lesica and Allendorf 1999; Anderson et al. 2011; Savolainen et al. 2007;

marine invertebrates: Sanford and Kelly 2011; lepidopterans: Aardema et al. 2011; salmonids: Taylor 1991; Garcia de Leaniz et al. 2007). For example, both colder and warmer temperatures relative to the local environment can reduce the survival and growth of translocated trees (Savolainen et al. 2007).

If source populations with adaptations— i.e. a high frequency of genotypes that confer high fitness— to the key environment features of the reintroduction location can be identified, targeting those populations for translocation can increase the success of reintroduction programs. Knowledge of local adaptation could therefore serve as a basis for identifying source populations with adaptations to the key environment features of the reintroduction location. Local adaptation is both taxonomically and geographically widespread, with fitness advantages of local populations observed in 71% of reciprocally translocated plants and animals and the fitness advantage averaging 45%, meaning that the fitness of local individuals was on average 45% greater than the fitness of foreign individuals (Hereford 2009). The fitness advantage tends to be positively correlated with the genetic similarity and environment similarity between the source and foreign locations (Raabová et al. 2007; Hereford 2009; Fraser et al. 2011). That is, genetically or environmentally dissimilar source populations tend to show lower individual fitness when translocated into a foreign location than similar source populations. Geographically close source populations also tend to show higher inidivudal fitness in foreign locations, although this relationship likely arises as a by-product of genetic and environment similarity, as both decrease with increasing geographic distance. Identifying source populations with adaptations to the key environment features of the reintroduction location can therefore be accomplished using genetic or environment similarity. I term

these two approaches (i) ancestry matching and (ii) environment matching, which are not mutually exclusive.

1.2.1 Ancestry Matching Approach

Using an ancestry matching approach, a source population is selected for translocation based on genetic similarity to the extirpated population. This approach is based on the premise that close genetic relatives could share genes that confer adaptations to the key environment features of the reintroduction location. The same genes may occur in both the source and extirpated populations because they were present in a recent common ancestor or were transferred between populations through gene flow (Moritz 1999). Reintroduction programs could use historical samples of the extirpated population, if available, and collect samples from source populations to directly measure genetic similarity. Similarity is typically estimated from phylogenetic relationships or historical gene flow using similarity at genetic markers (for methods see Goudet 1995; Holder and Lewis 2003). Often several unlinked genetic markers, such as microsatellite loci or single nucleotide polymorphisms (SNPs) need to be used to provide sufficient resolution for estimating the genetic similarity between populations (Beaumont and Nichols 1996; Parker et al. 1998). Alternatively, geographic distance between the source and foreign locations can be used as a proxy for genetic similarity as there is often a correlation between the two variables (e.g. r = 0.22-0.52 for two studies on plants, Montalvo and Ellstrand 2000; Raabová et al. 2007); albeit, direct estimates of genetic similarity had a stronger relationship with the fitness-related traits of translocated populations than geographic distance in these two studies.

1.2.2 Environment Matching Approach

Using an environment matching approach, a source population is selected for translocation based on environment similarity between the source and reintroduction locations. Locations containing similar key environment features tend to produce individuals with similar phenotypes, either through selection on the same genes (e.g. Campbell and Bernatchez 2004; Turner et al. 2010; Schumer et al. 2011) or on different genes that produce similar phenotypes (e.g. Hoekstra and Nachman 2003; Nachman et al. 2003; Campbell and Bernatchez 2004; Hoekstra et al. 2006). Regardless of the underlying mechanism, reintroduction programs could measure the similarity of key environment features between source and reintroduction locations. Analysis of similarity is typically accomplished using distance matrices constructed of measurements of the key environment features (for methods see Montalvo and Ellstrand 2000; Raabová et al. 2007; Lawrence and Kaye 2011). Geographic distance between the source and foreign locations can also be used as a proxy for environment similarity when there is expected to be a correlation between the two variables (e.g. $r = 0.22$-0.75 in Montalvo and Ellstrand 2000; Raabová et al. 2007); albeit, direct estimates of environment similarity had a stronger relationship with the fitness-related traits of translocated populations than geographic distance in these two studies (also see Lawrence and Kaye 2011).

1.3   Adaptive Potential Strategy

The second strategy for selecting source populations is to emphasize the potential to adapt to the key environment features of the reintroduction location. This strategy favours the translocation of source populations with high heritable genetic variation. The

evolutionary response (R) to selection is based on the selection pressure (S) and the amount of heritable genetic variation (h^2) underlying the phenotype ($R = Sh^2$; Falconer and Mackay 1996). That is, for a given selection pressure, such as that exerted by a key environment feature, there is a stronger evolutionary response (genetically induced change in phenotype) when there is a higher amount of heritable genetic variation underlying phenotypes. An association between the amount of heritable genetic variation and the potential to adapt is supported by laboratory populations of *Drosophila melanogaster* (Reed et al. 2003). Also, the amount of heritable genetic variation is associated with local persistence for metapopulations of butterflies (*Melitaea cinxia*) (Saccheri et al. 1998). Two approaches that provide high heritable genetic variation are translocations of individuals from (i) a single source population that has high heritable genetic variation and (ii) multiple source populations that are genetically or environmentally dissimilar from each other.

1.3.1 Single Source Population Approach

Using a single source population approach, a source population is selected for translocation because it possesses a high amount of heritable genetic variation. This approach typically assumes that heritable genetic variation scales with neutral genetic variation, which is supported in laboratory populations of *Drosophila* (Briscoe et al. 1992). Genetic markers can be used to estimate the amount of within-population neutral genetic variation using indices such as heterozygosity, allelic richness, or the proportion of polymorphic loci (for methods see Excoffier and Heckel 2006). Population size can sometimes be used as a proxy for the amount of neutral genetic variation because of a correlation between the two variables ($r = 0.7$ for animal populations, reviewed by

Frankham 1996), assuming the population has not experienced a bottleneck otherwise there may be a weak correlation between these two variables (Reed and Frankham 2001). However, one concern with using neutral genetic markers (or population size) is that there might be no relationship between the amount of neutral and heritable genetic variation (Reed and Frankham 2001). Alternatively, quantitative genetic methods can be used to estimate the amount of heritable genetic variation for survival and fitness-related traits using a parent-offspring correlation or an analysis of variance of offspring traits produced using specific breeding designs (for methods see Falconer and Mackay 1996; Lynch and Walsh 1998). Although, such analyses are often costly and infrastructure-intensive, they have an advantage of being able to target specific traits that are thought to be important for fitness (e.g. Puurtinen et al. 2009).

1.3.2 Multiple Source Populations Approach

Using a multiple source population approach, two or more source populations with distinctive genetic or environmental backgrounds are selected for translocation, which combined as a mixed-source group should produce a high amount of heritable genetic variation. Distinctive source populations can be identified based on genetic and environment dissimilarity, using methods similar to those described for identifying ancestry and environment matches. However, the multiple source population approach is associated with two major concerns.

First, translocations from multiple source populations may result in inter-population breeding, which can lead to outbreeding depression or hybrid breakdown (Lesica and Allendorf 1999; Weeks et al. 2011; IUCN 2013; Cochran-Biederman et al. 2015),

especially given the distinctive genetic or environmental backgrounds of the source populations (Edmands 2007). Outbreeding depression may arise in hybrids because of genetic incompatibilities between populations (Lynch 1991; Neff 2004; Neff et al. 2011) and may not be detected until at least the second generation of inter-population breeding (Edmands 2007). For example, outbreeding depression led to reduced growth of second-generation inter-population hybrids when multiple source populations of slimy sculpin (*Cottus cognatus*) were translocated into Minnesota as part of a reintroduction program (Huff et al. 2011).

Second, the multiple source population approach is essentially a bet-hedging strategy, and, as such, provides little mechanistic insight into the factors that influence the outcome of reintroduction programs. For example, a mixed-source group by chance may contain an ancestry match or an environment match that has high fitness not because of adaptive potential, but because of pre-existing adaptations in the reintroduction location. Post-translocation monitoring could reveal a single source population with higher fitness and might aid such mechanistic analysis. Although reintroduction programs would indeed benefit from focussing on this single source population after the initial translocation, if the knowledge of how to select the population was available *a priori*, the fitness of initially translocated individuals could be increased relative to using individuals from multiple source populations. Some caution is warranted when using the multiple source populations approach because of concerns of outbreeding depression and delayed or lack of identification of a single best source population.

1.4 Atlantic Salmon in Lake Ontario

Atlantic salmon (*Salmo salar*) in Lake Ontario provide an ideal study species to examine source population selection approaches for reintroducing extirpated populations. Reports suggest that Lake Ontario Atlantic salmon were so abundant that people could walk on their backs during upstream migration (MacCrimmon 1977), harvest individuals with pitchforks and clubs, and harvest over one thousand individuals in a night (Whitcher and Venning 1869), indicating that Lake Ontario Atlantic salmon was a large freshwater fishery. Lake Ontario Atlantic salmon were extirpated by 1898, mainly because of habitat degradation (Crawford 2001). Dams blocked adults from accessing suitable spawning habitat, thus forcing adults to spawn in unsuitable areas (Wright 1892). Pollution from agriculture and mill runoff increased siltation of the spawning sites causing the suffocation of developing eggs (Wilmot 1878; 1882). Deforestation increased water temperatures to intolerable levels (Wilmot 1882). Finally, overfishing with trap nets and other devices removed large amounts of adults that had the potential to reproduce (Wilmot 1869).

The Lake Ontario habitat has been revitalized such that many of the original factors leading to the extirpated have been largely addressed (Beeton 2002). Lake Ontario and its tirbutaries also currently supports ecologically-similar salmonid species, but recent attempts to reintroduce Atlantic salmon using one source population have yet to succeed in establishing a self-sustaining population (Stewart and Schaner 2002; COSEWIC 2006, 2010). Although there has been restoration to ameliorate the environment of Lake Ontario and its tributaries, the current environment is still quite different from its historical conditions (Beeton 2002, see summary in Table 1.1). Recent environmental

changes in Lake Ontario and its tributaries are likely impeding a successful reintroduction and two additional source populations are being used for reintroduction efforts (COSEWIC 2006). In particular, two key environment features of Lake Ontario and its tributaries have been identified as likely impediments to a successful reintroduction of Atlantic salmon: (1) the presence of introduced non-native salmonid species and (2) the presence of introduced high thiaminase-containing prey fishes that lead to a thiamine deficiency (Dimond and Smitka 2005; COSEWIC 2006, 2010).

Recently introduced non-native salmonids are likely to be detrimental to Atlantic salmon in Lake Ontario, its tributaries, and in general (Dimond and Smitka 2005; COSEWIC 2006, 2010). Beginning in the 1860s, millions of these non-native salmonids were introduced to Lake Ontario and its tributaries to provide a fishery and to decrease overpopulated prey fishes, specifically alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) (Parsons 1973; Crawford 2001; Beeton 2002; Kerr 2006). These include the Pacific salmonids– Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), rainbow trout (*O. mykiss*), and one European salmonid– brown trout (*S. trutta*) (Stanfield et al. 2006). Throughout their evolutionary history, North American populations of Atlantic salmon have not co-occurred with any of these non-native salmonid species until recently and although Atlantic salmon and brown trout are broadly sympatric in Europe, North American populations of Atlantic salmon diverged approximately 600,000 - 700,000 years ago (King et al. 2007). Because Atlantic salmon do not naturally coexist with these non-native salmonid species they may be exposed to stronger competition if living in sympatry (Hearn 1987; Fausch 1988).

Table 1.1. Summary of Environmental Changes in Lake Ontario and its Tributaries and their Anticipated Effect on Atlantic Salmon (*Salmo salar*). The presence of introduced non-native salmonid species and high-thiaminase containing prey fishes (i.e. alewife and rainbow smelt) have been identified as two key environment features of Lake Ontario and its tributaries that are likely impediments to a successful reintroduction of Atlantic salmon (Dimond and Smitka 2005; COSEWIC 2006, 2010).

Change in the environment	Additional details	Anticiapted effect on Atlantic salmon	Extent of the change to the environment
Eutrophication	-run-off from agriculture -sewage waste from cities -phosphate detergents	-increased adult mortality because of low dissolved oxygen -increased adult mortality because of increased risk of infection	habitat restoration has reduced the magnitude of change
Land-use	-dams -forestry -agriculture -urbanization	-increased juvenile mortality due to loss of tributary habitat and changes in hydrology	habitat restoration has reduced the magnitude of change
Overfishing	-recreational and commercial fishing	-increased adult mortality because direct fishing of salmon	reduced commercial fisheries has reduced the magnitude of change
Invasive species	-sea lamprey -zebra and quagga mussels -round goby -alewife -rainbow smelt	-increased adult mortality -thiamine deficiency in adults because of thiaminase in introduced prey fishes	currently a large change
Introduced species	-brown trout -rainbow trout -Chinook salmon -coho salmon	-increased mortality due to interspecific competition	currently a large change
Pollution	-chlorinated organics -mercury	-increased mortality because of reduced health	although there is limited current input, still a change because of persistent effects from historical input
Climate change	-temperature increase	-increased mortality because of low water levels and dissolved oxygen	projected change in the future

Of the introduced non-native salmonids, brown trout and rainbow trout have similar habitat preferences to Atlantic salmon for riffle microhabitats in nursery streams and tend to be more aggressive than Atlantic salmon (e.g. Gibson 1981; Scott et al. 2005). In contrast, Chinook salmon and coho salmon prefer pool microhabitats in nursery streams and exhibit comparable aggression as Atlantic salmon (e.g. Heland and Beall 1997; Holecek et al. 2009). Based on the high ecological overlap (Hutchinson 1957) and differences in levels of aggression (Holway and Suarez 1999), it is thus predicted that competition with brown trout and rainbow trout, rather than with Chinook salmon and coho salmon, will have the biggest impact on survival and fitness-related traits of juvenile Atlantic salmon.

In addition, the introduction of high thiaminase-containing prey fishes is likely to be detrimental to Atlantic salmon. Thiaminase is an enzyme that breaks down thiamine (vitamin B1) (Brown et al. 2005). Thiaminase occurs naturally and can be found in large quantities in certain prey fishes. Historically, low thiaminase-containing lake herring or cisco (*Coregonus artedi*) and bloater (*C. hoyi*) were the dominant prey fishes for Atlantic salmon in Lake Ontario (Fitzsimons et al. 1998). After cisco and bloater populations declined because of overfishing and environmental changes (Beeton 2002), high thiaminase-containing alewife and rainbow smelt were introduced to increase prey fish populations for predatory fishes (Fitzsimons et al. 1998; Crawford 2001). Alewife and rainbow smelt eventually replaced cisco and bloater as the dominant prey fishes in the diet of salmonids in Lake Ontario (Dimond and Smitka 2005). Similarly, the recently introduced round goby (*Neogobius melanostomus*) has been increasing in the diet of Lake Ontario salmonids and contains variable (low to high) thiaminase content (Tillit et al

2005; Honeyfield et al. 2012). Atlantic salmon consuming high thiaminase-containing prey fishes can develop a thiamine deficiency, which is associated with 'wiggling' behaviour and the loss of equilibrium that can be fatal because of a reduced ability to feed and migrate (Brown et al. 2005; Fitzsimons et al. 2005). Mature females may also pass on the thiamine deficiency to offspring via her eggs, resulting in significant offspring mortality and, in some cases, a complete reproductive failure (Fisher et al. 1996; Ketola et al. 2000). Similarly, mature males may have reduced reproductive function because of reduced spermatogenesis (Gangolf et al. 2010) and decreased offspring survival because of an unidentified change in sperm quality (Koski 2002). Interestingly, recent evidence suggests that alewife, present at the time of the historical Atlantic salmon population decline, may have contributed to the extirpation of this population because of a thiamine deficiency (Smith 1892; Smith 1995). These factors together may result in thiamine deficiency being the primary factor impeding a successful reintroduction of Atlantic salmon into Lake Ontario because it can cause high mortality and low reproductive success.

Certain populations of Atlantic salmon may be better able to cope with the two features that are likely impeding a successful reintroduction into Lake Ontario, i.e. non-native salmonids and high thiaminase-containing prey fishes. Populations of salmonid species may have genetic differences in behaviour because of differences in their local environments, such as the intensity of predation (Rosenau and McPhail 1987; Swain and Holtby 1989; Houde et al. 2010; Van Zwol et al. 2012), which may alter competitive ability. For example, populations that show increased aggression (Holway and Suarez 1999) or avoid agonistic interactions (Metcalfe 1986) may be better at competing with

non-native salmonids. Similarly, there may be differences among populations in their ability to process diets that are high in thiaminase. Although there is a clear link between the consumption of high thiaminase-containing prey fishes and the development of a thiamine deficiency (Honeyfield et al. 2005), it is less clear to what extent the ability to cope with ingested thiaminase varies within and among populations of salmonid species. For example, some freshwater resident populations of Atlantic salmon primarily consume rainbow smelt, yet do not appear to display a thiamine deficiency (Dimond and Smitka 2005). Also, the extent of thiamine deficiency symptoms varies among Atlantic salmon individuals from Saint-Mary's River, Michigan (Dimond and Smitka 2005), as well as coho salmon individuals from Platte River, Michigan (Brown et al. 2005) that typically consume alewife. These data suggest there may be some degree of variation in thiaminase tolerance both within and among populations.

1.5   Source Populations

Three source populations of Atlantic salmon are being used for reintroduction efforts into Lake Ontario: LaHave River (LaHave) from Nova Scotia, Sebago Lake (Sebago) from Maine, and Lac Saint-Jean (Saint-Jean) from Quebec (Dimond and Smitka 2005). A summary of the three source populations is presented in Table 1.2 and Figure 1.1. Because key environment features of Lake Ontario and its tributaries have changed relative to historical conditions, evolutionary and ecological theory suggests selecting source populations using an environment matching versus an ancestry matching approach. An environment match should possess a high frequency of genotypes that confer adaptations (i.e. high fitness) to the new conditions in the reintroduction location (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). An ancestry match may not

necessarily possess this high frequency of genotypes that confer adaptations if key environment features have changed from historical conditions (Krueger et al. 1981; Seddon and Soorae 1999; IUCN 2013). Greater details on a perspective source population selection framework are presented in Chapter 7. In addition, the adaptive potential strategy, such as the single source population approach or the multiple source population approach, could also be considered for the reintroduction efforts because of the ability of source populations to adapt to new selection pressures (Krueger et al. 1981; IUCN 2013). The simultaneous translocation of the three source populations is considered the multiple source population approach, given the divergent genetic and environment backgrounds of these populations (King et al. 2001; Dimond and Smitka 2005). At the time these populations were selected by the Ontario Ministry of Nartural Resources and Forestry (OMNRF), there was no information on the amount of within-population heritable genetic variation, which could be used for considering the single source population approach.

The LaHave population has been the focus of reintroduction efforts since the 1990s. However, this populations was primarily selected because it was readily available (Dimond and Smitka 2005; Kerr 2006) rather than based on any specific criteria. Due to this, the LaHave population may not be the most suited for translocation into Lake Ontario because it is not an environment match to both features, nor is it an ancestry match. That is, LaHave River does not contain non-native salmonids, alewife and rainbow smelt are not the primary diet in this population (Dimond and Smitka 2005), and it is not a close genetic relative to the historical population (King et al. 2001). The LaHave population is anadromous (Dimond and Smitka 2005) and anadromous Atlantic

salmon consume capelin (*Mallotus villosus*), sand eels (Ammodytidae), krill (Euphausiacea), and amphipods (Amphipoda) (Rikardsen and Dempson 2011), a more diverse diet which presumably contains low thiaminase concentrations. The LaHave population was imported into Ontario from 1989 to 1995 as fertilized eggs from single-pair matings of wild adult LaHave salmon in LaHave River (43°53'N, 70°27'W), a naturally reproducing river during the period of import.

On the other hand, the Sebago population may be more suitable for translocation into Lake Ontario. Although the Sebago population is not an ancestry match, this population could be an environment match to both features. That is, the Sebago population is not a close genetic relative to the historical Lake Ontario population (King et al. 2001), and stocked Sebago salmon appear to be doing well in Lake Champlain where there is rainbow trout and brown trout as well as rainbow smelt and alewife (LCSG 2006; Marsden et al. 2010). The Sebago population was selected for Lake Champlain because two independent assessments by New York and Vermont of stocked Atlantic salmon from three landlocked source populations (Sebago Lake, Lake Memephremagog in Vermont and Quebec, and West Grand Lake in Maine) found that the Sebago population had the highest performance (Dimond and Smitka 2005). Admittedly, Sebago Lake does not contain non-native salmonids or alewife (Dimond and Smitka 2005), so this population is not a direct environment match using the criterion of environment similarity. Also, the Sebago population is potamodromous and primarily consumes rainbow smelt in Sebago Lake (Dimond and Smitka 2005), as well as recently introduced alewife in Lake Champlain (LCSG 2006). The Sebago population was imported into Ontario in 2006 as fertilized eggs from single-pair matings of wild adult Sebago salmon

in Panther River (43°53'N, 70°27'W), a tributary of Sebago Lake and a hatchery supplemented river.

Similarly, the Saint-Jean population may also be more suitable for translocation into Lake Ontario. The Saint-Jean population is an environment match to one of the two features and is likely an ancestry match. That is, Lac Saint-Jean contains rainbow smelt but does not contain non-native salmonids or alewife (Dimond and Smitka 2005), and the Saint-Jean population, specifically Métabetchouane River and Rivière aux Saumons, is believed to share the same glacial refugium as the historical Lake Ontario population, albeit the populations would have been separated by at least 8,600 years following the colonization of the two different lakes (Tessier and Bernatchez 2000). The Saint-Jean population is potamodromous and primarily consumes rainbow smelt. The Saint-Jean population was imported into Ontario in 2007 as fertilized eggs from single-pair matings of wild adult Saint-Jean salmon in Rivière-aux-Saumons (48°41'N, 72°30'W), a tributary of Lac Saint-Jean and a naturally reproducing river.

Table 1.2. Ecological and Genetic Information on the Three Source Populations of Atlantic Salmon (*Salmo salar*). Competition with non-native salmonids and consuming high thiaminase-containing prey fishes that lead to a thiamine deficiency are identified as likely impediments to a successful reintroduction of Atlantic salmon into Lake Ontario (Dimond and Smitka 2005). Sebago Lake information is for the group that was stocked into Lake Champlain. An anadromous ability means that the adult Atlantic salmon have access to the Atlantic Ocean but the majority are believed to remain in the freshwater lake.

	Historical Lake Ontario	Lac Saint-Jean, Quebec	Sebago Lake, Maine	LaHave River, Nova Scotia
potamodromous	✓ (mostly)	✓	✓	✗
anadromous	ability	ability	✗	✓
genetic similarity	✓	✓	✗	✗
Competition				
rainbow and brown trout	✗	✗	✓	✗
coho and Chinook salmon	✗	✗	✗	✗
Thiamine deficiency				
rainbow smelt	✗	✓	✓	✗
alewife	✗	✗	✓	✗
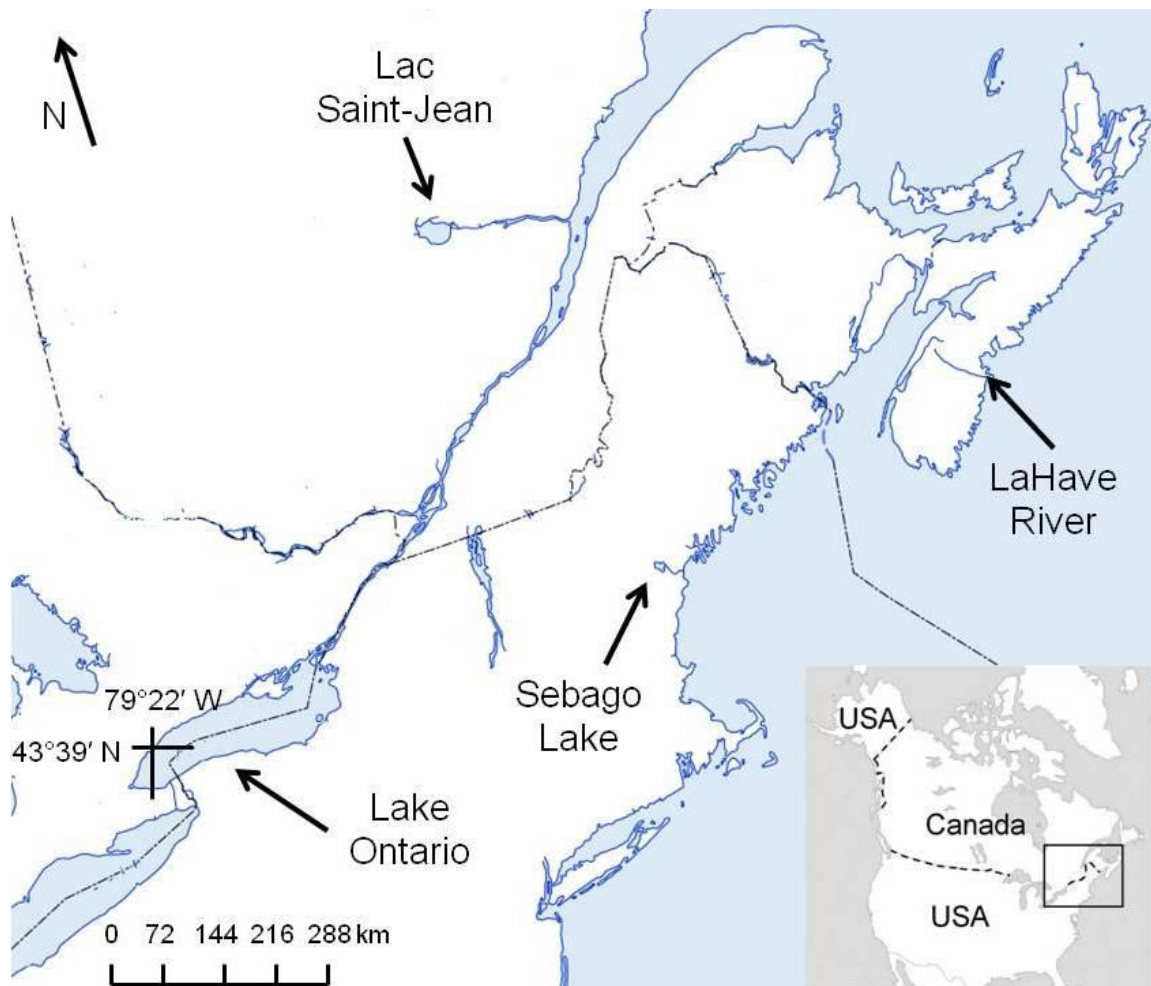
Figure 1.1. Locations of the Extirpated Lake Ontario Population and the Three Source Populations of Atlantic Salmon (*Salmo salar*) being Used for Reintroduction Efforts.

Based upon the environment matching approach for selecting source populations, it is predicted that overall the Sebago population will have the highest performance (i.e. survival and fitness-related traits), followed by the Saint-Jean population, then the LaHave population when exposed to both features in experimental settings. Specifically, the Sebago population will do well with non-native salmonids and a high thiaminase diet, the Saint-Jean population will do well with a high thiaminase diet but not non-native salmonids, and the LaHave population will not do well with both non-native salmonids and a high thiaminase diet. In more detail, stocked Sebago salmon appear to be doing well in Lake Champlain where there is brown trout and rainbow trout as well as rainbow smelt and alewife (LCSG 2006; Marsden et al. 2010). The Saint-Jean population primarily consumes rainbow smelt and should do just as well as the Sebago population that also consumes primarily rainbow smelt in Sebago Lake (Dimond and Smitka 2005), as well as recently introduced alewife in Lake Champlain (LCSG 2006), when exposed to a high thiaminase diet. However, it is unknown whether the Saint-Jean or LaHave population have the potential to do well with the presence of non-native salmonids in contrast to the Sebago population (Dimond and Smitka 2005). In addition, the LaHave population primarily consumes a diversity of prey species (Rikardsen and Dempson 2011), a diet that may be low in thiaminase, suggesting that it may not be do well if exposed to a high thiaminase diet.

Also, the adaptive potential strategy could be considered for selecting source populations. The OMNRF is currently translocating all three source populations into Lake Ontario (Wilson 2014), which is the multiple source population approach. However, at the time these populations were selected by the OMNRF, there was no information on the amount

of within-population heritable genetic variation of survival and fitness-related traits, which could be used for considering the single source population approach. Measuring the amount of heritable genetic variation of these traits could be used to predict which of the three source populations may have the potential to adapt to new selection pressures in Lake Ontario.

1.6   Objectives and Thesis Structure

My overall objective is to evaluate the relative performance (i.e. survival and fitness-related traits) of the three source populations of Atlantic salmon in the context of suitability for translocation into Lake Ontario. The two key environment features that are likely impeding a successful reintroduction of Atlantic salmon are: (1) the presence of non-native salmonids and (2) the presence of high thiaminase-containing prey fishes. Experiments are a useful way to compare the relative performance of different source populations exposed to key environment features at small scales, such as laboratory settings and natural sites in the reintroduction location, prior or simultaneously to considering the source populations for large scale reintroduction efforts (e.g. van Katwijk et al. 2009). Here, I examine three source populations that differ in the degree of environment match to two features of Lake Ontario and its tributaries. I measured the relative performance of these three source populations when exposed to non-native salmonids and a high thiaminase diet. The environment matching approach may be supported if overall the Sebago population has the highest performance, followed by the Saint-Jean population, then the LaHave population. I also measured the amount of within-population heritable (additive) genetic variation for survival and fitness-related traits at early-life history stages that were exposed to water from a tributary of Lake

Ontario. If considering the single source population approach for selecting source populations, this population would be identified as the one with the highest amount of heritable genetic variation.

This thesis contains six data chapters. In Chapter 2 ("Competitive Interactions among Multiple Non-Native Salmonids and Three Populations of Atlantic Salmon") I placed Atlantic salmon juveniles into artificial streams with four species of non-native salmonids to examine the effects of interspecific competition in a controlled environment. Because Atlantic salmon may be exposed to more than one non-native salmonid species in tributaries of Lake Ontario, in Chapter 3 ("Predictability of Multi-Species Competitive Interactions in Three Populations of Atlantic Salmon") I build on Chapter 2 by examining whether there are non-additive competitive interactions in a multi-species treatment, i.e. whether the observed multi-species effects can be predicted by a simple additive model of the effects from two-species treatments. In Chapter 4 ("Competitive Effects between Rainbow Trout and Two Populations of Atlantic salmon in Natural and Artificial Streams") I placed Atlantic salmon juveniles into two natural stream sites differing in the presence of rainbow trout to examine the effects of interspecific competition and also compare these results to the artificial streams (Chapter 2). In Chapter 5 ("Effects of Feeding High Dietary Thiaminase to Sub-Adult Atlantic Salmon from Three Populations") I fed sub-adult Atlantic salmon a diet mimicking the high thiaminase concentrations of prey fishes in Lake Ontario to examine the effects of thiamine deficiency. In Chapter 6 ("Genetic Architecture of Survival and Fitness-Related Traits in Three Populations of Atlantic Salmon") I describe a full-factorial quantitative genetic breeding design and analysis to quantify the amount of heritable (additive) genetic

variation for survival and fitness-related traits at early-life history stages. In Chapter 7 ("Restoring Biodiversity through Reintroductions: Approaches for Source Population Selection") I provide a literature review of studies examining the different source population selection approaches and provide a perspective source population selection framework.

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Chapter 2

2 Competitive Interactions among Multiple Non-Native Salmonids and Three Populations of Atlantic Salmon[*]

2.1 Introduction

The introduction of non-native species is one of the leading causes of native species extinctions and declines (Cox 2004; Clavero and García-Berthou 2005). Non-native species can negatively impact native species by increased predation, competition, parasites, habitat alteration, and hybridization (Gurevitch and Padilla 2004). For example, introductions of rabbit (*Oryctolagus cuniculus*) and red foxes (*Vulpes vulpes*) have caused extinctions of native rodent species in Australia (Smith and Quin 1996). Similarly, worldwide introductions of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) have caused declines in native salmonids (Korsu et al. 2010). In addition, life-history traits, such as body size and growth rate, are commonly impacted by non-native species. For example, non-native plants have reduced the body mass of native grasses in North America and Europe (Callaway and Aschehoug 2000). Similarly, the presence of non-native salmonids leads to a reduced growth and foraging rate of native salmonids (Korsu et al. 2010) and are considered an impediment to rehabilitation of native galaxiid fishes in the Southern Hemisphere (McDowall 2006).

Measures of the endocrine system have also been used to provide insight about the sublethal effects that non-native species can have on native species. Competitive

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[*] Versions of this chapter have been published (year one) or are in review (year two): Houde ALS, Wilson CC, Neff BD. 2015. Competitive interactions among multiple non-native salmonids and two populations of Atlantic salmon. *Ecol Freshw Fish* 24:44-55. Houde ALS, Wilson CC, Neff BD. 2015. Effects of competition with four non-native salmonid species on Atlantic salmon from three populations. *Trans Am Fish Soc*, in review.

agonistic interactions with non-native species can be a source of chronic stress for native species (Sloman et al. 2001). The endocrine response for dealing with stress is to increase circulating glucocorticoids (Nelson 2011), such as cortisol in fishes (e.g. Wendelaar Bonga 1997; Iwama et al. 2004). An increase in glucocorticoids can be adaptive in the short-term for acute stressors because of the benefits of increased cardiovascular tone and energy availability (Wendelaar Bonga 1997). However, the increase can be detrimental in the long-term for chronic stressors because of the costs of lower disease resistance, growth, and reproduction (Pickering and Pottinger 1989). Losing agonistic interactions can also lead to reduced circulating androgens (Wingfield et al. 2001), such as 11-ketotestosterone (11-KT) in fishes (Oliveira et al. 2009). A decrease in androgens can cause reductions in aggression level and social status that can subsequently lead to lower survival and growth (Huntingford et al. 1990; Nelson 2011).

Salmonid fishes are an important group to examine the effects of introduced or invasive non-native species on native taxa. Several salmonid species have been introduced globally to provide fisheries (Crawford and Muir 2008), which has created new competitive interactions with ecologically-similar native salmonids (Hearn 1987; Fausch 1988). In particular, the juvenile life stages of salmonids are highly competitive periods, as feeding territories are typically limited in nursery streams and individuals aggressively defend those territories (Kalleberg 1958). Survival in juvenile salmonids within nursery streams is often correlated with higher social rank and aggression level, as measured by circulating 11-KT (Oliveira et al. 2009), presumably because these traits are beneficial in acquiring better feeding territories (Fausch 1984; Metcalfe 1986; Ruzzante 1994; Harwood et al. 2003). Individuals with higher social status and aggression level also tend

to be larger (Huntingford et al. 1990) and have lower levels of circulating cortisol (Øverli et al. 1999; Consten et al. 2002; Øverli et al. 2004), suggesting that they are not chronically stressed.

Here, I examine the survival and fitness-related traits of juvenile Atlantic salmon (*Salmo salar*) from three source populations in artificial stream tanks with varying extents of competition from juveniles of four non-native salmonid species: brown trout (*S. trutta*), rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*), and coho salmon (*O. kisutch*). Natural stream sites may differ in environmental variables that can affect the outcome of competition but are not easily controlled, whereas artificial streams can control for these variables (see Fausch 1998). An artificial stream experiment was conducted over two independent years. In year one, I examined the effects of competition on age 0+ juveniles of the LaHave and Sebago populations only. In year two, I examined the third source population (Saint-Jean) at age 0+ for the first time. The Saint-Jean population was not included in year one because broodstock had not reached maturity. Examining the performance (i.e. survival and fitness-related traits) of all three populations may be useful for guiding reintroduction efforts because these efforts are currently stocking all three populations into Lake Ontario tributaries that contain non-native salmonids. In addition, Van Zwol et al. (2012a) examined the effects of competition on older (age 1+) juvenile Atlantic salmon in the same artificial stream tanks. I want to determine which non-native salmonids species are the most problematic over the two year duration freshwater stage to help strengthen Atlantic salmon translocation into Lake Ontario recommendations. My objective was to test three hypotheses: (1) Atlantic salmon performance in competition with non-native salmonid species will be

related to the degree of niche overlap and differences in aggression levels; (2) Sebago juveniles will have a better competitive ability and thus higher performance than LaHave and Saint-Jean juveniles and; (3) competition with non-native salmonid species will be a source of chronic stress and cause changes in the social status of Atlantic salmon.

2.2 Materials and Methods

2.2.1 Study Populations and Non-Native Salmonid Species

Juveniles of all salmonid species were provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF). Fertilized eggs from single-pair matings of wild adult LaHave (44°14′N 64°20′W) were received from 1989 to 1995 and captive generations were produced every year in Ontario starting in 1996 (OMNR 2005). Fertilized eggs from single-pair matings of wild adult Sebago in Panther River (43°53'N, 70°27'W), a hatchery supplemented river, were received in 2006. Fertilized eggs from single-pair matings of wild adult Saint-Jean in Rivière-aux-Saumons (48°41'N, 72°30'W), a naturally reproducing river, were received in 2007. For this study, LaHave and Sebago Atlantic salmon families were produced in early November 2010 and 2011 using mature individuals at the OMNRF Harwood Fish Culture Station, Harwood, Ontario. Five females and five males from each population were mated in all possible combinations to produce a 5×5 full factorial breeding design (Lynch and Walsh 1998). Fertilized eggs and the resultant offspring were reared at the OMNRF Codrington Research Facility, Codrington, Ontario. Saint-Jean families were also produced in early November 2011 using single-pair matings ($n = 66$) of mature individuals at Harwood. A random subset of

500 fry (age 0+ parr) from the Saint-Jean families was transferred to the Codrington Facility in the spring of 2012.

Rainbow trout and brown trout were from hatchery parents whose ancestry was derived from naturalized populations of both species in the Ganaraska River, Ontario (43°56'N 78°17'W) (OMNR 2005). Rainbow trout and brown trout families for this experiment were produced by eight single-pair matings at the OMNRF Tarentorus Fish Culture Station, Sault Ste. Marie, Ontario and OMNRF Harwood Fish Culture Station, respectively. Chinook salmon and coho salmon families were from wild parents from the Credit River, Ontario (43°33'N 79°34'W). Chinook salmon and coho salmon families were produced by 30-100 single-pair matings at the OMNRF Normandale Fish Culture Station, Vittoria, Ontario and OMNRF Ringwood Fish Culture Station, Ringwood, Ontario, respectively. Random subsets of 250 fry (age 0+ parr) each for brown trout, rainbow trout, Chinook salmon, and coho salmon were transferred from the various OMNRF fish culture stations to the Codrington Facility in the spring of 2011 and 2012. The fry of each species were held in two tanks (38 L, $n = 125$ fry) until used in the artificial stream tanks. All juveniles were of the same age and culture history as those currently stocked in Lake Ontario streams; thus, fry of these species differed in body size and are therefore representative of the size differences in natural streams (see Table 2.1).

Table 2.1. Summary of the Initial Sizes of Fry (age 0+ parr) for Three Populations of Atlantic Salmon (*Salmo salar*) and Four Non-Native Salmonid Species (Brown Trout- *S. trutta*, Rainbow Trout- *Oncorhynchus mykiss*, Chinook Salmon- *O. tshawytscha*, and Coho Salmon- *O. kisutch*). Presented are means ± 1SD. Different uppercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$). Sample sizes in year one are: $n = 256$ for each Atlantic salmon population; and $n = 144$ for each non-native salmonid species. Sample sizes in year two are: $n = 224$ for each Atlantic salmon population; and $n = 120$ for each non-native salmonid species. Saint-Jean juveniles were not examined in year one because mature individuals to produce offspring were not available.

Traits	Atlantic salmon populations			Non-native salmonid species			
	LaHave	Sebago	Saint-Jean	Brown trout	Rainbow trout	Chinook salmon	Coho salmon
Year one							
length (cm)	5.8 ± 0.4^{AD}	5.6 ± 0.5^{B}	-	6.0 ± 0.7^{C}	6.0 ± 0.6^{CD}	8.2 ± 0.7^{E}	8.5 ± 1.0^{F}
mass (g)	2.17 ± 0.49^{AB}	2.00 ± 0.51^{A}	-	2.43 ± 0.91^{B}	2.15 ± 0.69^{AB}	5.95 ± 1.74^{E}	6.68 ± 2.27^{F}
condition ($100 \times$ g / cm^3)	1.09 ± 0.06^{A}	1.14 ± 0.06^{B}	-	1.05 ± 0.05^{C}	0.98 ± 0.06^{D}	1.05 ± 0.10^{C}	1.05 ± 0.06^{C}
Year two							
length (cm)	6.5 ± 0.6^{A}	6.8 ± 0.6^{B}	6.8 ± 0.8^{B}	6.6 ± 0.7^{AB}	6.0 ± 0.6^{C}	9.8 ± 0.7^{D}	10.6 ± 0.8^{E}
mass (g)	2.97 ± 0.92^{AC}	3.70 ± 0.99^{B}	3.28 ± 1.32^{BC}	3.23 ± 1.08^{ABC}	2.28 ± 0.71^{D}	10.32 ± 2.18^{E}	14.22 ± 3.44^{F}
condition ($100 \times$ g / cm^3)	1.05 ± 0.05^{AD}	1.13 ± 0.05^{B}	0.99 ± 0.05^{C}	1.06 ± 0.05^{DF}	1.02 ± 0.07^{E}	1.07 ± 0.05^{F}	1.18 ± 0.05^{G}

2.2.2 Experimental Set-up

Artificial stream tanks (25 cm × 240 cm) were setup at the Codrington Facility and mimicked the natural stream environment by containing two types of microhabitats: a 160 cm riffle section (mean ± 1SD: high current 20 ± 6 cm s^{-1}, low depth 28 ± 3 cm) followed by a 80 cm pool section (low current 7 ± 3 cm s^{-1}, high depth 68 ± 3 cm). Substrate was composed of two parts gravel river rock (2 mm- 64 mm) and one part cobble river rock (65 mm- 256 mm). Fish were supplied water from a natural Lake Ontario tributary at natural temperatures (8.6 ± 2.6°C).

Seven different treatments were set up for juveniles from each Atlantic salmon population, each with a total of 32 juveniles, using a substitutive design to examine the effects of competition (see Fausch 1998). Treatments were: Atlantic salmon alone (32 LaHave only, Sebago only, or Saint-Jean only), two-species (16 Atlantic salmon with 16 of one non-native salmonid species), and multi-species (16 Atlantic salmon with 4 of all four non-native salmonid species). Each treatment was represented by two replicates. Because Saint-Jean Atlantic salmon families were not available in year one, an Atlantic salmon mixed (LaHave and Sebago together) and a non-native salmonid species 'alone' treatment (rainbow trout, brown trout, coho salmon, or Chinook salmon only) were setup in year one only. In September 2011 and 2012, fry (age 0+ parr) of each salmonid species were first anaesthetized with tricaine methanesulphonate (MS-222) and tagged by species with visual implant elastomers (Northwest Marine Technology, Washington) at the base of the dorsal and adipose fins (Olsen and Vollestad 2001). Random subsets of brown trout, rainbow trout, coho salmon, Chinook salmon, and Atlantic salmon fry were

selected for the treatments. Fry were measured for fork length (nearest 0.1 cm) and mass (nearest 0.01 g) before being transferred to the artificial stream tanks (Table 2.1).

The juveniles were kept in the artificial stream tanks for 10 months (September to July). Juveniles were subjected to a natural light cycle and fed a competition-inducing ration of 3% body mass per day (e.g. Garner et al. 2008) of commercial pellets at random times and amounts per day (Keenleyside and Yamamoto 1962; Symons 1968). The pellets were introduced at the upstream side of the artificial stream tanks because in natural streams juvenile salmonids compete for upstream positions to secure the first access to food (Metcalfe 1986). During the winter months, juvenile competition is typically reduced in natural streams because Atlantic salmon seek shelter underneath the substrate and reduce feeding in low water flow areas (Huntingford et al. 1988). Therefore, during the winter months (January to April), the food ration was reduced to 1% body mass per day.

2.2.3 Survival and Fitness-Related Traits

In year one, juvenile measurements in the artificial stream tanks were collected on October 28, November 29, and July 24, and in year two on November 11, December 17, May 29, June 26, and July 25. The dates coincide to when the juveniles were fed the ration of 3% body mass per day (i.e. September to December and May to July), but otherwise were left undisturbed (January to April). Juveniles were measured for survival and three fitness-related traits comprising body length, mass, and condition (Fausch 1984, 1998). I also measured riffle use (the preferred microhabitat of Atlantic salmon) (Morantz et al. 1987) in both years and downstream displacement (upstream positions are typically associated with the first access to food) in year two only (Metcalfe 1986). For body

length (fork length), mass, and condition, all juveniles were removed from the artificial stream tanks, lightly anaesthetized, measured and then allowed to recover before being returned to the artificial stream tank. Condition was calculated as $100 \times mass / length^3$ (Fulton 1904). In year one, for riffle use, a trained observer took counts of each salmonid species within the riffle section at 12:00 on the day after body size measurements, taking care to limit visual exposure to the juveniles. I also examined riffle use by taking photographs the day before body size measurements, but did not have the data for all measurement dates. I therefore concentrated my analysis on the observer data in year one. In year two, for riffle use and downstream displacement, digital photographs were taken three times during the day (morning, noon, and evening) every 80 cm within the artificial stream tanks using cameras (Sony HDRXR200V) supported on a rig. Photographs were analyzed using ImageJ version 1.38 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/). Riffle use was measured as the proportion of Atlantic salmon in the riffle section and downstream displacement was measured as the average of the distance downstream for each individual from the beginning of the riffle section.

2.2.4 Blood Samples and Circulating Hormone Concentrations

At the termination of the experiment, juveniles were starved for 24 hours and then quickly netted out of the artificial stream tanks. I collected as many Atlantic salmon individuals as possible within 2 minutes (median of 9, range 2 to 10 individuals per tank). Care was taken to minimally disturb the juveniles while netting. Atlantic salmon were quickly submerged in an overdose of anaesthetic (MS-222) until gill movement ceased, then immediately measured for length and mass, and blood collected from the caudal peduncle using a Heparin lined tube. The time from the initial disturbance of the

juveniles to blood collection was recorded for each Atlantic salmon. Care was taken to ensure that the entire process took less than 5 minutes per artificial stream tank (see Sumpter et al. 1986). Plasma was immediately separated in the blood by centrifugation (1,500 RCF for 5 minutes) and stored at -20°C until analysis (Van Zwol et al. 2012b).

For the hormone analysis, I randomly selected a median of 4 of the collected plasma samples (range 2-9) to be measured for hormone concentrations. Prior to the enzyme immunoassay for 11-ketotestosterone (11-KT), 10 μL plasma samples were extracted three times with 2.5 mL diethylether using a snap freeze method described by Van der Kraak et al. (1989). The diethylether was evaporated in a fume hood and then the samples were stored at -20°C until assayed (Van Zwol et al. 2012b). Plasma concentrations of cortisol and 11-KT were determined using the manufacturer's instructions for enzyme immunoassay kits (Cayman Chemical Company, Michigan). Briefly, 11-KT samples were reconstituted with assay buffer prior to the assay. Each sample was run in triplicate, with 50 μL (1/20 plasma dilution for cortisol and 11-KT) loaded into each well. Plates were read at an absorbance of 405 nm.

2.2.5 Statistical Analysis of Traits

Survival, length, mass, condition, riffle use, and circulating hormones concentrations of individual Atlantic salmon were analyzed in R 3.0.1 (available at http://www.r-project.org/). Statistical significance was set at $\alpha = 0.05$. In year one, there were no significant differences between the populations in the Atlantic salmon alone (LaHave only or Sebago only) and Atlantic salmon mixed (LaHave and Sebago together) treatments (data not shown); therefore, the juveniles from the mixed treatment were

pooled with their appropriate population in the "alone" treatment for comparisons to other treatments.

Due to the Atlantic salmon mortality over the winter, which led to differences in juvenile densities for May through July, individual traits were statistically examined at the 3 month mark (November 29 and December 17 in year one and two) and again at the 10 month mark (July 24 and July 25). Survival and riffle use data were logit transformed (Crawley 2005) and circulating hormones concentrations were natural log + 1 transformed to increase normality. Linear models were used to examine effects for survival and riffle use. Survival over time comparisons between the alone treatment and each inter-specific competition treatment were also examined using log-rank survival curve analysis (*survdiff* in the survival package of R) and the *p*-values for the multiple comparisons were corrected using false discovery rate. Linear mixed-effects models (*lmer* in the lme4 package of R) were used to examine effects for length, mass, condition, cortisol concentrations, and 11-KT concentrations of individuals. Because of the differences in the initial length, mass, and condition of each population in year two (see Table 2.1), these traits were standardized by subtracting the initial mean values for each population in all size analyzes for year two. In year one, initial sizes for the populations were more similar, thus standardization was not necessary. Atlantic salmon models contained fixed effects for *population*, *treatment*, and *population × treatment* and mixed-effects models contained a random effect for *artificial stream tank* identity.

2.3   Results

2.3.1 Survival

Significant treatment and population by treatment effects were detected for the survival of Atlantic salmon (Table 2.2 and 2.3; Figure 2.1 and 2.2). There was no difference among the populations in survival at either time point (3 or 10 months) or over time in either year (year one: $X^2 = 0$, df =1, $p = 0.831$ and year two: $X^2 = 1.2$, df =2, $p = 0.550$). Atlantic salmon had lower survival in the presence of brown trout at 3 months (year two only) and 10 months, as well as lower survival over time in either year (year one: $X^2 = 20.6$, df =1, $p < 0.001$ and year two: $X^2 = 39.7$, df =1, $p < 0.001$). LaHave juveniles had lower survival in the presence of rainbow trout at 10 months (year one only) and there was no significant effect of rainbow trout on survival over time for the remaining two populations in either year (year one: $X^2 = 1.2$, df =1, $p = 0.264$ and year two: $X^2 = 0$, df =1, $p = 0.877$). In year one, LaHave juveniles had lower survival in the multi-species treatment at 3 months but had the opposite effect for Sebago juveniles at 3 months. However, Atlantic salmon had lower survival in the multi-species treatment at 10 months and over time ($X^2 = 12.3$, df =1, $p = 0.001$). Sebago juveniles had higher survival in the presence of coho salmon at 3 months (relative to the alone treatment), but otherwise the presence of coho salmon had no effect over time ($X^2 = 0$, df =1, $p = 0.980$). In year two, there was no effect of the multi-species treatment and the presence of coho salmon on the survival of Atlantic salmon at either 3 or 10 months or over time ($p > 0.07$). In either year, there was no significant effect of the presence of Chinook salmon on the survival of Atlantic salmon at either 3 or 10 months or over time ($p > 0.947$).

Table 2.2. Summary of Model Results for Traits in Two Populations Atlantic Salmon (*Salmo salar*) in Year One. Displayed are linear model results for survival and riffle use and linear mixed-effects results for length, mass, condition, circulating cortisol concentrations, and circulating 11-ketotestosterone concentrations (11-KT). Population and treatment were coded as fixed effects in all models and mixed-effects models contained a random effect for artificial stream tank identity.

Trait	3 months			10 months		
	df	*F*-statistic	*p*-value	df	*F*-statistic	*p*-value
Survival						
population	1,16	0.05	0.824	1,16	0.48	0.500
treatment	5,16	1.74	0.182	5,16	18.46	< 0.001
population × treatment	5,16	4.15	0.013	5,16	2.16	0.110
Body length						
population	1,461.9	5.73	0.017	1,355	1.82	0.178
treatment	5,461.9	4.02	0.001	5,355	4.71	< 0.001
population × treatment	5,461.9	0.46	0.808	5,355	0.71	0.617
Body mass						
population	1,461.9	0.24	0.623	1,355	1.20	0.274
treatment	5,461.9	6.56	< 0.001	5,355	5.78	< 0.001
population × treatment	5,461.9	0.58	0.712	5,355	0.60	0.699
Body condition						
population	1,26.0	53.25	< 0.001	1,44.1	3.40	0.072
treatment	5,21.0	8.45	< 0.001	5,12.2	2.22	0.118
population × treatment	5,26.2	1.16	0.355	5,17.2	2.09	0.116
Riffle use						
population	1,16	0.00	0.988	1,16	0.01	0.938
treatment	5,16	2.87	0.049	5,16	9.29	< 0.001
population × treatment	5,16	1.75	0.181	5,16	2.75	0.030
Cortisol concentrations						
population				1,14.1	0.29	0.601
treatment				5,32.3	3.06	0.023
population × treatment				5,28.7	1.21	0.330
11-KT concentrations[1]						
population				1,23.9	7.57	0.011
treatment				5,27.6	0.67	0.652

[1] Sample size was too small to examine a population × treatment interaction.

Table 2.3. Summary of Model Results for Traits in Three Populations of Atlantic Salmon (*Salmo salar*) in Year Two. Displayed are linear model results for survival and riffle use and linear mixed-effects results for length, mass, condition, circulating cortisol concentrations, and circulating 11-ketotestosterone concentrations (11-KT). Population and treatment were coded as fixed effects in all models and mixed-effects models contained a random effect for artificial stream tank identity.

Trait	3 months			10 months		
	df	*F*-statistic	*p*-value	df	*F*-statistic	*p*-value
Survival						
population	2,18	1.32	0.291	2,18	0.33	0.721
treatment	5,18	3.17	0.032	5,18	6.75	0.001
population × treatment	10,18	1.93	0.107	10,18	1.61	0.183
Body length						
population	2,557.94	13.05	< 0.001	2,556.96	32.20	< 0.001
treatment	5,557.94	0.99	0.422	5,556.96	13.69	< 0.001
population × treatment	10,557.94	0.25	0.990	10,556.96	1.48	0.144
Body mass						
population	2,557.97	18.34	< 0.001	2,556.98	28.51	< 0.001
treatment	5,557.97	0.79	0.554	5,556.98	19.99	< 0.001
population × treatment	10,557.97	0.33	0.972	10,556.98	1.58	0.108
Body condition						
population	2,19.013	0.26	0.776	2,556.88	8.94	0.021
treatment	5,18.762	1.23	0.336	5,556.88	2.54	0.028
population × treatment	10,18.746	0.25	0.985	10,556.88	1.17	0.306
Riffle use						
population	2,90	2.88	0.062	2,90	0.48	0.618
treatment	5,90	1.03	0.406	5,90	3.86	0.003
population × treatment	10,90	1.18	0.316	10,90	1.83	0.066
Downstream displacement						
population	2,90	0.82	0.444	2,90	2.77	0.068
treatment	5,90	0.75	0.589	5,90	3.41	0.007
population × treatment	10,90	1.34	0.219	10,90	1.65	0.104
Cortisol concentrations						
population				2,16.66	0.39	0.686
treatment				5,16.67	0.98	0.459
population × treatment				10,16.72	1.19	0.365
11-KT concentrations						
population				2,135	1.00	0.371
treatment				5,135	1.22	0.304
population × treatment				10,135	1.84	0.060
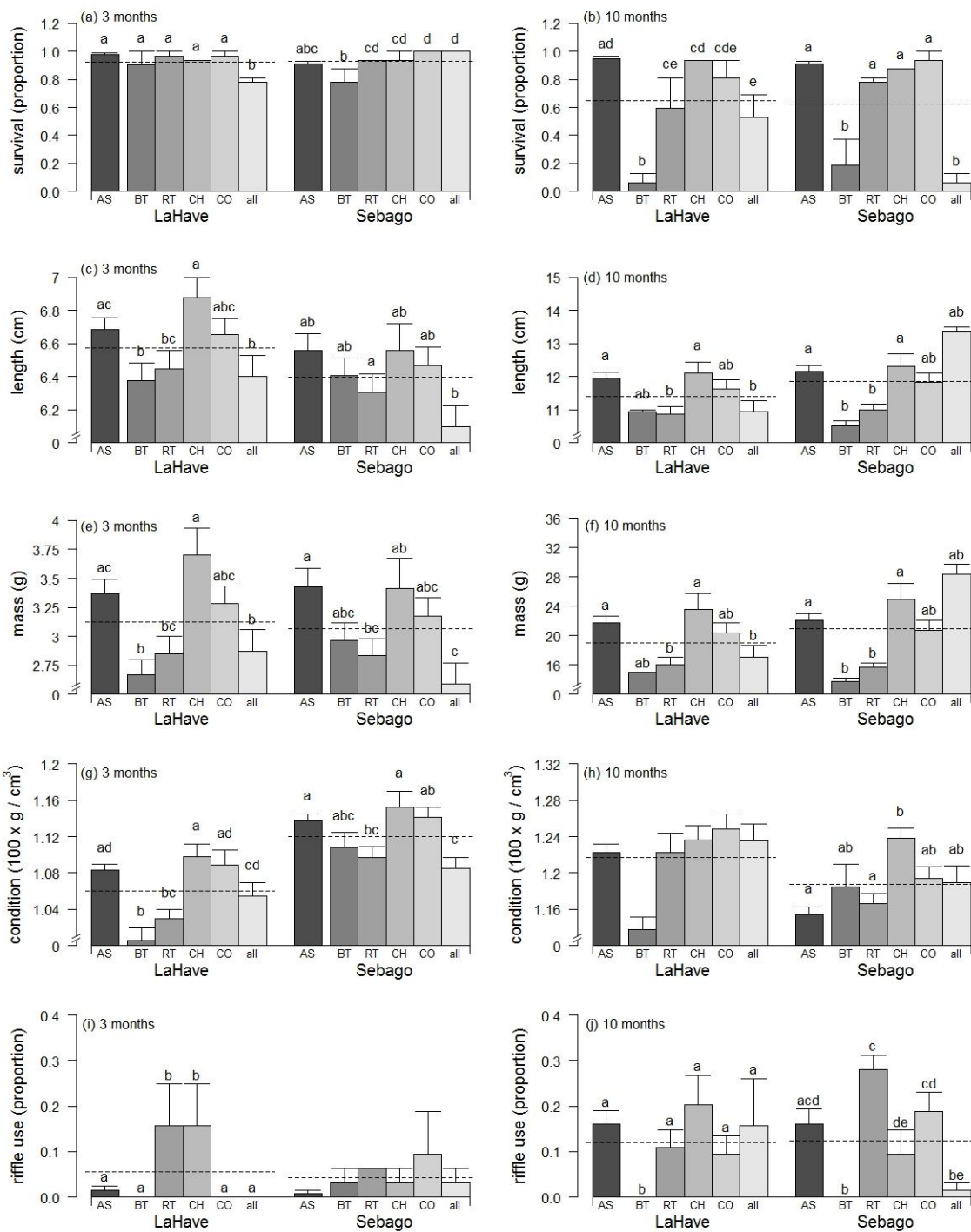
Figure 2.1. Traits in the Artificial Stream Tanks at 3 and 10 months for Two Populations of Atlantic salmon (*Salmo salar*) in Year One. Treatment symbols are AS = pooled Atlantic salmon alone and Atlantic salmon mixed, BT = Atlantic salmon with brown trout, RT = Atlantic salmon with rainbow trout, CH = Atlantic salmon with Chinook salmon, CO = Atlantic salmon with coho salmon, all = Atlantic salmon with all four non-native salmonid species. Displayed are means ± 1SE for treatments. Dashed lines are the means for the population across all treatments. Different lowercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$).
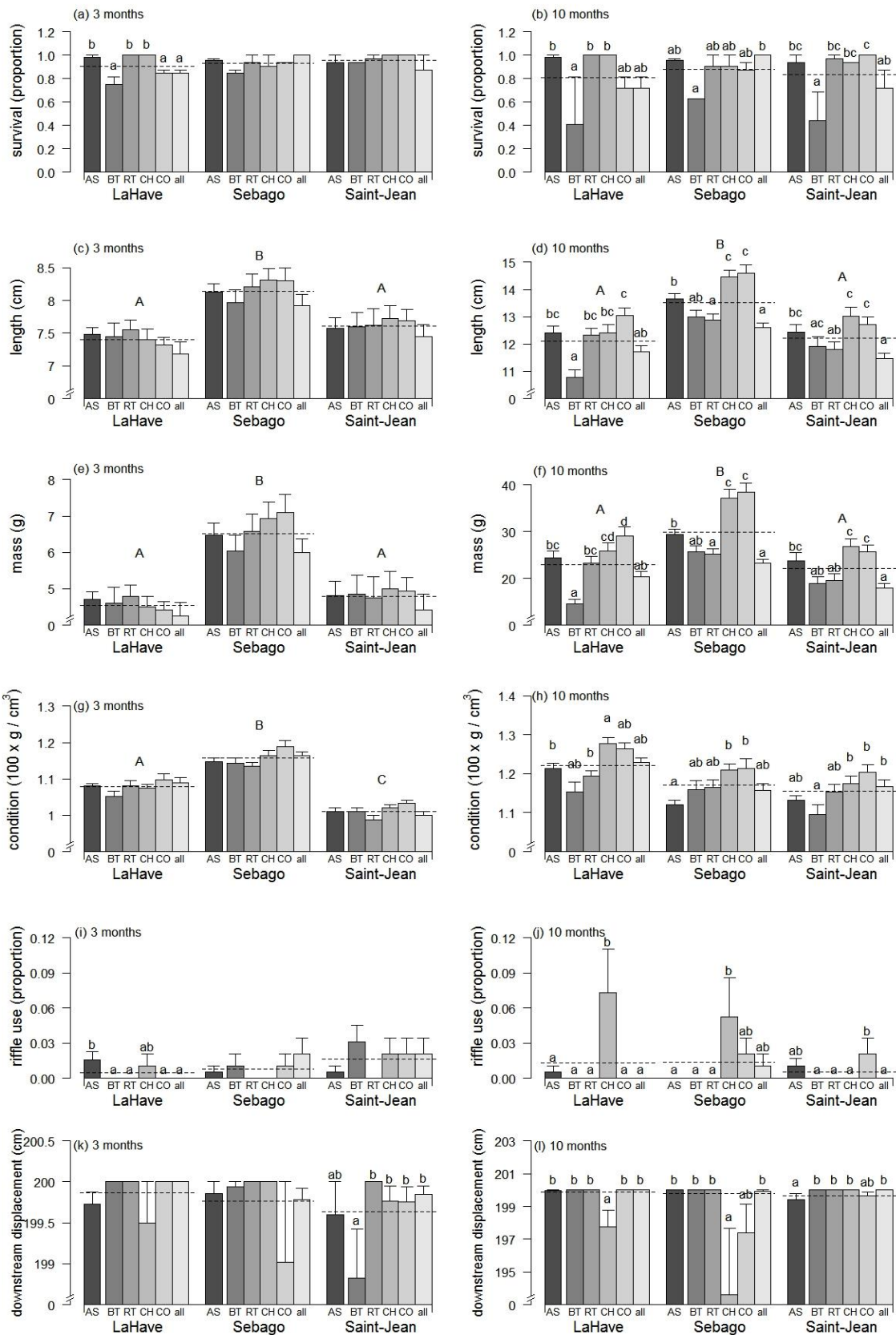
Figure 2.2. Traits in the Artificial Stream Tanks at 3 and 10 months for Three Populations of Atlantic salmon (*Salmo salar*) in Year Two. Treatment symbols are AS = Atlantic salmon alone, BT = Atlantic salmon with brown trout, RT = Atlantic salmon with rainbow trout, CH = Atlantic salmon with Chinook salmon, CO = Atlantic salmon with coho salmon, all = Atlantic salmon with all four non-native salmonid species. Displayed are means ± 1SE for treatments. Dashed lines are the means for the population across all treatments. Different uppercase and lowercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$).

2.3.2 Length, Mass, Condition, Riffle Use, and Downstream Displacement

The three Atlantic salmon populations initially differed in body length, mass, and condition (Table 2.1). In year one, LaHave juveniles were longer and in lower condition than Sebago juveniles. In year two, LaHave juveniles were shorter than Sebago and Saint-Jean juveniles. LaHave juveniles were also lighter and in lower condition than Sebago juveniles. Among the four non-native salmonid species, coho salmon were the largest in initial body size followed by Chinook salmon, brown trout, and rainbow trout (Table 2.1).

Significant population effects were detected for the body length, mass, and condition of Atlantic salmon at either 3 or 10 months (Table 2.2 and 2.3; Figure 2.1 and 2.2). In year one, Sebago juveniles were in higher condition than LaHave juveniles at 3 months, but this pattern was reversed at 10 months; although, when alone, there were no differences between the populations in length, mass, or condition at either time (one-way ANOVAs, $p > 0.14$ for all). In year two, although standardizing for differences in the initial body length, mass, and condition of the populations, Sebago juveniles grew more and put on more mass than both LaHave and Saint-Jean juveniles at 3 and 10 months. Also, LaHave and Saint-Jean juveniles had larger increases in condition relative to Sebago juveniles at 10 months, but not at 3 months.

Also, significant treatment effects were detected for the body length, mass, and condition of Atlantic salmon at either 3 or 10 months (Table 2.2 and 2.3; Figure 2.1 and 2.2). In year one, LaHave juveniles, but not Sebago juveniles, had lower length and mass in the presence of brown trout. However, Sebago juveniles had lower mass in the presence of

brown trout at 10 months. LaHave juveniles had lower mass and Sebago juveniles had lower condition in the presence of rainbow trout at 3 months as well as both populations had lower length and mass at 10 months. Atlantic salmon had lower length and mass and Sebago juveniles had lower condition in the multi-species treatment at 3 months. Also, LaHave juveniles had lower length and mass in the multi-species treatment at 10 months. There were no significant effects of the presence of Chinook salmon and coho salmon on Atlantic salmon length, mass, and condition at either 3 or 10 months. In year two, Atlantic salmon had lower length and mass in the presence of brown trout, rainbow trout, and the multi species treatment at 10 months, whereas the effect was opposite in the presence of Chinook salmon and coho salmon at 10 months. In addition, the Atlantic salmon had higher condition in the presence of Chinook salmon at 10 months. There was no significant effect of the presence of brown trout, rainbow trout, coho salmon, or the multi species treatment on Atlantic salmon condition at 10 months.

Significant treatment effects were detected for the riffle use of Atlantic salmon at 3 months and 10 months (Table 2.2 and 2.3; Figure 2.1 and 2.2). The populations did not differ in riffle use and downstream displacement across all treatments at either time in either year. In year one, Atlantic salmon had lower riffle use in the presence of brown trout at 10 months, but not at 3 months. Sebago juveniles had lower riffle use in the multi-species treatment at 10 months, but otherwise had no effect. LaHave juveniles had higher riffle use in the presence of rainbow trout and Chinook salmon at 3 months, but not at 10 months. There was no significant effect of the presence of coho salmon on the riffle use for Atlantic salmon at either 3 or 10 months. Similar riffle use results were found when analysing the photograph data at 3 months (data not shown). In year two,

Atlantic salmon had higher riffle use and lower downstream displacement in the presence of Chinook salmon at 10 months. There was no significant effect of the presence of brown trout, rainbow trout, coho salmon, or the multi species treatment on the riffle use and downstream displacement of Atlantic salmon at 10 months.

2.3.3 Circulating Hormone Concentrations

No population differences in circulating cortisol concentrations were detected in either year. However, significant treatment effects were observed in year one, but not year two (Table 2.2 and 2.3; Figure 2.3 and 2.4). In year one, LaHave juveniles, but not Sebago juveniles, had higher circulating cortisol concentrations in the multi-species treatment, whereas the presence of rainbow trout had the opposite effect. There was no significant effect of the presence of brown trout, Chinook salmon, and coho salmon on the circulating cortisol concentrations in Atlantic salmon, although the lack of effect detection for the presence of brown trout may have been limited by high variances (Figure 2.3 and 2.4).

Significant population effects were detected for circulating 11-KT concentrations in year one, but not year two (Table 2.2 and 2.3; Figure 2.3 and 2.4). No significant treatment effects were detected for circulating 11-KT concentrations in either year. In year one, Sebago juveniles had lower circulating 11-KT concentrations than LaHave juveniles across all treatments, but when alone, there was no difference between the populations ($F_{1,9}= 3.46$, $p = 0.096$).
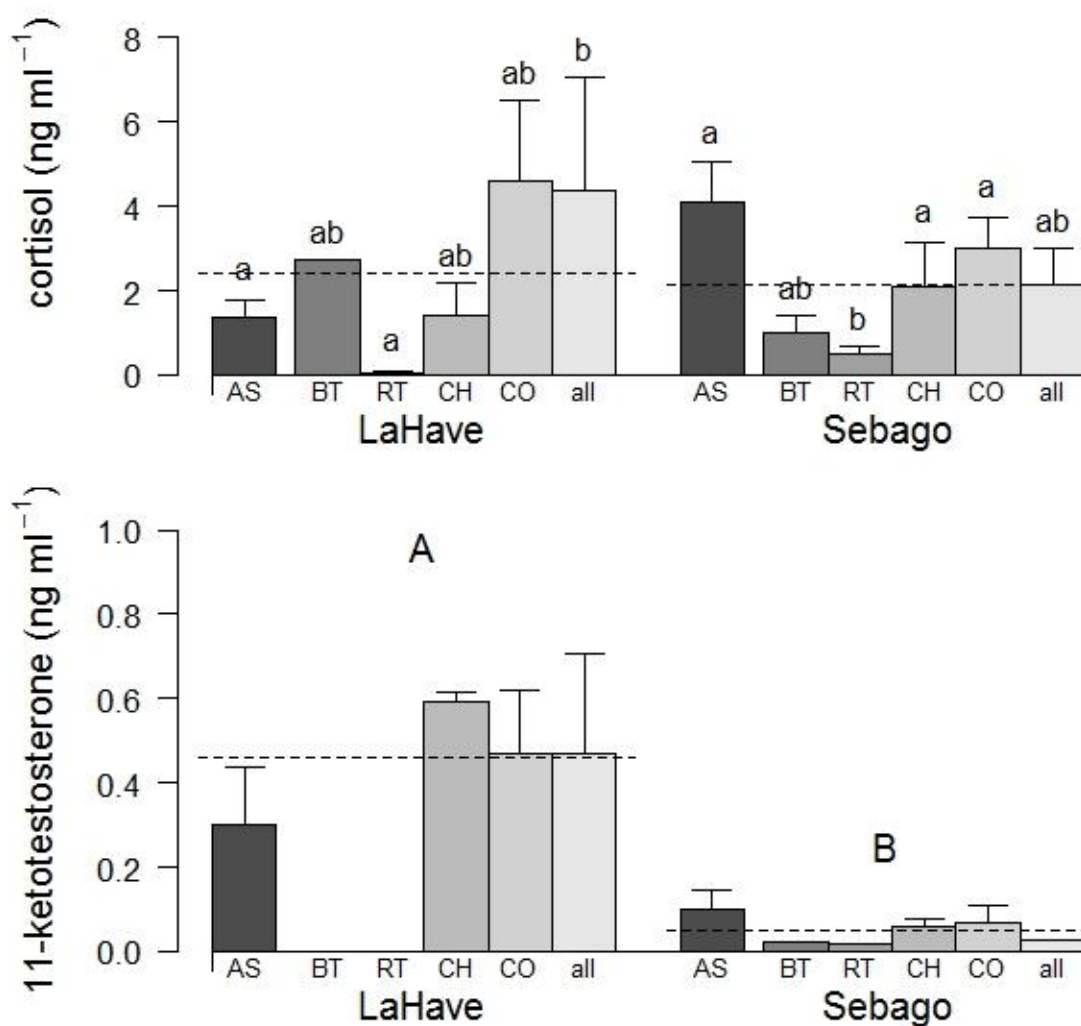
Figure 2.3. Circulating Hormone Concentrations in the Artificial Stream Tanks at 10 months for Two Populations of Atlantic salmon (*Salmo salar*) in Year One. The treatment symbols are the same as those described in the caption for Figure 2.1. Different uppercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$). There was insufficient plasma to examine circulating 11-KT concentrations for LaHave juveniles in the brown trout and rainbow trout treatments.
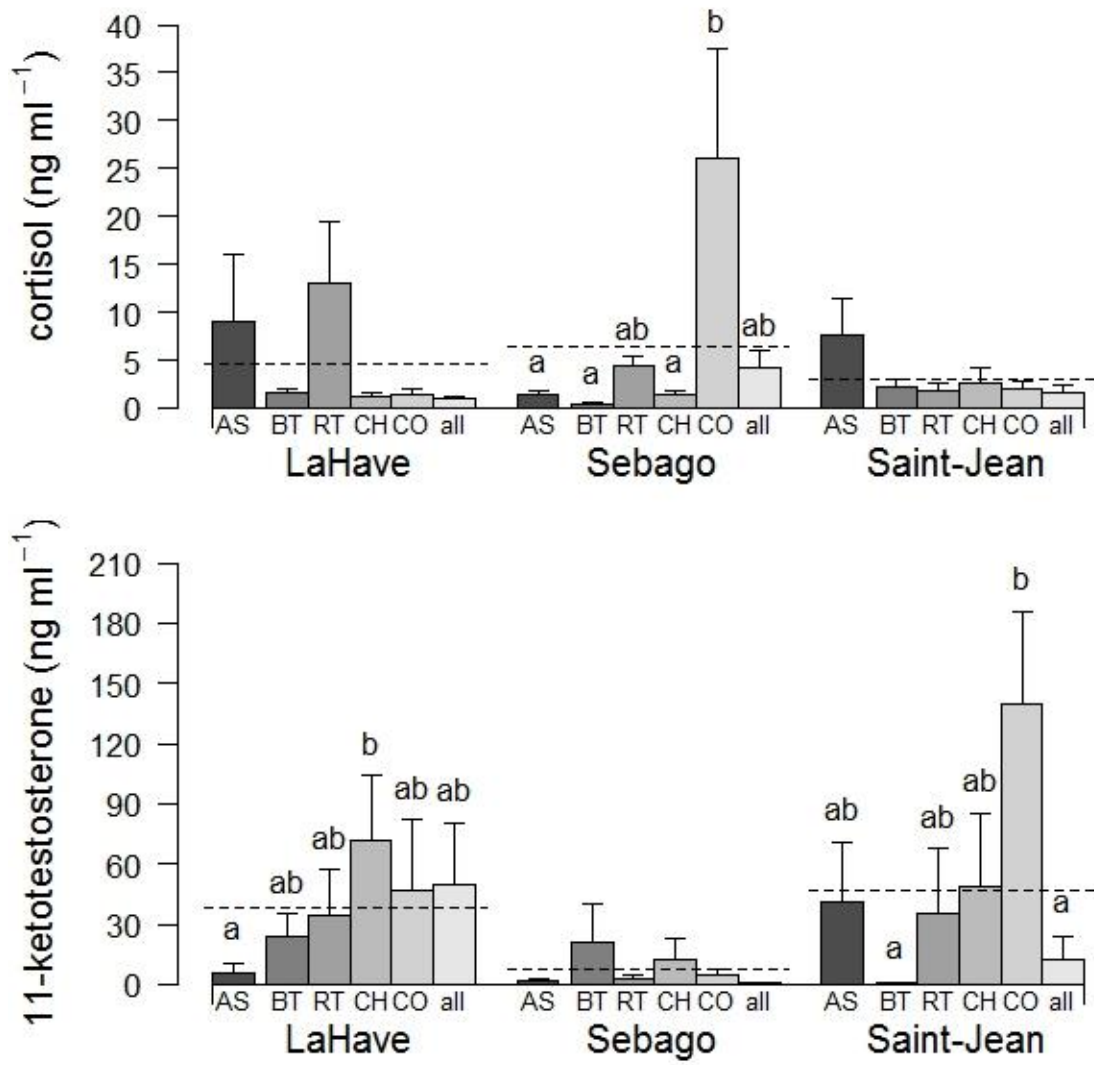
Figure 2.4. Circulating Hormone Concentrations in the Artificial Stream Tanks at 10 months for Three Populations of Atlantic Salmon (*Salmo salar*) in Year Two. The treatment symbols are the same as those described in the caption for Figure 2.2. Different uppercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$).

Circulating cortisol concentrations were correlated with the final (10 months) body size in either year. In year one, length and mass of Sebago juveniles were positively correlated with circulating cortisol concentrations (length: $r = 0.362$, df $= 66$, $p = 0.002$ and mass: $r = 0.345$, df $= 66$, $p = 0.004$), but length and mass of LaHave juveniles and condition for Atlantic salmon were not ($p > 0.64$ for all). In year two, length and condition of LaHave juveniles were correlated with circulating cortisol concentrations (length: $r = 0.350$, df $= 49$, $p = 0.011$ and condition: $r = -0.363$, df $= 49$, $p = 0.009$), as well as the length and mass of Saint-Jean juveniles (length: $r = 0.393$, df $= 49$, $p = 0.004$ and mass: $r = 0.371$, df $= 49$, $p = 0.007$). There were no correlations between these metrics for Sebago juveniles ($p > 0.07$ for all). Circulating cortisol concentrations were not correlated with time to blood collection in either year (year one: $r = -0.052$, df $= 128$, $p = 0.558$ and year two: $r = -0.094$, df $= 151$, $p = 0.249$).

Circulating 11-KT concentrations were not correlated with the final body size in year one ($p > 0.12$ for all), but correlated with the final size in year two. Circulating 11-KT concentrations were correlated with the length and condition of LaHave juveniles (length: $r = -0.358$, df $= 49$, $p = 0.010$ and condition: $r = 0.292$, df $= 49$, $p = 0.037$) and the condition of Saint-Jean juveniles ($r = 0.700$, df $= 49$, $p < 0.001$). There were no correlations between these metrics for Sebago juveniles ($p > 0.13$ for all). Circulating 11-KT concentrations were not correlated with time to blood collection in either year (year one: $r = -0.042$, df $= 41$, $p = 0.789$ and year two: $r = 0.051$, df $= 151$, $p = 0.532$).

2.4   Discussion

Non-native species have the potential to reduce the performance of native species, which could have significant consequences for reintroduction efforts. Examining the effects of competition with four non-native salmonids for two independent years in the artificial streams, I found that competition with brown trout, rainbow trout, and the multi-species treatment reduced the survival and fitness-related traits of Atlantic salmon. In contrast, in both years, Atlantic salmon survival and performance for fitness-related traits were not reduced in competition with Chinook salmon or coho salmon. I cannot rule out density effects at 10 months because of differential mortality across treatments. However, similar effects for body size traits were previously detected at 3 months in year one when treatment densities were more equal, indicating that density effects are not likely driving the results. Brown trout and rainbow trout may have reduced the performance of Atlantic salmon due to high ecological niche overlap in stream environments and are typically more aggressive than Atlantic salmon (Gibson 1981; Hearn and Kynard 1986; Volpe et al. 2001; Scott et al. 2005; Vehanen 2006). Chinook salmon and coho salmon, on the other hand, have little niche overlap with Atlantic salmon in streams (Gibson 1981; Beall et al. 1989; Heland and Beall 1997; Scott et al. 2005; Holecek et al. 2009).

Stress level and social status are commonly assessed in fishes using circulating levels of hormones. Measuring circulating levels of cortisol for two independent years in the artificial streams, I found that competition with non-native species did not appear to induce chronic stress in Atlantic salmon. Chronic stress for salmonids is indicated at cortisol concentrations above 10 ng ml^{-1} (Maule et al. 1987; Pickering and Pottinger 1989) and the Atlantic salmon juveniles concentrations were below this value in all but

two treatments in year two. Also, measuring circulating levels of 11-KT for both years in the artificial streams, I found that competition with non-native species did not appear to change the social status of Atlantic salmon juveniles. Some caution is warranted when interpreting the hormone results, however, as the juveniles I sampled were those that survived over the winter. Conceivably, the individuals that died may have been of lower social status and succumbed to chronic stress (see Wendelaar Bonga 1997; Gregory and Wood 1999). Alternatively, the Atlantic salmon may have adapted to the prolonged chronic stress (i.e. after 10 months of competition with non-native salmonid species). Prolonged exposure to a chronic stressor can decrease the production of cortisol (see Wendelaar Bonga 1997). In addition, different life stages tend to have different sensitivities to stressors, with younger juvenile salmonids typically being more tolerant of anthroprogenic handling, and possibly agonistic interactions, than older life stages (Wendelaar Bonga 1997). Indeed, Atlantic salmon that were a year older and exposed to a shorter period (8 days) of social interactions with non-native salmonid species had an increase in circulating cortisol concentrations to a level indicative of chronic stress (Van Zwol et al. 2012b).

It is also possible that the circulating hormone concentrations in the Atlantic salmon simply relate to metabolism (Wendelaar Bonga 1997; Mommsen et al. 1999). Larger juveniles typically have a higher metabolic rate (Metcalfe et al. 1995), and circulating cortisol concentrations may have increased proportionally to metabolic rate given the food deprived conditions (i.e. starvation for 24 hours in this study; see Wendelaar Bonga 1997; Mommsen et al. 1999). Indeed, in year one, I found that Sebago juveniles had a significant positive relationship between circulating cortisol concentration and body size.

In year two, I found that LaHave and Saint-Jean juveniles had a significant positive relationship between circulating cortisol and body length and between circulating 11-KT and body condition. Other hormones, such as growth hormone (Jonsson et al. 1998), testosterone (Desjardins et al. 2006), and arginine vasotocin (Dewan and Tricas 2011), may instead be involved in mediating aggression, social status, and ultimately stress in juvenile salmonids. These other hormones deserve further attention.

My results have implications for the reintroduction efforts of an extirpated species. Although it is still premature to comment on the relative suitability of the different source populations of Atlantic salmon for whole-lake restoration, my findings suggest that the three populations may exhibit differential performance during the juvenile stage. At least in Lake Ontario tributaries, depending on the resident local communities of non-native salmonids, the source populations showed differences in survival and growth. Juvenile Atlantic salmon from the Sebago population generally fared better than the other two populations, but there were exceptions. In year two, Sebago juveniles grew more than both LaHave and Saint-Jean juveniles (after standardizing for differences in initial body size). In year one, the presence of rainbow trout reduced the survival of LaHave juveniles but not Sebago juveniles, although LaHave juveniles had better survival in the multi-species treatment than Sebago juveniles. Interestingly, stocked Sebago juveniles also appear to do well in Lake Champlain, where there is competition with brown trout and rainbow trout (Marsden et al. 2010). These results suggest that a source population appearing to do well in a location with key environment features similar to the reintroduction location may possess adaptations important to fitness (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). In addition, the results presented here, as well as those

from previous studies (Van Zwol et al. 2012a,b), indicate that non-native salmonids can negatively affect the survival and performance of Atlantic salmon over the entire two year stream residency period, with brown trout in particular having a large impact. Adjusting stocking efforts to avoid tributaries with established brown trout populations may therefore increase the effectiveness of reintroduction efforts.

2.5   References

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Chapter 3

3 Predictability of Multi-Species Competitive Interactions in Three Populations of Atlantic Salmon[*]

3.1 Introduction

Non-native species are one of the top global threats to native species and biodiversity (Clavero and García-Berthou 2005). In particular, the fitness and health of native species can be reduced by competition with ecologically-similar non-native species (Hamilton et al. 1999; Maskell et al. 2006). As even small declines in population fitness can result in the extirpation of native species, particularly when confronted by multiple stressors, potential ecological pressures from non-native species are a significant conservation concern (Gause 1934; Hutchings 1991; Harig et al. 2000). Similarly, competition with non-native species can also impede a successful reintroduction of native species by limiting increases in population growth rate (Simberloff 1990; Vitousek 1990).

Globally, species introductions, whether planned or unintentional, have become so common that native species are often in competition with more than one ecologically similar non-native species (Cox 2004). For example, native galaxiid fishes are in competition with two or more introduced salmonid species in Chilean Patagonia (Young et al. 2009) and native seagrass are in competition with several introduced seaweeds in North America (Williams 2007). In the Laurentian Great Lakes, zooplankton and aquatic macroinvertebrates have been heavily impacted by the establishment of Ponto-Caspian invaders (Ricciardi and McIsaac 2000; Ricciardi 2001). In general, however, the

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combined effects of competition with multiple non-native species are largely unknown. Instead, a simple additive function of two-species competition effects is used to predict multi-species effects (Weigelt et al. 2007). Some studies examining multi-species competition support the simple additive function of two-species competition effects in plants (Fowler 1982; Weigelt et al. 2007) and animals (Vandermeer 1969; Pomerantz 1981; Young et al. 2009). Yet, other studies examining multi-species competition have found non-additive competitive interactions (plants: Miller 1994; Dormann and Roxburgh 2005; animals: Wilbur 1972; Neill 1974; Case and Bender 1981; Wilbur and Fauth 1990; Wootton 1993). Based on these latter studies, the influence that non-additive competitive interactions have on the performance of focal native species is highly variable, with native species performance increasing, decreasing, or remaining unchanged (Levine 1976; Stone and Roberts 1991).

Theory suggests that non-additive effects can arise in multi-species competition because of high variability in niche overlap, as well as synergistic effects from a higher number of species in the community. High niche overlap (i.e. when there is competition for three or more limiting resources) in multi-species competition can lead to the competitive exclusion of all but one species (Huisman and Weissing 1999, 2001, 2002). Conversely, low niche overlap can result in the stable coexistence of multiple species where each species is limited by different resources (Huissan and Weissing 1999). On top of this effect, if the dimensionality of species number in the community is greater than the number of limiting resources, species will be competitively excluded until the number of species matches the set imposed by limiting resources and carrying capacity (Huisman and Weissing 1999, 2001). The species that are not outcompeted are expected to be those

with traits most beneficial for acquiring the limiting resources (Huisman and Weissing 2001, 2002). Similarly, high diversity habitats, containing more resource gradients, tend to support higher species diversities than low diversity habitats (MacArthur and MacArthur 1961), possibly because the species have a greater capacity for niche separation in high diversity habitats resulting in less competition than in lower diversity habitats (Young 2001).

The reintroduction efforts of Atlantic salmon (*Salmo salar*) into Lake Ontario are a prime example of a native extirpated species whose restoration may be impeded by the presence of non-native competitors. Of these, rainbow trout (*Oncorhynchus mykiss*) are currently the most abundant salmonid in Lake Ontario tributaries (49% of sites sampled), followed by brown trout (*S. trutta*, 31%), then coho salmon (*O. kisutch,* 8%) (Stanfield et al. 2006). Chinook salmon (*O. tshawytscha*) have also been heavily stocked into Lake Ontario tributaries (OMNR 2014), and are thought to have established naturalized populations in the basin (Connerton et al. 2009).

Here, I examine the survival, body size, and riffle use of Atlantic salmon juveniles of the three populations in artificial streams containing four non-native salmonid species. Atlantic salmon and non-native salmonid species body sizes were representative of those stocked in Lake Ontario tributaries and thereby reflect the size differences in natural streams. Greater details on the Atlantic salmon survival and fitness-related traits in the artificial streams are described in Chapter 2; however, here I examine the predictability of multi-species competition effects based on the classic two-species additive models. My objectives were to test two hypotheses: (1) that multi-species competition effects can be

predicted by a simple additive model of two-species competition effects; and (2) that Sebago juveniles will have a better competitive ability and thus higher performance (i.e. survival and fitness-related traits) than LaHave and Saint-Jean juveniles in multi-species competition.

3.2   Materials and Methods

3.2.1 Study Species

Juveniles of all salmonid species were provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF). LaHave and Sebago Atlantic salmon families ($n = 25$ per population) were produced in early November 2010 and 2011 using mature individuals at the OMNRF Harwood Fish Culture Station, Harwood, Ontario. Families of fertilized eggs were transported the same day to the OMNRF Codrington Research Facility, Codrington, Ontario. Saint-Jean Atlantic salmon families ($n = 66$) were produced early November 2011 at Harwood and transferred to Codrington as fry (age 0+ parr, $n = 500$) in spring 2012. The Saint-Jean population was not included in 2010, as it was not possible to obtain sufficient numbers of fry. Rainbow trout, brown trout, Chinook salmon, and coho salmon fry ($n = 250$ for each species) were transferred from OMNRF Fish Culture Stations to Codrington in spring 2011 and 2012. Details on the broodstock and breeding of the salmonid species are described in Chapter 2.

3.2.2 Survival, Fitness-Related Traits, and Riffle Use

Juveniles were kept in the artificial stream tanks for 10 months (September to July) in each year. Details on the artificial stream tanks and experimental set-up are described in Chapter 2. Atlantic salmon were measured for survival and three fitness-related traits

(length, mass, and condition; Fausch 1984, 1998). Atlantic salmon riffle use was also examined, as it is the species' preferred microhabitat (Morantz et al. 1987). Measurements were collected in year one on October 28, November 29, and July 24, and in year two on November 11, December 17, May 29, June 26, and July 25. On these dates, all juveniles were removed from the artificial stream tanks, lightly anaesthetized, measured for body length and mass, and then allowed to recover before being returned to the artificial stream tank. Condition was calculated as $100 \times mass / length^3$ (Fulton 1904). The day after, for riffle use, a trained observer took counts of each salmonid species within the riffle section at 12:00. I also examined riffle use by taking photographs the day before body size measurements, but did not have the data for all measurement dates. I therefore concentrated my analysis on the observer data and similar riffle use results were found when analysing the photograph data (data not shown).

3.2.3 Statistical Analysis of Multi-Species Effects

The statistical analysis for the predictability of multi-species competition effects was performed in R 3.0.1 (available at http://www.r-project.org/). Statistical significance was set at $\alpha = 0.05$. In both years, survival was assessed at 10 months in the artificial streams (July 24 and July 25), whereas body length, mass, condition, and riffle use were examined at 3 months (November 29 and December 17) because overwinter mortality of juveniles caused differences in fish densities that may influence these later traits (e.g. Fausch 1998).

I compared the observed and predicted multi-species competition effects for the Atlantic salmon traits using the method described in Weigelt et al. (2007). First, observed effect

estimates (*OE*) for each Atlantic salmon replicate were extracted using linear models that contained a fixed effect for artificial stream tank identity and no intercept. Second, predicted effect estimates of multi-species competition (*PE*) on Atlantic salmon were calculated based on a simple additive function of the observed estimates for two-species treatment replicates, weighted by the number of artificial stream tanks ($n = 8$):

$$PE = \frac{1}{n} \times \sum_{i=1}^{n} \text{OE}_i$$

Where *i* denotes a replicate of a given two-species treatment. Third, the deviations between predicted and observed multi-species effects were tested for a significant difference from zero using one-sample Student's *t*-tests.

Confidence intervals (95%) for the deviations were generated using a modified bootstrapping method of Neff and Fraser (2010). First, data from Atlantic salmon individuals were resampled with replacement until the original sample size was reproduced. Using the resampled data set, the deviations were again calculated. The resampling process and calculations were repeated 1000 times for each of the two multi-species replicates per population, from which the 95% confidence interval (CI) was determined for each parameter. Pairwise population comparisons of the deviations were conducted by calculating, for one Atlantic salmon population, the proportion of deviations that were larger than the other Atlantic salmon populations. The proportion served as a one-tailed *p*-value testing for significant differences between the populations.

3.3   Results

Significant deviations between observed and predicted multi-species effects were detected for Atlantic salmon body length and mass, but not for survival, condition, or riffle use (Table 3.1). The deviations of length and mass were significantly more negative than expected. Negative deviations mean that the Atlantic salmon juveniles had worse performance (i.e. lower length and mass) than predicted by the simple additive model in the observed multi-species treatment. The Atlantic salmon populations were not significantly different in the deviations for the majority of traits, with the exception of riffle use in year two (Table 3.1). Sebago juveniles had the largest deviations followed by Saint-Jean juveniles then LaHave juveniles. Sebago juveniles also had larger deviations for survival than both LaHave and Saint-Jean juveniles in year two, but the opposite occurred in year one for LaHave and Sebago juveniles.

Table 3.1. Summary of the Deviations between Predicted and Observed Multi-Species Effects for Three Populations of Atlantic Salmon (*Salmo salar*). Stream 1 and 2 are the artificial stream tank identities representing replicates for the multi-species treatment. Significance of the deviations was determined by a one-tailed Student's *t*-test. Confidence intervals (95%) were created using resampling procedures. LaHave is LA, Sebago is SE, and Saint-Jean is SJ for pair-wise population comparisons.

Trait	Stream 1	Stream 2	95% CI	Pair-wise *p*-value
Survival				
Year 1				
LaHave	0.086	-0.226	-0.453, 0.469	LA-SE = 0
Sebago	-0.570	-0.218	-0.742, -0.430	
Year 2				
LaHave	0.031	-0.156	-0.359, 0.188	LA-SE = 0
Sebago	0.172	0.172	0.219, 0.328	LA-SJ = 0.462
Saint-Jean	-0.273	0.039	-0.469, 0.164	SE-SJ = 0
t-test *p*-value	0.172			
Body length (cm)				
Year 1				
LaHave	-0.221	-0.157	-0.566, 0.180	LA-SE = 0.313
Sebago	-0.218	-0.474	-0.797, 0.093	
Year 2				
LaHave	-0.252	-0.271	-0.793, 0.300	LA-SE = 0.484
Sebago	-0.300	-0.277	-0.765, 0.191	LA-SJ = 0.463
Saint-Jean	-0.317	-0.148	-0.889, 0.330	SE-SJ = 0.436
t-test *p*-value	< 0.001			
Body mass (g)				
Year 1				
LaHave	0.001	-0.003	-0.043, 0.041	LA-SE = 0.084
Sebago	-0.037	-0.045	-0.078, -0.003	
Year 2				
LaHave	-0.324	-0.365	-1.394, 0.870	LA-SE = 0.325
Sebago	-0.717	-0.697	-1.776, 0.471	LA-SJ = 0.423
Saint-Jean	-0.482	-0.480	-1.772, 1.003	SE-SJ = 0.420
t-test *p*-value	< 0.001			

Body condition ($100 \times$ g cm^{-1})				
Year 1				
LaHave	-0.285	-0.223	-0.809, 0.295	LA-SE = 0.277
Sebago	-0.354	-0.691	-1.117,0.131	
Year 2				
LaHave	-0.008	0.027	-0.038,0.062	LA-SE = 0.495
Sebago	0.003	0.009	-0.028,0.048	LA-SJ = 0.250
Saint-Jean	-0.012	-0.011	-0.043,0.020	SE-SJ = 0.221
t-test *p*-value	0.286			
Riffle use				
Year 1				
LaHave	-0.078	-0.078	-0.109, -0.031	LA-SE = 0.168
Sebago	0.008	-0.055	-0.094, 0.133	
Year 2				
LaHave	0.070	-0.055	-0.023, 0.250	LA-SE = 0
Sebago	-0.047	-0.109	-0.125, -0.039	LA-SJ = 0.016
Saint-Jean	-0.039	0.086	-0.063, -0.008	SE-SJ = 0.026
t-test *p*-value	0.180			

3.4   Discussion

Native species may have lower performance in sympatry with multiple non-native species than predicted by two-species competition effects. I found negative deviations, indicating reduced performance, for Atlantic salmon body length and mass in the multi-species treatment, such that Atlantic salmon juveniles had smaller body size in the multi-species treatment than predicted using a simple additive model of two-species treatment effects. Other studies have found similar non-additive competitive interactions in multi-species competition (Wilbur 1972; Neill 1974; Case and Bender 1981; Wilbur and Fauth 1990; Wootton 1993) and suggest that varying degrees of ecological niche overlap can lead to non-additive competitive interactions (Stone and Roberts 1991; Huisman and Weissing 1999, 2001, 2002). Similarly, there tends to be higher niche overlap among species in habitats with lower than higher environment diversity (Young 2001). Atlantic salmon have high niche overlap with brown trout and rainbow trout in streams for habitat resources such as depth, velocity, and substrate (Gibson 1981; Hearn and Kynard 1986; Volpe et al. 2001; Scott et al. 2005; Vehanen 2006), but have little stream niche overlap with Chinook salmon and coho salmon (Gibson 1981; Beall et al. 1989; Heland and Beall 1997; Scott et al. 2005; Holecek et al. 2009). Given that brown trout and rainbow trout are typically more aggressive than Atlantic salmon (Gibson 1981; Hearn and Kynard 1986; Volpe et al. 2001; Scott et al. 2005; Vehanen 2006), these species may displace Atlantic salmon from riffle to pool microhabitat (e.g. Hearn and Kynard 1986). In the multi-species treatment, those displaced Atlantic salmon would encounter competition with Chinook salmon and coho salmon, which might contribute to the non-additive effects that were observed.

Non-additive competitive interactions can also occur in communities with a higher number of species (Dormann and Roxburg 2005). Theoretical models suggest that species with niche overlap can co-exist until the number of species matches the number of limiting resources (Huisman and Weissing 1999, 2001). Once this threshold is exceeded, only the species with the best competitive abilities typically remain (Huisman and Weissing 2001, 2002). Similarly, a higher number of species can be supported in higher diversity habitats because of a greater capacity for niche separation (Young 2001). Despite the historical loss of Atlantic salmon from Lake Ontario, species richness of salmonids in the lake and its tributaries has greatly increased due to introductions of non-native salmonids (Webster 1982; Crawford 2001; Stanfield et al. 2006). Although brown trout and rainbow trout are typically more aggressive than Atlantic salmon, Chinook salmon and coho salmon show comparable aggression as Atlantic salmon (Gibson 1981; Beall et al. 1989; Heland and Beall 1997; Scott et al. 2005; Holecek et al. 2009). As aggression can be a beneficial trait for acquiring resources (Holway and Suarez 1999), Atlantic salmon in Lake Ontario tributaries may have reduced performance in multi-species competition with these four non-native species due to a combination of niche overlap and habitat saturation (Jones and Stanfield 1993; Crawford 2001; Van Zwol et al. 2012a,b).

It is also possible that frequency-dependent competitive interactions contribute to the non-additive competitive interactions observed in the multi-species treatment. My experimental design used a constant number of Atlantic salmon and non-native salmonids to compare the strengths of intraspecific and interspecific competition. Brown trout, in particular, are known to be highly aggressive relative to other salmonids (Scott et al.

2005; Vehanen 2006), and brown trout performance is more negatively impacted by intraspecific than interspecific competition (see Van Zwol et al. 2012b). In the two-species treatment, which had greater numbers of brown trout than in the multi-species treatment, there may have been a higher number of competitive interactions between brown trout individuals than between Atlantic salmon and brown trout. By contrast, the multi-species treatment, which had fewer brown trout, may have resulted in more interactions between brown trout and Atlantic salmon individuals. As I did not directly quantify behavioural interactions in this study, I cannot draw any definitive conclusions without more research examining the effect of the relative numbers of individuals across species in multi-species interactions. Nevertheless, from the results of this study and others (Van Zwol et al. 2012a,b; Chapter 2), it is clear that brown trout have a strong negative effect on juvenile Atlantic salmon in tributary habitats and contribute to negative non-additive growth effects.

My results may have implications for source population selection for reintroduction efforts of extirpated populations. The presence of four non-native salmonid species is an important environmental feature that may be impeding a successful reintroduction of Atlantic salmon into Lake Ontario (Jones and Stanfield 1993; Crawford 2001; COSEWIC 2006, 2010). I found that the Sebago population had lower survival in year one and higher survival in year two in the multi-species treatment relative to the other Atlantic salmon populations. The results in year two may be due to Sebago juveniles avoiding agonistic interactions with the non-native salmonids to a greater extent than LaHave and Saint-Jean juveniles (Van Zwol et al. 2012a). Avoiding agonistic interactions is a behavioural strategy that can conserve energy, which can instead be directed towards

survival and growth (Metcalfe 1986). Such a strategy may be particularly effective when resources or preferred habitats are not limited. Interestingly, stocked Sebago salmon also appear to co-exist with naturalized and stocked rainbow trout and brown trout in Lake Champlain (Marsden et al. 2010). The results from year two may support that a source population has adaptations important to the reintroduction location if it does well in a location with similar key environment features (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). However, given the differences in performance of the Atlantic salmon populations between years in the artificial streams, I suggest that reintroduction efforts could benefit from more research examining source population performance and the composition of non-native competitors in natural streams for different years.

In conclusion, non-additive competitive interactions were detected in the multi-species treatment which here caused reduced performance for native Atlantic salmon juveniles. These non-additive competitive interactions may be caused by high niche overlap with brown trout and rainbow trout, as well as an increase in the number of potentially competing species in stream communities. As reintroduction efforts become more necessary both locally and globally, source populations for these efforts should be examined in small scale natural settings that are similar to the reintroduction location, with particular consideration given to resident species assemblages.

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Chapter 4

4 Competitive Effects between Rainbow Trout and Atlantic salmon in Natural and Artificial Streams[*]

4.1 Introduction

Non-native species are recognized as one of the top threats to preserving native species (Clavero and García-Berthou 2005) in part because competition by ecologically similar non-native species may reduce the ecological performance of native species (Hamilton et al. 1999; Maskell et al. 2006). Non-native species that are more aggressive than native species also tend to be better at acquiring resources which can cause native species to shift their ecological niche to sub-optimal habitats and conditions (Holway and Suarez 1999), further reducing population growth and performance (Hearn 1987; Fausch 1988). Such competition with non-native species may also impede a successful reintroduction of native species (Simberloff 1990; Vitousek 1990).

Established populations of non-native salmonids have been identified as a potential concern for the re-establishment of formerly native Atlantic salmon into Lake Ontario (Jones and Stanfield 1993; Crawford 2001; COSEWIC 2006, 2010). Currently, Atlantic salmon in Lake Ontario streams may be competing with up to four species of non-native salmonids. Of these, rainbow trout and brown trout are the most abundant (Stanfield et al. 2006), and have similar microhabitat associations to, and are generally more aggressive than, Atlantic salmon (Gibson 1981; Hearn and Kynard 1986; Armstrong et al. 2003; Scott et al. 2005). Therefore, rainbow trout and brown trout have the potential to

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competitively displace Atlantic salmon to sub-optimal conditions in streams, such as a higher percentage of rocks and lower water depth microhabitats (Gibson 1981; Hearn and Kynard 1986; Volpe et al. 2001).

In the case of the reintroduction efforts of Atlantic salmon (*Salmo salar*) into Lake Ontario, three source populations are being used for reintroduction efforts: LaHave River, Sebago Lake, and Lac Saint-Jean. The performance (i.e. survival and fitness-related traits) of LaHave and Sebago Atlantic salmon in competition with non-native salmonid species in Lake Ontario has been recently examined in artificial streams (see Van Zwol et al. 2012b,c; Chapter 2). Artificial streams can provide important insights as they allow the manipulation of a number of conditions (e.g. combination of competitors, competitive levels, sediment types) in a controlled environment as well as for increased experimental replication in contrast to natural environments. The effectiveness of artificial environments for simulating natural environments may vary, however, and examining interspecific competition effects in natural streams can place the results into a larger management context (Fausch 1988, 1998). Relatively, few studies have contrasted interspecific competition effects between artificial and natural environments (e.g. Blanchet et al. 2007); a recent meta-analysis examining interspecific competition effects suggests that the direction of effects are similar, but that the magnitude of effects can differ across the two types of experiments (Korsu et al. 2010). A comparison between artificial and natural streams may therefore help to identify similarities and differences in the responses of Atlantic salmon to competition with non-native salmonids and allow improved application of the findings from controlled, artificial environments to natural environments.

Here, I examine LaHave and Sebago Atlantic salmon juveniles in two natural stream sites of Lake Ontario that differed in the presence and absence of non-native salmonids, mainly rainbow trout (*Oncorhynchus mykiss*). I also compare the performance of Atlantic salmon in the natural streams to artificial streams. The Saint-Jean population was not included in these experiments, as it was not possible to obtain sufficient numbers of juveniles. My objective was to test three hypotheses: (1) juvenile Atlantic salmon in competition with rainbow trout in streams will have sub-optimal microhabitat associations and have reduced survival and fitness-related traits; (2) Sebago juveniles will have a better competitive ability and thus higher performance than LaHave juveniles with rainbow trout; and (3) that results from competition with rainbow trout in artificial streams are similar in direction, but not in magnitude to results in natural streams.

4.2   Materials and Methods

4.2.1 Study Populations

LaHave and Sebago Atlantic salmon families were produced in early November 2010 at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Harwood Fish Culture Station (Harwood, Ontario). Five females and five males within each population were mated in all possible combinations to produce a 5×5 full factorial breeding design (Lynch and Walsh 1998) for each population. Offspring were then transported the same day as fertilization to the OMNRF Codrington Research Facility, Codrington, Ontario, where they were exposed to natural photoperiods and local stream temperatures (mean ± SD: 8.4 ± 2.6°C). The offspring of one Sebago female had very low survival; therefore, five of the 25 Sebago families were removed from the study.

4.2.2 Study Sites

Two sites within Duffins Creek, Ontario, were used to compare the performance of Atlantic salmon juveniles exposed to competition with non-native salmonids in natural conditions. I was only able to use two sites because of the challenges in getting landowner access to sites, appropriate permits to release fish in multiple locations, and minimizing the overlap in sites used for my experiment and the other stocking efforts of the OMNRF. My study nevertheless represents a rare opportunity to assess how generalizable the knowledge gained regarding the effects of competition in artificial streams is to natural systems. Because environment features may influence the outcomes of competition (Jones and Stanfield 1993; Fausch 1998; Stanfield and Jones 2003), the two sites were as similar as possible in temperature, productivity, and microhabitat, but differed in the presence of rainbow trout (Stanfield et al. 2006 and confirmed by my microhabitat surveys). The first site (Upper Duffins) did not contain rainbow trout and the second site (Lower Duffins) contained juvenile rainbow trout, but also low numbers of brown trout. Both sites contain native brook trout (*Salvelinus fontinalis*) and also have been used previously by the OMNRF for Atlantic salmon juvenile stocking.

Atlantic salmon fry were measured for body length (fork length) and mass, and families were pooled together by site (Table 4.1). Fry were released at the sites on 24 May 2011 using plastic bags filled with oxygen saturated water. At the sites, bags were held within the stream water until the temperature was similar between the water inside the bag and the stream. Fry were then gently dispersed into riffle habitats within a 200 m section of stream using plastic watering cans (stocking area was 1066 m^2 for Upper Duffins and 1341 m^2 Lower Duffins). Sebago salmon fry were initially larger in body length, mass,

and Fulton's condition (Fulton 1904) than LaHave salmon fry (Student's t-tests, $p <$ 0.001): Sebago salmon fry ($n = 540$) were 3.0 ± 0.2 cm (mean + SD), 0.26 ± 0.06 g, and had a condition of 1.00 ± 0.12, and LaHave salmon fry ($n = 1125$) were 2.9 ± 0.2 cm, 0.23 ± 0.07 g, and had a condition of 0.93 ± 0.15.

Table 4.1. Summary of Fry Releases and Captured Juveniles at Two Natural Stream Sites for LaHave and Sebago Atlantic Salmon (*Salmo salar*). Area sampled is the stream area sampled by electrofishing. The age 0+ and 1+ are the counts of juveniles that assigned to the families and in brackets are the counts of juveniles that assigned to a population (including other OMNRF-stocked juveniles of the target age classes). "Older" indicates the number of juveniles that were larger than the individuals that assigned to the families and were excluded from analyses.

Site	Population	Number of fry released	Area sampled (m²)	Fall number of juveniles		Spring number of juveniles	
				age 0+	older	age 1+	older
Upper Duffins	LaHave	1444	-	18 (22)	12	5 (14)	1
	Sebago	446	-	11 (13)	0	1 (1)	1
	Total	1890	1967	29 (35)	12	6 (15)	2
Lower Duffins	LaHave	1469	-	8 (41)	10	2 (13)	5
	Sebago	457	-	3 (18)	7	0 (11)	0
	Total	1926	3436	11 (59)	17	2 (24)	5

4.2.3 Capturing Juveniles and Population Assignments

Atlantic salmon juveniles were captured from the two sites using a backpack electrofisher (Halltech Aquatic Research, Guelph, Ontario) and a lip-seine net at 5 months (Fall: 7-10 November 2011) and 11 months after release (Spring: 10-11 April 2012). Electrofishing started 500 m downstream of the fry release point and moved upstream until about 50 m upstream of the fry release point following a single pass zigzag pattern to ensure the greatest sampling coverage. The entire stream area, including all habitats, was sampled. There was greater coverage sampling downstream than upstream because the majority of fry disperse downstream, usually within 500 m of the release point, within the first year (Webb et al. 2001; Einum et al. 2011). In addition, size-dependent dispersal should be captured within the first 150 m of the release point (Einum et al. 2011). Captured individuals were held in large buckets (10 L) filled with stream water until a predetermined stream section sample was completed. Stream sections were defined as areas roughly 30 m in length that contained homogenous habitat (riffle, runs, or pools). These stream section boundaries were confirmed by the microhabitat survey described below. Upper Duffins had 9 stream sections and Lower Duffins had 12 stream sections. Atlantic salmon juveniles from each section were lightly anaesthetized using food-safe clove oil (Hilltech Canada, Vankleek Hill, Ontario, 100 ppm) and measured for body length, mass, and Fulton's condition (Fulton 1904), traits which are considered relevant for future survival (Metcalfe and Thorpe 1992; Koskinen et al. 2002). A small fin clip (< 0.15 cm^2) was then collected from one of the caudal fin lobes and stored in 95% ethanol for later genetic assignment to family and population (see Appendix A). Juveniles were allowed to recover and were then returned to the section from where they were originally

captured. Non-target species from each section were identified to species, counted, and immediately returned to the site downstream of electrofishing.

4.2.4 Microhabitat Variables

Microhabitat variables were measured once in the fall and used for both fall and spring analyses. Microhabitat measurement were collected at 10 m intervals throughout the study sites (see Peres-Neto 2004 for additional details): (1) average cross-sectional stream water depth from measurements every 50 cm along the entire cross-section; (2) cross-sectional stream width from bank to bank along the entire cross-section; (3) average cross-sectional stream water velocity from measurements at 2-3 points along the cross-section using a 10 second average measurement for each point using a digital flowmeter (Höntszsch, Germany); (4) stream substrate coarseness estimated visually from the centre of the cross-section in the area bounded 1 m upstream and 1 m downstream along the cross-section by percentage composition of clay (< 0.002 mm), silt (0.002-0.05 mm), sand (0.05-2 mm), gravel (2-60 mm), pebbles (60-150 mm), and rocks (> 150 mm). Visual classification of substrate coarseness was based off of a modified Wentworth scale (Heggenes and Saltveit 1990) and was recorded by the same individual for all sites to ensure the consistency of measurements.

4.2.5 Statistical Analysis of Microhabitat Associations

Cumulative distribution functions described by Perry and Smith (1994) were used to describe the associations between each salmonid species (i.e. Atlantic salmon, brook trout, and rainbow trout) and the microhabitat variables for both fall and spring. Principal component analysis with the correlation matrix was used to simplify substrate

composition variables into a smaller number of variables (Coghlan et al. 2007). The availability of each microhabitat variable at each site was quantified using the following cumulative distribution function:

$$f(t) = 100 \sum_{i=1}^{n} I \quad \text{where } I = \begin{cases} 1 & \text{if } x_i \leq t \\ 0 & \text{otherwise,} \end{cases}$$

where t was a level of the microhabitat variable and x_i was the microhabitat variable measurement for stream measurement i (i.e. taken every 10 meters). Similar cumulative distribution functions were calculated for each salmonid species counts in relation to each microhabitat variable at each site for the fall and spring:

$$g(t) = 100 \sum_{i=1}^{n} \frac{y_i}{\bar{Y}} I \quad \text{where } I = \begin{cases} 1 & \text{if } x_i \leq t \\ 0 & \text{otherwise,} \end{cases}$$

where y_i was the salmonid species counts in stream section i and $\bar{Y}$ was the mean counts of the species in a given sampling site and season. Significance of the microhabitat association was determined using a randomization procedure. The test statistic D was the maximum absolute vertical difference between $g(t)$ and $f(t)$ (Perry and Smith 1994). This observed D was compared to the distribution values of D produced by 999 random permutations of the microhabitat data (a total of 1000 permutations including the observed data). That is, under the null hypothesis of random association, I randomly

paired salmonid species counts and microhabitat variables to create the distribution values of *D*.

4.2.6 Statistical Analysis of Recapture, Size, and Condition

Atlantic salmon recapture proportion (number recaptured divided by the number released) between sites and populations was examined using relative fitness analyses described by Kalinowski and Taper (2005; available at http://www.montana.edu/kalinowski/RFA/RFA_Home.htm). One-way ANOVAs compared the body length, mass, and condition of recaptured Atlantic salmon between sites, seasons, and populations in R 3.0.1 (available at http://www.r-project.org/). Binomial generalized linear ordinary least squares regressions were used to test for relationships between Atlantic salmon recapture proportion with body length, mass, or condition. The binomial regressions were weighted by the number of fry released. Poisson (or quasi-Poisson in cases of overdispersion, i.e. if residual deviance was much larger than the degrees of freedom) generalized linear ordinary least squares regressions were used to test for relationships between Atlantic salmon counts with the average microhabitat variables of each stream section. Linear models tested for relationships between Atlantic salmon body length, mass, and condition with average microhabitat variables of each stream section. Statistical significance was set at $\alpha = 0.05$.

4.2.7 Statistical Comparisons between Natural and Artificial Streams

Atlantic salmon water depth, body length, mass, and condition values from the natural stream sites were compared against those from artificial stream environments (Chapter 2). For Atlantic salmon water depth in the natural streams, I used the average water depth

of the section where individuals were captured. The artificial streams contained siblings from eight of the families per population that were released into the two Duffins Creek sites. Artificial stream treatments that were used in the comparisons were (1) Atlantic salmon alone and (2) Atlantic salmon with rainbow trout. To compare the two different environments (natural versus artificial), data from both environments were combined and standardized prior to analysis (mean = 0 and variance = 1 for each variable). Standardized data were analyzed using two-way ANOVAs that contained treatment (rainbow trout absent or present) and source (natural streams or artificial streams).

4.3   Results

4.3.1 Juvenile Captures and Assignments

About 50% more Atlantic salmon juveniles were captured in Lower Duffins than Upper Duffins (Table 4.1). Because the sites potentially contained older Atlantic salmon (i.e. fall age 1+ and spring age 2+) from prior OMNRF Atlantic salmon fry releases, bimodal histograms of Atlantic salmon length were used to separate different age classes. Atlantic salmon that were in the larger mode were considered older Atlantic salmon age classes and were excluded from my analyses. This consideration was further supported based on genetic analysis of samples from the older Atlantic salmon age classes, which confirmed their exclusion from the experimental released families (data not shown). The proportions of older Atlantic salmon were not significantly different between sites ($X^2 = 0$, df = 1, $p = 0.99$).

All Atlantic salmon of the target age classes (i.e. fall age 0+ and spring age 1+), except for two individuals, were assigned to the families or to the LaHave and Sebago

populations (including other OMNRF-stocked juveniles of the target age classes) based on genetic analyses (see Appendix A), and were included in my analyses. OMNRF-stocked juveniles in my sample were a small proportion of what was stocked; OMNRF-stocked juveniles in Upper Duffins originated from fry stockings at a site 500 m downstream in May 2011 ($n = 21,730$) and 2010 ($n = 19,990$) and OMNRF-stocked juveniles in Lower Duffins originated from two fry stocking sites 1.7- 4 km upstream in May 2011 ($n = 36,140$) and 2010 ($n = 30,575$). In addition, Upper Duffins contained 108 and 55 brook trout in the fall and spring sampling periods, respectively, but did not contain rainbow trout. By contrast, the Lower Duffins site contained 16 and 6 brook trout, 560 and 199 rainbow trout, and 9 and 1 brown trout in the fall and spring sampling periods, respectively.

4.3.2 Microhabitat Associations

Although efforts were made to select sites that were as similar in microhabitat as possible, there were significant differences in the microhabitat variables between the Upper and Lower Duffins sites (MANOVA, $p < 0.001$). The sites were significantly different in water velocity (mean ± 1SD, Upper Duffins: $68 ± 12$ cm s^{-1} and Lower Duffins: $81 ± 12$ cm s^{-1}, Student's t-test, $p < 0.001$) and the percentages of pebbles ($19 ± 10\%$ and $37 ± 22\%$, $p < 0.001$) and sand ($20 ± 14\%$ and $12 ± 13\%$, $p = 0.005$) (principal component 2, Table 2), but the sites were not significantly different in water depth ($23 ± 10$ cm and $25 ± 8$ cm, $p = 0.51$) and the percentages of gravel ($18 ± 12$ and $14 ± 13\%$, $p = 0.097$) and rocks ($29 ± 23$ and $26 ± 24\%$, $p = 0.62$) (principal component 1, Table 4.2). Upper Duffins had a lower water velocity, a lower proportion of pebbles, and a higher proportion of sand than Lower Duffins.

Salmonid species were significantly associated with microhabitat variables (Figure 4.1). In the absence of rainbow trout (Upper Duffins), Atlantic salmon were found in habitats with a higher percentage of gravel in the fall and with a lower water depth in the spring. On the other hand, in the presence of rainbow trout (Lower Duffins), Atlantic salmon were found in habitats with a higher percentage of pebbles in the fall and with higher percentages of rocks and sand in the spring (Figure 4.1). Similarly, in the absence of rainbow trout, brook trout were found in habitats with a higher percentage of gravel in the fall, but had no microhabitat associations in the spring (Figure 4.1). In the absence of rainbow trout, brook trout had no microhabitat associations in the fall, but were found in habitats with a higher percentage of rocks in the spring. Rainbow trout were found in habitats with a higher percentage of rocks in the spring, but had no specific microhabitat associations in the fall.

Table 4.2. Summary of Relationships Between Substrate Composition and the First Two Principal Components based on Two Natural Stream Sites. Relationships greater than 0.45 and lesser than -0.45 are displayed in bold.

Variable	PC 1	PC2
Clay	0.376	0.148
Silt	0.430	-0.415
Sand	0.122	**-0.590**
Gravel	**0.494**	0.237
Pebbles	0.147	**0.600**
Rocks	**-0.638**	-0.205
Proportion of variance explained	29.3%	25.5%
Cumulative proportion of variance explained	29.3%	54.8%

Fall
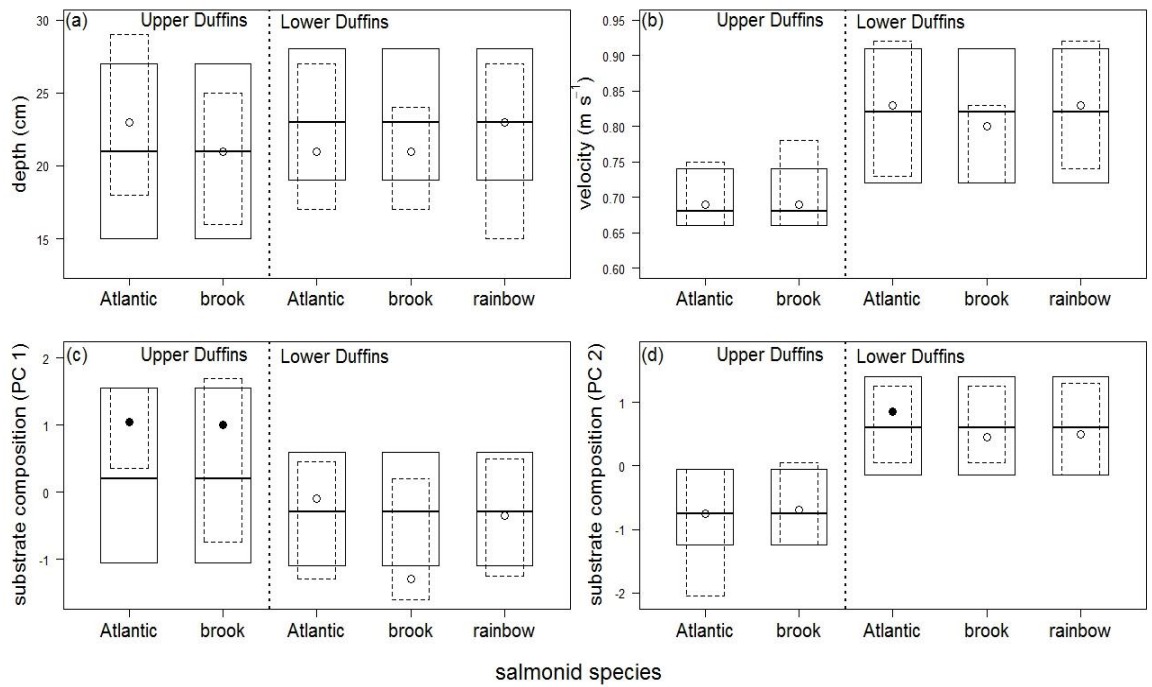
Spring
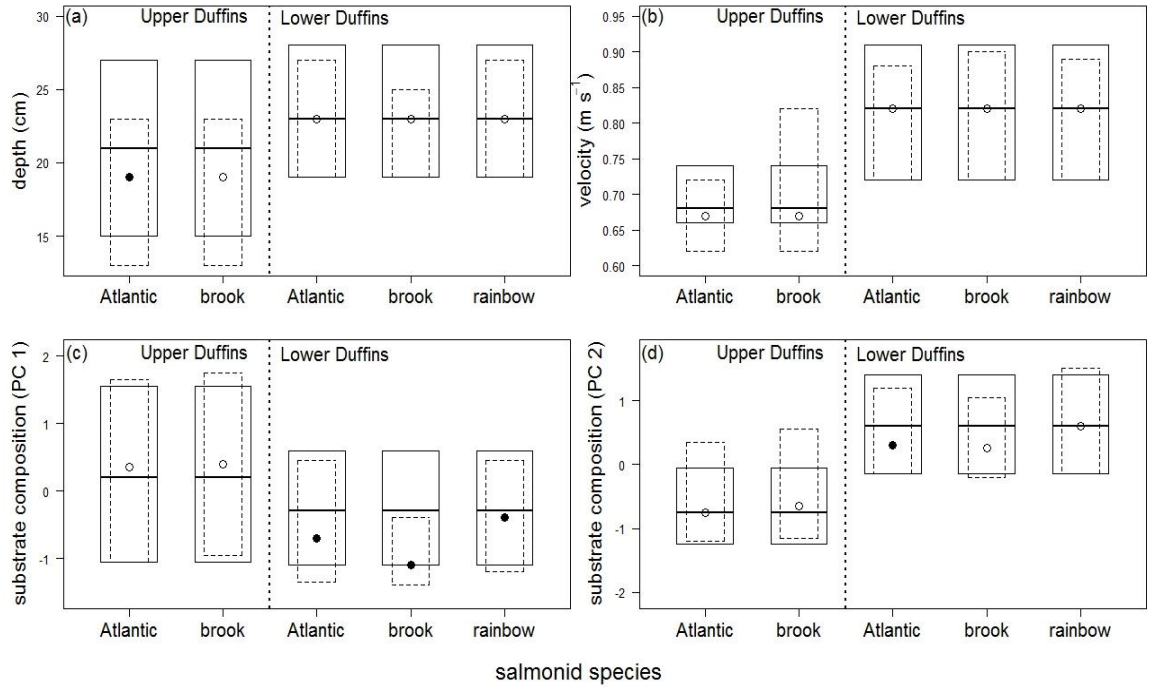
Figure 4.1. Microhabitat Associations of Three Species of Salmonid (Atlantic Salmon-*Salmo salar*, Brook Trout- *Salvelinus fontinalis*, Rainbow Trout- *Oncorhynchus mykiss*) at Two Natural Stream Sites. Shown are data from four microhabitat variables: (a) water depth, (b) water velocity, (c) principal component 1 of substrate composition (PC 1), (d) principal component 2 of substrate composition (PC 2). Solid lines and boxes display the median and 25th to 75th percentiles of available microhabitat; dots and dashed boxes display the median and 25th to 75th percentiles of associated (utilized) microhabitat. Filled dots indicate significant microhabitat associations ($p < 0.05$). The principal component loadings are presented in Table 4.2.

Between sites, Atlantic salmon associated with different microhabitat variables (Figure 4.1). Atlantic salmon were found in habitats with a lower water depth in the fall (one-way ANOVA, $p = 0.007$) (opposite in the spring, $p = 0.001$), and in both seasons were found in habitats with a higher water velocity (both $p < 0.001$) and higher percentages of rocks (fall, $p < 0.001$ and spring, $p = 0.026$) and pebbles (both $p < 0.001$) in the presence than in the absence of rainbow trout. Within sites, Atlantic salmon associated with different microhabitat variables in comparison to the other salmonid species that were present. In the absence of rainbow trout, Atlantic salmon were found in habitats with a greater water depth (one-way ANOVA, $p < 0.001$) and a larger percentage of sand ($p = 0.006$) than brook trout in the fall, and there were no significant differences in microhabitat associations in the spring ($p > 0.14$ for all). Conversely, in the presence of rainbow trout, Atlantic salmon were found in habitats with similar microhabitat variables as brook trout and rainbow trout for both seasons ($p > 0.13$ for all), with exception of water depth and the percentage of sand compared to brook trout (both $p < 0.001$) and water depth compared to rainbow trout ($p = 0.047$) in the fall. Atlantic salmon populations were not significantly different in microhabitat associations in both seasons ($p > 0.08$ for all; Figure 4.2), with exception that Sebago juveniles associated with a higher percentage of gravel than LaHave juveniles in the absence of rainbow trout ($p = 0.01$).
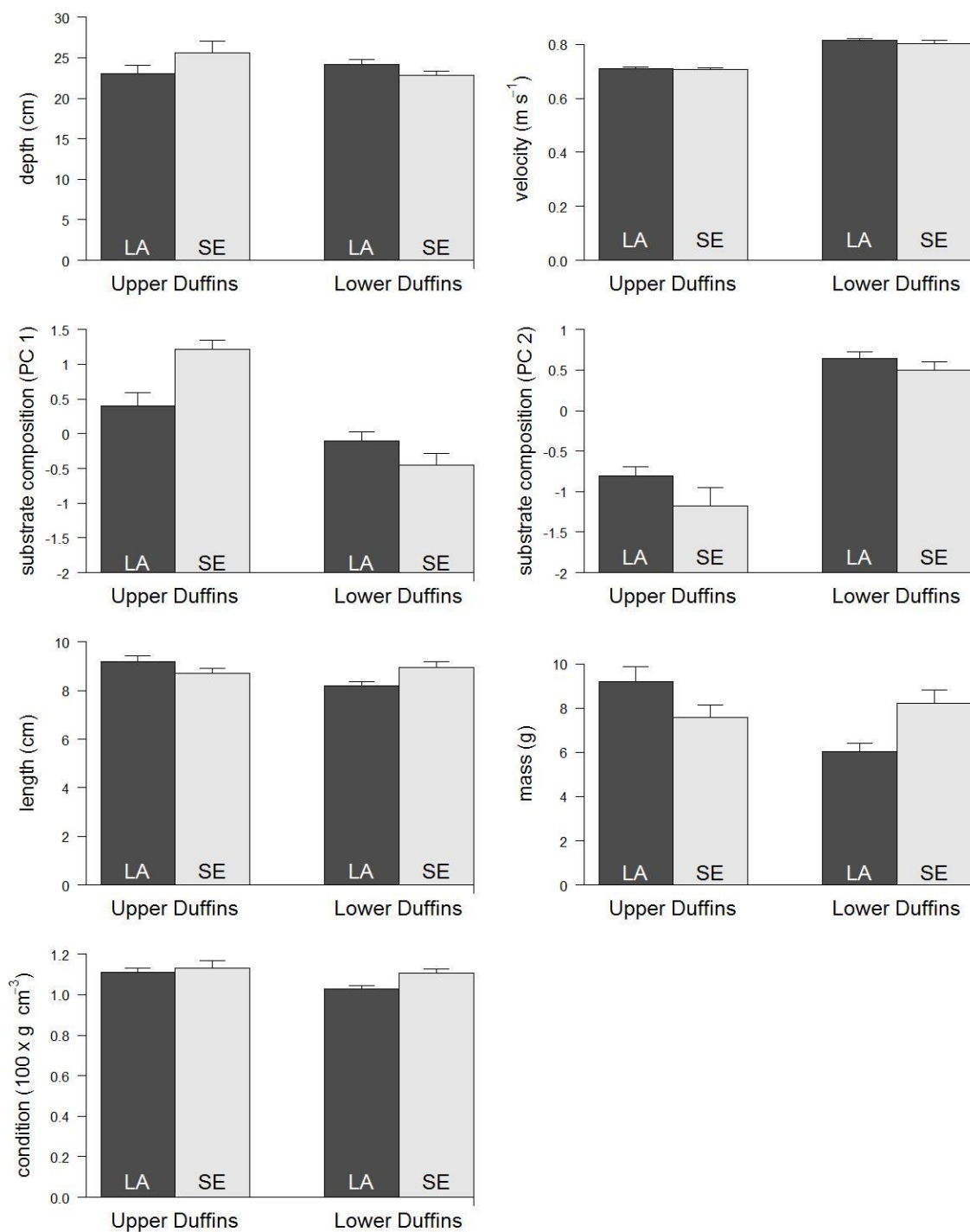
Figure 4.2. Microhabitat Associations, Body Length, Mass, and Condition for LaHave and Sebago Atlantic salmon (*Salmo salar*) in Two Natural Stream Sites. Displayed are means ± 1SE.

4.3.3 Recapture Proportion, Size, and Condition

Over the winter, the relative recapture proportion of Atlantic salmon was not significantly different between the two sites (0.95 [95% CI = [0.50, 1.85]); therefore, fall and spring Atlantic salmon counts were combined. Although one purpose of the stocking experiment was to assess fitness variation within as well as between the two source populations, the counts of juvenile Atlantic salmon were insufficient to assess family-level differences in recapture proportions (Table 4.1). Using the counts from Atlantic salmon that were assigned to specific families, the relative recapture proportion of Atlantic salmon was significantly different between sites (0.36 [0.19, 0.67]), which cannot be explained by the difference in sampling area (Table 4.1). On the other hand, using the counts of all Atlantic salmon (my experimental fish plus the OMNRF-stocked fish), the density was similar between the sites (0.017 Atlantic salmon m^{-2} for both sites). Also, the relative recapture proportion of the two Atlantic salmon source populations was not significantly different in both sites (Upper Duffins: 1.69 [0.81, 3.33] and Lower Duffins: 0.97 [0.22, 3.17]). There were no significant relationships between Atlantic salmon recapture proportion and initial release body length (binomial model, $p > 0.30$), mass ($p > 0.14$), and condition ($p > 0.26$) within sites (data not shown). Also, there were no significant relationships between Atlantic salmon recapture proportion and the microhabitat variables (quasi-Poisson models, $p > 0.12$ for all; data not shown) or the counts of older Atlantic salmon within sites ($p > 0.81$).

Body length, mass, and condition of Atlantic salmon were significantly different between sites and populations (Figure 4.2). Atlantic salmon were shorter (one-way ANOVA, $p = 0.005$), had lower mass ($p = 0.001$), and were in lower condition ($p = 0.007$) in the

presence than in the absence of rainbow trout. Sebago juveniles were longer ($p = 0.040$) and had higher mass ($p = 0.026$) than LaHave salmon in the presence of rainbow trout, whereas LaHave and Sebago juveniles were not significantly different in body length ($p = 0.12$) and mass ($p = 0.36$) in the absence of rainbow trout. Also, Sebago juveniles were in higher condition than LaHave juveniles in both sites ($p = 0.014$). For Upper Duffins, there was a significant correlation between these Atlantic salmon variables (i.e. body length, mass, and condition) and substrate composition (principal component 1) ($p < 0.04$); Atlantic salmon were larger in habitats with a higher percentage of rocks and in higher condition in habitats with a higher percentage of gravel. For Lower Duffins, there was a significant correlation between body condition and substrate composition (principal component 1 and 2) ($p < 0.03$); Atlantic salmon were in higher condition in habitats with higher percentages of rocks and sand. There were no significant relationships between the Atlantic salmon variables and the remaining microhabitat variables (linear models, $p > 0.09$ for all; data not shown) or the counts of older Atlantic salmon within sites ($p > 0.81$). There also were no significant relationships between body length (linear model, p > 0.11), mass ($p > 0.28$), or condition ($p > 0.27$) within sites at the time of release versus the time of recapture, based Atlantic salmon family means (data not shown).

4.3.4 Comparisons to Artificial Streams

The direction and magnitude of the response of the water depth that Atlantic salmon occupied as well as their body length and mass to the presence of rainbow trout did not significantly differ between natural and artificial streams (Table 4.3; Figure 4.3). On the other hand, the body condition response to the presence of rainbow trout was significantly different between natural and artificial streams; there was a greater

reduction in condition in the natural streams than in the artificial streams. In both artificial and natural streams, Atlantic salmon were not associated with different depths in the presence of rainbow trout. In addition, in both environments there was a reduction in Atlantic salmon body length, mass, and condition in the presence of rainbow trout.

Table 4.3. Two-Way ANOVA Results Comparing Habitat and Body Measurements of Juvenile Atlantic Salmon (*Salmo salar*) in Natural and Artificial Streams. Variables tested were treatment (rainbow trout absent or present) and source (natural streams or artificial streams). Samples sizes for the natural stream experiment were $n = 51$ individuals for the rainbow trout absent and $n = 83$ individuals for the rainbow trout present treatments. Sample sizes for the artificial stream experiment were $n = 32$ average values of individuals within streams in both the rainbow trout absent and present treatments for water depth, and were $n = 486$ individuals for the rainbow trout absent and $n = 225$ individuals for the rainbow trout present treatments for the body size variables.

Variable	df	Sum sq.	Mean sq.	F	p
Water Depth					
treatment	1	2.83	2.83	86.95	< 0.001
source	1	196.56	196.56	6036.91	< 0.001
treatment × source	1	0.00	0.00	0.05	0.827
residuals	203	6.61	0.03		
Body Length					
treatment	1	5.6	5.59	5.85	0.016
source	1	35.2	35.19	36.85	< 0.001
treatment × source	1	0.0	0.02	0.02	0.899
residuals	842	804.2	0.96		
Body Mass					
treatment	1	14.9	14.85	15.13	< 0.001
source	1	3.7	3.73	3.80	0.052
treatment × source	1	0.1	0.11	0.11	0.743
residuals	842	826.3	0.98		
Body Condition					
treatment	1	26.0	26.04	27.56	< 0.001
source	1	17.8	17.80	18.83	< 0.001
treatment × source	1	5.6	5.61	5.94	0.015
residuals	842	795.6	0.95		
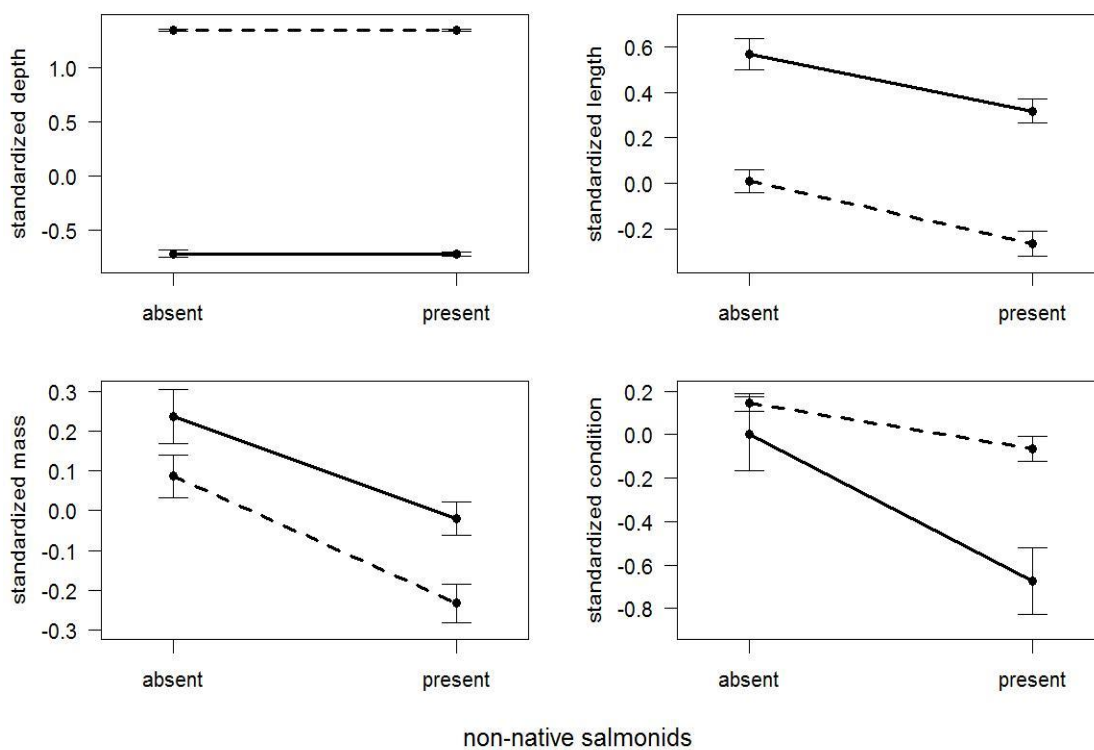
Figure 4.3. Standardized Water Depth, Body Length, Mass, and Condition of Atlantic Salmon (*Salmo salar*) in Natural and Artificial Streams. Displayed are means ± 1SE in the presence of rainbow trout. Solid lines represent natural stream data; dashed lines represent the artificial stream data.

4.4   Discussion

Ecological niche overlap among species has long been considered to lead to increased competition for similar resources (Hutchinson 1957). I found that Atlantic salmon and rainbow trout had similar microhabitat associations in a stream during the juvenile stage. I also found that the presence of rainbow trout led to reductions in Atlantic salmon body length, mass, and condition, but not the relative recapture proportion at this juvenile stage. My release sites were originally selected because they were similar in microhabitat composition, productivity, and temperature. Indeed, the sites were similar in water depth, and the percentages of gravel and rocks, but the sites differed in water velocity and the percentages of pebbles and sand. Nevertheless, the mean values for water velocity and the percentages of pebbles were within the optimal range for Atlantic salmon juveniles in both sites (Morantz et al. 1987; Guay et al. 2000; Beland et al. 2004; Hedger et al. 2005). Although, Atlantic salmon juveniles tend to avoid microhabitats with a high percentage of sand (e.g. Morantz et al. 1987), the difference in the percentage of sand between the two sites was small at 8%. Similarly, the sites both contained older Atlantic salmon, but the proportions were similar and the counts were not related to the changes in my focal Atlantic salmon numbers or sizes. Thus, the changes I observed in Atlantic salmon microhabitat association and size do not appear to be due to intraspecific competition with older Atlantic salmon. Instead my results suggest that the changes in Atlantic salmon microhabitat association and size at this site are due to competition with rainbow trout, as has been documented in other studies (Jones and Stanfield 1993; Stanfield and Jones 2003; Coghlan et al. 2007; Thibault and Dodson 2013).

Competition among ecologically-similar species may decrease by reducing the ecological niche overlap (Hutchinson 1957). I found that Atlantic salmon had optimal microhabitat associations in a natural stream site without rainbow trout but sub-optimal microhabitat associations in a site where rainbow trout were present. Specifically, Atlantic salmon were found in habitats with a higher percentage of gravel and lower water velocity, their optimal physical microhabitats (Morantz et al. 1987), when rainbow trout were absent, but were found in habitats with a lower water depth, lower percentages of pebbles, rocks, sand, and a higher water velocity in the presence of rainbow trout. Other studies have also found that Atlantic salmon shift to habitats with lower water depth and higher water velocity in the presence of rainbow trout, possibly because Atlantic salmon pectoral fins are better suited to holding position in faster water than rainbow trout (Gibson 1981; Hearn and Kynard 1986; Volpe et al. 2001). A shift in Atlantic salmon microhabitat associations may also be due to competitive displacement by the generally more aggressive rainbow trout (Gibson 1981; Hearn and Kynard 1986; but see Van Zwol et al. 2012a). The displacement could explain the reductions in Atlantic salmon body length, mass, and condition that I observed because of the increased energy expenditure or perhaps fewer available resources in the sub-optimal microhabitat (Hearn 1987; Fausch 1988). Native species that are displaced by ecologically-similar species may consequently have decreased fitness because of associations with sub-optimal microhabitats.

Salmonid populations may differ in their ability to cope with the competition imposed by non-native species. Examining my experimental families, I found no difference in the relative recapture proportion of the populations, but this result may reflect the small

sample size (driven partly by the high juvenile mortality in Lake Ontario tributaries; COSEWIC 2006, 2010). Indeed, I did detect differences between populations when examining all the Atlantic salmon caught. I found that Sebago salmon were longer, heavier, and had greater body condition than LaHave salmon in the natural stream site containing rainbow trout. Although Sebago salmon were initially larger at release, the difference was negligible and not likely to have driven the differences at recapture. For example the body length difference was 3% (0.1 mm) whereas at recapture the difference was 8% (7 mm). In addition, the LaHave and Sebago populations were similar in size in the natural stream site that did not contain rainbow trout. Similar results were reported for Atlantic salmon juveniles that were examined in artificial streams (Van Zwol et al. 2012b; Chapter 2). Van Zwol et al. (2012b) observed that Sebago salmon avoided agonistic interactions with rainbow trout relative to LaHave salmon. This difference in behavioural tactics may underlie the difference I detected in performance when in competition with a non-native species.

Effects of interspecific competition may be similar in natural and artificial environments. I examined the effects of competition with rainbow trout on the traits of Atlantic salmon in both natural and artificial streams (Chapter 2). I found that Atlantic salmon responses to competition were similar in both environments. A meta-analysis by Korsu et al. (2010) found that effects of competition were similar in direction, but differed in magnitude between environments. The direction and magnitude may have been more similar in my study (for three out of the four traits I examined) because I used a paired-family design, i.e. a subset of eight families per population in the artificial streams from those families that were released in the natural streams. My data suggest that there is merit in

performing controlled experiments first in artificial environments as a primary test for performance and fitness reductions due to interspecific competition (also see Fausch 1998). Artificial environments may also provide insight into target variables, such as the importance for controlling for physical habitat, before taking the research into the more complex natural environment.

My results have implications for the reintroduction efforts of native species. The presence of non-native salmonids has been identified as an important feature of the environment that may be an impediment to a successful reintroduction of Atlantic salmon into Lake Ontario (Jones and Stanfield 1993; Crawford 2001; COSEWIC 2006, 2010). I found that the Sebago population had better performance (i.e. larger body size and better condition) with rainbow trout in a natural stream than the LaHave population. Stocked Sebago salmon also appear to co-exist with naturalized and stocked rainbow trout and brown trout in Lake Champlain (Marsden et al. 2010), whereas the LaHave population has not previously been examined in wild sympatry with rainbow trout (Dimond and Smitka 2005). More broadly, my results suggest that source populations appearing to do well in a location with similar key environment features as the reintroduction location may possess important adaptations (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). Identifying ideal source populations may also require an examination of the performance of several populations in response to important features of the reintroduction location (van Katwijk et al. 1998). Finally, I found that the presence of ecologically similar non-native species reduced fitness-related traits of a native species in both natural and artificial environments. I suggest that native species reintroduction efforts minimize ecological

niche overlap with non-native species in an attempt to maximize the performance of translocated individuals.

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Chapter 5

5 Effects of Feeding High Dietary Thiaminase to Sub-Adult Atlantic Salmon from Three Populations[*]

5.1 Introduction

Anthropogenic impacts on natural environments are increasingly altering prey species composition and abundance. It is becoming apparent that these impacts can lead to deficiencies in essential nutrients formerly available in prey species (Barboza et al. 2009). Because essential nutrients cannot be synthesized *de novo*, deficiencies in these nutrients can leave predator species vulnerable to metabolic dysfunction and disease. For example, habitat changes have diminished the prey resources containing vitamin A for southern sea otters (*Enhydra lutris nereis*) (St Leger et al. 2011). Subsequent vitamin A deficiencies in sea otters resulted in abnormal bone growth and a reduction in survival (St. Leger et al. 2011). Furthermore, lipid deficiencies in *Daphnia magna* caused by human-induced cyanobacteria blooms reduced the number and quality of the eggs produced (Wacker et al. 2007). Nutrient deficiencies can have significant ecological effects, as even small reductions in individual fitness can lead to altered community dynamic, the extirpation of small populations (Hutchings 1991), and potentially impede a successful reintroduction of native populations (Dimond and Smitka 2005).

Thiamine (vitamin B1) is an essential, environmentally-obtained nutrient for many fish species (Halver and Hardy 2002). Thiamine is essential for metabolism as a coenzyme for several enzymes that breakdown carbohydrates and amino acids to produce energy (or

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adenosine triphosphate, ATP) (Kawasaki and Egi 2000). Many salmonid populations are currently experiencing thiamine deficiencies (Norrgren et al. 1993; Fisher et al. 1995; Fitzsimons et al. 1995). In the Laurentian Great Lakes and New York Finger Lakes, the source of the thiamine deficiency for salmonid fishes appears to be the consumption of introduced non-native prey fishes that contain high concentrations of thiaminase, an enzyme that degrades thiamine (Fitzsimons et al. 1998; Wistbacka et al 2002; Honeyfield et al. 2012). On the other hand, in the Baltic Sea, the thiamine deficiency in salmonids appears to be driven by a reduced thiamine transfer from lower to higher trophic levels because of eutrophication in the environment (Sylvander et al. 2013).

Salmonids within the Great Lakes and Finger Lakes historically consumed native prey fishes, such as cisco or lake herring (*Coregonus artedi*) and bloater (*C. hoyi*), which contain low thiaminase concentrations (Tillitt et al. 2005; Zajicek et al. 2005). Currently, within these lakes, the dominant prey fishes are now introduced non-native alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*), which contain high thiaminase concentrations (Tillitt et al. 2005; Zajicek et al. 2005; Honeyfield et al. 2012). A source of the thiaminase found in these introduced prey fishes is the non-pathogenic bacteria *Paenibacillus thiaminolyticus*, which has been isolated from Lake Michigan alewives (Honeyfield et al. 2002; Zajicek et al. 2009). Non-native prey fish can also produce thiaminase de novo within their bodies (Richter et al. 2012). Without consideration of the presence of thiaminase, the introduced non-native prey fishes themselves exceed the amount of dietary requirement of thiamine for fish (Fitzsimons et al. 1998; Tillitt et al. 2005). However, the high thiaminase concentrations of these prey

fishes can degrade any available thiamine in the digestive system of salmonid predators before it can be absorbed (Fitzsimons et al. 2007).

Here, I examine the performance (i.e. survival and fitness-related traits) of sub-adult (two-year-old) Atlantic salmon from three populations that were given prepared diets mimicking the historical diet (low thiaminase content) and the current diet (high thiaminase content) within the Great Lakes. I predict that potamodromous populations (i.e. the Sebago and Saint-Jean populations) that primarily consume rainbow smelt (Dimond and Smitka 2005) will have higher thiaminase tolerance than an anadromous population (i.e. the LaHave population) that has a more diverse diet (Rikardsen and Dempson 2011), which could be lower in thiaminase. Although several studies have examined the effects of thiamine deficiency in adult salmonids and their offspring, these effects have rarely been examined in smolt or sub-adult salmonids, the age when these fishes begin consuming high thiaminase-containing prey fishes (Morito et al. 1986; Ketola et al. 2008).

5.2 Materials and Methods

5.2.1 Study Populations

Families for the LaHave ($n = 37$), Sebago ($n = 14$), and Saint-Jean ($n = 66$) populations were produced in early November 2011 using single-pair matings of mature individuals at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Harwood Fish Culture Station, Harwood, Ontario. The LaHave mature individuals originated from fertilized eggs of single-pair matings of captive LaHave adults descended from the wild source population (44°14′N 64°20′W). The OMNRF LaHave broodstock was founded

from several years of wild spawn collections (1989 to 1995), and the captive adults used from the 2007 cohort were the product of two generations of post-founding hatchery breeding (OMNR 2005). The Sebago and Saint-Jean mature individuals originated from fertilized eggs of single-pair matings of wild Sebago from Panther River (43°53'N, 70°27'W) and wild Saint Jean from Rivière-aux-Saumons (48°41'N, 72°30'W); both founding wild spawn collections were carried out in 2007. Families were transported to the OMNRF Codrington Research Facility, Codrington, Ontario in spring 2012, where they were subjected to a natural light cycle and water from a surface stream (Marsh Creek) at natural temperatures. The salmon were fed commercial pellets (Corey Aquafeeds, Fredericton, New Brunswick) until used in the experiment.

5.2.2 Experimental Diets

Two experimental diets were formulated to be isoproteic, isoenergetic, and to contain different concentrations of bacterial thiaminase (*Paenibacillus thiaminolyticus*) isolated from Lake Michigan alewives (Honeyfield et al. 2002). These diets were control (no thiaminase) and high thiaminase (6,800 pmol min^{-1} per gram of feed, Honeyfield et al. 2005), similar to the thiaminase activity of alewife, rainbow smelt, and round goby (*Neogobius melanostomus*) in Lake Ontario (Honeyfield et al. 2012). The diets were formulated to mimic the naturally occurring symptoms of thiamine deficiency in lake trout (*Salvelinus namaycush*) (Honeyfield et al. 2005). Both diets (control and thiaminase) were fish meal based and contained all the nutrient requirements of fish, including thiamine measured at 19.8 ± 8.6 (mean $\pm$ 1SD) mg per kilogram of feed (Table 5.1).

All dry ingredients were thoroughly mixed (Hobart mixer, Hobart Ltd, Don Mills, Ontario, Canada) and then mixed again with the addition of thiaminase bacteria liquid culture (thiaminase diet only) and water (about 400 ml of liquid per kg of mash dry weight) at the University of Guelph Fish Nutrition Research Lab, Guelph, Ontario. The mix was immediately transported to the University of Western Ontario, London, Ontario. After 24 h, more water was then added until the feed was a dough-like consistency and the dough was screw pressed using a 5 mm diameter die. The resultant moist pellets were air dried at room temperature for 2 to 3 days and then transported and stored at -20°C at the Codrington Facility until used.

Table 5.1. Composition and Proximate Analysis of the Experimental Diets for Atlantic Salmon (*Salmo salar*). Greater details on the diet formulation are described in Honeyfield et al. (2005). Proximate analysis is based on dry matter basis. Thiaminase bacteria (*Paenibacillus thiaminolyticus*) cultures were prepared using liquid media (yeast extract 1.0 g L^{-1} and 8.0 g L^{-1} Difco nutrient broth, Becton Dickinson, Mississauga, Ontario) inoculated with the bacteria (3 ml inoculation for 1 L of media) and incubated for 96 h at 37°C. For the thiaminase diet, bacteria cultures were mixed into dry ingredients (300 ml per kilogram of feed) to produce a thiaminase activity of 6,800 pmol min^{-1} per gram of feed. Thiamine was measured at 19.8 ± 8.6 (mean $\pm$ 1SD) mg per kilogram of diet.

Variable	Control (%)	Thiaminase (%)
Diet composition		
fish meal, herring	32.0	32.0
starch	30.0	30.0
corn gluten meal	18.0	18.0
blood flour	8.6	8.6
fish oil	8.0	8.0
dextrin	1.0	1.0
choline chloride	0.5	0.5
vitamin premix	0.5	0.5
mineral premix	0.2	0.2
ascorbyl-2-polphosphate	0.2	0.2
betaine-HCl	1.0	1.0
bacterial thiaminase	none	trace
Proximate analysis		
dry matter	81.4	80.4
crude protein	38.7	39.4
crude lipid	10.4	10.3
total carbohydrates	25.2	24.0
ash	7.1	6.7

5.2.3 Experimental Set-up

Atlantic salmon were adapted to experimental conditions for one year before starting the trial. Groups of 48 individually marked salmon (16 fish per population, sub-adults that were two-year-olds) were randomly distributed into six (260 L) tanks. Experimental diets were assigned randomly to the tanks (three tanks per diet). Salmon were maintained on water from Marsh Creek at natural temperatures and subjected to a natural light cycle.

Trials began in October 2013 when salmon were anesthetized with buffered MS-222 (tricaine methanesulfonate, 0.1 g L^{-1}), measured for fork length (nearest 0.1 cm) and mass (nearest 0.1 g). Salmon individuals had an initial body mass of 56.3 ± 13.7 g (mean ± 1SD). Condition was calculated as $100 \times mass / length^3$ (Fulton 1904). While still anaesthetized, salmon were tagged with a 2 cm vinyl anchor tag on the left side just below the dorsal fin (Floy Tag & Mfg., Seattle, Washington) before being placed into the treatment tanks (Table 5.2). Tags were individually numbered and coloured for each population and were applied using a fine fabric gun (Avery Mark III Fine Fabric Pistol Grip) with a maximum needle insertion depth of 1.5 cm. The needle was disinfected with hydrogen peroxide between individuals. The same day as tagging, salmon were given a 1% (0.01 kg L^{-1}) sodium chloride bath for 20 minutes for additional disinfection.

After a 14 day recovery period during which fish were fed a commercial diet (Corey Aquafeeds, 3 mm pellet, once a day), individual salmon were lightly anaesthetized (MS-222, 0.05 g L^{-1}), placed on their right side and digitally photographed (10.3 MP Kodak Natural Color System) using a camera set at a fixed height. Each digital photograph contained a size and a colour standard. Salmon were allowed to recover and were

returned to their tank. A sample of extra salmon (not used in the experiment) were also sacrificed at this time point ($n = 12$ from each population) to serve as a baseline for the thiamine concentrations of red blood cells and plasma. These latter salmon were euthanized using an overdose of anaesthetic until gill movement ceased; blood samples (0.5-1 ml) were then collected from the caudal peduncle posterior to the anal fin using a Heparin lined tube. Blood samples were immediately separated into plasma and red blood cells by centrifugation (1,500 RCF for 5 minutes), frozen using dry ice and stored at -80°C until thiamine analysis.

Experimental salmon recovered for another 14 days, during which time they were fed a mixture of experimental diet and commercial diet (1:1). Afterward, salmon in the different treatment tanks were fed 100% their experimental diet for 8 months at 1% body mass per day from December to April and 2% body mass per day from June to August. Salmon survival was determined by removing mortalities daily from the tanks.

A subset of Atlantic salmon were sacrificed on June 10, 2014 ($n = 4$ from each population in each diet) to assess the thiamine concentrations of tissues. Baseline plasma total thiamine concentrations were at the lower end of the detection limit (mean ± 1SD, 0.18 ± 0.18 nmol ml^{-1}), so I also collected liver tissue at this time. Liver tissue is expected to be higher in total thiamine concentration (see Brown et al. 1998). Liver tissue was immediately frozen on dry ice and stored at -80°C until thiamine analysis.

Table 5.2. Summary of Body Traits and Total Thiamine Concentrations for Three Populations of Sub-Adult Atlantic Salmon (*Salmo salar*) at the Beginning of the Experiment. Presented are means ± 1SD. Different uppercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$). For morphology, *centroid size* (used as a covariate for morphology to control for potential allometric effects of body size, see Bookstein 1991) was included in the analysis. Morphology higher relative warp 1 (RW1) scores were associated with a more streamlined body shape. For skin pigmentation, higher principal component 1 (PC1) scores were associated with yellower body regions and higher principal component 2 (PC2) scores were associated with whiter body regions. Sample sizes are: $n = 12$ individuals for thiamine traits and $n = 96$ individuals for remaining traits for each Atlantic salmon population. The individuals used for thiamine traits were extra salmon (surplus) not used in the experiment (see Materials and Methods).

Traits	LaHave	Sebago	Saint-Jean
length (cm)	17.1 ± 1.2^A	17.6 ± 1.5^B	16.8 ± 1.5^A
mass (g)	52 ± 10^A	63 ± 14^B	54 ± 14^A
condition ($100 \times$ g cm^{-3})	1.03 ± 0.07^A	1.12 ± 0.05^B	1.12 ± 0.06^B
morphology (RW1)	0.018 ± 0.015^A	0.004 ± 0.011^B	0.002 ± 0.009^B
pigmentation (PC1)	-11.4 ± 13.2^A	-6.7 ± 12.2^B	2.1 ± 13.6^C
pigmentation (PC2)	-8.4 ± 10.3^A	-7.6 ± 10.7^A	-2.5 ± 10.9^B
red blood cells total thiamine (nmol g^{-1})	2.3 ± 1.2^A	1.9 ± 0.9^A	2.4 ± 1.0^A
plasma total thiamine (nmol ml^{-1})	0.12 ± 0.14^A	0.18 ± 0.19^A	0.26 ± 0.20^A

5.2.4 Thiamine Analysis

I focussed my thiamine analysis on the red blood cells and liver tissues; the total thiamine concentrations in plasma were nearly undetectable for the thiaminase diet (data not shown). Thiamine concentrations of red blood cells and liver tissues were determined using the method developed by Brown et al. (1998). Samples of red blood cells (100-200 mg) or liver (300 mg) tissue were mixed with tricholoracetic acid, boiled for 10 minutes, centrifuged (14,000 RCF for 15 minutes), washed with ethyl acetate and hexane, and kept at -20°C until oxidized. Washed extracts were oxidized with sodium hydroxide and potassium ferricyanide to their corresponding thiochromes. The thiochrome fluorescence of thiamine pyrophosphate, thiamine monophosphate, and free thiamine was measured using reverse-phase high-performance liquid chromatography with a Poroshell 120 column (100 × 4.6 mm, 2.7 μm mesh size; Agilent, Mississauga, Ontario) and a fluorescence detector at Agriculture Canada, London, Ontario.

5.2.5 Morphology and Skin Pigmentation

Photographs of the salmon were examined for body morphology and skin pigmentation using the methods described by Fraser et al. (2010) and Villafuerte and Negro (1998). For morphology, 21 landmarks related to aspects of head and body depth and caudal region lengths were measured using *tpsDig* software (Rohlf 2008) and these landmarks were subjected to a relative warp analysis using *tpsRelw* software (Rohlf 2009) to get the centroid sizes and principal relative warp scores. For skin pigmentation, the average colour of red, green, and blue pixels (RGB colour space) were measured for the dorsal, lateral, ventral, caudal peduncle, and caudal fin body regions using ImageJ version 1.47 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/). RGB colour space values for

skin pigmentation, i.e. dorsal, ventral, lateral, caudal peduncle, and caudal fin body regions, were converted into XYZ colour space values, and then converted into LAB colour space values using colour conversion formulas of EasyRGB (available at: http://www.easyrgb.com/). Principal component analysis (PCA) with the covariance matrix in R 3.0.1 (available at http://www.r-project.org/) was used to simplify LAB colour space values into a smaller number of variables.

For morphology, I considered only relative warp 1 which explained 30.4% of the variation among individuals and could be easily interpreted biologically: positive relative warp 1 scores were associated with a more streamlined body shape. For skin pigmentation, I considered principal components 1 and 2 which explained 39.0% and 22.6% of the variation among individuals, respectively. Principal component 1 was positively related to the yellowness of the lateral, ventral, and caudal peduncle body regions. Principal component 2 was positively related to the whiteness of the lateral, ventral, caudal peduncle, and dorsal body regions.

5.2.6 Swimming Performance

Atlantic salmon were measured for critical swimming speed between July 23 and August 4 using the methods described in Colborne et al. (2011). Briefly, an individual was placed into an acrylic swim flume (Loligo Systems, Denmark) and acclimated for a period of 3 minutes. Water flow speed was then increased incrementally at 0.3 m s^{-1} every 2 minutes until the individual displayed signs of fatigue. Critical swimming speed (U_{crit}) was calculated as $U_{crit}=U_i + (T_i / T_{ii} \times U_{ii})$, where U_i is the highest velocity maintained for a full 2 minute interval, T_i is the time of fatigue at last current velocity (minutes), T_{ii} is the

interval length (2 minutes), and U_{ii} is the velocity increment (0.3 m s^{-1}). To account for size influences on swimming performance, I used an Aitchinson (1986) log-ratio correction to produce relative swimming performance scores (also see Colborne et al. 2011) calculated as $rsp_i = [\ln(sp_i) - \ln(centroid_i)] / 2 + K$, where for individual i, rsp_i is the relative swimming performance, sp_i is the critical swimming speed, $centroid_i$ is the centroid size, and K is the minimum rsp_i included so that all rsp_i values are positive. Fatigued salmon were lightly anaesthetized, measured for length and mass, and then digitally photographed as described above. Thermal-unit growth coefficient (TGC) was calculated as $100 \times (S_2^{1/3} - S_1^{1/3}) / \Delta D$ (Cho 1992), where S_2 is the size at time 2, S_1 is the size at time 1, and ΔD is the growing degree-days ($\Delta D = \sum$ ˚C per day) from the initial body size measurements.

5.2.7 Statistical Analysis of Traits

Traits of individual Atlantic salmon were analyzed in R, using a significance threshold of $\alpha = 0.05$ for all statistical tests. Changes in traits (final – initial values for individuals) were used for analyses of condition, morphology, and skin pigmentation. Linear mixed-effects models (*lmer* in the lmerTest package of R) were used to examine effects for normally distributed data and binomial mixed-effects models were used for survival (coded as 1 for alive and 0 for dead). Mixed-effects models contained fixed effects for *population*, *diet*, and *population* × *diet* and a random effect for *tank* identity. A linear discriminant analysis (*lda* in the MASS package of R) was then used to examine the effect of diet on the three populations. Five traits were included in the analysis (liver thiamine concentrations; relative swimming performance; and changes in morphology, skin pigmentation, and body condition) because these traits displayed differences

between diets. Linear discriminant components were examined for correlations to variables and a two-way ANOVA was used to examine *population*, *diet*, and *population × diet* effects.

5.3 Results

5.3.1 Population Comparison of Initial Traits

The three Atlantic salmon populations initially differed in body length, mass, condition, morphology, and skin pigmentation (Table 5.2). Sebago salmon were longer and heavier than LaHave and Saint-Jean salmon. Both Sebago and Saint-Jean salmon had higher condition than LaHave salmon, whereas LaHave salmon had a more streamlined body shape than the other two populations. For skin pigmentation, Saint-Jean salmon had yellower and whiter body regions than LaHave and Sebago salmon. Despite these phenotypic differences, the three Atlantic salmon populations did not initially differ in baseline red blood cells or plasma total thiamine concentrations (Table 5.2). Total thiamine concentrations derivatives – thiamine pyrophosphate, thiamine monophosphate, and free thiamine – are presented in Appendix B.

5.3.2 Thiamine Concentrations

The baseline red blood cells total thiamine concentrations were not significantly different from that of salmon fed the control diet after 6 months ($t = -0.22$, df = 22, $p = 0.828$), however, they were significantly different and higher from those of the salmon fed the thiaminase diet at 6 months ($t = -6.22$, df = 45, $p < 0.001$; Table 5.2; Figure 5.1). Significant diet but not population effects were also detected for red blood cells and liver total thiamine concentrations (Table 5.3; Figure 5.1). Atlantic salmon fed the thiaminase

diet had lower red blood cells and liver total thiamine concentrations than those fed the control diet. I also detected a diet by population interaction for liver total thiamine concentrations with LaHave salmon having a larger decrease in liver total thiamine concentrations than Sebago and Saint-Jean salmon, although the diet by population interaction for total thiamine concentrations in red blood cells was not significant (Table 5.3; Figure 5.1). Despite this latter finding, there was a significant correlation between red blood cells and liver total thiamine concentrations across all fish ($r = 0.75$, df $= 22$, $p < 0.001$).

5.3.3 Diet Effect on Traits

Significant population but not diet effects were detected for the survival of sub-adult Atlantic salmon (Table 5.4; Figure 5.2) with the LaHave population exhibiting lower survival than the Sebago and Saint-Jean populations independent of diet treatment. Significant population effects were also detected for changes in skin pigmentation; LaHave salmon had whiter body regions than Saint-Jean salmon with Sebago salmon being intermediate (Table 5.4; Figure 5.2). There was a trend for all populations to have a less streamlined body shape and less yellow body pigmentation in the thiaminase diet. Significant diet effects were detected for the relative swimming performance of sub-adult Atlantic salmon; for all three populations, Atlantic salmon had lower relative swimming performance in the thiaminase than control diet (Table 5.4; Figure 5.2).

Table 5.3. Summary of Model Results Comparing Total Thiamine Concentrations of Red Blood Cells and Liver by Diet across Three Populations of Atlantic Salmon (*Salmo salar*). Displayed are linear mixed-effects results. Fixed effects were diet and population and a random effect was tank identity.

Tissue	df	F-statistic	p-value
Red blood cells			
population	2,18	0.72	0.498
diet	1,18	18.92	<0.001
population $\times$ diet	2,18	1.87	0.195
Liver			
population	2,18	0.48	0.625
diet	1,18	24.64	<0.001
population $\times$ diet	2,18	5.30	0.015
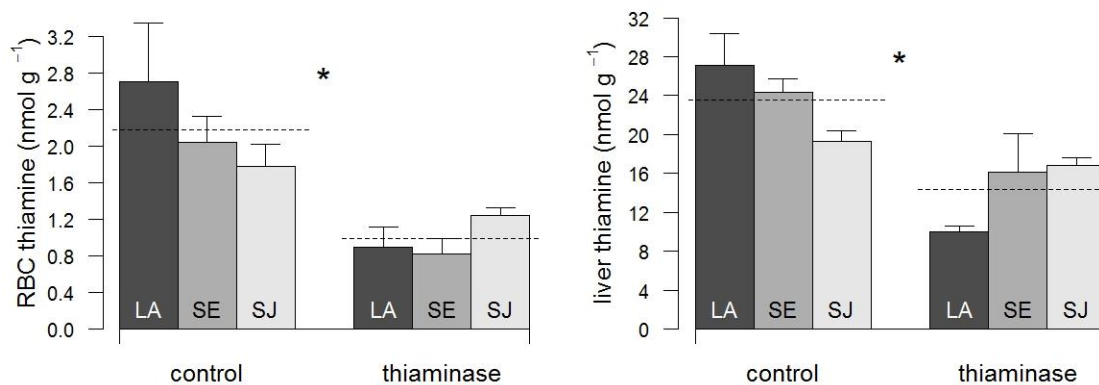
Figure 5.1. Total Thiamine Concentrations in Red Blood Cells and Liver by Diet for Three Populations of Atlantic Salmon (*Salmo salar*). RBC is red blood cells. Displayed are means ± 1SE for diets. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. Dashed lines show the means for the population across all diets. Star symbols denote indicate significant differences between diets ($p < 0.05$). Total thiamine concentrations derivatives– thiamine pyrophosphate, thiamine monophosphate, and free thiamine– are presented in Appendix B.

Table 5.4. Summary of Model Results Comparing Comparing Survival, Swimming Performance, and Body Traits by Diet across Three Populations of Atlantic Salmon (*Salmo salar*). Displayed are binomial mixed-effects results for survival and linear mixed-effects results for remaining traits. Changes in traits (final − initial values for individuals) were used for analyses of morphology, skin pigmentation, and condition. TGC is thermal-unit growth coefficient. Diet, population, and diet by population were treated as fixed effects; tank identity was treated as a random effect for the tests.

Trait	df	*F*-statistic	*p*-value
Survival			
population	2, 277.9	42.99	<0.001
diet	1, 4.0	0.00	1
population × diet	2, 277.9	0.00	1
Relative swimming performance			
population	2, 223.1	0.31	0.732
diet	1, 4.1	8.19	0.045
population × diet	2, 223.1	0.29	0.750
Morphology (RW1)			
population	2, 225.5	1.76	0.174
diet	1, 225.5	3.45	0.064
population × diet	2, 225.5	2.09	0.126
Pigmentation (PC1)			
population	2, 224.1	2.18	0.115
diet	1, 4.0	5.66	0.076
population × diet	2, 224.1	0.02	0.977
Pigmentation (PC2)			
population	2, 224.1	5.49	0.005
diet	1, 4.0	0.13	0.741
population × diet	2, 224.1	1.46	0.234
TGC of length			
population	2, 212.4	53.94	<0.001
diet	1, 4.1	0.54	0.503
population × diet	2, 212.4	3.03	0.050
TGC of mass			
population	2, 223.5	36.08	<0.001
diet	1, 4.1	0.02	0.713

population × diet	2, 223.5	2.34	0.015
Condition			
population	2, 223.9	17.33	<0.001
diet	1, 4.1	4.99	0.088
population × diet	2, 223.9	0.06	0.938
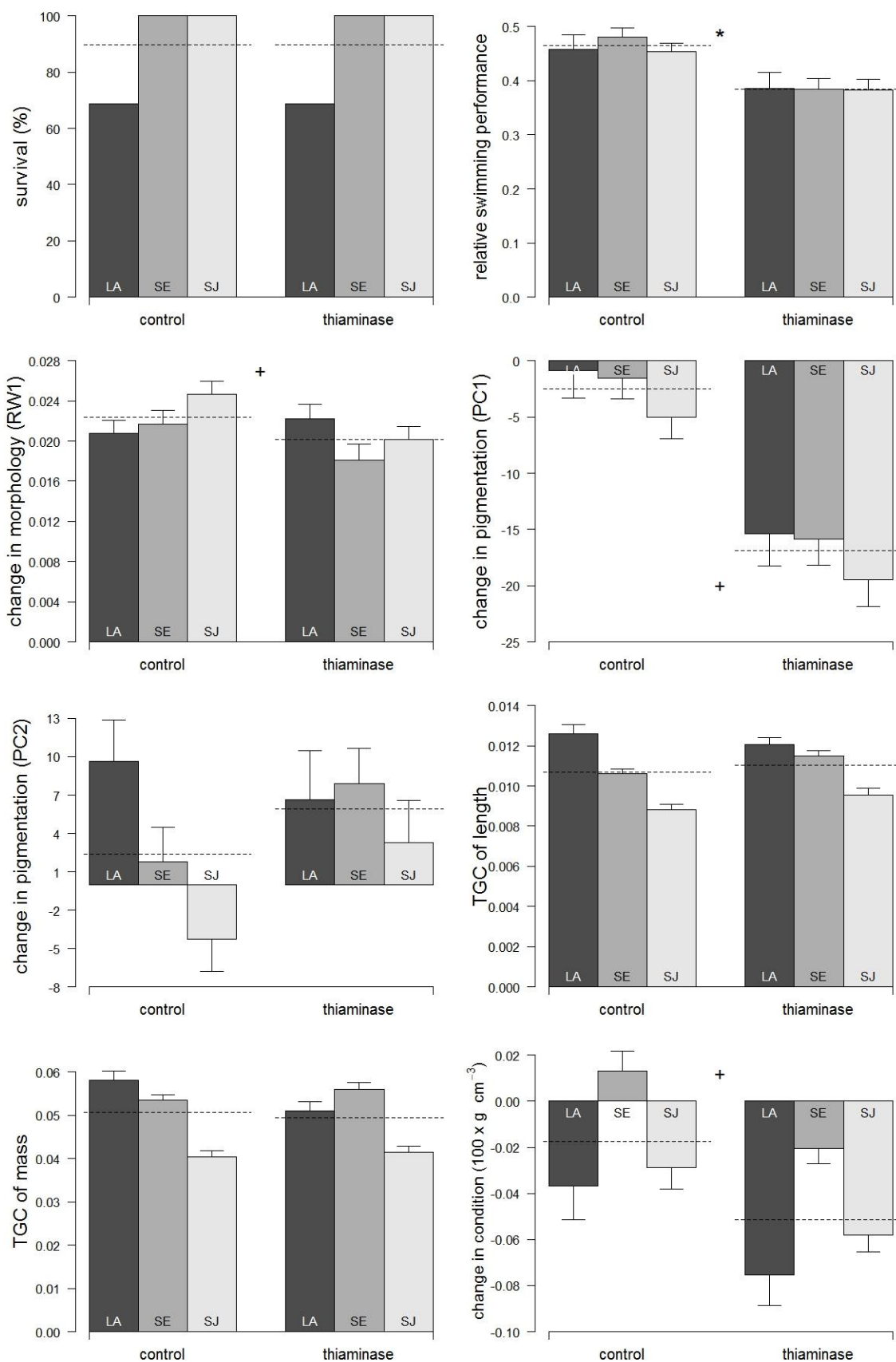
Figure 5.2. Survival, Swimming Performance, and Body Traits by Diet for Three Populations of Atlantic Salmon (*Salmo salar*). Displayed are means ± 1SE for diets. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. Dashed lines show the means for the diets across all populations. Star symbols indicate significant differences between diets ($p < 0.05$) and cross symbols indicate trends between diets ($p < 0.1$). For morphology, positive relative warp 1 (RW1) scores were associated with a more streamlined body shape. For skin pigmentation, principal component 1 (PC1) was positively related to the yellowness of the body regions, and principal component 2 (PC2) was positively related to the whiteness of the body regions.

Significant population but not diet effects were also detected for the thermal-unit growth coefficient of body length and mass and changes in body condition of sub-adult Atlantic salmon; although, there was a trend for Atlantic salmon to be in lower condition in the thiaminase than control diet, the differences were not significant (Table 5.4; Figure 5.2). Independent of diet, LaHave and Sebago salmon had a higher thermal-unit growth coefficient of length and mass than Saint-Jean salmon. Sebago salmon maintained a better condition relative to LaHave and Saint-Jean salmon.

There were no significant relationships between changes in morphology and changes in skin pigmentation within diets (Pearson correlations, $p < 0.12$ for all). There were also no significant relationships between relative swimming performance and body condition or skin pigmentation as measured by either PC1 or PC2 within diets (Pearson correlations, $p > 0.10$ for all).

5.3.4 Linear Discriminant Analysis

I considered linear discriminant components 1 and 2 (LD1, LD2), which explained 80.1% and 12.8% of the variation among the six groups (two diets by three populations), respectively. LD1 was positively related to liver thiamine concentrations, relative swimming performance, and changes in skin pigmentation (PC1) and body condition; LD2 was positively related to relative swimming performance and changes in morphology, skin pigmentation (PC1), and body condition.

Significant population, diet, and population by diet effects were detected for LD1 (two-way ANOVA, $p < 0.001$ for all) and significant diet and population by diet effects were detected for LD2 (two-way ANOVA, $p < 0.002$ for both; Figure 5.3). Generally, within

the control diet, LaHave salmon had higher LD1 values but lower LD2 values than Sebago and Saint-Jean salmon. The thiaminase diet also affected LaHave salmon more so than the other two populations, resulting in the opposite pattern – within the thiaminase diet, LaHave salmon had lower LD1 values and higher LD2 values than Sebago and Saint-Jean salmon (Figure 5.3).
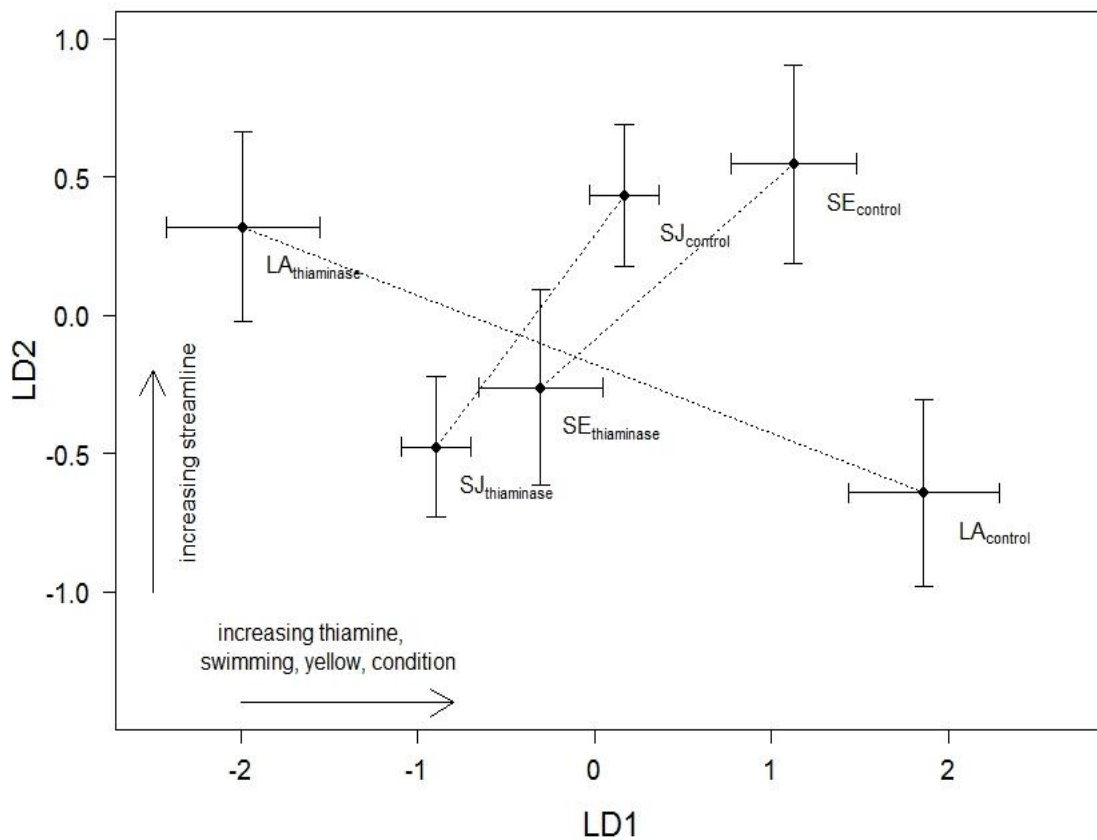
Figure 5.3. Canonical Plot of the First Two Linear Discriminant Components (LD1, LD2) separating Six Groups (Two Diets by Three Populations) for Atlantic Salmon (*Salmo salar*). Displayed are the centroids with 95% confidence intervals for the groups. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. Dashed lines connect the two diet centroids for each population.

5.4   Discussion

Atlantic salmon migrate into Lake Ontario as smolts and become sub-adults, remaining in the lake environment until they mature. During this time, high thiaminase-containing prey fishes may form a significant part of their diet due to the presence of alewife and rainbow smelt and near-absence of the historical coregonine prey assemblage (Tillitt et al. 2005; Zajicek et al. 2005; Honeyfield et al. 2012). I fed sub-adult (two-year-old) Atlantic salmon from three populations an artificial diet that mimicked the current high thiaminase content of prey fishes (Honeyfield et al. 2005) in an 8 month trial. These sub-adult Atlantic salmon had lower thiamine concentrations in tissues and lower swimming performance, but showed no change in survival or growth. This result is in contrast to Morito et al. (1986), who observed juvenile rainbow trout (*O. mykiss*) mortality after about 3 months of consuming low thiamine content diets (thiamine content of < 2 mg kg^{-1} feed). On the other hand, adult lake trout took more than two years on a similar bacterial thiaminase diet to mine to show an effect of thiamine deficiency (Honeyfield et al. 2005). Atlantic salmon thus appear to be able to tolerate a high thiaminase diet for at least 8 months without showing an effect on survival. On the other hand, there were trends for lower body condition, a less streamlined body shape, and less yellow body pigmentation when fed the thiaminase diet. These latter changes may be important because they have been shown to negatively impact Atlantic salmon survival (Taylor and McPhail 1985; Taylor 1991; Sutton et al. 2000; Garcia de Leaniz et al. 2007). A longer-term study is warranted to investigate survival across the entire lake-phase life stage (2 to 3 years).

Although there was no effect of the thiaminase diet on survival, there were several indicators of thiamine deficiency in the Atlantic salmon. I detected a decline in the

swimming performance of sub-adult Atlantic salmon fed the thiaminase diet. Morito et al. (1986) similarly found that the first signs of thiamine deficiency in the juvenile rainbow trout were changes in swimming behaviour (also see Amcoff et al. 1998; Brown et al. 2005; Fitzsimons et al. 2005). Thiamine is important for energy production, as it is required to enable pyruvate to enter the citric acid cycle to produce ATP (Morito et al. 1986; Koski et al. 2005). In addition, plasma lactate can increase as a result of thiamine deficiency in juvenile rainbow trout, which affects muscle performance (Morito et al. 1986; Fitzsimons et al. 2012). Because swimming is energetically costly, the Atlantic salmon fed the high thiaminase diet in the present study may have had lower swimming performance due to a reduction in ATP production or a build-up of lactate caused by a thiamine deficiency.

Other indicators of a thiamine deficiency may be changes in body appearance. I found a trend of sub-adult Atlantic salmon having less yellow body pigmentation when fed a thiaminase diet. Yellow pigmentation can be related to the amount of the carotenoid idoxanthin, a metabolite of astaxanthin (Hatlen et al. 1998). Because thiamine can act as an anti-oxidant (Lukienko et al. 2000), a thiamine deficiency may cause oxidative stress in the bodies of Atlantic salmon, resulting in the decline of other anti-oxidants such as astaxanthin (Pettersson and Lignell 1999). Body de-pigmentation may also be related to a lack of essential fatty acids (Leclercq et al. 2010). The lower liver thiamine concentration that I detected in the present study has been previously associated with lower liver lipid content in Chinook salmon (*O. tshawytscha*) (Honeyfield et al. 2008). Juvenile Chinook salmon fed diets lacking such fatty acids have decreased skin pigmentation (Nicolaides and Woodall 1962) and I also found a trend for lower condition and a trend for a less

streamlined body shape in the thiaminase diet. A less streamlined body shape may be a developmental effect related to reduced swimming activity (Taylor and McPhail 1985).

Although all three populations that I studied had similar responses to the thiaminase diet, I found that the LaHave population had a greater reduction in thiamine concentrations in the liver relative to the Sebago and Saint-Jean populations. The liver is a storage tissue for thiamine (Depeint et al. 2006), therefore the data may reflect fish from the LaHave population using more of their thiamine stores than the Sebago and Saint-Jean populations. I also found that the Sebago population was able to maintain better condition relative to the LaHave and Saint-Jean populations when fed a high thiaminase diet. Indeed, I predicted that freshwater resident populations, such as the Sebago and Saint-Jean populations, should have adaptations to higher thiaminase in their diets from consuming primarily rainbow smelt (Dimond and Smitka 2005), relative to anadromous populations, such as the LaHave population, that consume a more diverse diet (Rikardsen and Dempson 2011). Because I used a common garden experimental approach, my results indicate genetic differences in thiaminase tolerance among my study populations. Given that the LaHave population has been in captive breeding for longer than the Sebago and Saint-Jean populations (3 generations of captive breeding vs. single-pair matings using wild fish) the results from this present study might also reflect selection relaxation for thiaminase tolerance resulting from several generations of consuming a commercial diet that lacks any thiaminase.

Finally, my results have implications for the reintroduction efforts of an extirpated species. A successful reintroduction of Atlantic salmon into Lake Ontario may be

impeded by a diet of high thiaminase-containing prey fishes (Dimond and Smitka 2005; COSEWIC 2006, 2010). I found that a thiaminase diet mimicking a current Lake Ontario diet negatively impacted the swimming performance and body appearance of sub-adult Atlantic salmon relative to a control diet that mimicked a more historical diet of low thiaminase-containing prey fishes. Although I found no direct effect of the high thiaminase diet on survival during the 8 months trial, the Atlantic salmon fed a high thiaminase diet had less total thiamine in tissues, tended to be in lower condition and have a less streamlined body shape, all of which are indicators of lower survival (e.g. Taylor and McPhail 1985; Sutton et al. 2000; Taylor 1991; Garcia de Leaniz et al. 2007). The restoration of native prey fishes, containing lower thiaminase, may have to be considered for Lake Ontario to increase the health of salmonids in the lake (also see Fitzsimons and O'Gorman 2006). As the Sebago and Saint-Jean populations retained more thiamine in their tissues when fed the high thiaminase diet, they may have higher resistance to thiamine deficiency under natural conditions than the LaHave population. If so, this may have a significant effect on adult survival and recruitment in Lake Ontario, with significant implications for the reintroduction efforts. More broadly, source populations known to do well in locations with features similar to the reintroduction location may be suited for translocation because they may possess important adaptations (Krueger et al. 1998; Moritz 1999; Jones 2003, 2013).

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Chapter 6

6   Genetic Architecture of Survival and Fitness-Related Traits in Three Populations of Atlantic Salmon[*]

6.1   Introduction

The genetic architecture underlying phenotypic traits can be used to predict evolutionary trajectories. In particular, responses to selection are directly related to the amount of heritable (additive) genetic variance (Falconer and Mackay 1996). Non-additive genetic effects, on the other hand, have not been considered as important in part because they cannot be used to predict the response to selection (Lynch 1994). However, there is increasing evidence that non-additive genetic effects are key components of phenotypes (Crnokrak and Roff 1995; Roff and Emerson 2006). Furthermore, non-additive genetic effects are a cause of inbreeding depression (Crnokrak and Roff 1999; Keller and Waller 2002) and can be converted to additive genetic effects, for example during a bottleneck, which can then provide genetic variation for natural selection to act on (Carson 1990; also see Neff and Pitcher 2008).

Phenotypic variance can also be explained by maternal effects (maternal additive genetic and maternal environmental) (Falconer and Mackay 1996) and these effects can also affect evolutionary trajectories (Räsänen and Kruuk 2007). For example, maternal environmental effects can impact the rate and direction of change in response to natural selection and can generate rapid phenotypic changes in offspring traits as a result of the

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phenotypic plasticity of female traits (Mousseau and Fox 1998; Räsänen and Kruuk 2007). Also, additive genetic and non-additive genetic effects can also be used to understand mating systems (reviewed by Neff and Pitcher 2005). Traits that are mainly influenced by additive genetic effects indicate the importance of beneficial alleles present in only certain parents, whereas traits that are mainly influenced by non-additive genetic effects indicate the importance of the compatibility of alleles between parents. Such differences can govern mating patterns and affect the effective population size (e.g. Saccheri et al. 1998; also see Neff et al. 2011); for example, female mate choice for compatible gene combinations may be an important mechanism for maintaining genetic diversity (Neff and Pitcher 2005). Consequently, understanding the contributions of all of maternal environmental effects, additive genetic effects, and non-additive genetic effects is needed to fully understand evolutionary trajectories and mating systems in general for breeding programs.

Studies examining the architecture of traits have shown that the relative contributions of genetic and maternal environmental effects can change during development and may be influenced by the correlation between the trait and fitness. Traits expressed during the early-life history stages tend to be influenced mainly by maternal environmental effects, whereas traits expressed during later life stages are influenced increasingly by genetic effects (Kruuk et al. 2008). Initial egg investments are often fully utilized during early development, leaving later life stage traits that are influenced by genetic effects (e.g. Lindholm et al. 2006; Evans et al. 2010). For example, in mammals, maternal environmental effects typically decline, whereas additive genetic effects remain constant (e.g. Wilson and Réale 2006) or increase during development (e.g. Cheverud et al. 1983).

Additionally, life-history traits, such as survival, that have strong correlations with fitness typically have larger non-additive than additive genetic effects, whereas morphological traits, such as body size, that have weaker correlations with fitness typically have larger additive than non-additive genetic effects (Crnokrak and Roff 1995; Roff and Emerson 2006). Independent of trait type, directional selection, or to some extent stabilizing selection, on traits can erode additive genetic effects, fixing alleles across loci and leaving only non-additive genetic effects (Willis and Orr 1993). For example, morphological traits that are under strong directional selection in domestic species often have larger non-additive than additive genetic variances (Roff and Emerson 2006).

In this study, I examine the phenotypic variance of survival and fitness-related traits at three early-life history developmental stages (egg, alevin, and fry) in Atlantic salmon (*Salmo salar*) for two independent years. Atlantic salmon have declined sharply throughout their North American range over the past two centuries (Dunfield 1985). I used a full-factorial quantitative genetic breeding design to partition phenotypic variance in survival and fitness-related traits to maternal environmental, additive, and non-additive genetic effects for three source populations being used for reintroduction efforts of Lake Ontario and its tributaries. The resultant data were used to examine the relative contributions of additive and non-additive genetic effects to morphological and life-history traits, as well as any shift in contributions during early-life history stages. Also, using the adaptive potential strategy for reintroduction efforts, the amount of heritable (additive) genetic effects could be used to identifiy which of the three source populations may have the highest potential to adapt to new selection pressures in Lake Ontario and its tributaries (Lesica and Allendorf 1999; Weeks et al. 2011).

6.2   Materials and Methods

6.2.1 Families

Adult broodstock fish from each population were provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF). For this study, LaHave families ($n = 25$ in year one and 75 in year two), Sebago families ($n = 25$ in year one and 75 in year two), and Saint-Jean families ($n = 75$ in year two) were produced in early November 2010 and 2011, respectively, at the OMNRF Harwood Fish Culture Station, Harwood, Ontario following the methods of Pitcher and Neff (2006). Five females and five males from each population were mated in all possible combinations to produce full-factorial breeding design, with one block in the first year and three blocks in the second year (Lynch and Walsh 1998, p. 598). The Saint-Jean population was not included in the first year because broodstock had not reached maturity. Subsamples of eggs ($n = 7$ in year one and 20 in year two) from each female from only one family were measured for diameter (nearest 0.01 mm) using digital callipers and mass (nearest 0.0001 g) using a digital scale. For the first year only, those eggs were then frozen at -20°C, transported to the University of Western Ontario and kept frozen for subsequent energy content analysis. Remaining eggs were randomly placed into sections of Heath-style incubators and then tanks after hatching at the OMNRF Codrington Research Facility, Codrington, Ontario, using two to three sections (replicates) for each full-sibling family based on offspring numbers (i.e. to keep densities in sections equal). Digital photographs of the single layer of eggs in each section were taken and the number of eggs was calculated using ImageJ version 1.38 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/).

6.2.2 Survival and Fitness-Related Traits

I collected six measures of survival, as direct measures of early-life history stage fitness: egg survival (fertilized egg to hatch, also examined as a rate over time in year one only); alevin survival (post-hatch until yolk sac absorption, also examined as a rate over time in year one only); fry survival (yolk sac absorption until released into the wild); and overall survival (fertilized egg until released into the wild). In year one, I also measured 12 traits that are known to be related to fitness in salmonids (Metcalfe and Thorpe 1992; Berg et al. 2001; Pakkasmaa et al. 2001; Koskinen et al. 2002): egg diameter and mass; egg contents at fertilization (relative fat, protein, and energy); development time to hatch (also examined as a rate over time); body length at hatch; yolk sac volume at hatch; body length at yolk sac absorption; specific growth rate; and yolk sac conversion efficiency. In year two, I also measured six traits related to fitness in salmonids: egg diameter and mass; body length and mass at hatch; body length and mass at yolk sac absorption.

6.2.3 Statistical Analysis of Parental and Population Effects

All survival and fitness-related traits were examined for a population effect in addition to individual parental effects (dam and/or sire effects), position effects (tray and tank effects) and density effects using Akaike Information Criteria (AIC) forward step-wise model selection in R 3.0.1 (available at http://www.r-project.org/). Main effects were examined only, i.e. no interactions among effects. Statistical significance was set at $\alpha =$ 0.05 and all non-proportional data were checked visually for approximate normality using histograms before analysis with parametric statistics (Crawley 2005). Linear models were used for normally distributed data and binomial models were used for binary data (i.e. 1 for alive and 0 for dead and 1 for hatched and 0 for non-hatched). Effects that did not

cause a change in AIC of greater than 10 were considered to be poorly supported and were removed to produce the final model (Burnham and Anderson 2002). Remaining effects were tested for significance using an ANOVA of a linear model, or an analysis of deviance (ANODEV) of a binomial model. Non-significant effects, starting with non-significant interactions, were removed one at a time.

If individual parental effects were retained by the model selection process, the data were analyzed using mixed-effects models that treated individual parental effects as random intercepts and examined *population* as a fixed effect (in addition to the fixed effects of *density* if retained by the selection process). Any significant position effect if retained by the selection process was treated as a random intercept. Restricted Maximum Likelihood (REML) linear mixed-effects models were used for normally distributed data and Laplace approximation binomial generalized linear mixed-effects models were used for binary data in the lme4 package of R. The mixed-effects model output in the lme4 package does not produce significance values for fixed effects; therefore, significance for the *population* effect was determined using a likelihood ratio test between the full model and a reduced model without *population*.

6.2.4 Statistical Analysis of Genetic Architecture

In addition to parental and population effects, I examined nine out of the 18 survival and fitness-related traits in year one and seven of the 10 survival and fitness-related traits in year two for genetic architecture. The nine traits in year one that were not examined were the overall survival measure because I could not control for position effects, the five egg traits (i.e. diameter, mass, relative fat, protein, and energy) because data were collected

from only one family for each female, and the three traits examined as a rate over time (i.e. egg survival, alevin survival, and development time to hatch) because standard analyses cannot incorporate the inclusion of a time variable. The three traits in year two that were not examined were overall survival and two egg traits (i.e. diameter and mass) for the reasons described above.

First, the phenotypic variance was partitioned into random effects for *dam ID* (V_D, maternal environmental and maternal additive genetic variance), *sire ID* (V_S, paternal additive genetic variance), and *dam ID* × *sire ID* ($V_{D×S}$, non-additive genetic variance) components using a mixed-effects model. I used individual estimates of traits (e.g. individual survival and length) to account for within-family variation because means of family replicates overestimates genetic effects (see Puurtinen et al. 2009; Neff et al. 2011). Means of family replicates were used for specific growth rate and yolk sac conversion efficiency because individual estimates were not available. Regardless of the AIC criterion noted above, position effects were always included as a random effect to ensure that I did not overestimate non-additive genetic effects. Although position effects were treated as fixed effects for determining their influence on traits using model selection, in the present analyses, they were treated as random effects because they were a source of stochastic variation. Density effects were not included in the analysis because they came after individual parental effects for only two traits using model selection, suggesting that maternal environmental and genetic effects had larger influences on phenotypic variance than density effects (see results). Block effects were included as a random effect for egg survival in year two only because there was more than one block. Significances of the variance components were determined by likelihood ratio tests as

above. The additive, non-additive, and maternal environmental variance components were calculated based on (Lynch and Walsh 1998, p. 509): $V_D = \frac{1}{4} V_A + V_M$; $V_S = \frac{1}{4} V_A$; and $V_{D \times S} = \frac{1}{4} V_N$. Negative variance components were set to a value of zero.

Using a similar method outlined in Neff and Fraser (2010), bootstrap 95% confidence intervals were produced by first resampling with replacement the individuals within each replicate for each family until the original size was reproduced for trait assessments. I resampled individuals to account for within-family variation and ensure that the genetic effects were not overestimated (see Puurtinen et al. 2009). I resampled means per replicate for specific growth rate and yolk sac conversion efficiency because individual estimates were not available. Using the resampled data set, additive, non-additive, and maternal environmental variance components were calculated as a percentage of the phenotypic variance. The resampling and calculations were repeated 1000 times and the 95% confidence interval (CI) was determined for each parameter. Additionally, pair-wise population comparisons for each metric were done by calculating for one population the proportion of comparisons that were either larger or smaller than the other population. The proportions served as one-tailed p-values testing for differences between populations.

6.3   Results

Summary statistics of survival and fitness-related traits are presented in Table 6.1 and 6.2. There was nearly 100% offspring mortality for one Sebago female ($n = 5$ families) in year one and for the Saint-Jean families beyond the alevin stage in year two. Thus, the offspring from those Sebago families were not used in any of the analyses and the

offspring from Saint-Jean families were not used in analyses beyond the alevin stage. Individual parental effects and position effects (in the Heath trays and tanks) had significant influences on survival and fitness-related traits for model selection (Table 6.3 and 6.4). These effects were subsequently treated as random effects in the mixed-effects models. Density effects were also detected for body length and mass at hatch in year two, but came after individual parental effects in their influence on these traits (Table 6.4). The examination of genetic architecture revealed that maternal environmental and non-additive genetic effects explained most of the phenotypic variance in survival and fitness-related traits (Figure 6.1 and 6.2; Appendix C).

Table 6.1. Summary of Survival and Fitness-Related Traits from Two Populations of Atlantic Salmon (*Salmo salar*) in Year One. Presented are means ± 1SD, except for over time traits that are logit estimate ± 1SE. There were 25 LaHave families (5 females × 5 males) and 20 Sebago families (4 females × 5 males). Egg traits were based on 7 eggs per female. Survival, development time to hatch, and energy conversion numbers (*n*) represent the total number of replicates: 3 per LaHave family and 2 per Sebago family. Size traits were represented by 10 individuals per replicate. For example, *n* of 35 for LaHave egg traits is based on 7 eggs from each of the 5 females and *n* of 750 for LaHave size traits is based on 10 individuals from each of the 3 replicates from each of the 25 families.

Trait	*n*	LaHave	*n*	Sebago
Egg traits				
Diameter (mm)	35	5.72 ± 0.34	28	5.33 ± 0.40
Mass (g)	35	0.1051 ± 0.0133	28	0.0864 ± 0.0168
Relative fat (g / g of egg)	35	0.0031 ± 0.0077	28	0.0089 ± 0.0141
Relative protein (g / g of egg)	35	0.3702 ± 0.0321	28	0.3780 ± 0.0387
Relative energy (kJ / g of egg)	35	9.00 ± 0.76	28	9.42 ± 0.88
Egg survival (%)				
Over time	75	$-3.29 \times 10^{-3} \pm 2 \times 10^{-5}$	40	$-4.14 \times 10^{-3} \pm 3 \times 10^{-5}$
Day 0-83	75	69.1 ± 19.0	40	53.8 ± 19.9
Alevin survival (%)				
Over time	75	$-3.30 \times 10^{-2} \pm 6 \times 10^{-3}$	40	$-2.30 \times 10^{-2} \pm 5 \times 10^{-3}$
Day 84-138	75	84.0 ± 8.2	40	79.9 ± 8.8
Fry survival (%)				
Day 139-192	75	61.3 ± 19.5	40	58.0 ± 19.0
Overall survival (%)	25	35.7 ± 10.2	20	23.6 ± 14.1
Development time				
Over time	75	$2.42 \times 10^{-1} \pm 2 \times 10^{-3}$	40	$1.11 \times 10^{-1} \pm 1 \times 10^{-3}$
to hatch (degree-days)	75	479.8 ± 6.4	40	472.3 ± 12.1

Size traits				
Body length at hatch (mm)	750	16.3 ± 0.8	400	15.6 ± 0.8
Yolk sac volume (mm^3)	750	72 ± 17	400	64 ± 15
Body length at yolk sac absorption (mm)	750	25.8 ± 1.0	400	25.7 ± 1.2
Energy conversion				
Specific growth rate ($100 \times \ln$(mm) / degree-days)	75	0.146 ± 0.007	40	0.146 ± 0.009
Yolk sac conversion efficiency (mm / mm^3)	75	0.136 ± 0.016	40	0.158 ± 0.018

Table 6.2. Summary of Survival and Fitness-Related Traits from Three Populations of Atlantic Salmon (*Salmo salar*) in Year Two. Presented are means ± 1SD. There were 75 LaHave families (5 females × 5 males × 3 blocks), 75 Sebago families, and 75 Saint-Jean families. Egg traits were based on 20 eggs per female. Egg survival numbers (*n*) represent the total number of replicates: 2 per family. Alevin and fry survival numbers (*n*) represent the total number of replicates for one block per population (25 families): 2 per family. Size traits at hatch were represented by 5 individuals and at yolk sac absorption were represented by 15 individuals per replicate for one block per population. For example, *n* of 300 for LaHave egg traits is based on 20 eggs from each of the 15 females and *n* of 750 for LaHave size traits is based on 15 individuals from each of the 2 replicates from each of the 25 families.

Trait	*n*	LaHave	*n*	Sebago	*n*	Saint-Jean
Egg traits						
Diameter (mm)	300	5.42 ± 0.31	300	5.59 ± 0.33	300	5.63 ± 0.49
Mass (g)	300	0.0911 ± 0.0171	300	0.1002 ± 0.0182	300	0.1025 ± 0.0273
Egg survival (%)						
Day 0-74	150	53.3 ± 26.7	150	47.2 ± 20.2	150	22.9 ± 19.5
Alevin survival (%)						
Day 75-121	50	91.0 ± 10.2	50	93.1 ± 5.0	50	83.8 ± 11.6
Fry survival (%)						
Day 122-186	50	28.1 ± 17.7	50	55.6 ± 23.9	-	-
Overall survival (%)	25	13.8 ± 9.9	25	29.1 ± 17.6	-	-
Size traits						
Body length at hatch (mm)	250	24.8 ± 1.3	250	27.3 ± 1.5	200	27.1 ± 1.4
Body mass at hatch (g)	250	0.108 ± 0.017	250	0.154 ± 0.028	200	0.139 ± 0.023
Body length at yolk sac absorption (mm)	750	30.0 ± 2.5	750	33.8 ± 2.3	-	-
Body mass at yolk sac absorption (g)	750	0.262 ± 0.073	750	0.407 ± 0.088	-	-

Table 6.3. Model Selection and Population Effect Results for Survival and Fitness-Related Traits in Two Populations of Atlantic Salmon (*Salmo salar*) in Year One. All mixed-effects models contained a fixed effect for *population*. Mixed-effects models also contained fixed effects for *density* and *degree-days*, and random effects for *dam ID, sire ID, tray ID, and tank ID*, if these effects were identified during model selection.

Trait	Selected model	Mixed-effects model Population effect, *p*-value
Egg traits		
Diameter	*dam ID*	0.022
Mass	*dam ID*	0.021
Relative fat	no effects	
Relative protein	no effects	
Relative energy	*dam ID*	0.140
Egg survival		
Over time	*degree-days + dam ID + tray ID + sire ID + degree-days × dam ID + degree-days × sire ID + degree-days × tray ID*	< 0.001
Day 0- 83	*dam ID + tray ID + sire ID*	0.126
Alevin survival		
Over time	*degree-days + dam ID + sire ID + tank ID + degree-days × dam ID + degree-days × tank ID + degree-days × sire ID*	< 0.001
Day 84-138	*dam ID + tank ID + sire ID*	0.196
Fry survival		
Day 139-192	*dam ID + tank ID + sire ID*	0.451
Overall survival	*dam ID + sire ID*	0.104
Development time		
Over time	*degree-days + dam ID + tray ID + sire ID + degree-days × dam ID + degree-days × tray ID + degree-days × sire ID*	< 0.001
to hatch	*dam ID + tray ID + sire ID*	< 0.001
Size traits		
Body length at hatch	*dam ID + sire ID*	0.022
Yolk sac volume	*dam ID + sire ID*	0.226
Body length at yolk sac absorption	*dam ID + tank ID + sire ID*	0.117
Energy conversion		
Specific growth rate	*dam ID + tank ID + sire ID*	0.372
Yolk sac conversion efficiency	*dam ID + sire ID + tank ID*	< 0.001

Table 6.4. Model Selection and Population Effect Results for Survival and Fitness-Related Traits in Three Populations of Atlantic Salmon (*Salmo salar*) in Year Two. All mixed-effects models contained a fixed effect for *population*. Mixed-effects models also contained fixed effects for *density* and random effects for *dam ID*, *sire ID*, *tray ID*, and *tank ID*, if these effects were identified during model selection.

Trait	Selected model	Mixed-effects model Population effect, p-value
Egg traits		
Diameter	*dam ID*	0.048
Mass	*dam ID*	0.048
Egg survival		
Day 0-74	*dam ID + sire ID + tray ID*	< 0.001
Alevin survival		
Day 75-121	*dam ID + tank ID + sire ID*	0.016
Fry survival		
Day 122-186	*tank ID + dam ID + sire ID*	0.027
Overall survival	*dam ID + sire ID*	0.078
Size traits		
Body length at hatch	*dam ID + sire ID + density*	< 0.001
Body mass at hatch	*dam ID + sire ID + density*	< 0.001
Body length at yolk sac absorption	*dam ID + tank ID + sire ID*	< 0.001
Body mass at yolk sac absorption	*dam ID + tank ID + sire ID*	< 0.001
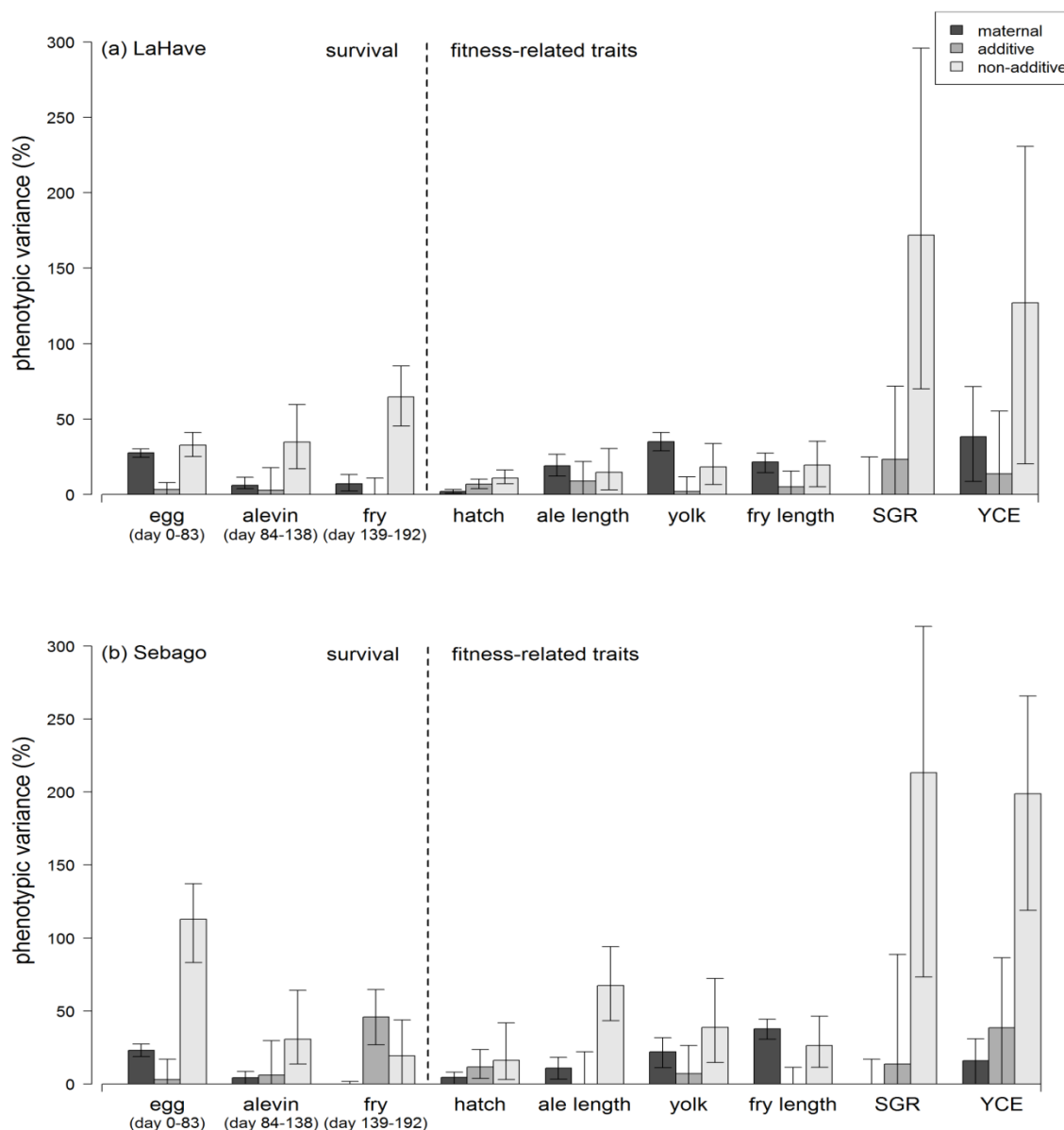
Figure 6.1. The Maternal Environmental, Additive, and Non-Additive Genetic Effects Underlying Phenotypic Variance of Survival and Fitness-Related Traits in Atlantic Salmon (*Salmo salar*) in Year One. Shown are data from two populations: (a) LaHave and (b) Sebago. Displayed are the median and 95% confidence intervals (CI) for maternal environmental, additive genetic, and non-additive genetic effects. Hatch is development time to hatch; ale length is body length at hatch; yolk is yolk sac volume; fry length is body length at yolk sac absorption; SGR is specific growth rate; and YCE is yolk sac conversion efficiency.
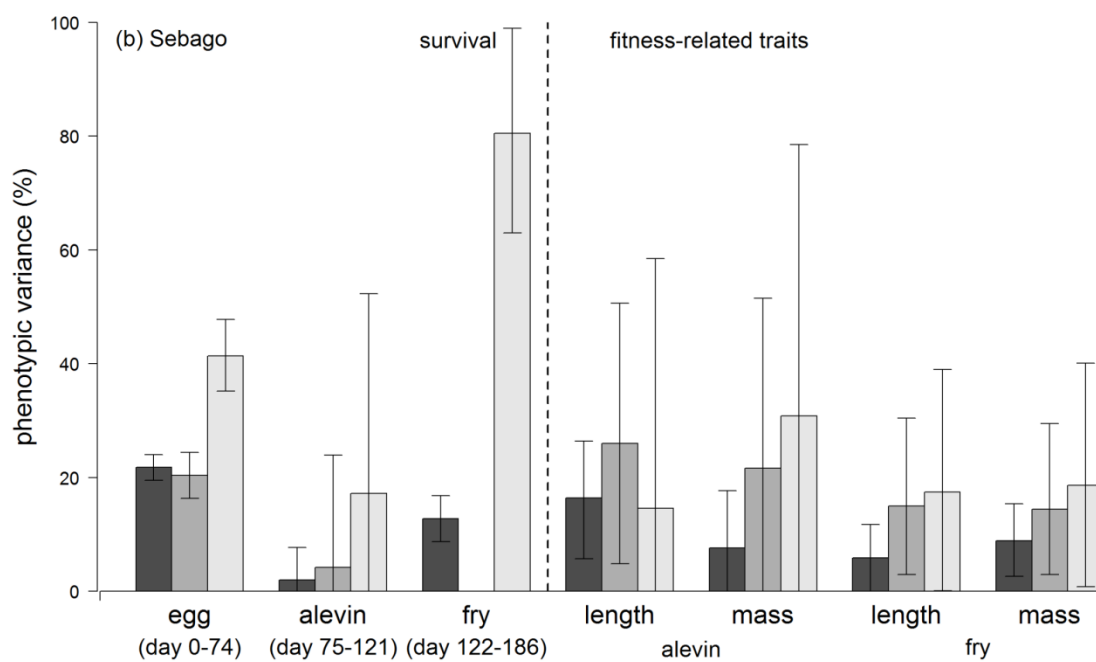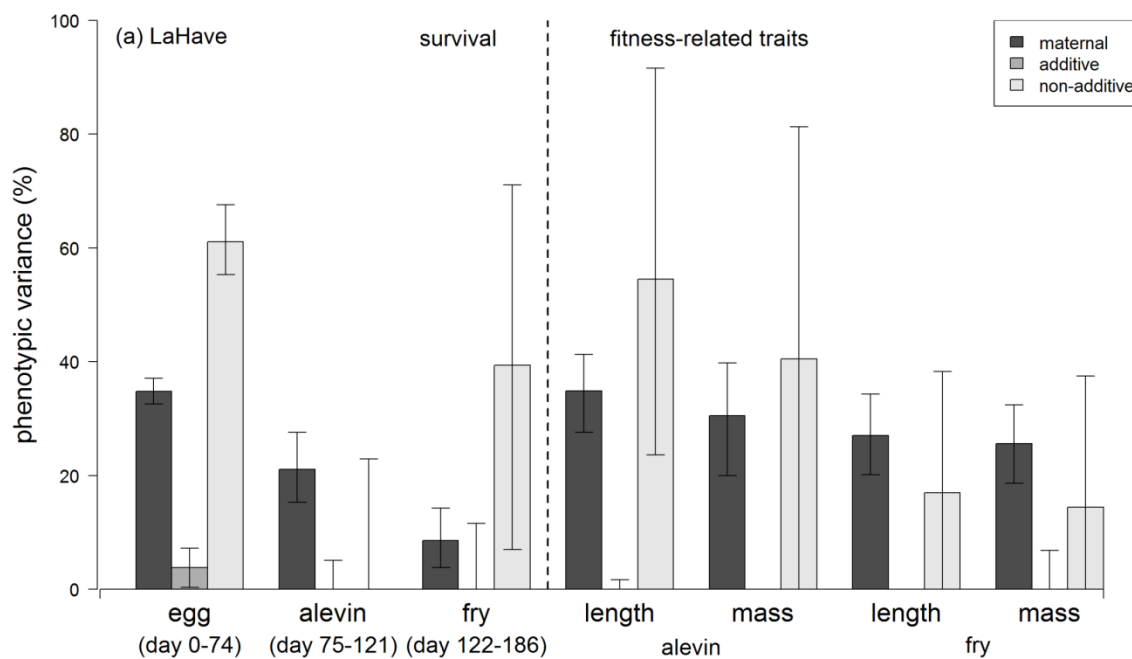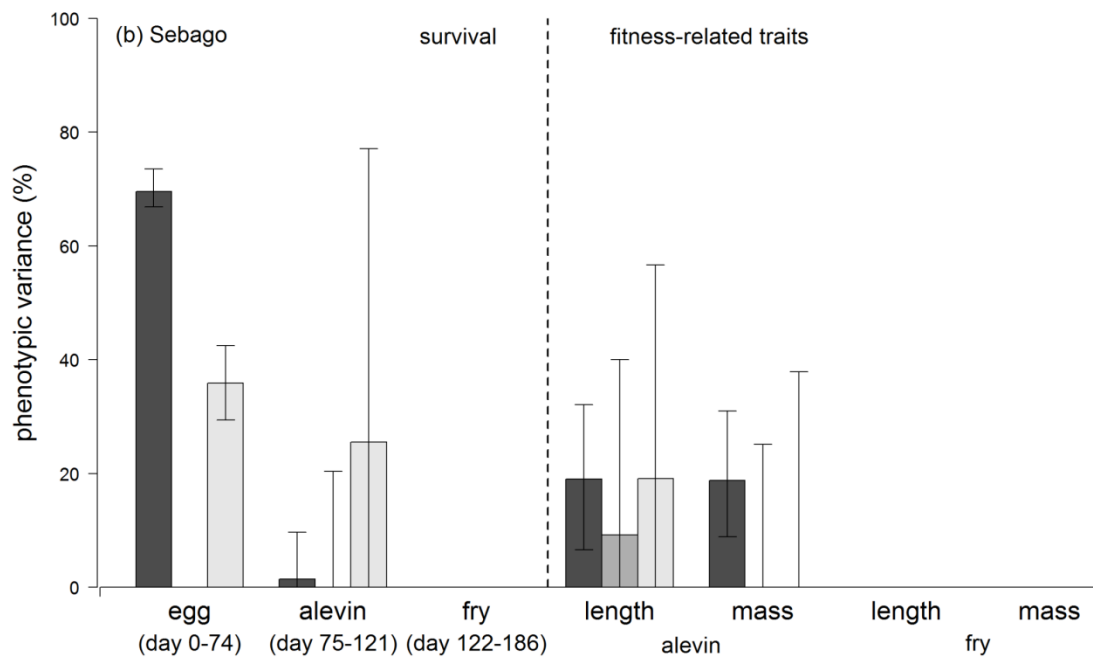
Figure 6.2. The Maternal Environmental, Additive, and Non-Additive Genetic Effects Underlying Phenotypic Variance of Survival and Fitness-Related Traits in Atlantic Salmon (*Salmo salar*) in Year Two. Shown are data from three populations: (a) LaHave, (b) Sebago, and (c) Saint-Jean. Displayed are the median and 95% confidence intervals (CI) for maternal environmental, additive genetic, and non-additive genetic effects.

6.3.1 Survival

In all three populations, dam effects were significant for egg survival, alevin survival (LaHave only), and fry survival (year two only) (Appendix C). Sire effects were not significant for any population, whereas dam × sire effects were significant for egg survival, but not alevin survival and fry survival (Sebago only in year one and LaHave only in year two). For the Saint-Jean population, maternal environmental effects were larger than genetic effects in their contribution to egg survival, but maternal environmental effects decreased during the alevin stage (Figure 6.2). On the other hand, for the LaHave (year two only) and Sebago populations, non-additive genetic effects were larger than maternal environmental effects in their contribution to egg survival, whereas maternal environmental effects similarly decreased during the alevin and fry stages (Figure 6.1 and 6.2). In year one, for the LaHave population, maternal environmental and non-additive genetic effects were similar in their contribution to egg survival. Also in year one, Sebago had significantly higher non-additive genetic effects for egg survival, but lower non-additive genetic effects for fry survival than LaHave (randomization routine one-tailed $p = 0.001$). In year two, Sebago had significantly higher additive genetic effects for egg survival than LaHave followed by Saint-Jean (randomization routine one-tailed $p = 0.001$). Differences were also observed among the populations for maternal environmental effects. In year one, LaHave had significantly higher maternal environmental effects for egg and fry survival than Sebago (randomization routine one-tailed $p = 0.001$). In year two, Saint-Jean had significantly higher maternal environmental effects for egg survival than LaHave followed by Sebago,

but lower maternal environmental effects for alevin survival than LaHave (randomization routine one-tailed $p = 0.001$).

6.3.2 Fitness-Related Traits

In year one, dam effects were significant for the LaHave and Sebago populations for development time to hatch and yolk sac volume, and for LaHave only specific growth rate and yolk sac conversion efficiency (Appendix C). Similarly, in year two, dam effects were also significant for all three populations for body length and mass at hatch and for LaHave and Sebago for body length and mass at yolk sac absorption. Sire effects on the fitness-related traits were not significant in any population, whereas dam $\times$ sire effects were significant for traits in year one (with exception of LaHave development time to hatch and body length at hatch) and in year two for LaHave body length at hatch only (Appendix C). In year one, non-additive genetic effects explained more of the phenotypic variance than maternal environmental effects for development time to hatch, body length at hatch (Sebago only), yolk sac volume (Sebago only), specific growth rate, and yolk sac conversion efficiency (Figure 6.1). On the other hand, maternal environmental effects explained more of the phenotypic variance than non-additive genetic effects for body length at hatch (LaHave only), yolk sac volume (LaHave only), and body length at yolk absorption. In year two, non-additive genetic effects explained more of the phenotypic variance than maternal environmental effects for body mass at hatch (except Saint-Jean), whereas the opposite was observed for body length at hatch (except LaHave) (Figure 6.2).

In year two, there were significant differences among the populations for all the genetic architecture values for the fitness-related traits. Sebago had higher additive genetic effects for all four fitness-related traits than LaHave, but not Saint-Jean (randomization routine one-tailed $p < 0.018$; Figure 6.2). On the other hand, in year one, there were no significant differences between populations in the majority of the genetic architecture values for the fitness-related traits (randomization routine one-tailed $p > 0.05$), with exception that Sebago had significantly higher non-additive genetic effects for body length at hatch than LaHave (randomization routine one-tailed $p = 0.012$; Figure 6.1). In either year, there were significant differences among the populations in maternal environment effects. In year one, LaHave had significantly higher maternal environment effects for body length at hatch, yolk sac volume, and yolk sac conversion efficiency, but lower maternal environmental effects for body length at yolk sac absorption when compared to Sebago (randomization routine one-tailed $p < 0.05$). Similarly, in year two, LaHave had higher maternal environmental effects for all four fitness-related traits than Sebago, but not Saint-Jean (randomization routine one-tailed $p < 0.040$).

6.3.3 Population Differences in Performance

In year two, the populations differed in survival (with exception of overall survival), but not in year one (with exception of the egg and alevin survival rates) (Table 6.3 and 6.4). For example, in year one, egg survival for the Sebago population declined at a faster rate than the LaHave population (Table 6.1). The opposite pattern was detected for alevin survival. Sebago had larger egg and alevin survival than Saint-Jean (25% and 10% of the mean, respectively), but not LaHave (6% and 3%) in year two (Table 6.2). However, in year two, Sebago had larger fry survival than LaHave (28%).

In addition, the populations differed in fitness-related traits in year one (6 of 12 traits) and year two (6 of 6 traits) (Table 6.3 and 6.4). In year one, LaHave and Sebago populations differed in egg diameter and mass, body length at hatch, development time to hatch (rate and degree-days), and yolk sac conversion efficiency (Table 6.3). However, the differences were generally small between the populations for egg diameter (0.4 mm, 7% of the mean) and mass (0.02 g, 21%), body length at hatch (0.7 mm, 4%), and development time to hatch (7 degree-days, 2%). LaHave hatched at a faster rate than Sebago. In year two, Sebago had larger body mass at hatch than both LaHave and Saint-Jean (0.03 g, 22.9%) and larger body mass at yolk sac absorption than LaHave (0.15 g, 44%) (Table 6.4). Similarly, the differences were generally small among populations for egg diameter, egg mass, body length at hatch, and body length at yolk sac absorption. Saint-Jean had larger egg diameter (0.2 mm, 4%) and mass (0.01 g, 12%) than LaHave, but not Sebago. Sebago had a larger body length at hatch than both LaHave and Saint-Jean (1.4 mm, 5%) and a larger body length at yolk sac absorption than LaHave (3.8 mm, 12%).

6.3.4 Population Differences in Additive Genetic Effects

Combining all survival and fitness-related traits values for both years, there was a significant difference in the additive genetic effects among the three populations (one-way ANOVA, $F_{2,35} = 4.50$, $p = 0.018$). Sebago had larger additive genetic effects (mean $\pm$ 1SD, 13.6 $\pm$ 13.4% of the phenotypic variance) than both LaHave (4.1 $\pm$ 6.3%) and Saint-Jean (2.3 $\pm$ 4.6%). The results were also similar using the trait values for which all three populations were represented in year two (one-way ANOVA, $F_{2,9} = 9.36$, $p = 0.006$): Sebago 18.1 $\pm$ 9.5%, LaHave 0.95 $\pm$ 1.9%, and Saint-Jean 2.3 $\pm$ 4.6%.

6.4   Discussion

My results detected maternal environmental and genetic effects that explained more than half (87% and 52% for year one and two) of the phenotypic variance in survival and fitness-related traits. In both years, maternal environmental effects were prominent at early (egg and alevin) life stages, decreased during development, and non-additive effects became most prominent at the later (fry) life stage. Similarly, in both years, I found that non-additive genetic effects were more prominent than additive effects. In contrast, the LaHave and Sebago populations were not significantly different in trait values and the genetic architecture of those traits in year one, but all three populations differed in the values for survival and fitness-related traits as well as the genetic architecture of those traits in year two.

Maternal environmental and genetic effects may be important in explaining the phenotypic variance of survival and fitness-related traits (Qvarnström and Price 2001). I found significant maternal environmental effects in the traits examined for architecture, and those effects explained a mean of 19% and 21% of the phenotypic variance across the traits in year one and two. I also found sire effects in the traits, with additive genetic effects explaining a mean of 12% and 5% of the phenotypic variance. Similarly, 16 other studies, examining some 60 different survival and fitness-related traits in natural populations, found maternal environmental effects explained a mean of $26 \pm 3\%$ (mean $\pm$ 1SD) of the phenotypic variance in the traits and that additive genetic effects explained a bit less at a mean of $18 \pm 3\%$ (see references in Table 1 in Puurtinen et al. 2009; also see Evans et al. 2010). Collectively, these data suggest that maternal environmental effects may be the primary factor contributing to survival and fitness-related traits during early

development, although additive genetic effects also contribute to phenotypic variance during this life stage.

The amount of phenotypic variance explained by maternal environmental and genetic effects may shift during development (Fox et al. 2003; Evans et al. 2010). Early-life history stages that rely on maternal investments such as egg nutrients often have phenotypic variances explained more by maternal environmental effects (reviewed by Wilson and Réale 2006). Later life stages instead have phenotypic variances largely explained by genetic effects because maternal investments have been fully utilized (Wilson and Réale 2006). I found that maternal environmental effects explained a mean of 23% and 24% of the phenotypic variance across the traits related to egg investments (egg and alevin) in year one and two, but that genetic effects also explained a similar amount of the variance in these traits (23% and 14%). I also found that genetic effects, largely influenced by non-additive effects, explained a mean of 40% and 19% of the phenotypic variance across the remaining traits that were collected at the later (fry) stage. Maternal environmental effects, on the other hand, captured only 17% and 14% of the variance in those traits. Similarly, other studies have found that maternal environmental and genetic effects explained about equal amounts of the phenotypic variance for early-life history stage traits (see references in Table 1 in Puurtinen et al. 2009; also see Evans et al. 2010). Furthermore, those studies also found that genetic effects explained $50 \pm 9\%$ and maternal environmental effects explained only $10 \pm 4\%$, on average, of the phenotypic variance for traits expressed during later life stages. Thus, the data suggest a shift with genetic effects becoming increasingly important with life stage, but also

suggest that non-additive genetic effects play an important role in survival and fitness-related traits.

Life-history and morphological traits may differ in the amount of genetic variance explained by additive and non-additive genetic effects. Life-history traits, which have strong correlations with fitness, typically have large non-additive genetic effects, whereas morphological traits, which have weak correlations with fitness, tend to have large additive genetic effects (Crnokrak and Roff 1995; Roff and Emerson 2006). However, a review recently suggested that additive and non-additive effects contribute about equally to both life-history and morphological traits (Puurtinen et al. 2009). I found that non-additive genetic effects were on average larger than additive genetic effects. Non-additive genetic effects explained means of 56% and 26% in year one and two, and additive genetic effects explained means of only 12% and 5% of the phenotypic variance across the traits. In my case, the morphological traits – body length at hatch, yolk sac volume, and body length at yolk sac absorption – may have possessed larger non-additive genetic effects because these traits typically have strong correlations with fitness in salmonids (see Koskinen et al. 2002); morphological traits in other mammal wild populations typically have weak correlations with fitness (see Crnokrak and Roff 1995; Roff and Emerson 2006). My data support the idea that non-additive genetic effects are larger than additive genetic effects for traits that have strong correlations with fitness and that this pattern may be independent of whether the traits are life-history or morphological in nature. Some caution is warranted when making these comparisons in my data set because my analysis is based on 5×5 crosses (albeit populations revealed analogous patterns).

The LaHave and Sebago populations were not significantly different in trait values and the genetic architecture of the traits in year one, but all three populations differed in the values for survival and fitness-related traits as well as the genetic architecture of those traits in year two. Because the rearing environments across the two years were nearly identical, the population differences in trait values may be associated with differences in the genetic architecture underlying the traits. Indeed, in year two, I found that the three populations differed in the genetic architecture, mainly non-additive genetic effects, of all seven traits that could be examined. Other studies have also found that populations can differ in the amount of non-additive genetic effects that explain traits (e.g. Waldmann 2001; Evans and Neff 2009). Given that the LaHave population has been in captive breeding longer than the Sebago and the Saint-Jean populations, the results might also reflect genetic changes caused by selection in a captive environment at least for that population. Because non-additive genetic effects result from specific pairings of gametes (e.g. genotype effects), large quantitative breeding designs are needed to fully detail their effects (see Lynch and Walsh 1998; Neff et al. 2011). Some caution is otherwise warranted because of the susceptibility to sampling error. The three Atlantic salmon populations also differed in the maternal environmental effects for six out of the seven traits. One important maternal environmental effect is dam age: older salmonid females generally produce larger offspring with higher survival relative to younger salmonids (Green 2008). In year one, the LaHave dams were a year older than the Sebago dams, whereas in year two, the dams were the same age in all populations. Differences in maternal environmental effects and non-additive genetic effects might thus explain the

variation in population comparisons of trait values across years. Moreover, they highlight the need for repeatability in studies of genetic architecture to make robust conclusions.

The large non-additive genetic effects in both years indicate the importance of the compatibility of alleles between parents on offspring fitness. Such compatibility has been of recent interest in the field of behavioural ecology in the context of mate choice (reviewed in Neff and Pitcher 2005). Observational mate choice studies comparing the offspring produced by natural matings with those produced by random matings have found increases in survival and fitness-related traits for the offspring produced by natural matings in Atlantic salmon (e.g. Consuegra and Garcia de Leaniz 2008; also see Agbali et al. 2010). Breeding programs should consider non-additive genetic effects in their mating designs as a way to increase offspring fitness.

My results have described the components explaining the phenotypic variance of survival and fitness-related traits during the early-life history stages of three Atlantic salmon populations. Both years support a shift from maternal environmental to genetic effects during development and highlight the importance of non-additive genetic effects in explaining the phenotypic variance of the traits. The variability in both the trait values and the genetic architecture of the traits across years may reflect effects of dam age (a maternal environmental effect) and non-additive genetic effects. This variability suggests some level of caution when interpreting results from one study.

Finally, the additive genetic effects were small, suggesting a weak adaptive potential of the traits (Falconer and Mackay 1996). There were also source population differences in the additive genetic effects: the Sebago population on average had larger additive genetic

effects than the LaHave and Saint-Jean populations. Although some caution is required because of the limited adaptive potential suggested of the traits, if considering the adaptive potential strategy for reintroduction efforts (Lesica and Allendorf 1999; Weeks et al. 2011), the Sebago population is predicted to better able to adapt to new selection pressures in Lake Ontario and its tributaries relative to the LaHave and Saint-Jean populations.

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Chapter 7

7   Restoring Biodiversity through Reintroductions: Strategies for Source Population Selection[*]

Despite potentially major effects on the outcome of reintroduction programs, few clear guidelines exist on how to optimally select source populations for translocation (see Cochran-Biederman et al. 2015). In Chapter 1, I presented the theoretical support for two source population selection strategies: the PRE-EXISTING ADAPTATION STRATEGY which focuses on populations with a high frequency of genotypes that confer adaptations (i.e. high fitness) in the reintroduction location, or the ADAPTIVE POTENTIAL STRATEGY, which focuses on populations with high heritable genetic variation that confer the potential to adapt (i.e. respond to new selection pressures) in the reintroduction location. The pre-existing strategy can be further divided into the ancestry matching approach and the environment matching approach. The adaptive potential strategy can be further divided into the single source population approach and the multiple source population approach. Here I review the empirical support for these two strategies and develop needed recommendations for selecting source populations.

7.1   Empirical Evaluation of the Approaches

Using the Web of Science, I conducted a literature search for studies that examined the fitness of different source groups translocated into foreign locations previously occupied by the target species or into locations containing small numbers of conspecifics. I included studies if they provided a coefficient of determination (r^2 or a Pearson

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[*] A version of this chapter is in review: Houde ALS, Garner SR, Neff BD. 2015. Restoring biodiversity through reintroductions: strategies for source population selection. *Restor Ecol,* in review.

correlation, *r*) between fitness-related traits (e.g. survival and reproductive traits) from different source groups and the genetic similarity to the group at the foreign location, the environment similarity between the source and foreign locations, or the amount of heritable genetic variation within the translocated groups. I also included studies that compared relative fitness-related traits among different source groups. Correlations between fitness-related traits and the genetic similarity and environment similarity are tests of the usefulness of the two approaches within the pre-existing adaptation strategy. Similarly, correlations between fitness-related traits and the amount of heritable genetic variation of the translocated groups are tests of the usefulness of the two approaches within the adaptive potential strategy. There were 15 studies that met these criteria with 11 studies that provided coefficients of determination (Table 7.1) and four studies that compared the relative fitness-related traits among different source groups.

Table 7.1. Summary of Studies that Measured the Effects of Ancestry Matching, Environment Matching, and Single Source Population Approaches on Survival and Fitness-Related Traits. The data comprise the species, the basis of the analysis, the traits measured, the effect size (r2), and the source reference. Effect sizes are significant ($p < 0.05$) unless denoted as non-significant using the symbol ns.

Species name	Basis	Trait	Effect size	Reference
Ancestry matching				
Lotus scoparius	genetic distance (allozymes)	individual fitness (juvenile survival and flower production)	38%	Montalvo and Ellstrand (2000)
Aster amellus	genetic distance (isozymes)	juvenile survival	4%	Raabová et al. (2007)
Lychnis flos-cucui	genetic distance (microsatellites)	juvenile survival	1% (ns)	Bowman et al. (2008)
Lychnis flos-cucui	genetic distance (microsatellites)	flower production	2% (ns)	Bowman et al. (2008)
Spartina alteriflora	genetic distance (AFLP)	clone size (stem diameter, number, height, width)	40%	Travis and Grace (2010)
Spartina alteriflora	genetic distance (AFLP)	flower production	30%	Travis and Grace (2010)
Environment matching				
Lotus scoparius	similar soil, temperature, and elevation	fitness (juvenile survival and height or flowers)	56%	Montalvo and Ellstrand (2000)
Lotus corinculatus	similar vegetation community	clone survival	3% (ns)	Smith et al. (2005)
Lotus corinculatus	similar vegetation community	reproductive biomass	4%	Smith et al. (2005)
Lotus corinculatus	similar vegetation community	seed number	4%	Smith et al. (2005)
Aster amellus	similar vegetation and elevation	juvenile survival	6%	Raabová et al. (2007)
Lychnis flos-cucui	soil, light, and temperature similarity	juvenile survival	16%	Bowman et al. (2008)
Lychnis flos-cucui	soil, light, and temperature similarity	flower production	27%	Bowman et al. (2008)
Castilleja levisecta	similar soil and vegetation functional group	juvenile survival	35%	Lawrence and Kaye (2011)
25 wetland species (e.g. *Anagallis, Spium, Eleocharis,* and *Oenanathe* sps.)	similar vegetation community	increase in population size	16%	Noël et al. (2011)
11 grassland species (e.g. *Anthoxanthum, Leontodon,*	similar temperature	cover	68%	Weißhuhn et al. (2012)

Trifolium sps.)

Single source population

Lychnis flos-cucui	population size proxy	juvenile survival	4% (ns)	Bowman et al. (2008)
Lychnis flos-cucui	population size proxy	flower production	19%	Bowman et al. (2008)

7.1.1 Empirical Tests of the Pre-Existing Adaptation Strategy

Of the 15 total studies, four studies examined the ancestry matching approach and seven studies examined the environment matching approach (Table 7.1). Comparing these two approaches, there was no significant difference between the effect sizes (Wilcoxon Rank Sum, $W = 0.35$, $p = 0.62$), albeit there was a large range of effect sizes (mean = 22%, range 1-68%, Table 7.1). Ancestry matching by genetic similarity was supported by four studies on several plant species that detected positive correlations with fitness-related traits (mean = 19%, range = 1-40%, Table 7.1). For example, genetic similarity using AFLPs explained 30% of the variation in plant flower production in *Spartina alteriflora* using 23 source groups translocated to a single foreign location (Travis and Grace 2010). Similarly, environment matching was supported by seven studies on several plant species that detected positive correlations with fitness-related traits (mean = 24%, range = 3-68%, Table 7.1). For example, environment similarity, using similarity of soil and vegetation, explained 35% of the variation in plant survival in *Castilleja levisecta* using six source groups translocated to 10 foreign locations (Lawrence and Kaye 2011).

Two of the 15 studies found support for environment matching but did not provide a coefficient of determination between fitness-related traits and environment similarity. Instead these studies compared the fitness-related traits between environment matches and environment non-matches. Smith and Bradshaw (1979) translocated individuals from four source groups (two environment matches and two environment non-matches) of grass plants (*Festuca*, *Agrostis*, and *Lolium* sps.) to 10 locations polluted with metalliferous waste in Great Britain. The two environment matches, based on lead and zinc tolerance in their local environment, had higher biomass than the two remaining

source groups in all 10 locations. Schneider (2011) translocated individuals from five source groups (one environment match and four environment non-matches) of Atlantic salmon (*Salmo salar*) into the Rhine River, Germany. The environment match, based on similar spawning time to the extirpated population (spawning time is linked to the water temperature similarity in the source and foreign locations), successfully reproduced in all 11 monitored locations, whereas the four environment non-matches successfully reproduced in only 5 of the 11 monitored locations. However, the four environment non-matches were translocated in different years separate from the environment match.

I found that only three studies examining ancestry matching or environment matching directly compared the effects of both approaches on fitness-related traits (i.e. Montalvo and Ellstrand 2000; Raabová et al. 2007; Bowman et al. 2008 in Table 7.1). In the first study, 60 individuals from 12 source groups were translocated to two foreign locations as seedlings (Montalvo and Ellstrand 2000). Environment similarity explained a larger amount of the variation in fitness than genetic similarity. In the second study, 18,000 individuals from six source groups were translocated to two locations as seeds (Raabová et al. 2007). Environment similarity by elevation similarity, but not vegetation similarity, explained a larger amount of the variation in juvenile survival than genetic similarity. In the third study, six individuals from 15 source groups were translocated to 15 locations as seedlings (Bowman et al. 2008). Environment similarity by soil and temperature similarity explained more variation in both juvenile survival and flower production than genetic similarity. Interestingly, in Montalvo and Ellstrand (2000) and Raabová et al. (2007), for some foreign locations, the ancestry match had the highest fitness and in other foreign locations the environment match had the highest fitness. In all three studies,

environment matching was a better predictor of fitness than ancestry matching; albeit, the single best population was sometimes an ancestry match and sometimes an environment match.

7.1.2 Empirical Tests of the Adaptive Potential Strategy

Of the 15 total studies, two studies examined translocations of source populations that differed in the amount of within-population genetic variation (Table 7.1). The two studies examined fitness-related traits as a function of source population size, a proxy of within-population neutral genetic variation (Frankham 1996), which has been shown to correlate with heritable genetic variation (Briscoe et al. 1992; but see Reed and Frankham 2001). Bowman et al. (2008) reciprocally translocated 15 populations of perennial herb *Lychnis flos-cuculi* in northeast Switzerland and measured survival and flower production; there was a positive correlation between these two variables and source population size. However, the authors noted that the higher fitness-related trait values for the larger relative to smaller source populations could also be explained by a lack of inbreeding depression rather than higher heritable genetic variation *per se* in the large source populations (Bowman et al. 2008). In the second study, Zeisset and Beebee (2013) translocated individuals from a large foreign source population of common toads (*Bufo bufo*) into Sussex, England after failed reintroduction attempts using two local small source populations. The translocation of the large source population successfully produced a self-sustaining population. Although there was no difference in the amount of within-population neutral genetic variation between the small and large population populations, the authors suggested that population size was positively correlated with heritable genetic variation (Zeisset and Beebee 2013). One potential caveat with the

interpretation of these studies is that neither directly estimated the amount of within-population heritable genetic variation. Thus, the fitness of translocated populations could not be clearly linked to adaptation following translocation, as these results are also consistent with the absence of inbreeding depression in larger relative to smaller source groups.

Three of the 15 studies examined translocations using multiple source populations. None of the studies provided a coefficient of determination between fitness-related traits and a direct quantity of the amount of heritable genetic variation within the translocated mixed-source group, although high heritable genetic variation was inferred because of the distinctive genetic and environmental backgrounds of each source. Instead these studies examined the contributions of each source populations to the reintroduced population. Tordoff and Redig (2001) translocated individuals from seven source groups of Peregrine falcons (*Falco peregrinus*) into the mid-western United States, and after one generation, five groups were detected in the reintroduced population. Wilson et al. (2007) translocated individuals from four source groups of walleye (*Sander vitreus*) into Nipigon Bay, and after two generations, a single source group largely contributed to the reintroduced population. Huff et al. (2010) translocated individuals from three source groups of slimy sculpin into nine foreign locations of southeastern Minnesota, and after two generations, a single source group had largely contributed to the reintroduced populations at eight of the nine locations. For all three studies, selection in the reintroduction location removed certain source groups, resulting in a single source group that disproportionally contributed to the reintroduced population. However, it is not clear

if this result was due to adaptation following translocation because there was no fitness comparison between the reintroduced population and its translocated group.

7.1.3 Summary of Empirical Support for the Strategies

Based upon my literature review, there was a difference in the level of support for the pre-existing adaptation and adaptive potential strategies. Most of the studies examining the pre-existing adaptation strategy found strong support for both the ancestry matching and environment matching approaches (Table 7.2). The strong support was inferred from positive correlations between fitness-related traits and direct measures of genetic similarity and environment similarity (Table 7.1). In contrast, most of the studies examining the adaptive potential strategy provided only ambiguous support for single or multiple source populations approaches (Table 7.2). This ambiguity arouse because neither the amount of heritable genetic variation within the translocated group nor the relationship between genetic variation and fitness-related traits were measured. Although the studies described successful population reintroductions, the explanation for the success could not be directly attributed to the high genetic variation within the translocated group. To provide less ambiguous tests of the effectiveness of the adaptive potential strategy, studies should directly examine the relationship between the amount of heritable genetic variation within the translocated group and fitness in the reintroduction location.

Table 7.2. Summary of Support for the Approaches within the Pre-Existing Adaptation and Adaptive Potential Strategies.

Level of support	Pre-existing adaptation strategy		Adaptive potential strategy	
	Ancestry matching approach	Environment matching approach	Single source population approach	Multiple source populations approach
strong	Montalvo and Ellstrand (2000) Raabová et al. (2007) Travis and Grace (2010)	Smith and Bradshaw (1979) Montalvo and Ellstrand (2000) Smith et al. (2005) Raabová et al. (2007) Bowman et al. (2008) Lawrence and Kaye (2011) Noël et al. (2011) Weißhuhn et al. (2012)		
weak	Bowman et al. (2008)			
ambiguous		Schneider (2011)	Bowman et al. (2008) Zeisset and Beebee (2013)	Tordoff and Redig (2001) Wilson et al. (2007) Huff et al. (2010)

Note: displayed are the references for studies that provided either strong support for the approaches, weak support, or that were ambiguous. Strong support was a significant positive relationship between fitness-related traits and either genetic similarity (ancestry matching), environment similarity (environment matching), or amount of heritable genetic variation (adaptive potential strategy). Weak support was a non-significant but positive relationship between fitness-related traits and an approach. Ambiguous support was an increase in fitness-related traits or a successful population reintroduction that was not clearly linked to an approach.

7.2   A Source Population Selection Framework

Building upon previous recommendations (Krueger et al. 1981; Seddon and Soorae 1999; Weeks et al. 2011; IUCN 2013; Cochran-Biederman et al. 2015), I constructed a novel source population selection framework (Figure 1). My framework has an *a priori* expectation that the habitat can support the target species, otherwise habitat restoration is recommended before considering a reintroduction (Beck et al. 1994; Dobson et al. 1997; Palmer et al. 1997; Cochran-Biederman et al. 2015). The framework is presented as a guide to selecting source populations with the highest probability of possessing adaptations to the key environment features of the reintroduction location. Cost, difficulty, and time constraints may be issues for certain steps and such steps can be skipped; however, skipping steps is not recommended because it may lower the probability that the source populations possess the needed adaptations to ensure successful reintroduction. My framework offers three key advantages and clarifications to previous recommendations: (1) it highlights the importance of identifying and measuring key environment features between the source and reintroduction locations prior to selecting source populations; (2) it offers guidelines for choosing between ancestry and environment matching; and (3) it prioritizes the pre-existing adaptation strategy above the adaptive potential strategy.
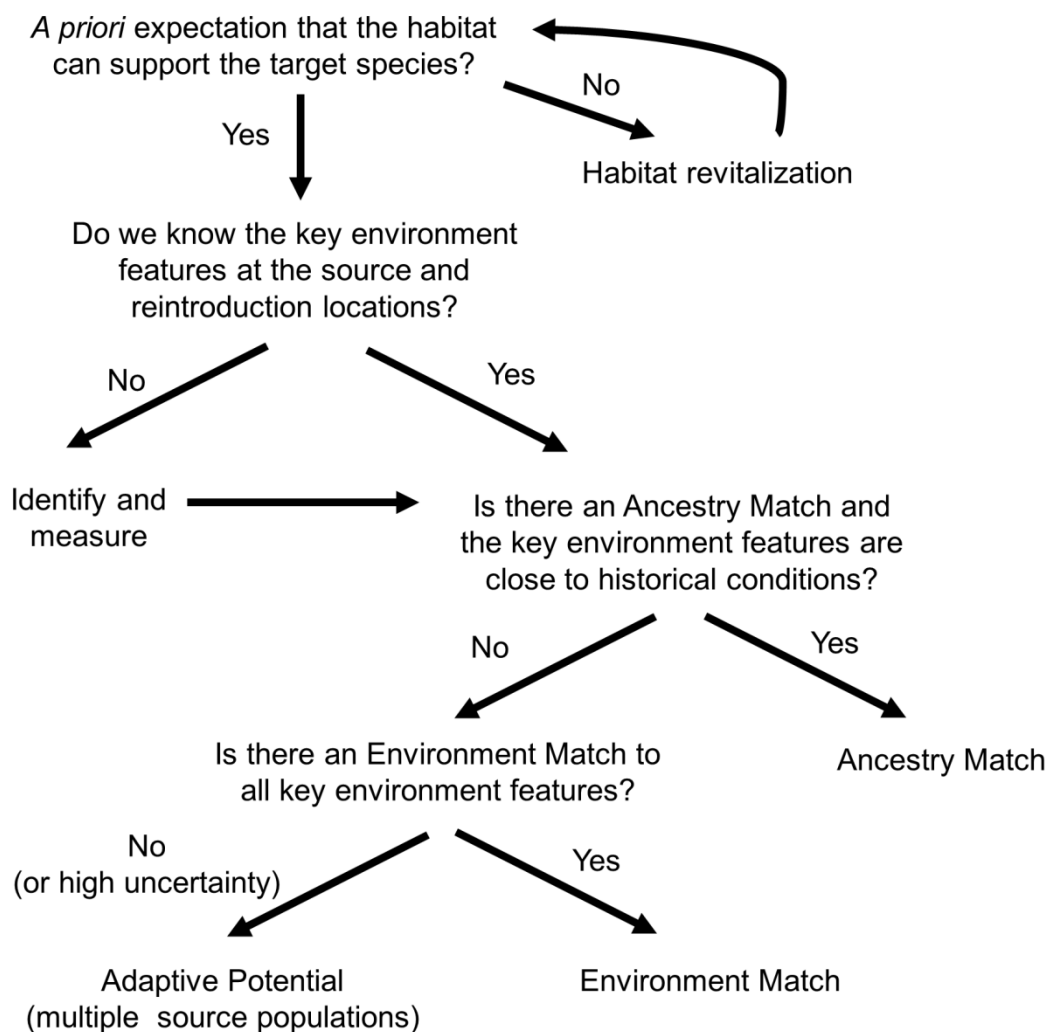
Figure 7.1. A Framework for Selecting Source Populations for Reintroduction. The framework is an optimized guide for selecting source populations. Steps may be skipped due to cost, difficulty, and time constraint issues; however, skipping such steps may reduce the probability of a successful reintroduction.

First, given the influence of key environment features on the fitness of different source populations in a location, these features should be identified and measured in the source and reintroduction locations. Key environment features may include temperature, competitors, predators, prey type, parasites, and pathogens. Second, the placement of ancestry matching and environment matching is dependent on the state of current key environment features relative to historical conditions. If there is an ancestry match, and the current key environment features are close to historical conditions (i.e. not largely changed), then the ancestry match should be translocated into the reintroduction location (also see Krueger et al. 1981). The ancestry match may possess adaptations to unidentified (cryptic) key environment features that may be absent in a source population chosen using environment matching (see Krueger et al. 1981; Garcia de Leaniz et al. 2007; Fraser 2008). In addition, ancestry matching has the greatest potential to restore an extirpated population closest to its original state, which may be particularly important for restoring populations of cultural or evolutionary significance (Moritz 1999). However, if the current key environment features have changed relative to historical conditions, and there is an environment match to those features, then the environment match should instead be translocated into the reintroduction location. An environment match to the new or otherwise changed key environment features may possess the necessary adaptations to these features. Third, if there is no ancestry match (for an environment close to historic conditions), no environment match to current conditions, or high uncertainty in the key environment features, then multiple source populations should be translocated as a bet-hedging strategy; preferably source populations with high heritable genetic variation or source populations from diverse genetic and environmental backgrounds. The fitness of

the translocated individuals should then be monitored to determine whether a single source population (or group of individuals) has higher fitness. That source population (or group of individuals) should then be the focus of future reintroduction efforts should further translocations be necessary.

Using this framework, if translocations do not establish a self-sustaining population, post-translocation monitoring should be used to determine any outstanding key environment features that could be preventing a successful reintroduction. Additional habitat restoration should be considered to address these environment features whenever possible. Trying another source population is cautioned, without identifying the key environment features first, because there is a high chance that a new source population will also lack the necessary adaptations and will not establish a self-sustaining population (e.g. Cochran-Biederman et al. 2015).

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Chapter 8

8 General Discussion[*]

My overall objective was to evaluate the relative performance (i.e. survival and fitness-related traits) of the three source populations of Atlantic salmon (*Salmo salar*) in the context of suitability for translocation into Lake Ontario. To this end, using experimental settings, I compared the relative performance of the three source populations exposed to two key environment features of Lake Ontario and its tributaries. Specifically, I exposed Atlantic salmon to: (1) four species of non-native salmonids (i.e. brown trout- *S. trutta*, rainbow trout- *Oncorhynchus mykiss*, Chinook salmon- *O. tshawytscha*, and coho salmon- *O. kisutch*) in artificial and natural streams and (2) a high thiaminase diet in a controlled setting. I also quantified the amount of within-population heritable (additive) genetic variation for early-life history traits when exposed to water from a Lake Ontario tributary. This heritable genetic variation can be used to predict the potential of traits to adapt to new selection pressures (Falconer and Mackay 1996), such as those of key environment features in Lake Ontario and its tributaries.

Because key environment features of Lake Ontario and its tributaries have changed relative to historical conditions, evolutionary and ecological theory suggests an environment matching approach for selecting source populations relative to an ancestry matching approach (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). Source populations can also be selected using an adaptive potential strategy, such as the single source population approach or multiple population approach (Lesica and Allendorf 1999;

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[*] A part of this chapter (knowledge gaps) is in review: Houde ALS, Garner SR, Neff BD. 2015. Restoring biodiversity through reintroductions: strategies for source population selection. *Restor Ecol,* in review.

Weeks et al. 2011). I discuss a perspective source population selection framework in greater detail in Chapter 7. Using the relative performance of the three source populations exposed to both features, I discuss the support for the environment matching approach. Using the amount of heritable (additive) genetic variation, I discuss which of the three source populations may be suitable for Lake Ontario using the single source population approach. In this chapter, I also present knowledge gaps and research needs for validating and potentially revising a source population framework presented in Chapter 7. I build on the framework and the research gaps by discussing their relevance for the reintroduction efforts of Atlantic salmon into Lake Ontario.

8.1 Relative Performance of the Three Source Populations

Three source populations of Atlantic salmon are being used for reintroduction efforts into Lake Ontario: LaHave from Nova Scotia, Sebago from Maine, and Saint-Jean from Quebec (Dimond and Smitka 2005). The source populations could possess genetic differences in their competitive ability (e.g. Rosenau and McPhail, 1987; Swain and Holtby 1989; Houde et al. 2010; Van Zwol et al. 2012a) and thiaminase tolerance (e.g. Brown et al. 2005; Dimond and Smitka 2005), which large scale reintroduction efforts could draw upon if the relative performance of the three source populations were evaluated using experimental settings (e.g. van Katwijk et al. 1998, 2009).

Based upon my studies, the three source populations differed in their performance when exposed to non-native salmonids as age 0+ juveniles. Although there was a decrease in performance for all three populations exposed to brown trout, rainbow trout, and the multi-species treatment, in the artificial streams, Sebago juveniles had higher growth in

the presence of non-native salmonids than LaHave and Saint-Jean juveniles. In the natural stream site containing rainbow trout, Sebago juveniles had higher growth but not recapture proportion relative to LaHave juveniles. Also, Sebago juveniles had higher growth but not survival relative to LaHave juveniles in four other natural stream sites containing non-native salmonids (Bowlby 2014). Although the survival (or recapture proportion) was similar for these two populations in the natural streams, the higher growth of Sebago juveniles can be associated with higher future survival (Metcalfe and Thorpe 1992; Koskinen et al. 2002). Unfortunately, Saint-Jean juveniles could not be examined in the natural stream sites because there were not enough individuals for both the artificial and natural streams. The Sebago juveniles may have higher growth in the presence of non-native salmonids relative to the other two populations because of avoiding agonistic interactions with the non-native salmonids (Van Zwol et al. 2012a). Avoiding agonistic interactions is a behavioural strategy that can conserve energy, which can instead be directed towards survival and growth (Metcalfe 1986).

The three source populations also differed in their performance when fed a high thiaminase diet. Although there was a decrease in performance for all three populations consuming a high thiaminase diet, Sebago salmon had higher condition than LaHave and Saint-Jean salmon and retained a higher concentration of liver thiamine than LaHave salmon. Saint-Jean salmon also retained a higher concentration of liver thiamine compared to LaHave salmon, but did not grow as well as Sebago and LaHave salmon. Other studies have found that individuals that typically consume high thiaminase-containing alewife (*Alosa pseudoharengus*) can differ in the thiamine concentrations of tissues. This has been observed for Atlantic salmon from Saint-Mary's River, Michigan

(Dimond and Smitka 2005) and coho salmon from Platte River, Michigan (Brown et al. 2005). The Sebago and Saint-Jean populations may be better at coping with a high thiaminase diet relative to the LaHave population because of higher thiaminase tolerance. Sebago and Saint-Jean salmon primarily consume rainbow smelt (*Osmerus mordax*), a high thiaminase-containing prey fish, and do not display a thiamine deficiency in their native lakes (Dimond and Smitka 2005), whereas LaHave salmon are anadromous with a more diverse diet (Rikardsen and Dempson 2011), which could be low in thiaminase. The results may indicate a genetic basis to thiaminase tolerance among Atlantic salmon populations.

Overall, the Sebago population had the best performance, for example highest growth, relative to the Saint-Jean and LaHave populations. Also, the Saint-Jean population had intermediate performance, higher concentration of liver thiamine than the LaHave population but lower growth than the Sebago population. In addition, other studies have found that the Sebago population had higher performance for fitness-related traits relative to the LaHave population. Sebago juveniles (age 0+) had no change in lactic acid (probiotic) bacteria in response to the presence of non-native salmonids, whereas there was a decrease in these bacteria for LaHave juveniles (Xiaoping He, University of Windsor, unpublished data). The Sebago juveniles also had higher immunity gene expression and swimming performance (because of a more streamlined body morphology) relative to the LaHave population (He et al. 2015; Andrew Smith, University of Quebec at Montreal, unpublished data). In addition, the Sebago population had the highest thermal tolerance, followed by LaHave population, and then the Saint-Jean population (Kayla Gradil, University of Western Ontario, unpublished data).

However, there are also indications that the Saint-Jean population may do better than the LaHave and Sebago populations at a different life stage. Saint-Jean juveniles that were one year older (age 1+) initated the most aggression and lost the least mass in response to brown trout and rainbow trout relative to LaHave and Sebago juveniles (Van Zwol et al. 2012a); albeit, Saint-Jean juveniles had an increase in chronic stress (based on elevated cortisol concentrations) relative to the remaining to populations (Van Zwol et al. 2012b). Interestingly, the source population that had the worst performance was the LaHave population, which has been the focus of previous reintroduction efforts (Dimond and Smitka 2005).

8.2   Pre-Existing Adaptation Strategy

Of the two approaches within the pre-existing adaptation strategy (i.e. ancestry matching and environment matching), evolutionary and ecological theory suggests that if the key environment features of the reintroduction location have changed, an environment match to the new conditions should possess the genes important to fitness relative to an ancestry match (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). Using experimental settings, the relative overall performance of the three source populations may support environment matching for selecting source populations in a changed environment. Stocked Sebago salmon appear to be doing well in Lake Champlain where there is also brown trout and rainbow trout as well as rainbow smelt and alewife (LCSG 2006; Marsden et al. 2010). Saint-Jean salmon are exposed to rainbow smelt but not non-native salmonids in Lac Saint-Jean (Dimond and Smitka 2005). LaHave salmon are not exposed to non-native salmonids in LaHave River (Dimond and Smitka 2005) and have a diverse diet (Rikardsen and Dempson 2011) that may be low in thiaminase. Overall, the Sebago

population had the highest performance, followed by the Saint-Jean population, then the LaHave population when exposed to both features. Specifically, the Sebago population had higher growth in the presence of non-native salmonids relative to the two remaining populations. The Sebago and Saint-Jean populations both had higher concentrations of liver thiamine when consuming a high thiaminase diet relative to the LaHave population. Given that the Sebago population is a match to both features, the Saint-Jean population is a match to a high thiaminase diet and not competition, and the LaHave population is not a match to either feature, the pattern of the relative overall performance of the three source populations may be explained by their degree of environment match to both features.

The Sebago population also appears to be doing well in other locations with similar features as Lake Ontario. Stocked Sebago salmon appear to be doing well in Lake Champlain where there is also brown trout and rainbow trout as well as rainbow smelt and alewife (LCSG 2006; Marsden et al. 2010). On the New York side of Lake Ontario there is stocking of the Sebago population and recently there has been an increase in Sebago salmon catches in Lake Ontario as well as adult returns and natural reproduction in Salmon River (Johnson 2014). The New York side of Lake Ontario also has all four non-native salmonid species (Johnson 2008) as well as alewife and rainbow smelt (Urban and Brandt 1993). However, the increase in Atlantic salmon survival and reproduction on the New York side of Lake Ontario could also be explained by environmental changes in the lake, such as a reduced proportion of alewife in the diet (Johnson 2014). Regardless, there appears to be merit to considering the Sebago population for translocation into Lake Ontario because of its performance in Lake Champlain and the New York side of Lake Ontario.

One recognized confounding issue with examining the LaHave population is its longer history of captive breeding than the Sebago and Saint-Jean populations. Captive rearing can reduce the fitness of populations when exposed to natural conditions because of domestication selection, such as reduced anti-predator response, and this reduction in fitness typically increases with the greater number of generations in captivity (reviewed by Fraser 2008). The LaHave population has been in captive breeding in Ontario since the 1990s (OMNR 2005) and is currently in its third and fourth generation (Gord Durant, Ontario Ministriy of Natural Resources and Forestry (OMNRF), personal communication). The Sebago and Saint-Jean populations are in their first generation of captive breeding in Ontario (Gord Durant, OMNRF, personal communication). Although the LaHave population is not an environment match to either non-native salmonids or a high thiaminase diet, the lower performance of this population relative to the Sebago and Saint-Jean populations when exposed to these two features of the natural environment could also be explained by a reduction in performance due to domestication selection.

8.3   Adaptive Potential Strategy

Source populations can also be selected for reintroduction efforts using the adaptive potential strategy. Given the divergent genetic and environmental backgrounds of the three source populations (King et al. 2001; Dimond and Smitka 2005), simulatenous translocation of the three source populations into Lake Ontario is considered the multiple source population approach. One concern is that the different source populations translocated into the same location may naturally inter-breed. Such inter-breeding between genetically and environmentally dissimilar populations can produce hybrid offspring with outbreeding depression (Edmands 2007), i.e. the hybrid offspring have

lower fitness than either parental population (Lynch 1991). Indeed, there has been an indication that the LaHave and Sebago populations are inter-breeding in Lake Ontario tributaries based on DNA microsatellite population assignments (Wilson 2014b). Although there has been no indication of outbreeding depression in the first generation hybrids of the LaHave and Sebago populations based on survival and fitness-related trait data collected from the egg to juvenile (age 0+) life stages (Chantal Audet, University of Windsor, unpublished data), genetic incompatibilities resulting in lower fitness can first arise in the second generation hybrids of genetically different Atlantic salmon populations (e.g. McGinnity et al. 2003; Fraser et al. 2010). Thus, some caution is warranted using the multiple source population approach for translocating Atlantic salmon into Lake Ontario because outbreeding depression may occur for inter-population hybrid offspring.

The single source population approach could also be considered for selecting source populations. Until recently (Chapter 6), there was no information on the amount of within-population heritable genetic variation for survival and fitness-related traits to consider this approach. My measurement of the amount of within-population heritable (additive) genetic variation of these traits at early-life history stages was low: on average 8% across both years. There were also differences among the three source populations. The Sebago population had a higher amount of heritable genetic variation (average of 14% across both years) than the LaHave (4%) and Saint-Jean populations (2%). The Sebago population could be selected for translocation into Lake Ontario if the single source population approach is considered in the future. However, the amount of heritable genetic variation for the traits was low, indicating a limited potential of the traits to adapt

to new selection pressures (Falconer and Mackay 1996). Another consideration is that the amount of heritable genetic variation for the traits was measured at early-life history stages (egg to first-feeding fry) in a hatchery environment, whereas Atlantic salmon are first exposed to non-native salmonids as juveniles and a high thiaminase diet as smolts in the natural environment. Concievably, there could be a higher amount of heritable genetic variation for survival and fitness-related traits at these later life stages which could be used to adapt to these features. For example, selection pressures in the natural environment can favour the survival of certain genotypes, thus changing the frequency of alleles such that now rare beneficial domiant alleles may increase the heritability for traits (Allendorf et al. 2013). Further research should consider quantifying the amount of within-population heritable genetic variation for traits at these later life stages exposed to the two features in natural settings. Because of the predicted limited potential of the early-life history traits to adapt to new selection pressures, using the single source population approach should be considered with caution. All together, the pattern of overall performance and the amount of heritable genetic variation of the three source populations generally supports environment matching over adaptive potential.

8.4   Knowledge Gaps and Research Needs

By examining the empirical literature on translocations, I have identified four major knowledge gaps (Table 8.1). Filling these gaps is critical to validate, and potentially revise, my source population selection framework. First, most studies have not measured fitness as per capita growth rate or intrinsic r but have measured fitness-related traits that do not necessarily capture population growth rate (see Hendry and Gonzalez 2008). For reintroduction programs, there is a large interest in establishing a self-sustaining

population with a growing ($r > 0$) or stable ($r = 0$) population size in the reintroduction location. Thus, per capita growth rate is a more useful measure than fitness-related traits and should be estimated in translocation studies. Additionally, there may be benefits to comparing different candidate source populations in experimental settings prior to large scale reintroduction efforts. For example, experiments could measure the relative fitness of different candidate source populations exposed to key environmental features in laboratory settings (e.g. van Katwijk et al. 1998, 2009; Chapter 5) or small scale natural settings of the reintroduction location (e.g. Chapter 4).

Second, environment matching is likely the most challenging of the source population selection approaches to implement because identifying key environment features can be difficult, time consuming, and costly. Most of the studies that examined environment matching in my analysis were on plants, possibly because of the better understanding of the key environment features for these taxa. The plant studies supported competitors (e.g. vegetation community) and temperature as key environment features that influence fitness. A better understanding of the key environment features for other taxa, such as animals, could increase the usefulness of environment matching. Identifying key environment features can be accomplished using local adaptation methods, e.g. common-garden and reciprocal translocation experiments (Kawecki and Ebert 2004), or assessing the influence of select features on the fitness of individuals in natural populations.

Table 8.1. Summary of Four Knowledge Gaps and the Benefit of the Knowledge for Selecting Source Populations.

Knowledge gap	Details	Benefit of knowledge
1. Can fitness-related traits predict reintroduction outcome?	Measure per capita growth rate instead of fitness-related traits	Per capita growth rate is a better predictor of population growth in the reintroduction location
2. What are the key environment features for environment matching?	Determine the features that have major influences on fitness (e.g. competitors and temperature) which should be used for the environment matching criteria	A better understanding of key features may enhance the implementation of the environment matching approach
3. What is the effect of current key environment features relative to historical conditions?	Distinguish between an ancestry match, which may have higher fitness exposed to historical key environment features, and an environment match, which may have higher fitness if matched to current key environment features	Evidence to support the selection of an ancestry match versus an environment match based on the state of the current key environment features
4. Does the adaptive potential strategy affect the outcome of translocations?	Compare the fitness of the reintroduced population and its translocated group in the new location to identify adaptation following translocation	Will determine if high heritable genetic variation is beneficial because of adaptive potential

Third, within the pre-existing adaptation strategy, the selection of an ancestry match versus an environment match is based on the state of the current key environment features relative to historical conditions. Although, the empirical support for ancestry and environment matching approaches appears to be similar, it is based on few studies and those studies show a large range in effect sizes, highlighting the need for more data. The three studies that examined both ancestry matching and environment matching (Montalvo and Ellstrand 2000; Raabová et al. 2007; Bowman et al. 2008) did not indicate if the current key environment features in the foreign locations had changed from historical conditions. An environment match is predicted to have higher fitness than an ancestry match when current key environment features have changed significantly from historical conditions. In contrast, an ancestry match is predicted to have higher or equivalent fitness as an environment match when historical key environment features have not changed substantially. To provide empirical data that addresses selecting an ancestry match versus an environment match, translocation studies should assess how source populations respond to the current key environment features relative to historical conditions at the reintroduction location, when known. Also, using similar local adaptation methods for identifying key environment features, researchers could experimentally manipulate environment features (e.g. historical versus current conditions) and examine the fitness of individuals from ancestry and environment matches (e.g. Chapter 4).

Fourth, it is not yet clear if the adaptive potential strategy is of practical benefit in reintroduction programs. This strategy aims to translocate a group with high heritable genetic variation, with the goal of facilitating adaptation from this variation through evolutionary processes. However, even when this strategy works as intended, many

individuals from the translocated group will likely have low fitness in the reintroduction location (Krueger et al. 1981). Consequently, the benefits of the adaptive potential strategy will be fully-realized only after multiple generations, once selection has acted on the translocated group to remove individuals with genotypes that confer low fitness in the reintroduction environment. No studies have directly compared the fitness of a reintroduced population and its translocated group, so it is difficult to estimate the magnitude of the fitness benefits resulting from the adaptive capacity strategy (i.e. adaptation following translocation). Further research is needed to determine the role of adaptive capacity in translocation outcome and whether populations with high heritable genetic variation are more likely to re-establish a population in the reintroduction location than populations with low heritable genetic variation. At this time there is limited evidence that the adaptive potential strategy affects translocation outcome.

8.5   Research Recommendations

The source population selection framework (Figure 7.1) may have relevance for the reintroduction efforts of Atlantic salmon into Lake Ontario. For the first step, there is an *a priori* expectation that the Lake Ontario habitat should now support Atlantic salmon because there has been habitat restoration such that the original factors leading to the extirpation have been largely addressed (Beeton 2002). The Lake Ontario habitat also supports ecologically-similar salmonids species (Beeton 2002). For the second step, key environment features for Atlantic salmon have largely been identified (reviewed by Taylor 1991; Garcia de Leaniz et al. 2007) and in particular two features (i.e. non-native salmonids and high-thiaminase containing prey fishes) have been implicated as likely

impediments to a successful reintroduction of Atlantic salmon into Lake Ontario (Dimond and Smitka 2005; COSEWIC 2006, 2010). In addition, there are measurements of these two features in the source and reintroduction locations. For the third step, although there is likely an ancestry match (i.e. the Saint-Jean population, based on Tessier and Bernatchez 2000), the two identified features of Lake Ontatio are not close to historical conditions, i.e. non-native salmonids and high-thiaminase containing prey fishes are recent changes (Beeton 2002), suggesting that the ancestry matching approach is not appropriate. For the fourth step, there may be an environment match to both features (i.e. the Sebago population based on its performance in Lake Champlain, LCSG 2006; Marsden et al. 2010), suggesting the environment matching approach is appropriate.

In addition, there is post-release monitoring of the three source populations by the Ontario Ministry of Natural Resources and Forestry (OMNRF) to evaluate the relative fitness of the three source populations in Lake Ontario and its tributaries. Furthermore, there is research testing for outbreeding depression, specifically for the first generation hybrids of the LaHave and Sebago populations (Chantal Audet, University of Windsor, unpublished data). Ideally, outbreeding depression research would examine inter-population hybrids of all three populations for at least two generations, because outbreeding depression may not be detected until the second generation in Atlantic salmon (e.g. McGinnity et al. 2003; Fraser et al. 2010). All together, the experiments measuring the relative fitness of different source populations or their inter-population hybrids in laboratory or small scale natural settings can be beneficial as a guide prior to large scale reintroduction efforts (e.g. van Katwijk et al. 1998, 2009). Not enough time

has passed to fully evaluate the relative fitness of the three populations over the entire life cycle and in small scale natural settings of Lake Ontario and its tributaries (Wilson 2014b). In the future, if the translocation of the three source populations into Lake Ontario has not resulted in a self-sustaining population, the post-release monitoring or further research could be used to determine any outstanding key environment features and habitat restoration could be considered to address these limiting features.

The knowledge gaps and research needs (Table 7.3) may also have relevance for the reintroduction efforts of Atlantic salmon into Lake Ontario. For the first gap, in my studies, I measured survival and fitness-related traits to compare among the three source populations. Admittedly, these measures do not necessarily capture per capita growth rate or intrinsic r (Hendry and Gonzalez 2008), which would be a more useful estimate of whether the source populations may provide a growing population ($r > 0$) or stable population ($r = 0$). Further research should consider measuring the per capita growth rate of the three source populations over the entire life-cycle and in natural settings. For the second gap, it is suggested that the key environment features have largely been identified for Atlantic salmon in Lake Ontario (Dimond and Smitka 2005; COSEWIC 2006, 2010), suggesting that an environment matching approach is appropriate if there is an environment match. However, additional key environment features may be identified from post-release monitoring or further research and this approach may no longer be appropriate if there is no environment match. For the third gap, environment features in Lake Ontario and its tributaries have changed relative to historical conditions (Beeton 2002), suggesting that an environment match may have higher performance than an ancestry match (Krueger et al. 1981; Moritz 1999; Jones 2003, 2013). Futher research

could test this prediction in natural settings using the three source populations. For the fourth gap, there is post-release monitoring (Wilson 2014a,b) and other research comparing the three source populations. Further research could also measure the amount of heritable genetic variation for the three populations to test whether source populations with a higher amount of this variation have higher fitness in the reintroduction location relative to source populations with a lower amount of this variation.

Admittedly, in my studies, the relative performance of the three populations was not examined in a fully natural setting or over the entire life cycle of the Atlantic salmon. Currently, the OMNRF is evaluating the relative performance of the three source populations over the entire life-cycle and in natural settings (Wilson 2014a,b). In particular, the Saint-Jean population is of interest as a source population because it is a presumed ancestry match to the extirpated Lake Ontario population (Tessier and Bernatchez 2000). Conceivably, an ancestry match, rather than an environment match, may be more likely to possess genes that are important to dealing with unidentified (cryptic) key environment features of Lake Ontario that may have been there historically (see Krueger et al. 1981; Garcia de Leaniz et al. 2007; Fraser 2008). Similarly, although the LaHave population had the worst performance when exposed to both features, the LaHave population may have better relative performance over the entire life cycle or certain Lake Ontario tributaries. For example, in year one of the artificial streams, LaHave juveniles had higher survival than Sebago juveniles in the multi-species treatment. Although a similar result did not occur in year two of the artificial streams, the results from year one suggest that the LaHave population may be more suitable than the Sebago population for natural streams containing all four species of non-native

salmonids. However, given the differences between years, further research should consider examining the three populations exposed to different compositions of non-native salmonid species in natural streams for different years. All together, the post-release monitoring and other research comparing the three source populations can have its benefits prior to large scale reintroduction efforts (e.g. van Katwijk et al. 1998, 2009).

8.6   Conclusion

Based on my experimental evaluations of the three populations using two key environment features of Lake Ontario and its tributaries (i.e. non-native salmonids and a high thiaminase diet), the pattern of overall performance and the amount of heritable genetic variation of the three source populations generally supports environment matching over adaptive potential. It is predicted that the Sebago population would be the most suited out of the three source populations for translocation into Lake Ontario. However, some caution is warranted, because all three source populations were not examined over the entire life cycle or in a fully natural setting. Conceivably, future information from post-release monitoring and further research of the three source populations over the entire life cycle and natural settings could reveal a different source population (i.e. the LaHave or Saint-Jean population) that is the most suited for translocation into Lake Ontario. Notably, given the concerns of outbreeding depression for naturally-produced inter-population hybrid offspring of Atlantic salmon, especially in the second generation (e.g. McGinnity et al. 2003; Fraser et al. 2010), identifying a single source population for future large scale reintroduction efforts should be considered.

8.7   References

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Appendices

Appendix A. Details of The Genetic Assignments for LaHave and Sebago Atlantic Salmon (*Salmo salar*).

Adipose fin tissue samples of the parents were previously collected by the Ontario Ministry of Natural Resources and Forestry (OMNRF) and stored in 95% ethanol for DNA microsatellite genotyping. Atlantic salmon genotype information of all samples was collected at the OMNRF DNA Profiling and Forensic Centre, Peterborough, Ontario. Genomic DNA was extracted from Atlantic salmon tissue samples using a crude lysis extraction method (see Wilson et al. 2007). DNA samples were amplified at eight DNA microsatellite loci (i.e. *Ssa197*, *Ssa202*- O'Reilly et al. 1996; *SSsp1605, SSssp2201*, *SSsp2213*, *SSsp2215, SSsp2216*, *SSspG7*- Paterson et al. 2004). The heat cycle parameters were amplification at 95°C for 3 min, 35 denaturation cycles at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 60 s. The extension time on the final cycle was 5 min. Amplified products were electrophoresed using an AB 3730 DNA Sequencer along with LIZ 500 size standards (Applied Biosystems). Genotypes at each locus were scored using GenoTyper 4.0 (Applied Biosystems) and confirmed by manual proofreading.

Atlantic salmon individuals were assigned to the families using likelihood-based parentage pair assignments in Cervus 3.0 (Marshall et al. 1998). A parentage assignment simulation in FAP 3.6 (Taggart 2007) estimated a 97.5% success rate of assignment to a single family given the known 5×5 full factorial families that were released. Individuals were allowed to mismatch at a single locus for the assignment to experimental families in Cervus. Individuals that could not be assigned to the experimental families were assigned

to either the LaHave or Sebago population in Structure 2.1 (Pritchard et al. 2000) using

the genotype information of all broodstock.

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Appendix B. Summary of Baseline Thiamine Concentrations comparing Red Blood Cells and Plasma across Three Populations of Atlantic Salmon (*Salmo salar*).

Table B1. Summary of baseline thiamine concentrations comparing red bloods cells and plasma across three populations of Atlantic salmon (*Salmo salar*). Presented are means ± 1SD. Thiamine symbols are TPP = thiamine pyrophosphate, TMP = thiamine monophosphate, TH = free thiamine, and TTH = total thiamine. Sample size is $n = 12$ from each population.

Tissue	TPP	TMP	TH	TTH
Red blood cells (nmol g^{-1})				
LaHave	2.1 ± 1.2	0.2 ± 0.2	0.0 ± 0.0	2.3 ± 1.2
Sebago	1.7 ± 0.8	0.2 ± 0.2	0.0 ± 0.0	1.9 ± 0.9
Saint-Jean	2.2 ± 0.9	0.3 ± 0.2	0.0 ± 0.0	2.4 ± 1.0
Plasma (nmol ml^{-1})				
LaHave	0.03 ± 0.05	0.02 ± 0.06	0.06 ± 0.07	0.12 ± 0.14
Sebago	0.06 ± 0.07	0.03 ± 0.05	0.09 ± 0.08	0.18 ± 0.19
Saint-Jean	0.07 ± 0.06	0.03 ± 0.05	0.15 ± 0.12	0.26 ± 0.20

Table B2. Summary of Thiamine Concentrations comparing Red Blood Cells and Liver after 6 Months of Diet across Three Populations of Atlantic Salmon (*Salmo salar*). Presented are means ± 1SD. Thiamine symbols are TPP = thiamine pyrophosphate, TMP = thiamine monophosphate, TH = free thiamine, and TTH = total thiamine. Sample size is $n = 4$ from each population in each treatment.

Tissue	TPP	TMP	TH	TTH
Control diet				
Red blood cells (nmol g^{-1})				
LaHave	1.8 ± 0.8	0.8 ± 0.5	0.1 ± 0.1	2.7 ± 1.3
Sebago	1.5 ± 0.4	0.4 ± 0.2	0.1 ± 0.1	2.0 ± 0.6
Saint-Jean	1.4 ± 0.4	0.3 ± 0.1	0.0 ± 0.0	1.8 ± 0.5
Liver (nmol g^{-1})				
LaHave	13.5 ± 2.5	10.8 ± 3.6	2.8 ± 1.0	27.1 ± 6.6
Sebago	12.1 ± 2.5	9.7 ± 2.1	2.4 ± 0.5	24.3 ± 2.8
Saint-Jean	10.1 ± 1.5	7.0 ± 0.8	2.2 ± 0.6	19.3 ± 2.2
Thiaminase diet				
Red blood cells (nmol g^{-1})				
LaHave	0.8 ± 0.5	0.1 ± 0.1	0.0 ± 0.0	0.9 ± 0.5
Sebago	0.7 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	1.2 ± 0.3
Saint-Jean	1.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	1.2 ± 0.2
Liver (nmol g^{-1})				
LaHave	7.6 ± 1.3	2.1 ± 0.7	0.3 ± 0.2	10.0 ± 1.3
Sebago	12.8 ± 5.9	3.0 ± 1.5	0.3 ± 0.4	16.1 ± 7.8
Saint-Jean	11.5 ± 0.8	4.5 ± 0.5	0.9 ± 0.2	16.9 ± 1.3

Appendix C. Summary of the Results for the Genetic Architecture of Survival and Fitness-Related Traits in Three Populations of Atlantic Salmon (*Salmo salar*).

Table C1. Summary of Results for the Genetic Architecture of Survival and Fitness-Related Traits in Two Populations of Atlantic Salmon (*Salmo salar*) in Year One. Presented are the results on the observed data for the populations using mixed-effects models containing random effects for *dam ID, sire ID, dam ID × sire ID*. All mixed-effects models contained a random effect for position effects (i.e. *tray ID* or *tank ID*). Significance of the effects was determined using likelihood ratio tests. The maternal environmental, additive, and non-additive variance components were calculated as: $V_D = \frac{1}{4} V_A + V_M$; $VS = \frac{1}{4} V_A$; and $V_{D×S} = \frac{1}{4} V_N$.

Trait	n	p-value	σ^2 (% total variance)	phenotypic variance	% phenotypic variance
Egg survival (Day 0-83)					
LaHave					
dam	5	< 0.001	0.499 (28.7)	maternal	27.9
sire	5	0.5654	0.015 (0.9)	additive	3.5
dam × sire	25	< 0.001	0.132 (7.6)	non-additive	30.6
tray	13	< 0.001	0.086 (5.0)		
Residual			1.002 (57.7)		
Sebago					
dam	4	< 0.001	0.988 (24.5)	maternal	23.5
sire	5	0.8746	0.039 (1.0)	additive	3.9
dam × sire	20	< 0.001	1.125 (27.9)	non-additive	111.6
tray	13	< 0.001	0.887 (22.0)		
Residual			0.992 (24.6)		
Alevin survival (Day 84-138)					
LaHave					
dam	5	0.036	0.100 (7.7)	maternal	6.8
sire	5	0.719	0.012 (0.9)	additive	3.7
dam × sire	25	0.286	0.089 (7)	non-additive	27.6
tank	38	0.044	0.126 (9.8)		
Residual			0.967 (74.8)		
Sebago					
dam	4	0.524	0.093 (5.9)	maternal	5.4
sire	5	0.692	0.019 (1.3)	additive	5.5
dam × sire	20	0.500	0.076 (6.5)	non-additive	22.1
tank	31	0.084	0.236 (15.5)		
Residual			0.950 (70.9)		
Fry survival (Day 139-192)					
LaHave					
dam	5	0.090	0.173 (8.6)	maternal	8.6
sire	5	1	0 (0.0)	additive	0.0
dam × sire	25	< 0.001	0.308 (15.3)	non-additive	61.2

tank	51	< 0.001	0.550 (27.3)		
Residual			0.984 (48.8)		
Sebago					
dam	4	0.161	0.137 (7.1)	maternal	0
sire	5	0.114	0.235 (12.1)	additive	48.3
dam × sire	20	0.287	0.078 (4.0)	non-additive	16.1
tank	32	< 0.001	0.526 (27.0)		
Residual			0.970 (49.8)		

Development time to hatch

LaHave					
dam	5	0.003	1.58 (3.8)	maternal	2.1
sire	5	0.046	0.72 (1.7)	additive	6.9
dam × sire	25	< 0.001	1.00 (2.4)	non-additive	9.6
tray	13	< 0.001	1.82 (4.3)		
Residual			36.72 (87.8)		
Sebago					
dam	4	0.004	12.53 (8.0)	maternal	5.4
sire	5	0.057	4.03 (2.6)	additive	10.3
dam × sire	20	0.025	3.42 (2.2)	non-additive	8.7
tray	13	< 0.001	4.22 (2.7)		
Residual			132.49 (84.5)		

Body length at hatch

LaHave					
dam	5	< 0.001	0.134 (21.6)	maternal	18.7
sire	5	0.038	0.018 (2.9)	additive	11.7
dam × sire	25	0.365	0.006 (1.0)	non-additive	4.0
tray	13	0.121	0.007 (1.1)		
Residual			0.453 (73.3)		
Sebago					
dam	4	0.124	0.078 (12.1)	maternal	11.3
sire	5	0.969	0.005 (0.8)	additive	3.3
dam × sire	20	< 0.001	0.092 (14.4)	non-additive	57.4
tray	13	0.194	0.017 (2.7)		
Residual			0.448 (70.0)		

Yolk sac volume

LaHave					
dam	5	< 0.001	117.3 (36.4)	maternal	35.9
sire	5	0.536	1.73 (0.5)	additive	2.2
dam × sire	25	0.028	7.41 (2.3)	non-additive	9.2
tray	13	0.728	0.67 (0.2)		
Residual			194.7 (60.5)		
Sebago					
dam	4	0.001	60.6 (25.2)	maternal	23.2
sire	5	0.461	4.83 (2.0)	additive	8.0
dam × sire	20	0.007	14.4 (6.0)	non-additive	24.0
tray		0.604	1.52 (0.6)		
Residual			159.4 (66.2)		

Body length at yolk sac absorption

LaHave					
dam	5	<0.001	0.273 (24.2)	maternal	22.9
sire	5	0.347	0.015 (1.3)	additive	5.2

dam × sire	25	0.022	0.036 (3.2)	non-additive	12.8
tank	38	<0.001	0.042 (3.8)		
Residual			0.760 (67.5)		
Sebago					
dam	4	< 0.001	0.578 (39.2)	maternal	39.2
sire	5	0.993	0 (0.0)	additive	0.0
dam × sire	20	0.002	0.086 (5.8)	non-additive	23.3
tank	31	< 0.001	1.29 (8.8)		
Residual			0.680 (46.2)		
Specific growth rate					
LaHave					
dam	5	0.022	1.9e-5 (35.1)	maternal	22.1
sire	5	0.195	6.9e-6 (13.0)	additive	51.9
dam × sire	25	0.036	1.2e-5 (22.5)	non-additive	90.1
tank	38	0.943	2.4e-7 (0.5)		
Residual			1.6e-5 (29.3)		
Sebago					
dam	4	1	2.8e-6 (3.6)	maternal	3.4
sire	5	1	0 (0.0)	additive	0.0
dam × sire	20	0.004	4.5e-5 (57.2)	non-additive	228.7
tank	31		0 (0.0)		
Residual			3.1e-5 (39.2)		
Yolk sac conversion efficiency					
LaHave					
dam	5	< 0.001	2.1e-4 (71.9)	maternal	69.3
sire	5	0.564	7.4e-6 (2.5)	additive	10.0
dam × sire	25	0.0002	5.2e-5 (17.7)	non-additive	70.8
tank	38	1	0 (0.0)		
Residual			2.3e-5 (7.9)		
Sebago					
dam	4	0.274	9.0e-5 (25.7)	maternal	16.0
sire	5	0.618	3.4e-5 (9.7)	additive	38.7
dam × sire	20	0.0001	1.7e-4 (48.9)	non-additive	195.6
tank	31	1	0 (0.0)		
Residual			5.5e-5 (15.8)		

Table C2. Summary of Results for the Genetic Architecture of Survival and Fitness-Related Traits in Three Populations of Atlantic Salmon (*Salmo salar*) in Year Two. Presented are the results on the observed data for the populations using mixed-effects models containing random effects for *dam ID, sire ID, dam ID × sire ID*. All mixed-effects models contained a random effect for position effects (i.e. *tray ID* or *tank ID*). Egg survival mixed-effects models contained a random effect for block effects. Significance of the effects was determined using likelihood ratio tests. The maternal environmental, additive, and non-additive variance components were calculated as: $V_D = \frac{1}{4} V_A + V_M$; $VS = \frac{1}{4} V_A$; and $V_{D×S} = \frac{1}{4} V_N$.

Trait	n	p-value	σ^2 (% total variance)	phenotypic variance	% phenotypic variance
Egg survival (Day 0-120)					
LaHave					
dam	15	< 0.001	1.043 (36.0)	maternal	35.0
sire	15	0.5346	0.028 (1.0)	additive	3.9
dam × sire	75	< 0.001	0.427 (14.7)	non-additive	58.9
tray	29	< 0.001	0.177 (6.1)		
block	3	0.4273	0.224 (7.8)		
Residual			0.998 (34.4)		
Sebago					
dam	15	< 0.001	0.506 (27.1)	maternal	22.0
sire	15	0.0021	0.096 (5.2)	additive	20.5
dam × sire	75	< 0.001	0.180 (9.6)	non-additive	38.5
tray	28	< 0.001	0.089 (4.8)		
block	3	0.9966	0 (0)		
Residual			0.994 (53.3)		
Saint-Jean					
dam	15	< 0.001	3.420 (69.8)	maternal	69.8
sire	15	0.9980	0 (0)	additive	0.0
dam × sire	75	< 0.001	0.403 (8.2)	non-additive	32.9
tray	28	< 0.001	0.149 (3.0)		
block	3	1	0 (0)		
Residual			0.926 (18.9)		
Alevin survival (Day 121-143)					
LaHave					
dam	5	< 0.001	0.422 (21.7)	maternal	21.7
sire	5	1	0 (0)	additive	0.0
dam × sire	25	1	0 (0)	non-additive	0.0
tank	46	< 0.001	0.579 (29.8)		
Residual			0.941 (48.5)		
Sebago					
dam	5	0.246	0.055 (4.3)	maternal	2.4
sire	5	0.522	0.024 (1.8)	additive	7.3
dam × sire	25	0.737	0.021 (1.6)	non-additive	6.5
tank	45	< 0.001	0.255 (19.7)		
Residual			0.941 (72.6)		

Saint-Jean

dam	5	0.370	0.039 (3.1)	maternal	3.1
sire	5	1	0 (0)	additive	0.0
dam × sire	25	0.604	0.158 (3.2)	non-additive	12.7
tank	38	< 0.001	0.219 (17.6)		
Residual			0.948 (76.1)		

Fry survival (Day 144-187)
LaHave

dam	5	0.091	0.195 (9.3)	maternal	9.1
sire	5	0.963	0.004 (0.2)	additive	0.8
dam × sire	25	0.060	0.198 (9.4)	non-additive	37.8
tank	46	< 0.001	0.745 (35.5)		
Residual			0.953 (45.5)		

Sebago

dam	5	0.079	0.389 (13.2)	maternal	13.2
sire	5	1	0 (0)	additive	0.0
dam × sire	25	< 0.001	0.577 (19.7)	non-additive	78.6
tank	45	< 0.001	0.995 (33.9)		
Residual			0.976 (33.2)		

Body length at hatch
LaHave

dam	5	0.003	0.718 (34.8)	maternal	34.5
sire	5	1	0 (0)	additive	0.0
dam × sire	25	0.015	0.236 (11.4)	non-additive	45.8
tank	46	< 0.001	0.440 (21.3)		
Residual			0.671 (32.5)		

Sebago

dam	5	0.002	0.417 (23.5)	maternal	17.5
sire	5	0.125	0.570 (6.0)	additive	24.0
dam × sire	25	0.866	0.065 (0.7)	non-additive	2.7
tank	45	< 0.001	0.412 (17.3)		
Residual			1.251 (52.5)		

Saint-Jean

dam	4	0.006	0.441 (20.5)	maternal	17.5
sire	5	0.373	0.065 (3.0)	additive	12.0
dam × sire	20	0.280	0.082 (3.8)	non-additive	15.3
tank	38	1	0 (0)		
Residual			1.569 (72.7)		

Body mass at hatch
LaHave

dam	5	0.003	8.9×10^{-5} (29.9)	maternal	29.9
sire	5	1	0 (0)	additive	0.0
dam × sire	25	0.176	2.8×10^{-5} (9.6)	non-additive	38.3
tank	46	< 0.001	4.9×10^{-5} (16.4)		
Residual			1.3×10^{-4} (44.1)		

Sebago

dam	5	0.036	1.1×10^{-4} (13.7)	maternal	9.0
sire	5	0.288	3.9×10^{-5} (4.7)	additive	18.7
dam × sire	25	0.464	3.2×10^{-5} (3.8)	non-additive	15.3
tank	45	0.002	1.2×10^{-4} (14.1)		
Residual			5.3×10^{-4} (63.7)		

Saint-Jean

dam	4	0.001	1.1×10^{-4} (19.2)	maternal	19.2
sire	5	1	0 (0)	additive	0.0
dam × sire	20	1	0 (0)	non-additive	0.0
tank	38	0.351	2.4×10^{-5} (4.4)		
Residual			4.2×10^{-4} (76.4)		

Body length at yolk sac absorption

LaHave

dam	5	< 0.001	0.172 (25.9)	maternal	25.9
sire	5	1	0 (0)	additive	0.0
dam × sire	25	0.217	0.168 (2.5)	non-additive	10.1
tank	44	< 0.001	0.514 (7.8)		
Residual			4.224 (63.8)		

Sebago

dam	5	0.003	0.681 (12.5)	maternal	9.2
sire	5	0.156	0.176 (3.2)	additive	12.9
dam × sire	25	0.245	0.148 (2.7)	non-additive	10.9
tank	45	< 0.001	0.398 (7.3)		
Residual			4.061 (74.3)		

Body mass at yolk sac absorption

LaHave

dam	5	< 0.001	1.5×10^{-3} (27.1)	maternal	27.1
sire	5	1	0 (0)	additive	0.0
dam × sire	25	0.167	1.9×10^{-4} (3.3)	non-additive	13.3
tank	44	< 0.001	4.6×10^{-4} (8.1)		
Residual			3.5×10^{-3} (61.5)		

Sebago

dam	5	0.007	7.9×10^{-4} (9.4)	maternal	5.9
sire	5	0.109	3.0×10^{-4} (3.6)	additive	14.2
dam × sire	20	0.336	1.7×10^{-4} (2.0)	non-additive	7.9
tank	45	< 0.001	6.2×10^{-4} (7.4)		
Residual			6.5×10^{-3} (77.6)		

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Appendix E. Experimental Protocol Approval Records

The experimental protocols used in the thesis research were developed in accordance with the guidelines of the Canadian Council on Animal Care, the Animal Care Committee at the University of Western Ontario, the Committees of the Ontario Ministry of Natural Resources and Forestry, and Environment Canada.

University of Western Ontario
Animal Use Protocol #2010-2014 (2010- present) for Chapters 2-6
"Behavioural and molecular ecology of fishes"

eSirius: Protocols
List of Protocols

Principal Investigator	Function	Department	Organization	AUP #	AUP Yr	Version	AUP Title	AUP Status	Status Date	Approval Date	Expire Date	Review Date	Submit Date
Neff, Bryan	Principal Investigator	Biology	Faculty Of Science	2010-214		1	Behavioural and molecular ecology of fishes	Approved (w/o Stipulation)	06/07/2011	05/26/2010	05/31/2014	06/01/2011	12/09/2010
Neff, Bryan	Principal Investigator	Biology	Faculty Of Science	2010-214		2	Behavioural and molecular ecology of fishes	Approved (w/o Stipulation)	06/01/2012	05/26/2010	05/31/2014	06/01/2012	12/09/2010
Neff, Bryan	Principal Investigator	Biology	Faculty Of Science	2010-214		3	Behavioural and molecular ecology of fishes	Approved (w/o Stipulation)	06/06/2013	05/26/2010	05/31/2014	06/01/2013	12/09/2010
Neff, Bryan	Principal Investigator	Biology	Faculty Of Science	2010-214		4	Behavioural and molecular ecology of fishes	Approved (w/o Stipulation)	06/06/2013	05/26/2010	05/31/2014	05/31/2014	12/09/2010
Neff, Bryan	Principal Investigator	Biology	Faculty Of Science	2010-214		5	Behavioural and molecular ecology of fishes	Approved (w/o Stipulation)	06/09/2014	06/09/2014	06/09/2018	06/09/2015	03/21/2014

Ontario Ministry of Natural Resources and Forestry
Aquatic Research and Monitoring Section

Animal Use Protocol #93 (2010-2011) for Chapters 2, 3, and 6
"Performance of early-life stages and juveniles of Atlantic salmon in competition with non-native salmonids"

Animal Use Protocol #94 (2011-2013) for Chapter 4
"Performance of juvenile Atlantic salmon in natural streams of Lake Ontario"

Animal Use Protocol #103 (2011-2013) for Chapters 2, 3, and 6
"Performance of early-life stages and juveniles of Atlantic salmon in competition with non-native salmonids"

Animal Use Protocol #115 (2013-2016) for Chapter 5
"Genetic adaptations to current thiaminase diets in candidate strains of Atlantic salmon for reintroduction into Lake Ontario"

Animal Use Protocol #128 (2014-2016) for Chapter 5
"Thiaminase diet effects on the swim performance of juvenile Atlantic salmon"

Ontario Ministry of Natural Resources
Aurora District Office
Licence to Collect Fish for Scientific Purposes #1065095 (2011) for Chapter 4

Environment Canada
Notification and Processing Control Unit
New Substance Notification #16996 (2013) for Chapter 5
"*Paenibacillus thiaminolyticus* in salmon diet research"

Curriculum Vitae

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Post-secondary Education and Degrees:

University of Western Ontario
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2010-2015 Ph.D.

Dalhousie University
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2007-2010 M.Sc.
2003-2007 B.Sc. (Honours Co-op)

Honours and Awards:

Queen Elizabeth II Ontario Graduate Scholarship
2013-2014 Ph.D.

Natural Sciences and Engineering Research Council
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Related Work Experience

Teaching Assistant
University of Western Ontario
2011-2014
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Published Publications:

Houde ALS, Smith AD, Wilson CC, Peres-Neto PR, Neff BD. 2015. Effects of competition between rainbow trout and Atlantic salmon in natural and artificial streams. *Ecol Freshw Fish.* doi: 10.1111/eff.12206.

Houde ALS, Black CA, Wilson CC, Pitcher TE, Neff, BD. 2015. Genetic and maternal effects on juvenile survival and fitness-related traits in three populations of Atlantic salmon. *Can J Fish Aquat Sci* 72: 751-758.

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In Review Publications:

Houde ALS, Wilson CC, Neff, BD. 2015. Effects of competition with four non-native salmonid species on Atlantic salmon from three populations. *Trans Am Fish Soc*, in review.

Houde ALS, Saez PJ, Wilson CC, Bureau DP, Neff BD. 2015. Effects of feeding high dietary thiaminase to sub-adult Atlantic salmon from three populations. *J Great Lakes Res*, in review.

Houde ALS, Garner SR, Neff BD. 2015. Restoring biodiversity through reintroductions: strategies for source population selection. *Restor Ecol,* in review.