GENOMIC AND EXPERIMENTAL EVIDENCE OF RAPID EVOLUTION IN AN INTRODUCED POPULATION OF ATLANTIC SALMON (*SALMO SALAR*) IN ROCKY RIVER, NEWFOUNDLAND

by

© Gwynhyfar Mason

A Thesis submitted to the

School of Graduate Studies

In partial fulfillment of the requirements of the degree of

Master of Science

Department of Biology

Memorial University of Newfoundland

January 2018

St. John's, Newfoundland and Labrador

Abstract

We characterized the presence of recent divergence in a population of Atlantic salmon (*Salmo salar*) introduced from Little Salmonier River into Rocky River, Newfoundland ~ 5 generations previously, by quantifying genomic divergence and conducting reciprocal transplant experiments. Genomic evidence based on Bayesian clustering and hybrid characterization using genome-wide single nucleotide polymorphisms support the presence of two populations, one at Little Salmonier, and both at Rocky River with hybridization with non-anadromous residents. In conjunction with evidence of adaptive divergence at ~90 loci, this finding supports the hypothesis of rapid evolution. Reciprocal transplants from controlled lab crosses revealed no significant growth differences between rivers, but higher survivorship in Rocky River. Purebreds outperformed hybrids in both rivers and the laboratory, a finding consistent with outbreeding depression. Overall, the results support the hypothesis of rapid evolution of salmon in Rocky River; with both adaptive evolution and introgression with residents as likely causes.

Key words: rapid evolution, Atlantic salmon, genomics, reciprocal transplant, microsatellites, single nucleotide polymorphism, SNP array, RAD-seq, outbreeding depression

Acknowledgements

First and foremost, I would like to thank my supervisors Dr. Ian Fleming and Dr. Ian Bradbury. Their guidance, support, advice and patience throughout my degree was invaluable, and I am forever grateful. Ian Fleming guided me through my first experience working with salmon, and taught me vital lessons in field work and fish rearing. Ian Bradbury exercised remarkable patience as I entered the world of genomic research equipped with seemingly endless questions. They were both readily available to provide edits and advice, and I cannot thank them enough for all they've done for me. I would also like to thank Dr. Dawn Marshall of my advisory committee. She was always able to provide input and advice in all areas, particularly with the genomic research, which was a new and somewhat daunting field for me. I would also like to thank my examiners for their insight and helpful advice in shaping this thesis.

I would like to thank all current and former members of the labs I was in. In the Fleming lab, Corinne Conway provided invaluable support, advice and friendship; Kristin Bøe, Nate Wilke, Brendan Wringe and Angelisa Dennis were extremely helpful both with field work and with working through various challenges I faced. In the Bradbury lab, thanks to Mallory Van Wyngaarden; who quickly became a good friend, was the recipient of my many questions, and partner-in-crime in general silliness. I am also grateful to Marion Sinclair-Waters and post-docs Ryan Stanley and Nick Jeffery, who provided advice on various genomic tests and statistics, and how to navigate my way through grad school. I would like to extend a special thank-you to Dr. Daniel Ruzzante and his lab at Dalhousie University, who graciously allowed me to conduct parental

analyses with their lab in early 2015. Thanks as well to Vicki Morris (DFO) for all her help and expertise in sample collection at both rivers. Thank you to all the volunteers who aided in conducting fish recaptures in September 2014. Special thanks to the Aquatic Biotechnology Lab at the Bedford Institute of Oceanography as well, for quickly analyzing unexpected fish samples.

Because I split my time between St. John's and Halifax for this degree, I must thank all the staff at the OSC who helped my travels run smoothly. JoAnn Greening made sure all my paperwork for conferences was in order and had patience for all my questions. I would also like to thank the OSC community, especially the Friend Farm, for providing support, friendship and endless entertainment to keep us all grounded. Finally, a huge thank you to my family and to Isaac for their endless love and support. I wouldn't have been able to make it through this degree without their patience, support, and ability to provide me with motivation when I needed it the most.

This work builds upon previous work documenting the transatlantic secondary contact in Atlantic salmon on the southern coast of Newfoundland, which also used the three genomic datasets in the present study (Bradbury *et al.* 2015). This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and Genomics Research and Development Initiative (DFO). Travel to conferences, meetings and workshops was provided by the Memorial University of Newfoundland (MUN) School of Graduate Studies, the MUN Department of Biology, and the MUN Graduate Students' Union.

Table of Contents

List of Tables

List of Figures

List of Common Terms, Abbreviations, and Symbols

Term	Description
Anadromous	Born in fresh water, spending the majority of life in salt water; returning to fresh water to spawn
ANOVA	Analysis of variance
Atlantic Salmon	*Salmo salar* (Linnaeus)
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
DAL	Dalhousie University, Halifax, NS
ddH_20	Double-distilled water
DFO	Fisheries and Oceans Canada
DNA	Deoxyribonucleic Acid
LSR	Study site: Little Salmonier River
MAF	Minor allele frequency
Mature male parr	Male salmon that mature and remain in fresh water; smaller than anadromous males
MCMC	Markov Chain Monte Carlo algorithm
MGPL	Marine Gene Probe Lab (Dalhousie University, Halifax, NS)
Microsatellite	Tandem repeats in non-coding regions of DNA
MS-222	Tricaine Methanesulfonate
MSV	Multisite variants; polymorphisms within duplicated regions
MUN	Memorial University of Newfoundland
NL	Newfoundland and Labrador, Canada
NS	Nova Scotia, Canada
NSERC	Natural Sciences and Engineering Research Council of Canada

OSC	Ocean Sciences Centre
Outlier	Locus putatively under selection
PC	Principal component
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PSV	paralogous sequence variations
RAD-seq	Restriction-site associated DNA sequencing
Rapid Evolution	Evolution occurring over short time spans or few generations
Reciprocal Transplant	Experiment introducing organisms from two environments to the other for a set period of time
RKR	Study Site: Rocky River
RKRO	Rocky River ouananiche (resident salmon)
SAEN	Salmon Association of Eastern Newfoundland
SNP	Single nucleotide polymorphism

List of Appendices

Co-authorship Statement

This work was completed, and this thesis was written by Gwynhyfar Mason with help and guidance from Drs. Ian Fleming and Ian Bradbury. Fish were collected by Ian Fleming, Ian Bradbury, Vicki Morris and Corinne Conway for the reciprocal transplant experiment. Microsatellite, SNP array and RAD-seq sample collection was conducted by Ian Bradbury with the assistance of Fisheries and Oceans staff and sequencing was completed by Dr. Lorraine C. Hamilton and Praveen N. Ravindran.

Chapter 1: Introduction

1.1 Rapid evolution

The concept of adaptive evolution, when species or populations adapt over time to suit their environment, is well established. Until recently, adaptive evolution was thought to take place over prolonged periods of time (Darwin 1859). Recent evidence, however, suggests that adaptive changes can occur in much shorter time spans than originally hypothesized, occurring often in just a few generations (Thompson 1998). This phenomenon has become known as rapid evolution, and it generally occurs when a population adapts quickly to a sudden change in environment, such as an introduction event, natural or anthropogenic changes to the ecosystem, or the addition or removal of a species (Hendry & Kinnison 2001; Reznick & Ghalambor 2001). Rapid evolution occurs most often when a population has an opportunity to grow and/or colonize (Hendry & Kinnison 2001; Reznick & Ghalambor 2001). Human-induced changes, introduced predators or prey, and changes to the biophysical environment can all contribute to rapid evolution and divergence (Hendry & Kinnison 2001).

Rapid evolution has become an increasingly popular area of study, with documented evidence in many taxa (Reznick and Ghalambor 2001), including peppered moths (Berry 1990), Trinidadian guppies (Reznick *et al.* 1997), several salmonids (Bourret *et al.* 2011; Hendry *et al.* 1998; Hendry *et al.* 2000; Kovach *et al.* 2013; Westley *et al.* 2013), and many others. Historically, some studies have utilized a reciprocal transplant approach to assess the presence of rapid evolution within a population. However, with advancing

biotechnology, the use of genetic and genomic data are also key to understanding whether populations are adapting to changing environmental conditions and how they are doing so.

1.2 Methods for assessing rapid evolution: reciprocal transplants and genomic data

Many studies assess changes in phenotypic traits over time as evidence of rapid evolution or adaptation (Hendry & Kinnison 1999). However, not all changes in phenotypic traits are evident to researchers or can be quantified reliably (Hendry & Kinnison 1999; Merilä & Hendry 2014). Some phenotypic studies acknowledge this shortcoming (Losos *et al.* 1997), while others assume that the phenotypic changes assessed are inherently due to genetic changes (Hendry & Kinnison 1999; Johnston & Selander 2008). A major driver behind observed phenotypic changes could be phenotypic plasticity, when a given phenotype adjusts on the basis of present environmental conditions (Freeland *et al.* 2011; Merilä & Hendry 2014). To mitigate the uncertainty of whether phenotypic differences are due to plasticity or adaptation, two main approaches can be used: reciprocal transplants and genetic comparisons.

Reciprocal transplants, an experimental design where individuals of two or more populations are placed both in their home and in the other population's environment for a set period of time, have been used as a method to assess local adaptation and rapid evolution for some time (Barrett *et al.* 2010; Handelsman *et al.* 2013; Hendry & Kinnison 1999; Stelkins *et al.* 2012; Westley *et al.* 2013). Despite this option, there have been

surprisingly few assessments of rapid evolution in newly established populations *in situ* (Westley *et al.* 2013*).*

Alternatively, molecular genetic approaches can be used to assess whether a population is experiencing divergence, most commonly using microsatellites and single nucleotide polymorphisms (SNPs) as markers. Microsatellites, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs), are non-coding, short tandem repeats in the genome (Ball *et al.* 2010; Fernández *et al.* 2013; Freeland *et al.* 2011). Single nucleotide polymorphisms, also referred to as SNPs, are loci where single bases vary between populations and individuals (Freeland *et al.* 2011). Unlike microsatellites, SNPs are much more prevalent and can occur in both coding and non-coding regions of the genome (Freeland *et al.* 2011) and are well suited for genome-wide scans of both neutral and adaptive variation (Freeland *et al.* 2011; Morin *et al.* 2009). Given differences among approaches and genetic marker types, an integrated approach utilizing both microsatellites and SNPs, combined with reciprocal transplant experiments, is ideal for assessing the degree of recent divergence within a population (Stockwell *et al.*, 2003; Williams *et al.*, 2008).

1.3 The Atlantic salmon (Salmo salar)

The Atlantic salmon is an anadromous fish species that spans the east and west coasts of the North Atlantic Ocean. Born in rivers, juveniles (parr) can remain in fresh water for 3-5 years before developing into smolt, a transformation in preparation for marine life, and migrating into the open ocean, where they remain for 1-5 years before

returning to their natal river to spawn (Klemetsen *et al.* 2003a). In some areas, particularly in Newfoundland, male Atlantic salmon can have two reproductive morphs; either anadromous males or mature male parr, the latter of which are smaller and may remain in freshwater throughout their lifespans or smoltify after maturing as parr (Fleming 1996; Hutchings & Myers 1994; Jones 1959; Weir *et al*. 2005). It is also possible for individuals (both sexes) within populations, known as residents or ouananiche, to remain in freshwater for the duration of their lifetime; a phenomenon that is often associated with barriers preventing successful upstream migration (Berg 1985; Fleming & Einum 2011; Verspoor & Cole 1989; Webb *et al.* 2007).

Salmon populations, particularly in the southern portion of their range, are at greater risk for population declines than similar species in the area, due to poor marine survival, illegal fishing activity, and changes in marine ecosystems (COSEWIC 2010). As such, determining the ability of Atlantic salmon to adapt to changing environmental conditions is required to properly manage their populations in the future. Atlantic salmon abundance in southern Newfoundland has declined over the past several decades, due to declining numbers returning from the sea, which has resulted in the classification of a designatable unit, a group of populations that are discrete and evolutionarily significant, being listed as 'threatened' (COSEWIC 2010). Stocking efforts or enhancement projects that have occurred in the past offer a unique opportunity to assess the ability of Atlantic salmon populations to adapt to new environments, and, in turn, determine how they will likely respond to changing environments. Of particular interest is the St. Mary's Bay Atlantic Salmon Enhancement Project that took place in Rocky River, NL in 1984,

implemented by the Salmon Association of Eastern Newfoundland (SAEN). This project aimed to establish a returning anadromous Atlantic salmon run in the river that previously could not exist due to an impassible waterfall at the mouth of the river (Greene 1986). After the river was stocked for several years and a fish ladder installed in 1986, a yearly returning population became established in Rocky River. As such, this system represents a case study by which to examine the presence of rapid evolution in Atlantic salmon in the wild.

1.4 Goals of this thesis

The aim of this work was to characterize the presence of recent adaptive divergence in the Rocky River (RKR) Atlantic salmon population from its founder population in Little Salmonier River (LSR) by conducting reciprocal transplants, while also using genetic and genomic analyses to quantify divergence. The reciprocal transplants consisted of the release of F1 pure (RKR x RKR) and hybrid (RKR x LSR) crosses and were recaptured after approximately 80 days. A subset of fish was also kept under laboratory conditions throughout the transplant period to examine potential differences within a controlled environment. Genetic and genomic analyses consisted of comparing contemporary samples from the two rivers using a microsatellite panel, a genome-wide single nucleotide polymorphism (SNP) array, and restriction-site associated DNA (RAD) sequencing derived SNPs to quantify divergence between the populations. We predicted that (1) a measurable degree of divergence has occurred in the RKR population since the introduction from LSR, (2) RKR purebred fish would outperform hybrids in Rocky River, but both purebreds and hybrids would likely perform similarly in

Little Salmonier River, and (3) more distinct differences would be evident from genomic analyses than from the reciprocal transplants, with RAD-seq SNPs showing the highest degree of resolution. This work highlights the importance of combining *in situ* experimental studies with large-scale genomic analysis to give insight into how quickly a population can evolve and potentially adapt locally within a limited geographical range with similar environments.

1.5 Thesis format

Four chapters comprise this thesis, including this introduction (Chapter 1) and a conclusion chapter (Chapter 4). Chapters 2 and 3 were prepared in manuscript format in preparation for publication, resulting in some overlap between the two. Both chapters are in preparation for submission to as yet undetermined journals.

Chapter 2: Experimental evidence of rapid evolution in a recently introduced Atlantic salmon (*Salmo salar*) population: Potential effects of outbreeding depression in Rocky River, Newfoundland

2.1 Abstract

The capacity of populations to evolve quickly is central to population-scale responses to climatic change and anthropogenic stress. Here, we characterize the presence of recent adaptive divergence in a population of Atlantic salmon (*Salmo salar*) introduced from Little Salmonier River (LSR) into Rocky River (RKR), Newfoundland in the 1980s by conducting reciprocal transplants of F1 crosses. Pure (RKR) and hybrid (RKR♀ x LSR♂) crosses were created from adults (11 females, 44 males) caught at each river and resulting juveniles were released reciprocally in June 2014 for ~80 days, holding fish from each family in laboratory conditions throughout. Recaptured fish were assigned to the family level using five microsatellite markers, and differences in size at recapture and survival between rivers and between cross type were assessed. There was no difference in growth between release sites, however, length differed significantly between cross types (pure RKR vs. hybrid). Recapture rate differences were significant among mothers, but marginally non-significant between cross types (pure RKR vs. hybrid) and sites. Purebreds were slightly heavier, longer, and had higher recapture rates in both locations than hybrids. Purebred fish kept under laboratory conditions were slightly heavier and longer than hybrids, but exhibited a much smaller and not statistically significant difference in size than those under field conditions. Overall, results suggest that outbreeding depression may be contributing to differences in growth

and survival between purebreds and hybrids, allowing pure Rocky River fish to outperform hybrids in both wild environments, which suggests potentially adaptive differences between the two populations.

2.2 Introduction

Adaptive evolution, until recently, was thought to take place over long periods of time (Carroll *et al.* 2007; Darwin 1859; Franks & Munshi-South 2014; Hendry & Kinnison 1999; Thompson 1998). However, recent evolutionary and adaptive evidence suggests that adaptive changes can occur over much shorter time spans, often in only a few generations (Thompson 1998), known as rapid evolution. Rapid evolution occurs most often when a population experiences a sudden change in environmental conditions, such as exposure to human-induced changes, introduced predators or prey, and changes to the biophysical environment (Hendry & Kinnison 2001). The introduction to a new environment, natural or anthropogenic changes to the ecosystem, or the addition or removal of a species all contribute to rapid evolution and divergence (Hendry & Kinnison 2001; Reznick & Ghalambor 2001). When a new species or population is introduced to an environment, the introduced individuals may breed with the local population, creating hybrid offspring. Sometimes, this hybridization results in a phenomenon called outbreeding depression, which is a reduction in fitness or survival in the hybrid offspring of genetically dissimilar parents (Gharrett & Smoker 1991; Edmands & Timmerman 2003). Outbreeding depression often occurs with increases in anthropogenically caused

introduction events (Edmands & Timmerman 2003), which have become common in aquatic species experiencing changes in their environments at an unprecedented rate.

Rapid evolution has become an important area of ecological study (Hendry & Kinnison 1999). Evidence of rapid evolution has been documented in many taxa, reviewed by Reznick and Ghalambor (2001), including peppered moths (Berry 1990), Trinidadian guppies (Reznick *et al.* 1997), sockeye salmon (Hendry *et al.* 1998), Italian wall lizards (Herrel *et al.* 2008), and many others. Research has shown increasing evidence of rapid evolution in nature, particularly within species currently experiencing accelerated declines in population size due to environmental change (Stockwell *et al.* 2003; Williams *et al.* 2008). Of particular interest are aquatic species of both economic and ecological importance that have experienced population declines largely due to overfishing, such as the Atlantic salmon (*Salmo salar*).

The Atlantic salmon is an anadromous fish species that spans the east and west coasts of the North Atlantic Ocean. Born in rivers, juveniles (parr) can remain in fresh water for 3-5 years before developing into smolt, a transformation in preparation for marine life, and migrating into the open ocean, where they remain for 1-5 years before returning to their natal river to spawn (Klemetsen *et al.* 2003a). Male Atlantic salmon are known to have two reproductive morphs, either anadromous males or mature male parr, the latter of which are smaller and may remain in fresh water throughout their lifespans or smolt after maturing as parr (Fleming 1996; Hutchings & Myers 1994; Jones 1959; Weir *et al*. 2005). It is also possible for individuals (both sexes) within populations,

known as residents or ouananiche, to remain in freshwater for the duration of their lifetime; a phenomenon often associated with barriers preventing successful upstream migration (Berg 1985; Fleming & Einum 2011; Verspoor & Cole 1989; Webb *et al.* 2007). Salmon populations are at greater risk for population declines due to poor marine survival, illegal fishing activity, and changes in marine ecosystems (COSEWIC 2010), with declines in southern Newfoundland documented over the past several decades, resulting in a 'threatened' listing (COSEWIC 2010). As such, determining the ability of Atlantic salmon to adapt to changing environmental conditions is required to effectively manage their populations in the future. Stocking efforts or enhancement projects that have occurred in the past offer a unique opportunity to assess the ability of Atlantic salmon populations to adapt to new environments, and, in turn, determine how they will likely respond to future changing environments.

Rocky River, located on the Avalon Peninsula in Newfoundland, encompasses a watershed area of nearly 300 km^2, making it the largest on the peninsula (Greene 1986). In 1984, the St. Mary's Bay Atlantic Salmon Enhancement Project, implemented by the Salmon Association of Eastern Newfoundland (SAEN) began stocking the river with fry (early-stage juveniles) in order to establish an anadromous Atlantic salmon run (Greene 1986). Prior to this implementation, an anadromous population was not present in the system due to an impassible waterfall at the mouth of the river. In 1986, a fish ladder was installed to allow returning adults to migrate upstream to spawn (Greene 1986). For the four consecutive years of stocking, fry were obtained from the artificial breeding of broodstock of Little Salmonier River, southwest of Rocky River, which has a well-

established natural anadromous population (Greene 1986). Since that time, an anadromous population has successfully established in Rocky River, and annual counts can reach up to 500 returning adults (DFO 2015). As such, the Rocky River system represents a case study by which to examine the presence of rapid evolution in Atlantic salmon in the wild.

In this study, we characterized the presence of recent adaptive divergence in the Rocky River (RKR) Atlantic salmon population from its founder population in Little Salmonier River (LSR) by conducting reciprocal transplants of F1 pure (RKR x RKR) and hybrid (RKR x LSR) crosses for approximately 80 days. A subset of fish was also kept under common-garden laboratory conditions throughout the transplant period to examine potential differences within a controlled environment. Our specific goals were to: (1) assess growth and survival differences in juvenile salmon between the two rivers, (2) assess growth and survival differences between cross types, and (3) compare the observations with those obtained from the lab-raised fish. We predicted that a measurable degree of divergence has occurred in the RKR population since the introduction from LSR and that RKR purebred fish would outperform hybrids in Rocky River, but both purebreds and hybrids would likely perform similarly in Little Salmonier River. We hoped to provide insight into the degree to which a population may evolve and adapt locally in 5-6 generations within a limited geographical range with similar environmental conditions.

2.3 Methods

2.3.1 Study site

The study took place in two river systems: Little Salmonier River (LSR), near North Harbour (43.120914° N, -53.731512° W) and Rocky River (RKR), near Colinet (47.251624° N, -53.568495° W), both located within St. Mary's Bay on the Avalon Peninsula of Newfoundland (Figure 2.1). LSR encompasses a drainage area of approximately 122 km^2 (Bourgeois 1998); spawning grounds are abundant but difficult to access, and the river was reported to have the highest production rate in St. Mary's Bay in the mid-1980s (Bourgeois 1998; Greene 1986; Porter *et al.* 1974). Following the enhancement project and the installation of the fish ladder, RKR has been used recreationally for angling purposes (Greene 1986). Adequate spawning grounds exist along the entirety of the main river above the falls, as well as in nearby, accessible tributaries (Porter *et al.* 1974). A Fisheries and Oceans Canada (DFO) counting fence placed just above the falls records annual adult fish returns in order to assess population size and overall health (DFO 2015).

In the present study, ~8000 fish were released in two tributaries at both LSR and RKR for a total of four release sites. LSR sites (47.122934° N, -53.730341° W; 47.123082° N, -53.731745° W, respectively) were accessed via a bridge that crossed the main river on Highway 92. LSR site 1 is a tributary located off the east side of the river and LSR site 2 is a side channel of the main river located off the west side. Fish were released at four separate areas, ranging from 116.8 to 363.7 m above the mouth of the

stream at site 1, and from 45.7 to 126.4 m at site 2. RKR sites (47.262456° N, -53.544964° W; 47.303923° N, -53.540261° W, respectively) were accessed by the Markland Rd. (Highway 81), under which several tributaries cross. RKR site 1 was located upstream of a large culvert at one tributary, and RKR site 2 was upstream of a small bridge in another tributary. Fish were released at four separate areas, ranging from 48.1 to 359.9 m above the culvert at site 1, and from 75.1 to 408.4 m above the bridge at site 2. General river characteristics were observed and recorded using data provided by the Water Resource Management Division in the Department of Environment and Conservation with the Government of Newfoundland and Labrador (Table 2.1). The RKR monitoring station (47.226944° N, -53.568611° W) is located approximately 4.3 and 8.8 km from RKR site 1 and site 2, respectively. The LSR monitoring station (47.121667° N, -53.731667° W) is located approximately 0.17 and 0.16 km from LSR site 1 and site 2, respectively. While these data loggers provided a general overview of river conditions, their distance from the release sites (particularly from RKR sites) likely did not allow for an accurate representation of habitat conditions.

2.3.2 Experimental crosses and rearing

In September 2013, 11 wild females were caught from the RKR counting fence managed by DFO and held in 4 m^2 tanks with continuous water flow at the Ocean Sciences Centre (OSC) in Logy Bay, NL. Adult female collections were attempted at LSR, but were unsuccessful. In early November 2013, mature male parr were collected from both LSR and RKR using a backpack electrofisher. Parr were held in separate 4 m^2 tanks at the OSC according to river until the crosses took place November 14 – 28, 2013.

Each female was crossed with four males: two from LSR, two from RKR. Females were anaesthetized one at a time by placing them in an aqueous solution of MS-222 (Topic Popovic *et al.* 2012) and were weighed, photographed, and then stripped of their eggs. They were then re-weighed and fin-clips (preserved in 95% ethanol) and scale samples were measured. Females were then returned to the holding tanks. Total egg weight was taken and a sample of ten eggs removed to be weighed individually. Eggs were divided in approximately equal proportions among four separate holding containers to be crossed with the corresponding male. Mature male parr were placed in an overdose of MS-222 (~ 0.4g/L) (Topic Popovic *et al.* 2012), then stripped of their milt, which was added to the corresponding batch of eggs. Males were then weighed and photographed for length measurement, and fin clips taken (preserved in 95% ethanol). Once fertilized, each batch of eggs was placed in a mesh basket within a Heath tray incubator with common source flow-through water at ambient temperatures. Unfertilized or decaying eggs were removed 1-2 times per week from each family basket to reduce the risk of fungal infections.

Hatching began approximately 90 days post-fertilization from 21 February to 2 March 2014. Once 50% of each family had hatched, 10 alevins per family were randomly selected to be weighed (accurate to 0.01 g) and photographed for length (accurate to 0.001 mm). Fish were removed from the incubator trays and placed in tanks by family between 7 – 21 May 2014, when most of their yolk-sacs had been reabsorbed and they were swimming freely. Tanks were 35.5 x 25.4 x 17.8 cm, with a water depth of ~ 11.5 cm and a common source flow-through water system at ambient temperatures from a

local water source. To stimulate feeding behaviour, families were introduced to brine shrimp *(Artemia salina*) nauplii, which are used frequently to boost larval fish appetites (Brown *et al.* 2003; Sorgeloos *et al.* 2001). Approximately 10 mL of nauplii, concentrated in fresh water, were deposited in each family tank 3 times per day. After approximately 7 days of brine shrimp feed, fish were introduced to a commercial starter diet (0.5 mm starter feed, Corey Aquafeeds) and subsequently weaned off the nauplii. Once approximately 50% of fish in each tank were actively feeding on dry food, ten individuals from each family were randomly sampled for weight (0.01 g) and fork length measurements (0.001 mm). As the fish grew, they were fed multiple times per day initially with 0.5 mm starter feed, and then with 1.0 and 1.5 mm standard salmonid hatchery feed (also from Corey Aquafeeds).

2.3.3 Reciprocal transplants

Prior to release on 29 June 2014, all experimental fish were adipose fin-clipped between 23-26 June 2014 for identification in the field at the time of recapture, and a sample of 10 fish from each family was measured for weight (0.01 g) and fork length (0.001 mm). Small groups of juveniles were anaesthetized in MS-222, fin-clipped, and allowed to recover in fresh water with continuous air flow. Once recovered, they were then divided evenly into four, circular 1 m^2 diameter tanks. On 29 June 2014, these fish were released at each of four study sites (one tank per site), with two sites at each river (n = 4844 RKR x LSR hybrids, 3220 RKR purebreds). Within each study site, approximately equal numbers of fish were released in four locations (1211 hybrids, 804

purebreds per location), approximately 20-50 m apart, in sheltered areas of the streams. Family size differences led to slight differences in the number of fish per family released at each site, with LSR family sizes ranging from 128 to 656 individuals (440.4 ± 145.5) and RKR families ranging from 80 to 648 individuals (292.7 ± 202.8).

Recapture sampling took place 17 – 23 September 2014, 80-86 days post release (DPR). This resampling was conducted by electrofishing upstream at each tributary in which fish were released in, beginning approximately 150-200 m below the most downstream release point and travelling upstream of the highest release point, until no recaptures were made (between 50 – 100 m). Between five and six passes were made at each site throughout the recapture sampling period. Upon recapture, fin-clipped individuals were placed in holding buckets according to the section of river in which they were caught, and then transported back to the measuring station. Fish were euthanized, assigned an identification number, weighed (0.01 g) and photographed for subsequent fork length measurement (0.001 mm). A clip was taken from each individual's tail and placed in 95% ethanol for later parentage assignment purposes.

2.3.4 Laboratory fish

While the majority of fish (n = 8064) were released into LSR and RKR, between 10 and 30 individuals from each family were kept at the Ocean Sciences Centre (OSC) of Memorial University to be raised under common laboratory conditions (n = 204). After the experimental fish were released in June 2014, the remaining fish from each family were divided evenly between two 1 m^2 tanks with common source flow-through water.

Automatic feeders were set up at each tank to deposit approximately 2 g of food (combination of 1.0 mm and 1.5 mm standard salmonid hatchery feed, Corey Aquafeeds), five times per day, to satiation. Artificial lighting was set to reflect natural light conditions; tanks were cleaned and deceased fish were removed twice per week. Fish were euthanized between 25 - 27 September 2014, weighed (0.01 g), photographed for later length measurement (0.001 mm), and had a fin clip taken and stored in 95% ethanol for later parentage assignment (47 hybrids, 60 purebreds).

2.3.5 Parentage assignment

All recaptured and laboratory fish were assigned at the family level to analyze size and survivorship differences. This assignment was completed by assessing five microsatellite markers specific to Atlantic salmon: SSa85 (O'Reilly 1997), SSsp2210 SSsp2213, SSsp2215 (Paterson *et al.* 2004), and SSaD486 (King *et al.* 2005) (Table 2). Parentage analysis was conducted at the Marine Gene Probe Lab (MGPL) at Dalhousie University (DAL). DNA from offspring and parents was extracted using a Perkin Elmer Multiprobe II Plus liquid handler using a glassmilk protocol modified from Elphinstone (2003). Extracted DNA was then re-suspended in 120 μL of low TE (Tris-HCl and Ethylenediaminetetraacetic acid (EDTA)) and kept refrigerated. DNA quantity and quality was assessed by electrophoresing on a 1% agarose gel using a PicoGreen protocol and then fluoresced with a Perkin Elmer Fusion DNA Quantifier (Perkin Elmer, Waltham, Massachusetts (King *et al.* 2001; McCracken *et al.* 2014).

DNA amplification using polymerase chain reactions (PCR) utilizing 1 μL of genomic DNA, 2.3 μL ddH2O, 0.5 μL 10X PCR buffer (100 mM Tris-HCl, pH 8.3 at 25°C; 500 mM KCl; 15 mM $MgCl_2$, 0.01% gelatin; Bio Basic Inc., Markham, Ontario), 0.5 μL $MgSO_4$ (Bio Basic Inc., Markham, Ontario), 0.5 μL dNTPs (Bio Basic Inc., Markham, Ontario), 0.05 μL forward and reverse primers (labelled with fluorescent M_{13}) (Bio Basic Inc., Markham, Ontario), and 0.05 μL TSG DNA polymerase (Bio Basic Inc., Markham, Ontario). All amplifications were completed using either a 96-well or 384-well thermocycler (Eppendorf, Ep gradient S model). Individuals were amplified using the following procedure: a 3 minute denaturation (95°C), then 35 cycles of 94°C for 45 seconds, annealing temperature specific to the marker added for 45 seconds, 72 °C for 45 seconds, and an extension at 72°C for 5 minutes after the cycles had finished (McCracken *et al.* 2014).

Resulting fragments were electrophoresed and imaged using Li-COR 4200/4300 DNA analyzers (Li-COR Biosciences, Lincoln, Nebraska) (McCracken *et al.* 2014). Genotypes were obtained and scored using SAGA software (Li-COR Biosciences, Lincoln, Nebraska), and then manually scored to ensure accuracy. After all individuals were successfully genotyped, parentage was assessed using COLONY software (Zoological Society of London, London, England) that uses full-pedigree likelihood methods to infer parental relationship among individuals with multilocus genotype data (Jones & Wang 2010).

2.3.6 Statistical analyses

All statistical analyses were conducted using R software v3.2.3. Quadratic regressions were used to explore the relationship between length and weight among the recaptured fish and lab-raised fish. We examined the differences in size at recapture (length and weight) between mothers, cross types, and transplant sites using ANOVA. Cross type and transplant site were included as fixed factors, and mother was included as a random factor. In the weight (g) comparison, the interaction between cross type and mother was significant; and in the fork length (mm) comparison, the interaction between mother and transplant site was significant, thus they were both included in their respective models. Similarly, growth differences among lab-raised fish were also investigated using ANOVA. Cross type and tank were included as fixed factors, mother was included as a random factor and interaction terms were only included in the models when they were significant predictors of variance.

We examined the relationship between offspring recapture rates (survival) per mother, between cross type and between sites using ANOVA. Cross type and transplant site were included as fixed factors. No interaction terms were significant predictors of variance; thus they were not included in the model.

2.4 Results

Changes in weight and length after fish hatched (at hatch, start feed, and prior to release) were relatively similar between pure RKR fish and hybrids, with nearly identical

mean values (Table 2.3). However, weight differed significantly ($F_{1, 741} = 5.585$, $p < 0.05$) between cross types (pure RKR and hybrid), with hybrids slightly heavier at hatch and start feed time points (Figure 2.2). Weight increased slightly at each measurement point, and variability increased as fish aged. Size at hatch, 50% start feed, and prior to release varied slightly amongst families from each river. Length did not vary significantly between cross types (Figure 2.2). A large difference in mean length between hatch and the start of feeding was not reflected in the weight measurement, likely due to the re-absorption of the yolk-sac. Thus, the majority of growth resulting from the re-absorption of the yolk-sac was dedicated to increasing body length. Length differed little between the start of feeding and the time of release, though variability increased slightly.

2.4.1 Size and survival differences between rivers and cross type

General river characteristics showed that mean daily river flow (m^3s^{-1}) differed significantly between rivers, between months, and between rivers within each month ($F_{1, 220} = 23.364$, $p < 0.005$; $F_{3, 220} = 6.676$, $p < 0.005$; $F_{3, 220} = 4.522$, $p < 0.005$, respectively) (Table 2.1). RKR experienced higher flow throughout the experiment and the highest mean flow in each river was recorded in August 2014 (14.2 m^3s in RKR and 2.4 m^3s in LSR). Average daily river stage (the change in water levels within a river; m) varied significantly amongst rivers and amongst release months, but the interaction between the two was not significant. ($F_{1, 223} = 11149.51$, $p < 0.005$; $F_{3, 223} = 16.26$, $p < 0.005$, respectively) (Table 2.1). LSR experienced higher stages throughout the experiment and the highest average stage in each river was recorded in August 2014 (0.848 m in RKR

and 2.347 in LSR). River temperature (˚C) also differed significantly between rivers and months, as did the interaction between the two ($F_{1, 220} = 145.07$, $p < 0.005$; $F_{3,220} = 41.17$, $p < 0.005$; $F_{3, 220} = 17.87$, $p < 0.005$, respectively) (Table 2.1). Temperature was consistently higher in RKR than in LSR throughout the experimental period, with the highest mean temperature of 23.6°C in August at RKR, and 19.7°C in July at LSR.

At the time of recapture, 40 (0.992%) fish were retrieved from LSR, and 70 (1.736%) from RKR. The relationship between weight and length followed a similar positive, quadratic regression in both rivers, and for both cross types ($R^2 > 0.939$), with no significant differences (Figure 2.3). However, recapture length varied significantly between cross type in both rivers ($F_{1, 91} = 4.295$, $p < 0.05$) with purebreds slightly longer (mean ± SD: 49.95 ± 4.63 mm) than hybrids (mean ± SD: 48.21 ± 4.60 mm), which was not reflected significantly in the recapture weights between purebreds (mean ± SD: 1.21 ± 0.38 g) and hybrids (mean ± SD: 1.08 ± 0.36 g) ($F_{1,91} = 3.071$, $p = 0.0831$). There were no significant differences in size at recapture by mother alone (Figure 2.4), but the interaction between mother and cross type varied significantly for both weight and length measurements amongst recaptures ($F_{4, 91} = 3.225$, $p < 0.05$; $F_{4, 91} = 3.574$, $p < 0.01$ respectively). This difference indicates that cross type effects on offspring size at recapture varied amongst mothers. Size at recapture was also not significantly affected by release site (weight $F_{1, 91} = 0.013$, $p = 0.9081$; fork length $F_{1, 91} = 0.248$, $p = 0.620$).

Recapture rates ((number of fish recovered / number of fish released) * 100%) differed significantly amongst mothers (Figure 2.5A) ($F_{8, 33} = 14.483$, $p < 0.005$).

Interestingly, recapture rate differences were marginally non-significant according to cross type and with respect to river ($F_{1, 33} = 4.137$, $p = 0.050$; $F_{1, 33} = 3.82$, $p = 0.059$, respectively; Figure 2.5B, Figure 2.6), although pure RKR fish fared slightly better in both environments.

2.4.2 Comparison of size in the laboratory

Size differences among experimental fish by mother at experiment termination were marginally non-significant ($F_{8, 96} = 1.969$, $p = 0.0585$; $F_{8, 96} = 1.777$, $p = 0.0909$, weight and length, respectively) (Figure 2.7). There were no significant weight or length differences between cross type ($F_{1,96} = 0.645$, $p = 0.424$, $F_{1,96} = 0.147$, $p = 0.702$, respectively) or between tanks ($F_{1,96} = 0.069$, $p = 0.7931$, $F_{1,96} = 0.011$, $p = 0.9157$, respectively) in the lab-raised fish (Figure 2.8). The growth trajectory followed a positive, quadratic curve ($R^2 = 0.972$), similar to that seen in the recaptured individuals (Figure 2.9).

2.5 Discussion

The establishment of a returning Atlantic salmon population in Rocky River, Newfoundland, appears to represent the implementation of a successful enhancement strategy. Reciprocal transplants, combined with continuous monitoring in a laboratory setting, showed no significant differences in growth or survival in transplanted fish, indicating that the introduced population at Rocky River has not yet significantly diverged from its parent population. However, in both transplant and laboratory results, it

appeared that purebreds tended to slightly outperform their hybrid counterparts, which could suggest outbreeding depression.

There is some evidence of a low level of outbreeding depression as a result of hybridization between the LSR and RKR Atlantic salmon populations, despite a lack of statistically significant results. Prior to transplant release, both cross types had similar growth trajectories, with slightly heavier hybrids than purebreds. There were no size differences between rivers at the time of recapture, although length at recapture was significantly greater for purebreds than hybrids. Recapture rates were marginally higher at RKR for both cross types, likely due to more suitable habitat at the release sites (more shelter, slower flow rates, etc.). Purebreds tended to be heavier and longer than their hybrid counterparts regardless of recapture location. This finding somewhat meets the 'local vs. foreign' criterion for testing for evidence local adaptation in RKR, where native populations have higher fitness than non-native individuals (Kawecki & Ebert 2004; Stelkens *et al* 2012). However, without being able to have pure LSR offspring, we were not able to fully test this model. Not surprisingly, laboratory-raised fish were larger than recaptures as they had a reliable source of food and did not experience the environmental challenges of the wild, such as predation risks. Among the laboratory raised fish, there were no significant differences in growth between tanks or between mothers. Purebreds appeared to slightly outperform hybrids under laboratory conditions, but not significantly so. As was expected, lab fish were longer and heavier than recaptured fish, due to a constant and abundant food source. In the 5-6 generations since the introduction of anadromous fish into RKR, more distinct differences in growth and survival between

cross types might have been expected. The low levels of differentiation could be due to overall similarities in river environments, or that phenotypic evidence of rapid evolution has not yet become observable, possibly due to insufficient time for evolutionary differences to become apparent. More notable differences may have been apparent had we been able to compare purebreds of each river. However, due to the limited accessibility to field sites, LSR females were not able to be acquired.

2.5.1 How early can rapid evolution be detected in introduced populations?

Significant evidence of rapid evolution has been demonstrated in relatively short time frames in aquatic species, though some can adapt more quickly than others. On the one hand, adaptation can become evident in as little as three generations, such as the threespine stickleback (*Gasterosteus aculeatus*) rapidly developing the ability to survive in cold winter temperatures (Barrett *et al.* 2010). On the other hand, reproductive isolation in two sockeye salmon (*Oncorhyncus nerka*) populations due to changes in breeding ground type became apparent, only after approximately 13 generations (~56 years), which is not necessarily equivalent to rapid evolution (Hendry *et al.* 2000). In Atlantic salmon, evidence of rapid evolution has been documented in an introduced population that showed molecular differences in both microsatellite and enzymatic loci and in the age of maturation between the introduced and parent rivers, 5-6 generations after the introduction event (Martinez *et al.* 2001). In the present study, although anadromous fish were introduced to RKR approximately 30 years (5-6 generations) previously, we found little significant evidence that the two populations differ in juvenile

size at age (i.e. growth). This was also seen by Stelkin *et al* (2012) in brown trout (*Salmo trutta*) reciprocal transplants, where no evidence for local adaptation was reported at the embryonic stage from transplants using five populations. They speculated that this could possibly be due to a lack of environmental differences at the release sites. Though data loggers at the rivers in our study showed that overall, the rivers differed, the actual release sites were likely similar. Weight and length were similar in fish recaptured in our study at both rivers with a marginally significant paternal effect on length, as purebred cross types tended to perform slightly better than hybrids. Moreover, slightly more purebreds were recaptured than hybrids across both rivers, demonstrating potential adaptive differences in survival amongst these populations.

The few significant results could be caused by several factors, including the small number of generations since the introduction. Stockwell *et al*. (2003) cite several contingencies upon which rapid evolution, or "contemporary evolution", as it is referred to in their review, depends, including the number of generations required for adaptive changes to become evident. They outline a simplistic scenario predicting approximately 25 generations for adaptation to occur (Stockwell *et al*. 2003). However, the authors recognize that this estimate cannot fully reflect of what occurs in a natural population, as selective forces rarely remain consistent throughout time. Atlantic salmon populations in eastern Newfoundland have a typical generation time of approximately five years (COSEWIC 2010; O'Connell *et al*. 2006), so at the time of the reciprocal transplant experiment, only approximately 5-6 generations had passed since anadromous fish were introduced into RKR. Potentially, the introduced population has not been present in RKR

long enough to yield distinct and measurable adaptive differences compared to the parent population. Stockwell *et al.* (2003) also suggest that when conservation efforts occur in the form of creating 'refuge' populations (populations that are established when a particular species or population is endangered or at risk of extirpation/extinction), refuge populations may show reduced genetic diversity. However, they argue that this reduction would cause the refuge population to diverge from the ancestral population, and that it would be difficult for it to perform in the ancestral habitat; this prediction contradicts our study findings, with RKR purebreds tending to slightly outperform hybrids at both the introduced and ancestral habitats.

2.5.2 Outbreeding depression in recently introduced populations

Several studies document outbreeding depression in salmonids, when hybrid offspring of two different populations experience lowered fitness compared to their purebred counterparts (Edmands & Timmerman 2003), has been documented several times in salmonids (Côté *et al.* 2014; Gharrett *et al.* 1999; Gilk *et al.* 2004; Lehnert *et al.* 2014; O'Toole *et al.* 2015). Many underlying causal factors may cause hybrids to underperform; one such example is when subtle environmental changes have different effects on purebreds and hybrids. Côté *et al*. (2014) assessed the effects of oxygenation during embryonic development in Atlantic salmon purebreds and hybrids. Although they detected some degree of heterosis amongst the hybrids under normal conditions, outbreeding depression was evident among offspring that developed in a hypoxic environment, both in terms of growth and survival after hatching (Côté *et al*. 2014). This

finding shows that even early in development, outbreeding can have detrimental effects on a population's overall health if the environmental conditions are not suitable for both crosses. In the present study, offspring were exposed to a common water source, which may have been more conducive to development in hybrids than in purebreds, as hybrids tended to be heavier in early development prior to exogenous feeding. It is also possible that hybrid offspring may have been able to process the artificial feed better than the purebred offspring. A similar trend was seen in a study by Green & Rawles (2010), where hybrid catfish crosses were heavier and grew more quickly than purebreds when fed to satiation with a commercial feed. A similar finding was reported in cultured stocks of walleye (*Sander vitreus*), with walleye x sauger (*Sander canadensis*) hybrid crosses converting feed more efficiently and growing faster than purebred walleye (Barry *et al* 2003). This trend reversed itself in our study once fish began feeding exogenously, with purebred individuals tending to perform slightly better.

Environmental differences, even across relatively small spatial or temporal scales, can have adverse effects on hybrids and transplants. O'Toole *et al*. (2015) conducted reciprocal transplant experiments with Atlantic salmon crosses from neighbouring rivers to assess the fitness of local, foreign, and hybrid fish. A major flood that occurred during the release period provided a unique opportunity for the researchers to assess cross type survival in unstable environmental conditions. Purebred foreign individuals were the most represented in the pre-flood estimates, however, when the fish began to smoltify, both purebred crosses (local x local, foreign x foreign) outperformed the hybrids, and local fish represented the majority of adult returns (O'Toole *et al*. 2015). Our study,

although conditions differed among the two river systems, the within-river environments remained relatively stable seasonably, except for the presence of high flow rates in RKR in August 2014 and low water levels at the LSR sites at the time of recapture. O'Toole *et al*. (2015) suggested that evidence of local adaptation between the two river systems in their study may have been more pronounced had a massive flood not occurred during the study period, which could explain the low levels of outbreeding depression seen between cross types in our study, as conditions were relatively stable at the release sites throughout the experimental period.

Even outbreeding depression in the absence of significant results can provide insight on salmonid population health. A study on Chinook salmon (*Oncorhynchus tshawytscha*) found little evidence of outbreeding depression in either F1 hybrids or F2 backcrosses, despite significant parental genotypic differences (Lehnert *et al.* 2014). They also acknowledged the incomplete cross design for their F2 backcrosses as a potential factor in the lack of significant differences between populations. Likewise, if a complete cross design had been possible our study, effects of outbreeding and hybridization may have been more easily observed. However, incomplete cross designs demonstrate significant evidence of outbreeding depression, as shown by Gilk et al. (2004), quantifying outbreeding depression in two spatially separated populations of pink salmon. Their crosses were conducted in a "blocked incomplete-factorial" design, crossing one female with four males, two from each population; similar to that in our study. Their study took place over two generations, creating F1 and F2 offspring, and they observed significant outbreeding depression amongst hybrids in both locations in

both brood years (Gilk *et al.* 2004). While our study revealed no extensive differences due to hybridization, it provides insight on the adaptive potential of the RKR population.

2.5.3 Limitations and management implications

While our study provides suggestive evidence of rapid evolution in the RKR anadromous population from its parent population in LSR in the form of purebreds consistently outperforming hybrids in reciprocal transplant and laboratory experiments, we acknowledge limitations of our findings. Results may have been clearer if the release period had been longer. Westley et al. (2013) conducted reciprocal transplant experiments on brown trout (*Salmo trutta*) in three river systems in Newfoundland to assess the degree of rapid evolution of this invasive species. They recaptured fish over a two-year period to assess over-winter survival, producing evidence indicating higher levels of survival among local individuals, and individuals that were reared within their home environments (Westley *et al.* 2013). Had our study's release period been longer, distinct differences in survival between crosses may have been more prominent. Gilk *et al*. (2004) also saw significant evidence of outbreeding depression and local adaptation in pink salmon after two generations. However, a longer release period does not always produce significant evidence of rapid evolution or outbreeding depression, as was seen in the Lehnert *et al*. (2014) study of chinook salmon (*Oncorhynchus tshawytscha*), reared in a semi-natural environment (salt water pens) across two generations. Their study took place under controlled laboratory conditions, rather than in a reciprocal transplant design where significant differences may have been more apparent. Using full cross designs

when creating pure and hybrid lines, as in Lehnert *et al.* (2014), yields the most conclusive results when assessing rapid evolution.

Due to limited accessibility to field sites, we were not able to acquire adult females from LSR. Thus, our design crossed RKR females only, mated with mature male parr from both LSR and RKR. Because of this constraint, we were not able to assess whether purebreds of both lineages would outperform hybrids or perform better within their home environment. We were also only able to create hybrids of RKR females and LSR males, so we cannot confirm that LSR female/RKR male hybrids would have performed similarly, though we suspect as much. Gilk *et al.*'s (2004) crosses, similar to that of ours, showed significant evidence of outbreeding depression amongst the hybrids upon their recapture and in the F2 backcrosses, demonstrating that it is possible to determine the presence of rapid evolution with an incomplete cross design. The ability to assess the effects of outbreeding depression when two populations have the potential to hybridize is essential when implementing enhancement strategies.

Rocky River represents a successful enhancement strategy with the aim of creating an anadromous run that did not previously exist in the ecosystem (Bourgeois 1998). However, to date, the RKR population has not met the conservation egg requirement designated by Fisheries and Oceans Canada, which is defined as the number of eggs deposited per fluvial habitat unit (O'Connell *et al*. 1997), and the number of returning adults has clearly declined in South Newfoundland (Bourgeois 1998; COSEWIC 2010; DFO 2015). As such, threatened populations must be considered

carefully when developing management strategies and enhancement programs. However, the RKR Atlantic salmon population has established itself successfully and does not appear to be under any imminent threat of extirpation, despite lower than desired yearly egg counts (Bourgeois 1998).

2.5.4 Conclusions

The establishment of an anadromous Atlantic salmon population in Rocky River, Newfoundland illustrates a successful enhancement strategy implemented in this species. Reciprocal transplants, combined with growth and survival monitoring of pure and hybrid F1 crosses in a common laboratory environment, showed that pure crosses tended to perform slightly better than their hybrid counterparts. It is possible that the hybridization between populations created a low degree of outbreeding depression, suggesting that the introduced RKR fish have started to locally adapt to their new environment in the 5-6 generations post-introduction. This study provides valuable insight into the relative success of enhancement strategies in Atlantic Canada, as well as the ability of Atlantic salmon and other salmonids to adapt to rapidly changing environments, often caused by anthropogenic actions. We acknowledge that it is difficult to conclude that hybridization and potential resulting outbreeding depression is occurring in this population from a single one-generation study, but it coincides with other research in similar species. Future studies should examine the RKR population over a longer period of time with a full parental cross design; assessing over-winter survival and the number of adult returns, in

order to gain a more in-depth understanding of the degree to which rapid evolution has shaped the population.

2.6 Tables

Table 2.1: Monthly summaries of stage (m), flow (m^3s^{-1}) and temperature (°C) in Little Salmonier River and Rocky River from June - September 2014. Data were not available for minimum and maximum stage for July 2014 (accessed from Water Resources Management Division, Dept. of Environment and Conservation, Government of Newfoundland and Labrador).

		Stage			**Flow**			**Temperature**		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
LSR	June	2.293	2.339	2.314	1.628	2.384	1.946	12.693	18.107	15.148
	July	N/A	N/A	2.282	1.173	1.924	1.579	17.197	23.276	19.703
	August	2.315	2.382	2.347	1.87	3.015	2.396	16.839	20.779	18.546
	September	2.218	2.238	2.229	0.833	1.024	0.941	14.08	18.756	16.132
RKR	June	0.699	0.74	0.718	3.755	5.833	4.576	14.713	21.227	17.782
	July	N/A	N/A	0.718	3.685	7.215	5.608	18.145	24.316	20.943
	August	0.819	0.91	0.848	11.484	21.669	14.202	20.829	24.982	22.856
	September	0.663	0.679	0.671	2.677	3.091	2.886	20.74	26.628	23.581

Table 2.2: Characteristics of five Atlantic salmon (*Salmo salar*) microsatellite DNA loci used to determine parentage among recaptured and laboratory-raised offspring. *- denotes tagged primer sequence.

Name	Primer Sequence (5' - 3')	Repeat Motif	Allele Size Range (bp)	Annealling Temperature	Accession Number	Reference
SSa85	*(a)*AGG TGG GTC CTC CAA GCT AC	$(GT)_{14}$	110-138	60°C	U43692	O'Reilly et al. (1996)
	(b) ACC CGC TCC TCA CTT AAT C*					
SSsp2210	*(a)* AAG TAT TCA TGC ACA CAC ATT CAC TGC	$(GTTA)_{11}$	124-164	55°C	AY081808	Paterson et al. (2004)
	(b) CAA GAC CCT TTT TCC AAT GGG ATT C*					
SSsp2213	*(a)* ATG TGG AGG TCA ACT AAC CAG CGT G	$(GTTA)_{22}$	151-191	61°C	AY081809	Paterson et al. (2004)
	(b) CAT CAA TCA CAG AGT GAG GCA CTC G*					
SSsp2215	*(a)* ACT AGC CAG GTG GTC CTG CCG GTC	$(GTTA)_{14}$	110-142	65°C	AY081810	Paterson et al. (2004)
	(b) AGG GTC AGT CAG TCA CAC CAT GCA C*					
SSaD486	*(a)* TCG CTG TGT ATC AGT ATT TTG G	$(TAGA)_{11}$	162-210	53°C	AF525208	King et al. (2005)
	(b) ACT CGG ATA ACA CTC ACA GGT C*					

Table 2.3: Weight (g) and fork length (mm) of juvenile Atlantic salmon (*Salmo salar*) at three stages prior to transplant release: 50% hatch, 50% start feed, and prior to release.

Purebred							
	Weight (g)				Fork Length (mm)		
	n	Range	Mean	S.D.	Range	Mean	S.D.
Hatch	120	0.0738 - 0.1348	0.09412	0.0126	14.28 - 19.66	17.2586	1.2671
Feed	110	0.08 - 0.1602	0.1104	0.0181	25.122 - 30.088	27.0571	1.0596
Release	81	0.0704 - 0.2529	0.1208	0.0412	24.386 - 32.163	27.3723	1.7345
Hybrid							
	Weight (g)				Fork Length (mm)		
	n	Range	Mean	S.D.	Range	Mean	S.D.
Hatch	159	0.0112- 0.1348	0.0995	0.0153	15.895 - 19.088	17.5223	0.7412
Feed	160	0.0755 - 0.1642	0.1166	0.0208	24.212 - 29.712	27.1158	1.0521
Release	105	0.0556 - 0.2812	0.1236	0.0392	23.871 - 32.368	27.4.883	1.4151

2.7 Figures

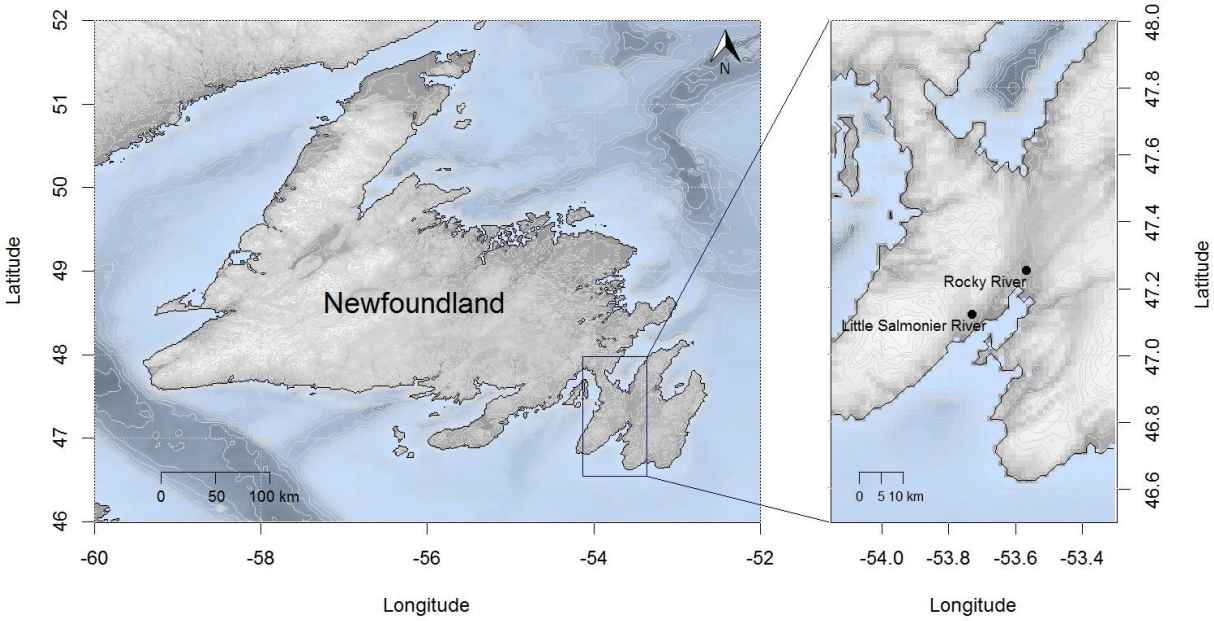

Figure 2.1: Location of the study sites, Little Salmonier River and Rocky River, on the Avalon Peninsula in Newfoundland where reciprocal transplants of juvenile Atlantic salmon (*Salmo salar*) took place in summer 2014.

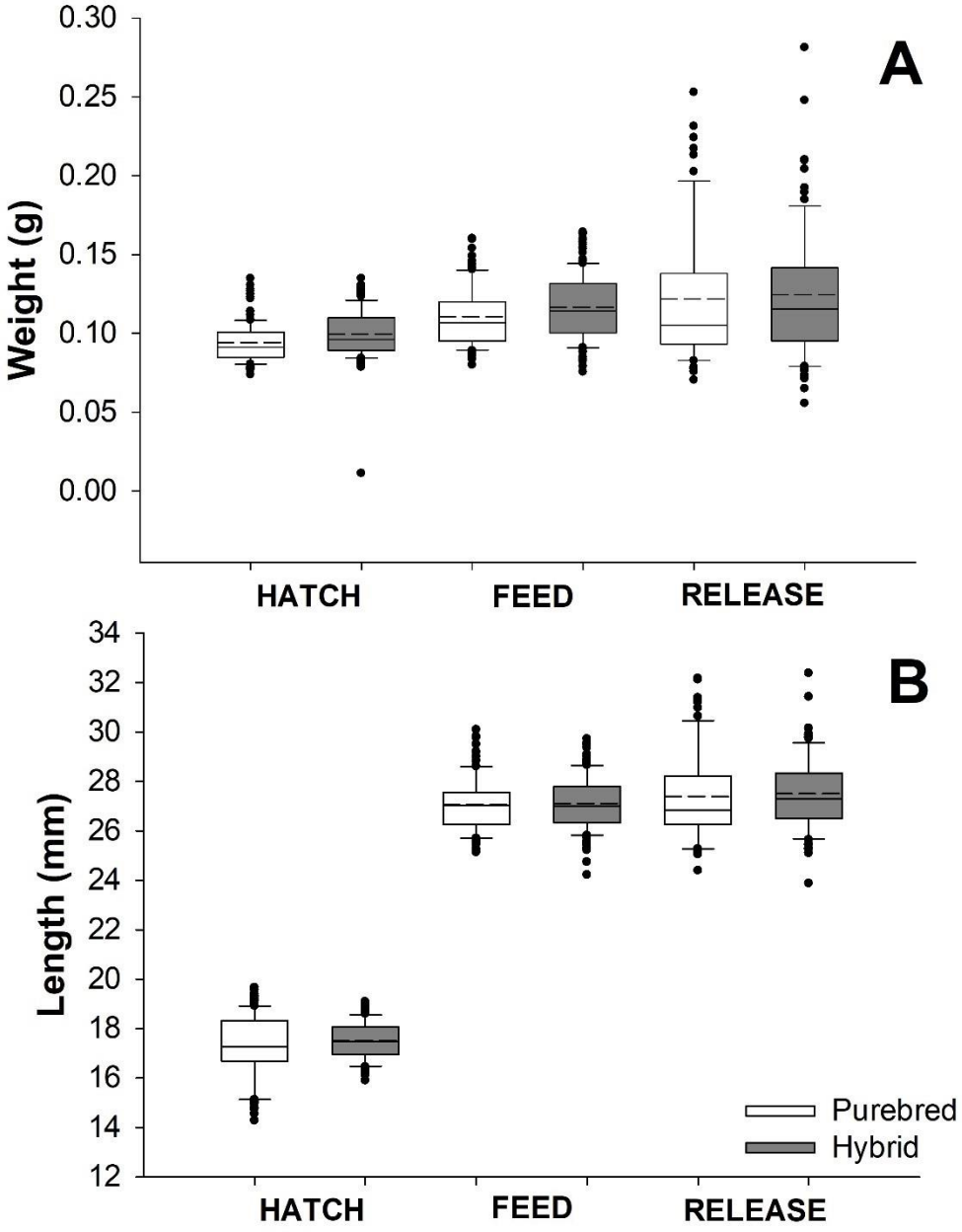

Figure 2.2: Weight (g; panel A) and fork length (mm; panel B) differences between juvenile Atlantic salmon (*Salmo salar*) cross types (purebred: RKR x RKR, hybrid: LSR x RKR) at three measurement periods (50% hatch, start feed, at release) prior to being released reciprocally at Little Salmonier River (LSR) and Rocky River (RKR). Plot indicates median (solid line), mean (dashed line), 1st and 3rd quartiles (top and bottom of box), 5th and 95th percentiles (whiskers), and outliers.

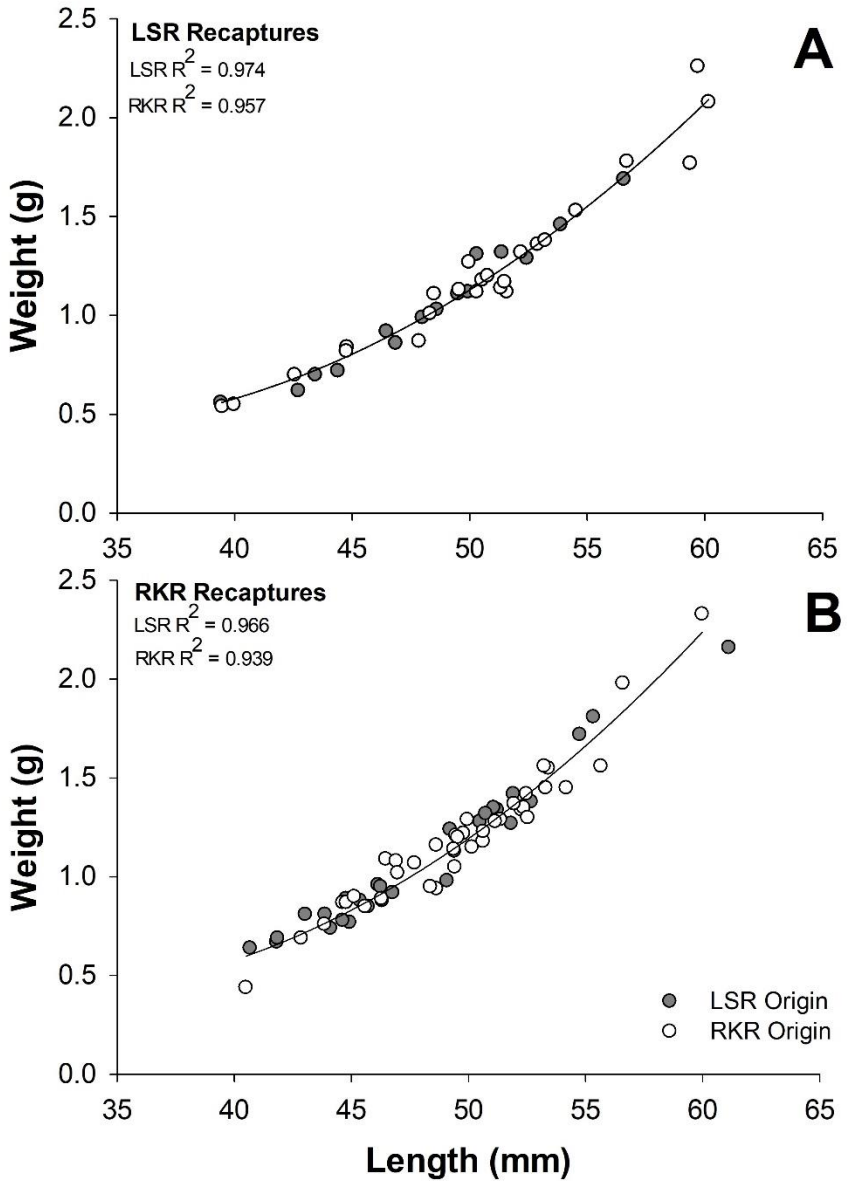

Figure 2.3: Quadratic relationships between length (mm) and weight (g) of recaptured juvenile Atlantic salmon (*Salmo salar*). Top panel (A) indicates fish recaptured at Little Salmonier River (LSR) ($R^2 = 0.965$, $y = 2.327 – 0.123x + 0.002x^2$), bottom panel (B) indicates fish recaptured at Rocky River (RKR) ($R^2 = 0.953$, $y = 2.432 – 0.133x + 0.002x^2$)

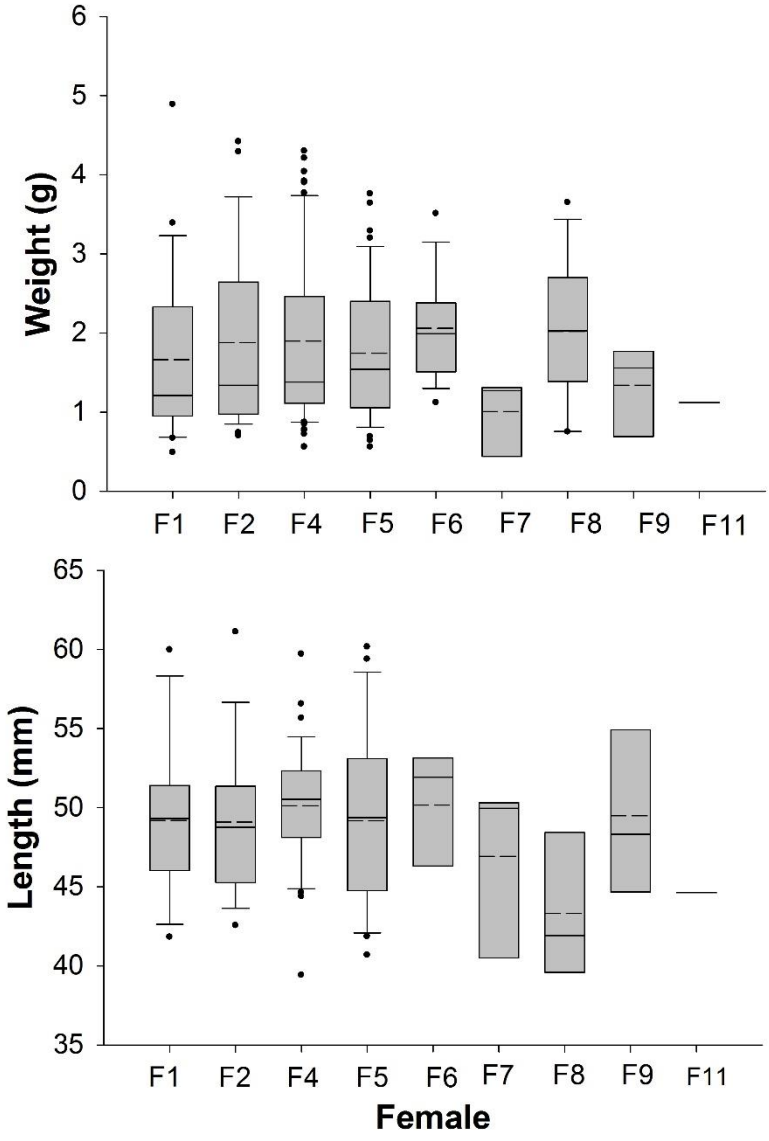

Figure 2.4: Weight (g; panel A) and length (mm; panel B) comparisons of all recaptured juvenile Atlantic salmon (*Salmo salar*) according to female (mother). Recaptures include fish from both Little Salmonier River (LSR) (40 recaptured fish) and Rocky River (RKR) (70 recaptured fish). Plot indicates median (solid line), mean (dashed line), 1st and 3rd quartiles (top and bottom of box), 5th and 95th percentiles (whiskers), and outliers.

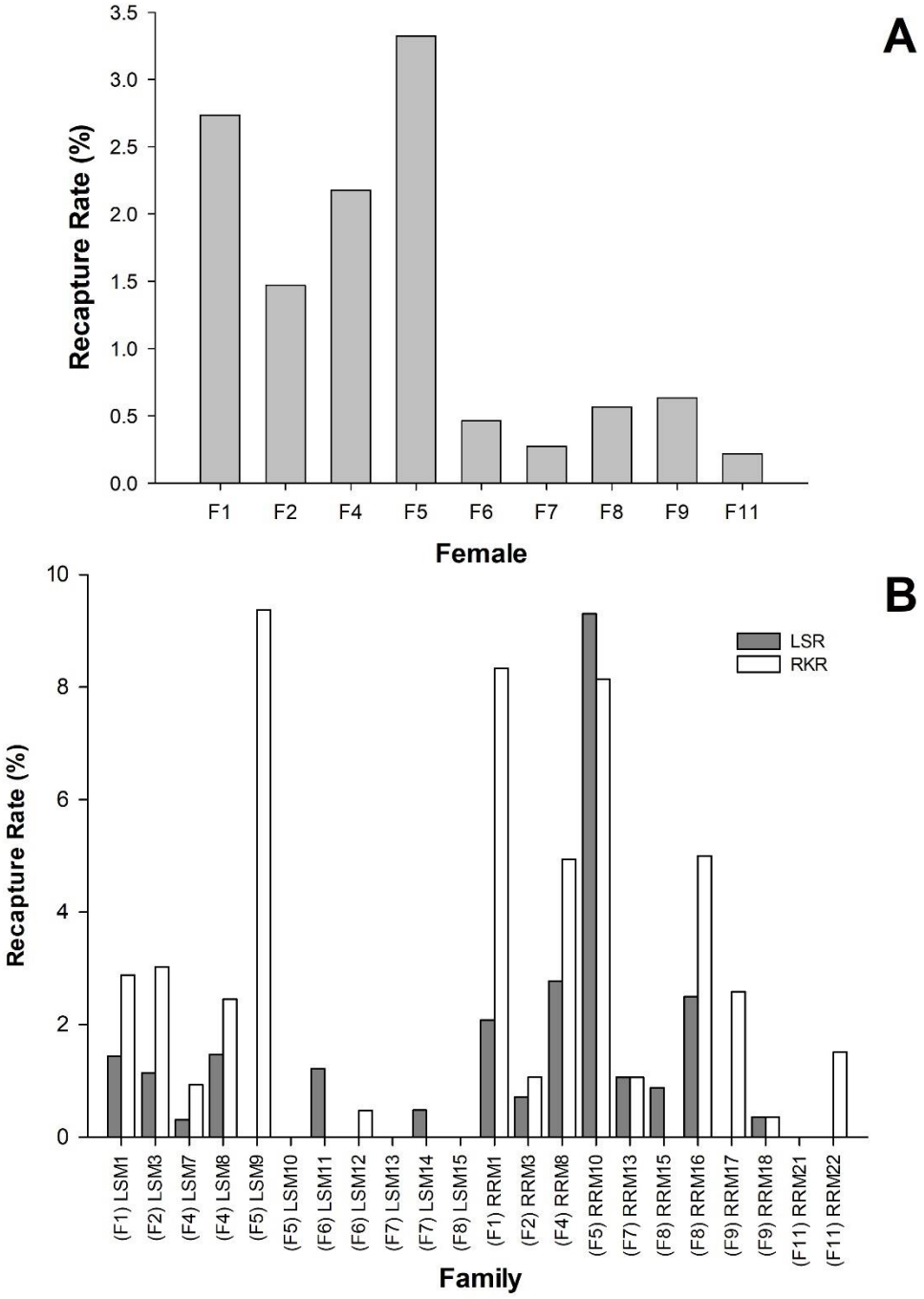

Figure 2.5: Recapture rates (%) by female (mother, panel A) and by individual family (panel B) of juvenile Atlantic salmon (*Salmo salar*) released in Little Salmonier River (LSR; grey) and in Rocky River (RKR; white)

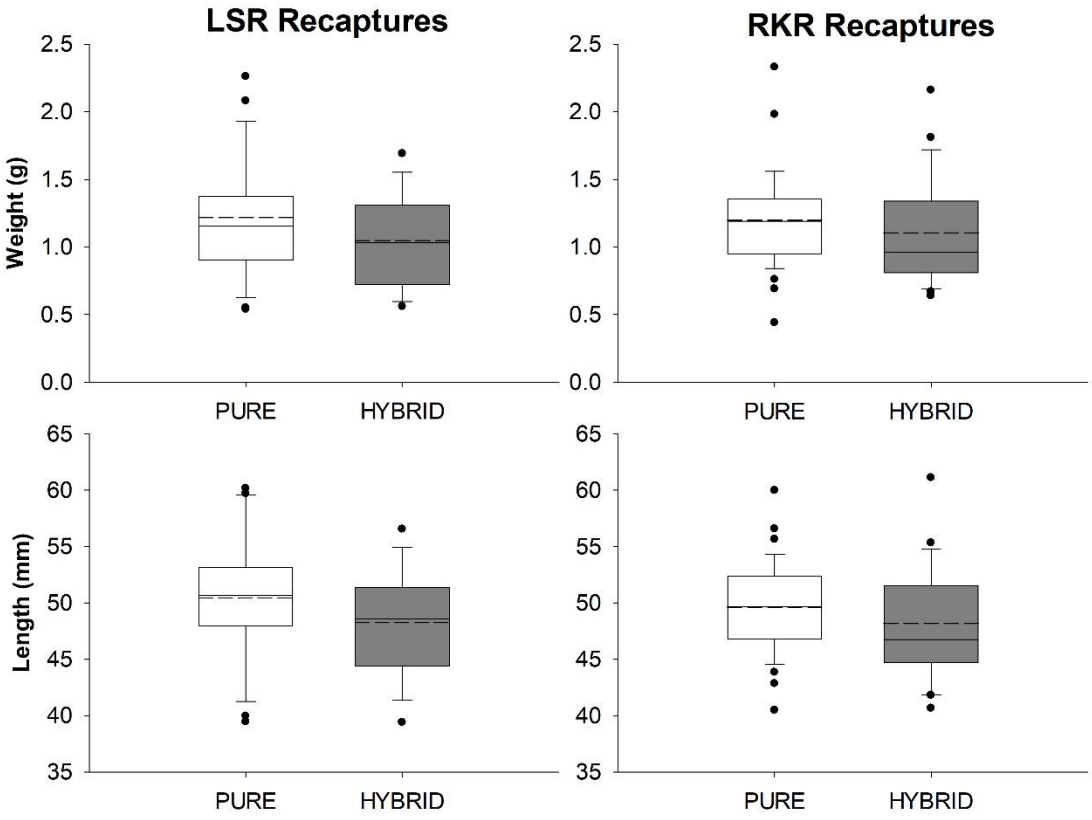

Figure 2.6: Weight (g) and length (mm) comparisons between recaptured juvenile Atlantic salmon (*Salmo salar*) cross types recaptured from Little Salmonier River (LSR, 40 recaptured fish, left) and Rocky River (RKR, 70 recaptured fish, right). Plot indicates median (solid line), mean (dashed line), 1st and 3rd quartiles (top and bottom of box), 5th and 95th percentiles (whiskers), and outliers.

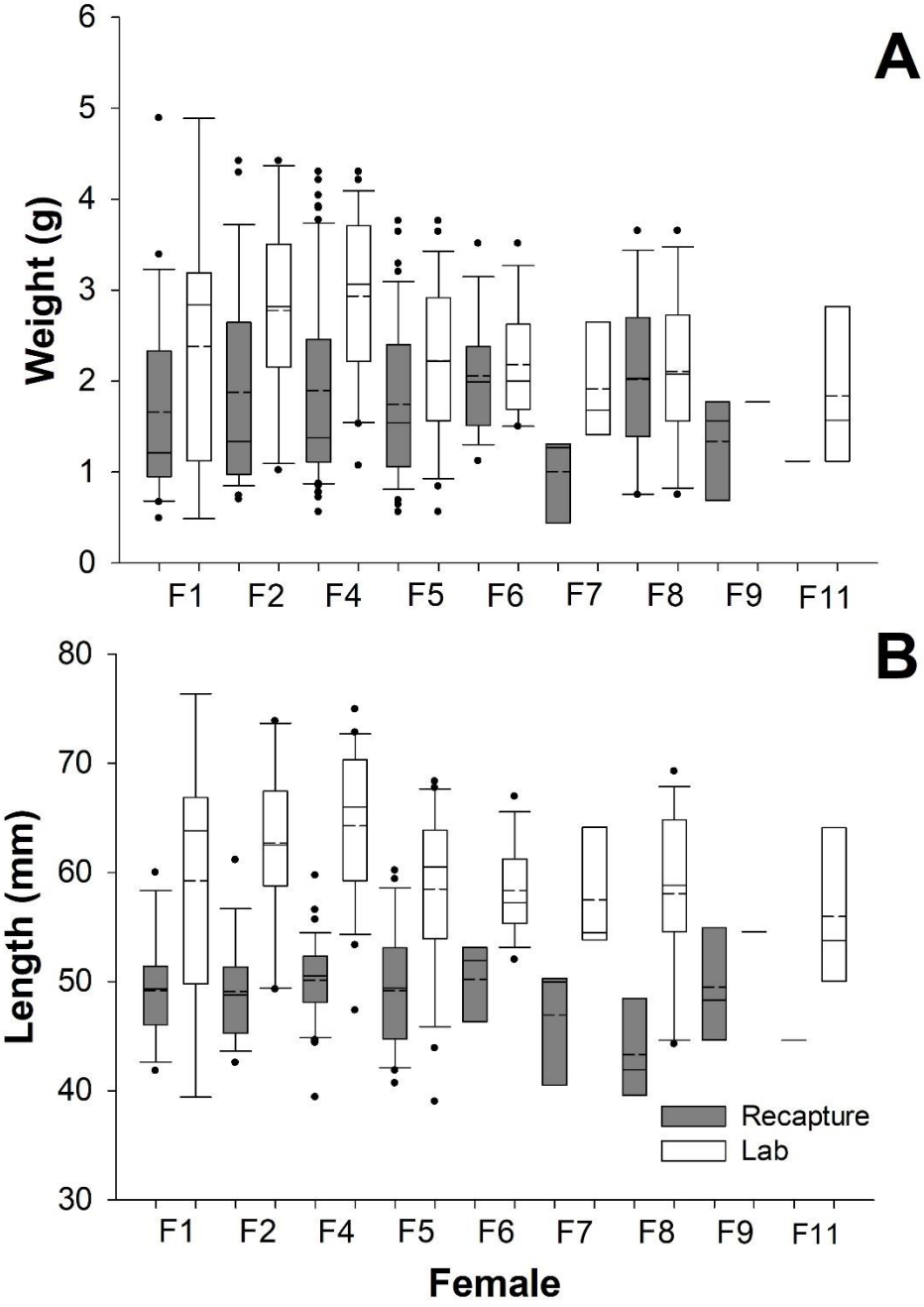

Figure 2.7: Weight (g; panel A) and length (mm; panel B) comparisons of recaptured (grey) and laboratory-raised (red) juvenile Atlantic salmon (*Salmo salar*), separated by female (mother). Recaptures include fish from both Little Salmonier River (LSR) and Rocky River (RKR). Plot indicates median (solid line), mean (dashed line), 1st and 3rd quartiles (top and bottom of box), 5th and 95th percentiles (whiskers), and outliers.

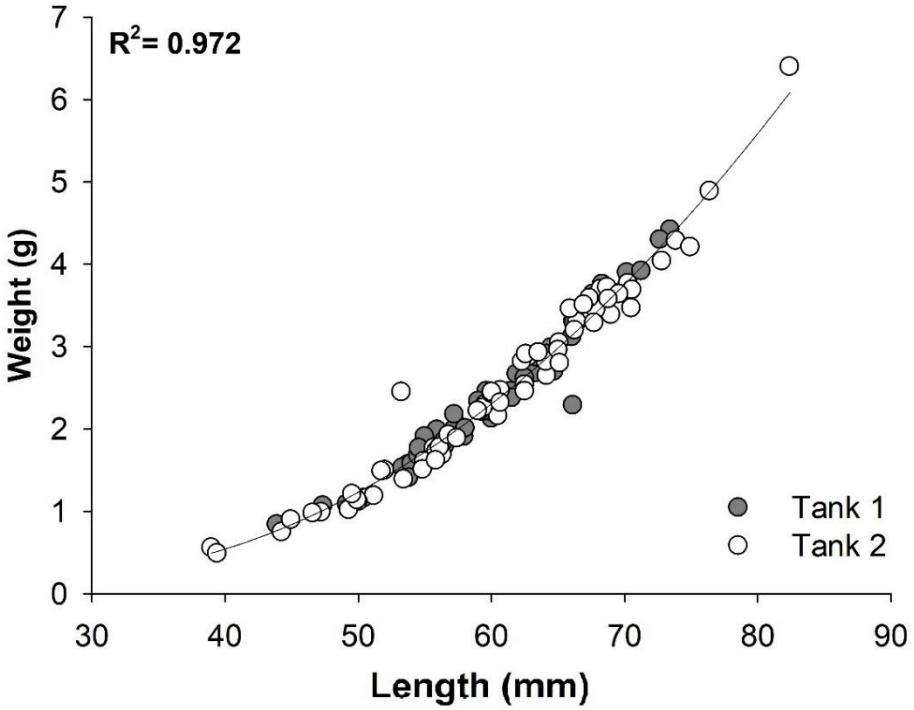

Figure 2.8: Quadratic relationship between length (mm) and weight (g) of juvenile Atlantic salmon (*Salmo salar*) raised under common laboratory conditions at the Ocean Sciences Centre (OSC) in Logy Bay, Newfoundland ($R^2 = 0.972$, $y = 2.785 – 0.144x + 0.002x^2$).

Chapter 3: Genomic evidence of rapid evolution in an introduced Atlantic salmon (*Salmo salar*) population revealed using microsatellites, a single nucleotide polymorphism array and restriction-site associated DNA sequencing

3.1 Abstract

The capacity of populations to evolve quickly is central to population-scale responses to climate change and anthropogenic stress. Here, we characterize the presence of recent genomic divergence in an introduced population of Atlantic salmon (*Salmo salar*) using (1) a microsatellite panel (15 loci), (2) a genome-wide single nucleotide polymorphism (SNP) array (5568 loci), and (3) restriction-site associated DNA (RAD) sequencing derived SNPs (8495 loci). Comparisons of individuals from the source (Little Salmonier River, LSR) and recipient system (Rocky River, RKR) more than 20 years following the introduction revealed F_{ST} values > 0.05 in 15.5% (F_{ST} = 0.017) and 21.2% (F_{ST} = 0.027) of the markers in the SNP-array and RAD-seq SNPs, respectively. Both Bayesian clustering and principal coordinate analyses identified two populations using the SNP array and RAD-seq data, but only one using the microsatellite data. Outlier tests identified ~90 loci putatively under selection despite only 4-5 generations since introduction. The results support a hypothesis of rapid change with two possible non-mutually exclusive alternatives for this rapid change: (1) rapid adaptive evolution, and (2) introgression with non-anadromous RKR salmon. Hybrid analysis revealed that RKR samples were largely backcrosses or F2 hybrids, supporting a role for hybridization and introgression in the observed rapid divergence. Ultimately, the genomic results support hypotheses of rapid evolution of salmon in Rocky River, possibly through adaptive

evolution and/or recent introgression with resident individuals. This work highlights the importance of several genomic approaches in analyzing and understanding rapid evolution in Newfoundland salmon populations, and the increased resolution in identifying population differences with RAD-seq techniques.

3.2 Introduction

Adaptive evolution, until recently, was thought to take place over long periods of time (Carroll *et al.* 2007; Darwin 1859; Franks & Munshi-South 2014; Hendry & Kinnison 1999; Thompson 1998). However, recent evidence supports the possibility of evolutionary and adaptive changes over much shorter time periods, often occurring in only a few generations (Thompson 1998). Rapid adaptive evolution, adaptive change within a species over a short period of time, has historically been assessed by measuring changes in phenotypic or morphological traits (Hendry & Kinnison 1999). However, with this approach, it can be difficult to distinguish adaptive evolution from phenotypic plasticity (Hendry & Kinnison 1999). Common garden and/or reciprocal transplant experiments largely mitigate this uncertainty, but these methods cannot distinguish what genes might be experiencing selective forces and what their function might be (Hendry & Kinnison 1999). Genomic analyses can allow further insight into whether a population is experiencing adaptive change due to rapid evolution, and possibly, the biological functions under selection. Furthermore, examining the genetic and genomic components of rapid evolution is of critical in assessing whether a species can adapt to changing environmental conditions.

While historically many studies assessing rapid evolution have focused on differences in phenotypic traits, determining whether observed differences reflect adaptive evolution or phenotypic plasticity can be difficult (Freeland *et al.* 2011; Hendry & Kinnison 1999; Merilä & Hendry 2014). Genetic and genomic examinations of adaptation have proven powerful in quantifying rates of evolutionary change and linking them to changes in phenotype (Franks & Munshi-South 2014; Kovach *et al.* 2013; Martinez *et al.* 2001). The recent advent of genomic molecular technology has allowed studies using higher genomic resolution of population structuring and adaptation not previously possible (Bourret *et al.* 2013). For example, Kovach *et al.* (2013) found a change in migration timing due to an increase in stream temperature in a population of pink salmon (*Oncorhynchus gorbuscha*) in 16 years, by using 23 microsatellite markers to assess patterns of genetic divergence and allele frequency temporal autocorrelation. Besnier *et al.* (2014) found increased pesticide resistance in salmon lice (*Lepeophtheirus salmonis*) in North American aquaculture farms after only 10 years by developing a ~6000 marker SNP array that revealed strong population-wide selective sweeps towards increased resistance. Furthermore, introgression, the transfer of genetic information between populations that occurs from hybridization and consequent backcrossing between an introduced and a local population, can also accelerate local adaptation caused by rapid evolution (Taylor 1991). Many studies have also shown significant rapid evolution in Atlantic salmon (*Salmo salar*), including a loss of local adaption in wild populations as a result of introgression by escaped farmed salmon (Bourret *et al* 2011; Glover *et al* 2013). Genetic changes in an introduced population from their source

population have also been documented 30 years after their introduction (Martinez *et al.* 2001).

Atlantic salmon (*Salmo salar*) is an anadromous fish that spans the northeast and northwest coasts of the North Atlantic Ocean. The species is known for its complicated life cycles, natal homing abilities, and large-scale ocean migrations (COSEWIC 2010; Keefer & Caudill 2014; Reddin 1988; Thorstad *et al.* 2010). Newfoundland has many landlocked, or resident Atlantic salmon populations (also called ouananiche), which is rare in other areas of the species range (Adams *et al.* 2014; Burton & Idler 1984; Klemetsen *et al.* 2003b; Verspoor & Cole 1989). Salmon populations, particularly in the southern portion of their range, are at greater risk for extinction than their northern counterparts and are presently experiencing population declines (COSEWIC 2010; WWF 2001). As such, determining the ability of Atlantic salmon to adapt to changing environmental conditions is required to properly manage their populations in the future. Atlantic salmon abundance in southern Newfoundland has declined over the past several decades, which has resulted in its listing as 'threatened' (COSEWIC 2010). Management and enhancement activities conducted in the area over the past several decades now provide a unique opportunity to estimate the degree of adaptation at fine-scale distances (< 50 km). Beginning in 1984, stocking occurred for four consecutive years in the Rocky River, located on the Avalon Peninsula, with fry from the nearby Little Salmonier River (Greene 1986). Rocky River, the largest watershed on the peninsula, with an area of nearly 300^2 km, lacked an anadromous salmon population due to an unpassable waterfall at the mouth of the river (Greene 1986). In 1986, a fish ladder was constructed to bypass

the falls so that returning adults could travel upstream to spawn (Greene 1986). Since that time, an anadromous population has successfully become established, with annual counts averaging 350-400 returning adults, though the range of annual counts could feasibly be greater (DFO 2015). As such, the Rocky River system represents an ideal case study to examine the presence of adaptation from rapid evolution in Atlantic salmon in the wild.

Here, we used genetic and genomic analyses to assess the potential for rapid evolution between source (Little Salmonier River, LSR) and introduced (Rocky River, RKR) Atlantic salmon populations. We compare contemporary samples from the two rivers using a microsatellite panel, a genome-wide single nucleotide polymorphism (SNP) array, and restriction-site associated DNA (RAD) sequencing derived SNPs to quantify divergence between the populations. Specifically, we aimed to (i) determine whether the Rocky River population has diverged from the Little Salmonier population by comparing different genetic and genomic approaches, (ii) identify and annotate potential loci under selection using the SNP array, and (iii) evaluate the role selection has played in population divergence from the source population, versus introgression with resident RKR salmon. We build directly on a previous study documenting transatlantic secondary contact in Atlantic salmon on the southern coast of Newfoundland, which also used microsatellite panel, SNP array, and RAD-seq SNPs, to determine differences in resolution of complex spatial structuring (Bradbury *et al.* 2015). Adding to that research, our current study highlights the usefulness of multiple genetic and genomic approaches, in particular RAD-seq based approaches, in assessing rapid evolution between recently separated populations. Lastly, it also provides insight into how quickly a population can

evolve and potentially adapt locally within a limited geographical range over similar environments.

3.3 Methods

3.3.1 Sampling

Samples were collected between July – September 2008-2010 by electrofishing at two locations per river (Figure 3.1; see Bradbury *et al.* [2015] for further details). Tissue samples were collected from parr of various ages (between age 0-3) in tributaries of LSR and RKR, generally < 5 m wide and 2 m deep. Fin clips were taken from each captured individual, and stored in 95% ethanol. Further angling was conducted to collect an additional 80 mature individuals from RKR resident salmon populations, located above obstructions 18.6 km into the watershed, in July 2015.

3.3.2 Microsatellite genotyping

A total of 175 individuals were sampled and genotyped for 15 microsatellite loci. Microsatellite polymorphisms were quantified for 15 loci as follows: SSa85, SSa202, SSa197 (O'Reilly *et al.* 1996), SSOSL417 (Slettan *et al.* 1995), SSaD85 (T. King, unpublished data), SSaD58, SSaD71, SSaD144, SSaD486 (King *et al.* 2005), MST-3 (henceforth referred to as U3) (Presa & Guyomard 1996), SSsp201, SSsp2210, SSsp2215, SSsp2216 and SSspG7 (Paterson *et al.* 2004). Details describing the microsatellite genotyping protocol have been described elsewhere (Bradbury *et al.* 2015; Bradbury *et al.* 2013). To summarize, DNA extraction was completed using a Qiagen DNeasy 96 Blood and Tissue extraction kit (Qiagen) following manufacturers guidelines.

DNA quantification used QuantIT PicoGreen (Life Technologies) with a final concentration of 10 ng/µL after dilution in 10 mM Tris (Buffer EB, Qiagen). All loci were multiplexed (Bradbury *et al.* 2013), and PCR was performed (10 µL total volume) composed of 10 ng DNA, 1x Type-it Microsatellite PCR master mix (Qiagen) and the corresponding primer mix for each panel. PCR products were then size separated on an ABI 3130xl (Life Technologies) capillary electrophoresis system with Gene Scan 500 as the internal size standard (labelled in LIZ; Life Technologies). Gene Mapper 4.0 (Life Technologies) was used to analyze the resulting electropherograms. Two types of control samples were included on each extraction plate, redundants and cross-plate controls. Any individual that did not amplify for more than two loci was removed from subsequent analysis.

3.3.3 SNP array genotyping

A subset of individuals that were microsatellite genotyped were also analyzed with a 5568 loci SNP array developed by the Centre for Integrative Genetics (CIGENE, Norway) for which many of the loci are annotated and mapped. The subset was analyzed using the Illumina infinium assay (Illumina, San Diego, CA, USA) following manufacturer's instructions (see Bourret *et al.* 2013a,b for details). A total of 59 individuals were sampled and genotyped (40 from RKR, 19 from LSR). The array was primarily composed of nuclear loci, but included eight mtDNA loci as well. Loci were classified visually into one of several categories: single locus SNPs, paralogous sequence variants (PSVs), and multisite variants (MSVs) that arise from genome duplication (for more information, see Lien *et al.* 2011; Bourret *et al.* 2013b). SNPs were filtered for a >

95% call rate (the proportion of genotyped SNPs) and a minor allele frequency (MAF) < 5%.

3.3.4 RAD-seq analysis

Again, a subset of individuals used for the microsatellite genotyping was analyzed for RAD sequencing. A total of 40 individuals were sampled and genotyped (20 from LSR, 20 from RKR), 2.5µL of spectrophotometrically quantified DNA was submitted to FLORAGENEX, Oregon, where RAD tags were generated and sequenced following methods outlined by Baird *et al.* (2008), Hohenlohe *et al.* (2010) and Emerson *et al.* (2010). To summarize, individual barcodes and sequencing adaptors were ligated to *Sbf* I-digested genomic DNA, and the resulting fragments were then sequenced from the restriction sites. RAD samples that were barcoded individually were sequenced on the Illumina GAIIx platform with single-end 1 x 100-bp chemistry. RAD tags of approximately 90-100 bp in length were created after reads were separated by individual, and sequencing barcodes were removed after the run. Identification of SNP candidates and RAD reference mapping was completed using the available *S. salar* genome (http://genomicasalmones.dim.uchile.cl). Both BOWTIE (version 0.11.3; Langmead *et al.* 2009) and SAMTOOLS (version 0.1.12a; Li *et al.* 2009) algorithms, as well as custom scripts, were used to call candidate SNPs (for more information regarding the SNP calling protocol, see Bradbury *et al.* 2015). Resulting SNP candidates were filtered for PSVs, MSVs and SNPs with three or more alleles. Only SNPs that were unambiguously mapped to the *S. salar* reference genome were used in the analysis.

3.3.5 Data analysis

The microsatellite data were checked and filtered for any scoring errors and null alleles using MICROCHECKER (Van Oosterhout *et al.* 2004). Both the SNP array and RAD-seq SNPs were filtered for relatedness using COLONY v2.0.5.9 (Jones & Wang 2010), and all half-siblings were removed from further analysis (no other relationship was present in the data). Data were then filtered at a maximum marker missingness rate of < 0.05, a minor allele frequency of < 0.05, a maximum individual missingness rate of < 0.25 and at a Hardy-Weinberg disequilibrium p value of < 0.001 using PLINK v1.07 (Purcell *et al.* 2007). Observed heterozygosity, and individual and pairwise F_{ST} values were calculated in ARLEQUIN v3.5.2.2 (Excoffier *et al.* 2005). An F_{ST} frequency distribution of the locus-specific F_{ST} values was created for both the SNP array and RAD-seq data sets to visualize the degree of genetic differentiation between the two populations using GENALEX (Peakall & Smouse 2006). Principal coordinate analyses (PCoA) were also conducted for these two data sets using GENALEX and the locus-specific F_{ST} values.

Population clustering was then determined for each data set using STRUCTURE v2.3.4 (Pritchard *et al.* 2000). This analysis uses a Bayesian clustering method to approximate the number of distinct groups present in the data. STRUCTURE assumes Hardy-Weinberg and linkage equilibrium amongst all loci, and uses a MCMC (Markov chain Monte Carlo) algorithm to assign individuals that do not meet the equilibrium requirements, and estimate the number of populations within the data (denoted by the value *k*). The algorithm was run three times for each value of *k* (1-5) with a burn-in of

100 000 repetitions, and 300 000 repetitions after burn-in. All result replicates were consolidated using CLUMPAK (Kopelman *et al.* 2015).

The genomic distribution of differentiation between populations was mapped using the linkage map for North American Atlantic salmon published by Brenna-Hansen *et al.* (2012), and a LOWESS second-order filter. Outliers were identified for both the SNP array and RAD-seq data using a non-hierarchical island model at both the 95% and 99% confidence intervals in ARLEQUIN. The outliers were annotated using BLAST2GO (Conesa *et al.* 2005) by Bourret *et al.* (2013).

To explore any possible influence of introgression between the RKR population and resident fish, both the SNP array and RAD-seq data sets were analyzed using NEWHYBRIDS software (Anderson & Thompson 2002), which assigns genetically sampled individuals into one of several hybrid categories (pure, F1 hybrid, F2 hybrid, F1 backcross) based on posterior probability using a Gibbs sampler method. To further explore the relationship between anadromous and resident salmon, an additional PCoA was conducted with the microsatellite data, incorporating data from resident salmon in RKR (n = 80, collected above obstructions, 18.6 km inland from the main watershed).

3.4 Results

3.4.1 Evidence for rapid evolution

After quality control filtering, the microsatellite data set consisted of 14 loci for 175 individuals, the SNP array contained 2574 loci for 52 individuals and the RAD-seq data consisted of 8495 loci for 33 individuals. Average observed heterozygosity for the

microsatellite data was 0.841 (± 0.121, SD), 0.273 (± 0.162, SD) in the SNP array, and 0.310 (± 0.175, SD) in the RAD-seq data (Figure 3.2A). The F_{ST} frequency distribution (Figure 3.2B) was similar for the SNP array and RAD-seq data sets with global F_{ST} values of 0.0172 (15.5% > 0.05) and 0.0265 (21.2% > 0.05), respectively. In contrast, the global F_{ST} value for the microsatellites was 0.009, much lower than the data sets composed of SNPs.

The pairwise microsatellite F_{ST} value was 0.008. The PCoA of the microsatellite data revealed little to no spatial clustering (Figure 3.3A). LSR and RKR overlapped across much of the plot, with PC1 and PC2 axes explaining 5.0% and 3.9% of the variation, respectively. In contrast, both SNP datasets displayed some separation of the populations. The SNP array PCoA showed more defined differentiation between the two populations (Figure 3.3B). The pairwise F_{ST} value was double that of the microsatellite at 0.016, with the first two principal coordinates explaining 4.1% and 3.0%. Although RKR was still wide-spread across the coordinates and overlapped with LSR, LSR itself appeared to be more tightly clustered. Lastly, the RAD-seq PCoA differentiated the populations most, with a pairwise F_{ST} value of 0.025, the highest amongst the three types of data (Figure 3.3C). The principal coordinates explained 5.3% and 4.3% of variation, also the highest of the three datasets. There was little overlap between the two populations, with LSR and RKR individuals clearly distinguished from one another.

3.4.2 Outlier analysis and annotation

Outlier tests using both the SNP array and RAD-seq SNPs identified multiple outliers at both the 95 and 99% confidence intervals (CI; Figure 3.4). Using the SNP array, there were 90 markers above the 95% CI, and 83 from the RAD-seq SNPs. The genomic distribution of differentiation among the two populations was examined using the SNP array and a published linkage map (Brenna-Hansen *et al*. 2012). Of the 2456 loci used in the SNP array, 2193 (89.2%) could be placed on the linkage map, and were distributed across all 27 linkage groups (Figure 3.5). However, there were slight peaks at six of the linkage groups: ssa01q-fission, ssa07, ssa08/29, ssa11, ssa16 and ssa20. The 90 outliers identified in the SNP array were examined for published annotations. Of these 90, there were 32 hits from a previous BLAST2GO analysis (Bourret *et al*. 2013). Although ontologies varied, some patterns were apparent (Table 3.1). The highest number of hits was with reproduction and embryonic development (21), however identified several loci that are involved with cellular transport and signaling (14), immune responses (11), protein synthesis and associated processes (17), metabolic processes (16) and programmed cell death (4). Linkage group ssa10 had the most associated biological processes, mainly embryonic development and growth or metabolic processes. Linkage group ssa08/29 also produced numerous hits, again mostly with embryonic development, as well as apoptosis. Linkage groups in higher map positions were generally associated with cellular signaling and protein processes, while reproductive and embryonic development were mainly associated with lower map positions. Metabolic processes appeared widespread throughout linkage groups.

3.4.3 Influence of hybridization with resident salmon

Bayesian clustering with STRUCTURE using the microsatellite data revealed no population differentiation (Figure 3.6A). However, SNP array and RAD-seq SNPs both offered evidence of structuring (Figure 3.6B, C) with K = 2 supported in both instances. LSR individuals belonged to one homogenous group, while the RKR individuals were a mixture of two groups, with some intermediate admixture coefficients (q-values) present. The nature of these admixed individuals was explored further using NEWHYBRIDS (Anderson & Thompson 2002) which assigned individuals to various hybrid classes (Figure 3.7). For both data sets, again LSR was entirely composed of one pure group and RKR consistently showed evidence of hybridization among LSR and a second group, mostly F1 backcrosses and some F2 hybrids.

To further test the hypothesis that the observed hybridization was the result of interbreeding between recently introduced individuals and known resident Atlantic salmon inhabiting the RKR watershed, additional samples taken from resident populations higher up the watershed were analyzed using the microsatellite panel (see above). These individuals were collected above barriers that prevented upstream migration of anadromous individuals, ensuring that they were resident individuals. PCoA comparing the resident population (RKRO) with both anadromous runs (Figure 3.8) indicated little evidence of structuring between RKR and LSR anadromous populations. However, the resident population (RKRO) apparently overlapped more closely with the RKR anadromous samples, which were more widely spread across the PC1 axis than LSR fish. The frequency of PC1 values for RKR and RKRO samples, again, seemed

more similar, compared to that of LSR (Figure 3.8B). Similarly, pairwise F_{ST} values indicated less divergence among RKR and RKRO individuals (0.007), than among LSR and RKRO (0.014) (Figure 3.8A). This analysis supports the possibility that hybridization between the RKR anadromous fish and the resident population may be contributing to divergence of the recently introduced anadromous individuals in Rocky River.

3.5 Discussion

The capacity of populations to evolve quickly is central to population-scale responses to change, long-term stability, and persistence. In this study, we used several genetic and genomic approaches to characterize the presence of recent potentially adaptive divergence in the introduced population of Atlantic salmon in Rocky River, from the source population in Little Salmonier River. Our observations of clear differentiation using two SNP datasets, and the detection of loci potentially under selection, support hypotheses of rapid evolution in the introduced Rocky River population. Interestingly, additional comparisons with resident individuals within Rocky River also indicate a possible role for introgression in driving this rapid change. This work highlights the importance of several genomic approaches in analyzing and understanding rapid evolution, and increased resolution in identifying population differences with RAD-sequencing techniques. The intriguing aspect of this enhancement project was that it was successful (Bourgeois 1998; Greene 1986), and an anadromous population of Atlantic salmon at RKR now exists, with yearly adult returns averaging approximately 350-400 individuals (DFO 2015). This work highlights the processes

involved in a successful introduction and establishment of an anadromous Atlantic salmon run.

3.5.1 Evidence for rapid evolution

Genetic and genomic studies of rapid evolution in the wild allow the determination of adaptive capacity to changing environments, and can detect selection that phenotypic studies cannot. The presence of one, pure group at LSR, and two groups with a degree of admixture at RKR suggests that the introduced population at RKR evolved from the source population. A study conducted by Martinez *et al.* (2001) with six microsatellites yielded similar results in an introduced population of Atlantic salmon, with significant genetic difference evident ~30 years (5-6 generations) after the introduction event. Hendry *et al.* (2000) also showed reproductive isolation in two populations of sockeye salmon (*Onchorhynchus nerka*) with common ancestry in approximately 56 years (13 generations), which may explain the selective forces potentially acting on genes involved in reproduction and embryonic development observed in the present study. Another study conducted on Atlantic salmon also documented significant genetic changes when fish were introduced continually into a system (Perrier *et al.* 2013). The differentiation between LSR and RKR populations using the SNP-array enabled detailed analysis of which markers were experiencing selection, and what biological processes they are potentially involved in.

3.5.2 Outlier analysis and annotation

The outlier tests using the SNP array yielded approximately 90 SNP loci potentially influenced by selection. Of these 90 loci, 32 have known gene ontologies. Although several of the noted biological processes are typical of rapidly evolving populations (i.e., protein synthesis and related processes, growth and metabolic differences, etc.) (Aykanat *et al.* 2015; Garcia de Leaniz *et al.* 2007; Morinville & Rasmussen 2003), of particular interest was the finding that the most prominent biological processes selected for related to reproduction/embryonic development (associated outliers found in six linkage groups). Genes responsible for the development of the heart, cell differentiation, and brain and neural crest/tube formation were most common among the biological processes, all associated with embryonic development. A similar scenario was observed in a comparison between anadromous and resident forms of brook trout (*Salvelinus fontinalis*), where significant signs of directional selection were detected in early development, including directional selection for size traits in embryonic development (Perry *et al.* 2005) Another study comparing anadromous and resident brook trout showed a higher metabolic rate in the anadromous form prior to migration, indicating lower growth efficiency in these individuals (Morinville & Rasmussen 2003). The differences between the parental population at LSR, and the resident RKR population that hybridized with the introduced anadromous RKR population, in linkage groups associated with maturation, reproductive timing, and embryonic development likely reflected life-history differences.

3.5.3 Influence of hybridization with resident salmon

This work supports a hypothesis of recent evolution in the RKR anadromous population which is rapidly adapting to its introduced environment, in part by hybridizing with the resident non-anadromous salmon. A previous microsatellite study (8 loci) conducted on these sympatric anadromous and resident populations concluded that, due to long-term segregation between the two populations, they had maintained their differentiated genotypes (Adams *et al.* 2014). Our study used several more microsatellite loci (n=14) than this previous study, and only four of these loci were common between the two studies. It is also possible that the resident and anadromous populations interbreed more than previously thought, as was seen in a comparison between resident and anadromous brown trout (*Salmo trutta*), where there was no significant genetic difference between the two life-history types (Hindar *et al.* 1991). Also, Adams *et al.* (2014) collected their samples approximately 12 years prior to the samples collected for this study, which may have allowed two additional generations of hybridization to occur between the anadromous population at RKR and their resident counterparts.

The SNP introgression analysis, which determined the degree of hybridization between the LSR and RKR populations, indicated interbreeding and introgression between the anadromous and resident RKR populations. Although not considered the typical form of rapid evolution, introgression with locally adapted populations is actually quite common. Seehausen (2013) discusses several situations when hybridization may facilitate adaptation or speciation, including when constraints that previously prevented

populations from hybridizing are no longer present, which was seen in our study. Similarly, there has been a well-known, widespread hybridization between native Atlantic salmon and introduced brown trout (*Salmo trutta*) in eastern Newfoundland, an area where trout were not previously found until the late 1800s (Verspoor 1988). Another study documented hybridization between native westslope cutthroat trout (*Oncoryhnchus clarki lewisi*) and introduced rainbow trout (*Oncorhynchus mykiss*), where many of the hybrid individuals were classified as backcrosses (Rubidge & Taylor 2004), similar to what we observed. However, these hybrids tended to not be viable, unlike in our study. While these examples may be more closely related to invasive species studies, they demonstrate that hybridization between conspecifics or closely related species can potentially occur within a few generations. Abbott *et al.* (2013) likens the adaptive potential of hybridization between populations to that of the sexual recombination of alleles within populations that allows for local adaptation. They, along with several other publications, also suggest that hybridization may contribute to adaptive variation more frequently than mutation (Arnold & Martin 2009; Grant & Grant 1994; Kim & Rieseberg 1999; Kunte *et al.* 2011; Whitney *et al.* 2010). However, it is also possible that hybridization between two previously allopatric populations may not be beneficial in the long-term, as was reported by Seehausen (2013) and by Bourret *et al.* (2011), who assessed the genetic integrity in a population of Atlantic salmon after it began hybridizing with escaped farm fish. There were significant decreases in genetic differentiation using both SNP and microsatellite markers of the anadromous population after it had introgressed with escaped farmed fish in the area. While unlikely, it is

possible that the anadromous population at RKR, while able to maintain the population in the river, may be losing differentiation that it had in the original population, LSR, prior to the introduction event. It is more probable that after the introduction, the anadromous fish at RKR began hybridizing with resident salmon that migrated into the main watershed, creating a locally adapted, introgressed anadromous population.

3.5.4 Comparison of marker types

This study demonstrated the advantages of using multiple genomic approaches to assess rapid evolution in a natural setting. By assessing the RKR introduced population using a combination of approaches, we were able to determine that the anadromous population appears to be rapidly evolving, gained insight into the underlying mechanisms of that evolution, and what traits are possibly being acted upon by selection. The microsatellite analysis, while initially showing no differentiation between the LSR and RKR populations, did support the observation that the RKR population is likely interbreeding with the resident individuals, evident by the slight differentiation observed in the principle coordinates analysis. The SNP analyses revealed the presence of a separate group at RKR, determined the degree of introgression within the population, and allowed us to pinpoint outliers, their position on the genome, and determine which traits are putatively being selected for.

Microsatellites, while easily developed and increasingly cost-effective, are limited by inconsistencies in allele size calling and tendency for high error rates (Fernández *et al.* 2013; Freeland *et al.* 2011; Morin *et al.* 2004). While initially, Adams *et al.* (2014) found

significant differences between anadromous and resident populations of Atlantic salmon at RKR using microsatellites, our study did not find such differentiation despite a larger panel of microsatellites. Furthermore, there was no difference between the founder and introduced populations, suggesting that, in this case, microsatellites were insufficient to determine the presence of rapid evolution. As part of a larger study across the island of Newfoundland, Bradbury *et al.* (2015) reported an eastward loss in diversity between populations, due to increasing levels of genetic drift between small, eastern populations (Bradbury *et al.* 2013), which could contribute to the lack of differentiation seen here with the same panel.

The high prevalence and presence of SNPs in both coding and non-coding regions, allow for genome-wide scans of both neutral and adaptive variation (Freeland *et al.* 2011). Our study used the SNP data, particularly the SNP array, was used to map outlier loci on the existing genome, which allowed a fuller understanding of which loci are potentially being acted upon by selection. Both the SNP array and RAD-seq SNPs used in this study showed increased diversity levels and heterozygosity between populations in the southeast portion of Newfoundland in a larger-scale study conducted by Bradbury *et al.* (2015). They also noted that both SNP data sets were able to detect introgression and hybridization, unlike the microsatellites, which is also true for our current study. Ultimately, our study shows the importance of using multiple marker types to assess the presence and degree of rapid evolution occurring between populations.

3.5.5 Limitations and future work

While our study provided evidence of genomic rapid evolution of the RKR anadromous Atlantic salmon population, we acknowledge limitations in our findings. It was not possible to genotype the RKR resident salmon samples with either the SNP array or RAD-seq SNPs. Originally, we did not expect that the RKR anadromous population would be rapidly evolving by hybridizing with the resident population, as previous studies had suggested that these two populations were significantly different genetically (Adams *et al.* 2014). However, when preliminary analyses revealed intermediate admixture coefficients in the RKR samples, rather than be completely separate or uniform with LSR, it warranted further investigation. Due to time constraints when sampling occurred, we could only genotype resident samples using the microsatellite panel. In future studies, these samples should be genotyped using the SNP array and RAD-seq SNPs to further investigate the hybridization between them and the anadromous population at RKR. Of particular interest, is further research into how the resident population may be influencing genes associated with embryonic development that are being acted upon by selection in the introduced RKR population.

3.5.6 Conclusions

This study provided insight into rapid evolution occurring in Rocky River by analyzing and comparing three marker types. Principal coordinates analyses revealed that RAD-seq SNPs detected the highest level of differentiation between LSR and RKR. Approximately 90 outliers were detected with the SNP array, which were generally

widespread throughout the genome, and with varying functions. Bayesian clustering revealed one group at RKR with microsatellites, and two with both SNP data sets. Hybridization analysis determined that the second group present at RKR likely resulted from hybridization with the resident river population. Hybridization between these two populations in RKR is potentially allowing the anadromous population to become adapted in their new environment. Ultimately, the results support hypotheses of rapid evolution of Atlantic salmon in RKR, and both adaptive evolution and recent introgression with resident individuals seem likely. This work highlights the importance of several genomic approaches in detecting and understanding rapid evolution in Newfoundland, the utility of SNP arrays when they are annotated and mapped, and the increased resolution in identifying population differences with RAD-sequencing techniques.

3.6 Tables

Table 3.1: Major biological processes of detected outlier loci from the Atlantic salmon (*Salmo salar*) SNP array, and their location on the published North American Atlantic salmon genome (linkage group) (Brenna-Hansen *et al.* 2012). Annotation of SNP loci is from supplementary material found in Bourret *et al.* (2013).

Linkage Group	Hits	Major processes
ssa01q-fission	1	protein synthesis/processes, cellular signalling, embryonic development
ssa08/29	2	embryonic development, growth, metabolism, immune responses, apoptosis
ssa09	3	cellular signalling, structural processes
ssa10	3	embryonic development, growth, protein synthesis/processes, immune responses, metabolism, apoptosis
ssa11	2	embryonic development, apoptosis, protein synthesis/processes
ssa13	3	metabolism, protein synthesis/processes, immune responses, cellular signalling
ssa14	1	protein synthesis/processes
ssa15	3	reproductive processes, embryonic development, protein synthesis/processes, metabolism, cellular signalling
ssa16	3	metabolism, cellular signalling, protein synthesis/processes, cellular signalling, transcription/translation
ssa18	2	structural processes, cellular signalling, protein synthesis/processes
ssa20	3	metabolism, growth, apoptosis
ssa21	1	immune responses
ssa22	1	protein synthesis/processes
ssa01p/23	1	protein synthesis/processes
ssa24	2	embryonic development, cellular signalling, metabolism, growth, immune responses, behavioural responses

3.7 Figures

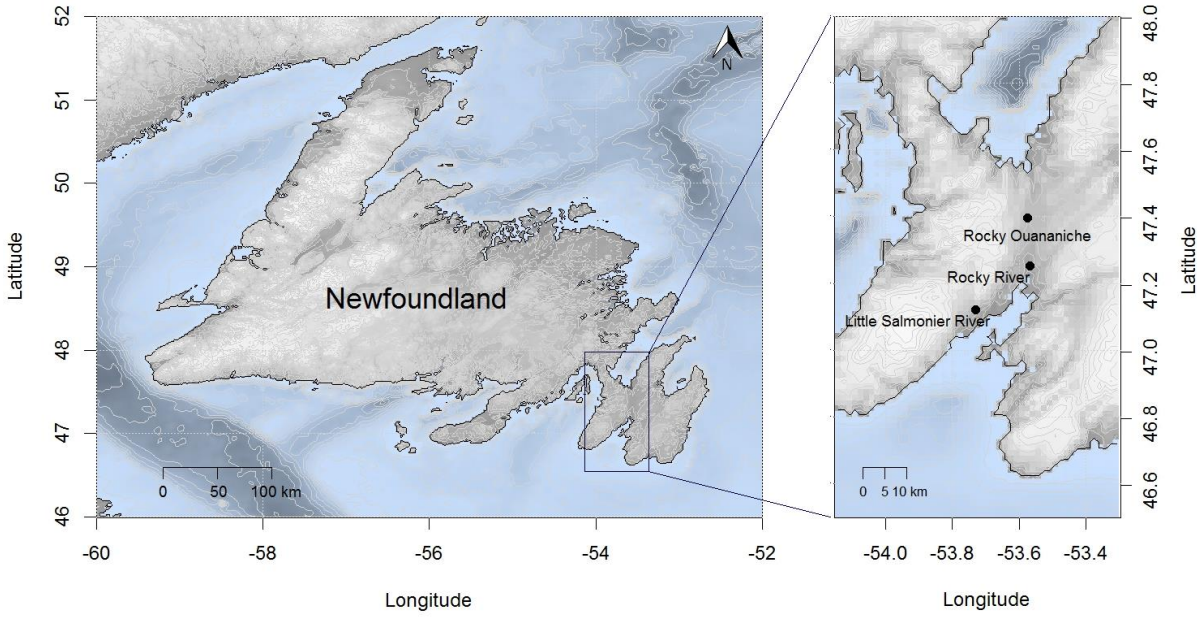

Figure 3.1: Location of sampling site for anadromous salmon in Little Salmonier River (LSR) and Rocky River (RKR), as well as the sampling location of RKR resident (ouananiche) salmon (RKRO) on the Avalon Peninsula in Newfoundland.

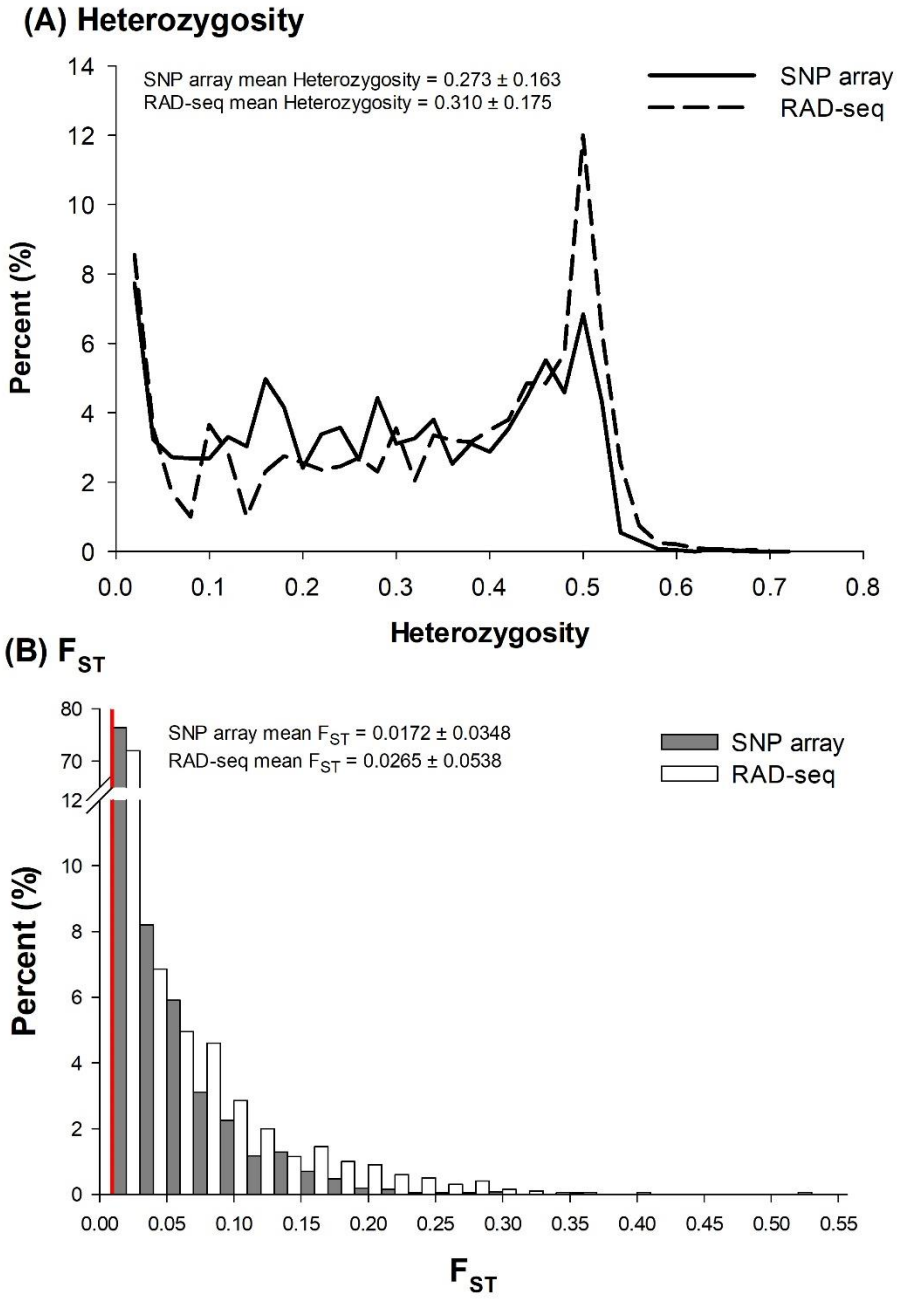

Figure 3.2: Comparison of observed heterozygosity percentages between the SNP array and RAD-seq SNPs (A) and comparison of locus specific F_{ST} frequency distribution percentages for the SNP array, RAD-seq SNPs and average F_{ST} microsatellite data (B). The red bar indicates the microsatellite average F_{ST} value.

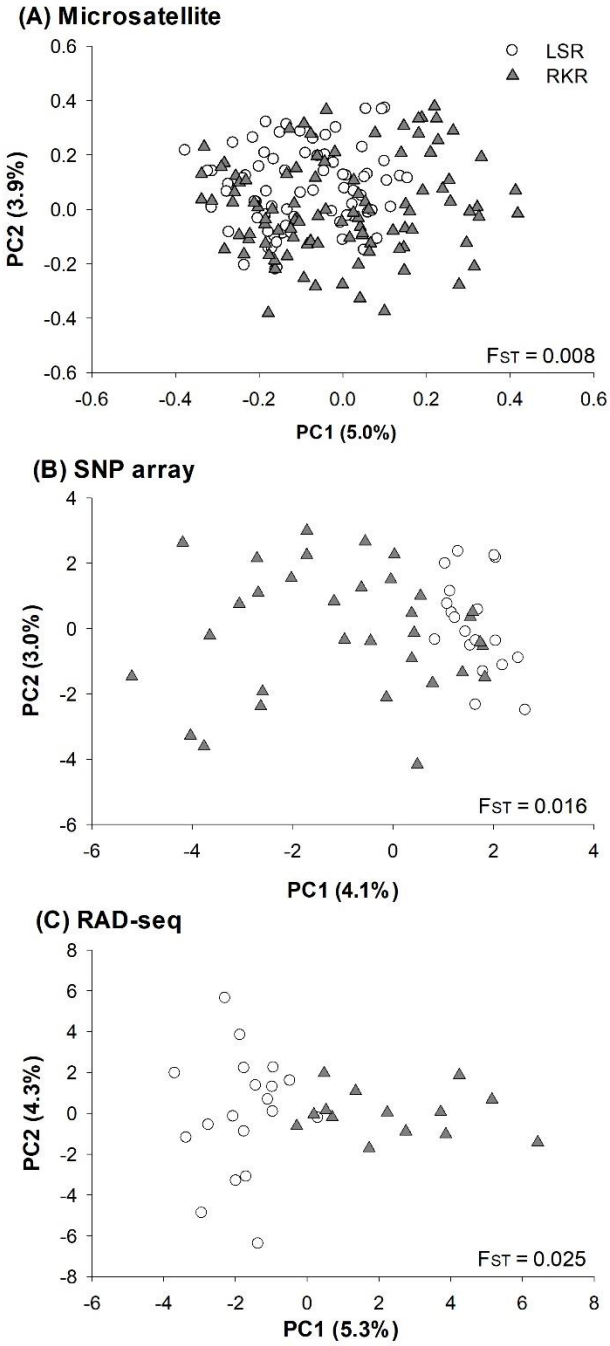

Figure 3.3: Comparison of principal coordinates analyses for microsatellites (A), the SNP array (B), and RAD-seq SNPs (C), with their associated pairwise F_{ST} values. Circles represent LSR samples, and triangles represent RKR samples. The percent variation explained by each axis is included.

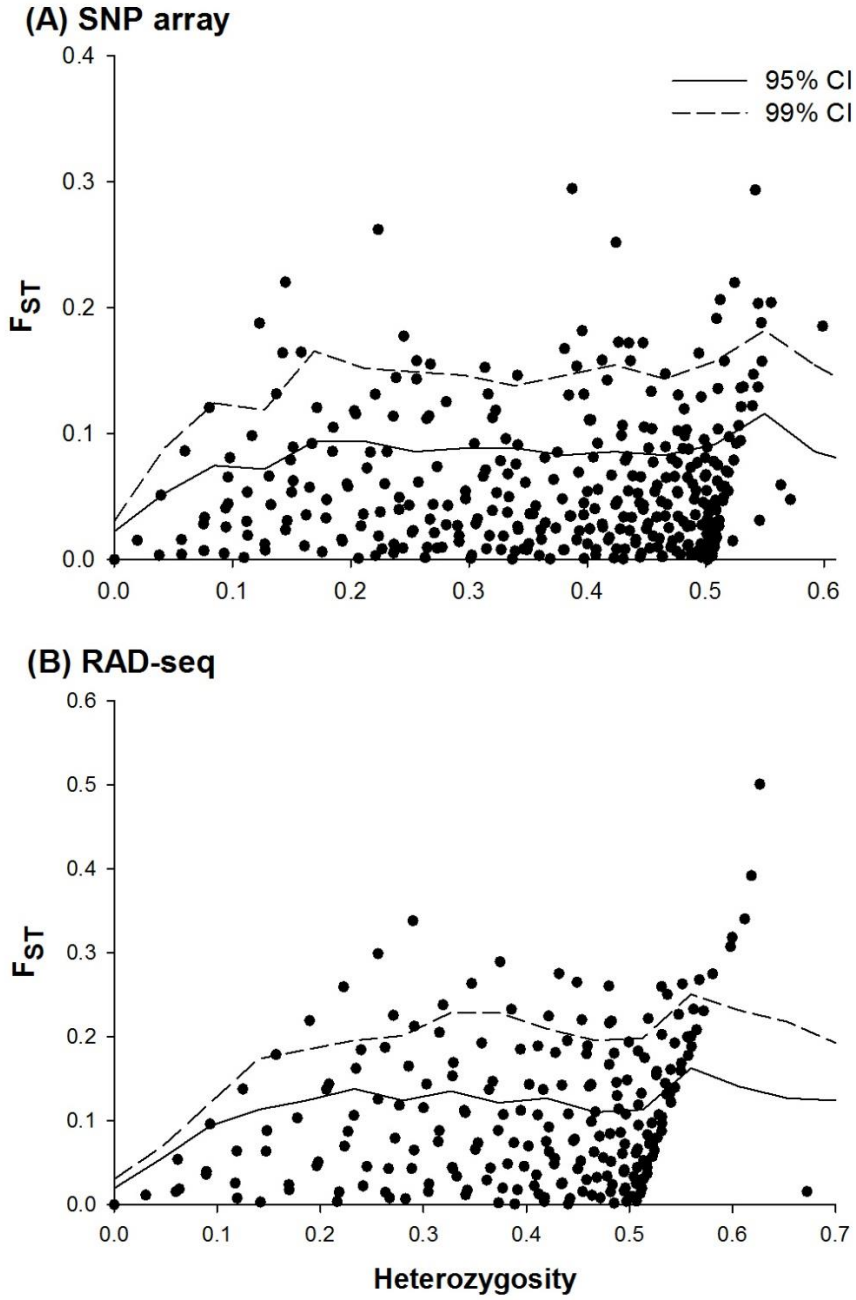

Figure 3.4: Comparison of outlier loci for the SNP array (A) and RAD-seq SNPs (B) based on observed heterozygosity and locus-specific F_{ST} values. Solid lines represent the 95% confidence interval; dashed lines represent the 99% confidence interval. Any marker above these lines are considered outliers.

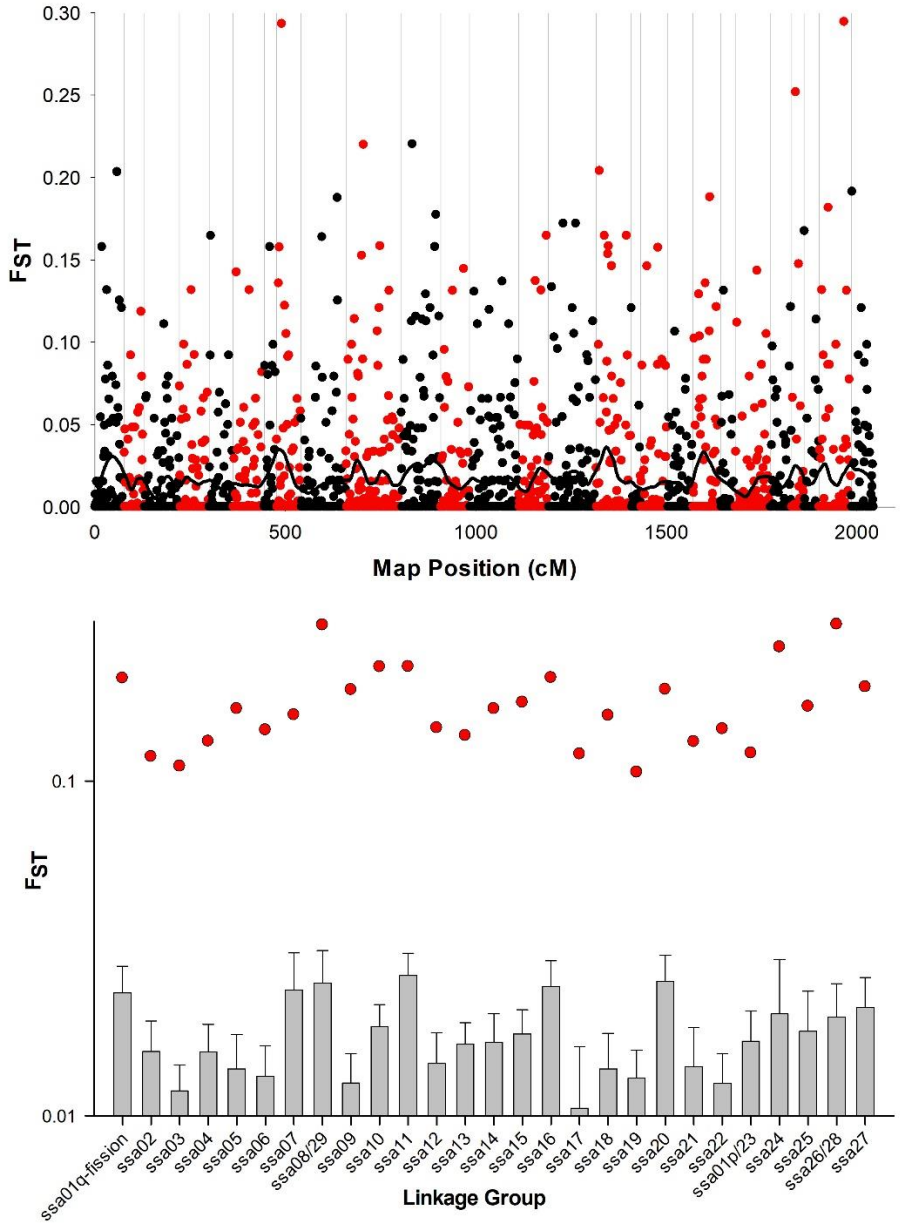

Figure 3.5: Genomic distribution of differentiation between LSR and RKR Atlantic salmon populations in Newfoundland. Map information is based on a published linkage map for North American Atlantic salmon (Brenna-Hansen *et al.* 2012). (A) Estimates of F_{ST} between the two populations across the North American Atlantic salmon genome; grey lines indicate different chromosomes. Solid line represents the results of a LOESS second order filter. (B) Average F_{ST} per linkage group with standard deviation (error bars) and maximum value (red circle).

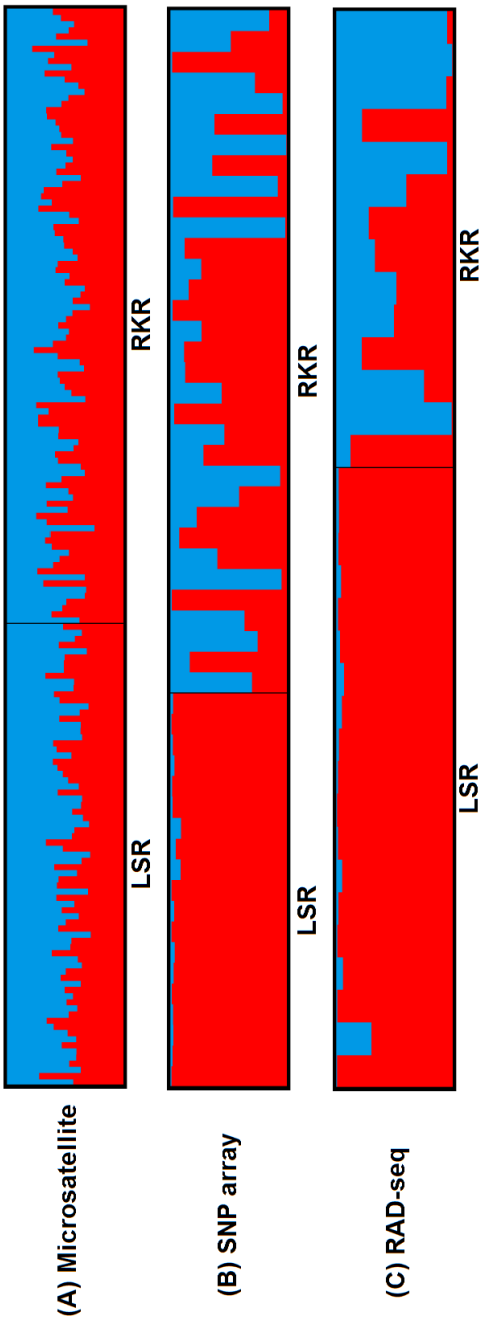

Figure 3.6: Bayesian clustering (i.e. STRUCTURE) analysis of the microsatellite and single nucleotide polymorphism (SNP) data sets for LSR and RKR populations: (A) microsatellite, (B) SNP array, (C) RAD-seq SNPs. All analyses shown represent optimal value of *k* in each instance.

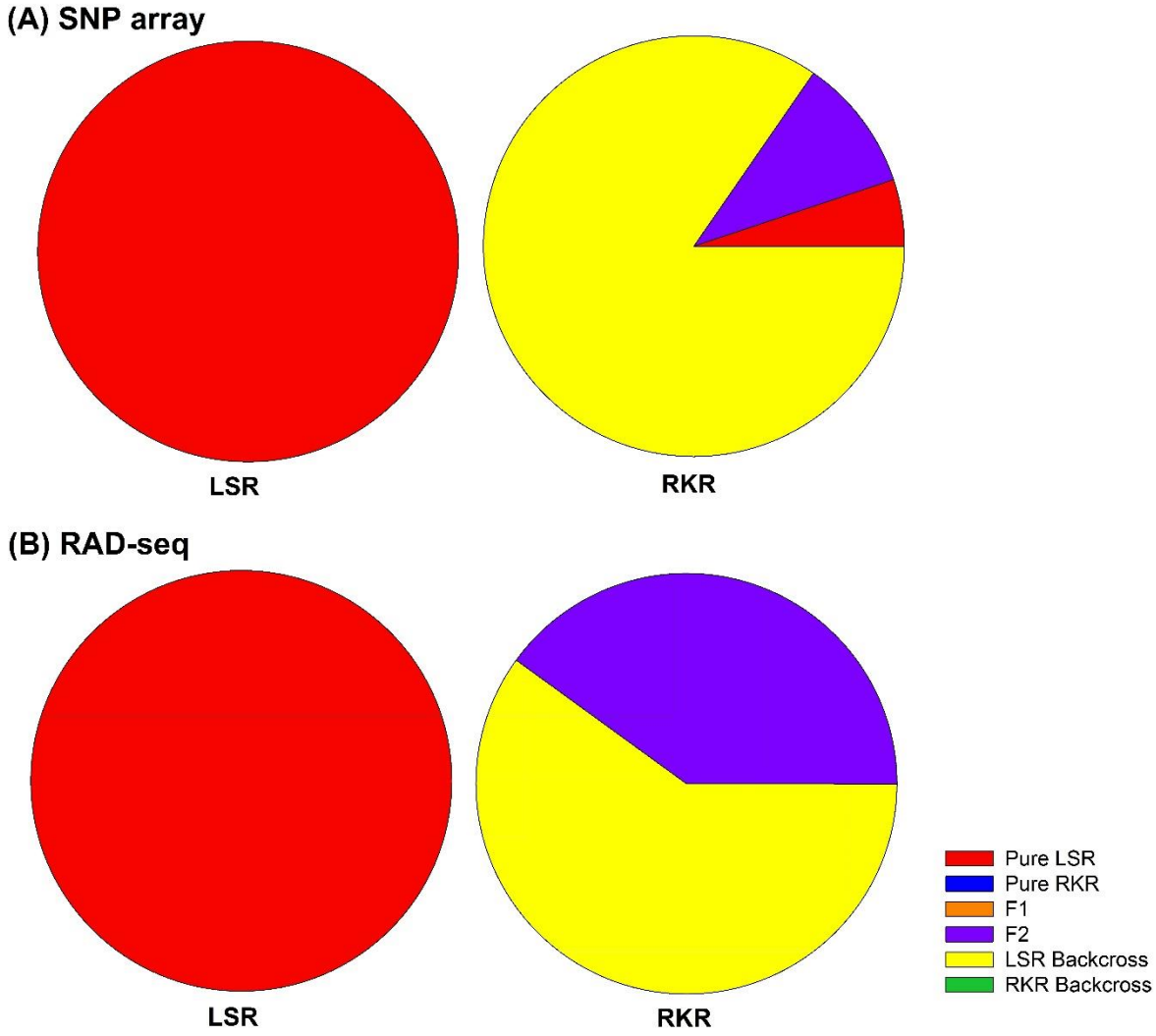

Figure 3.7: New Hybrids analysis with both LSR and RKR populations for the single nucleotide polymorphism (SNPs) data sets: (A) SNP array, (B) RAD-seq SNPs. LSR samples are in the left column; RKR samples are in the right column. Status was determined when an individual had > 50% assignment to a particular classification.

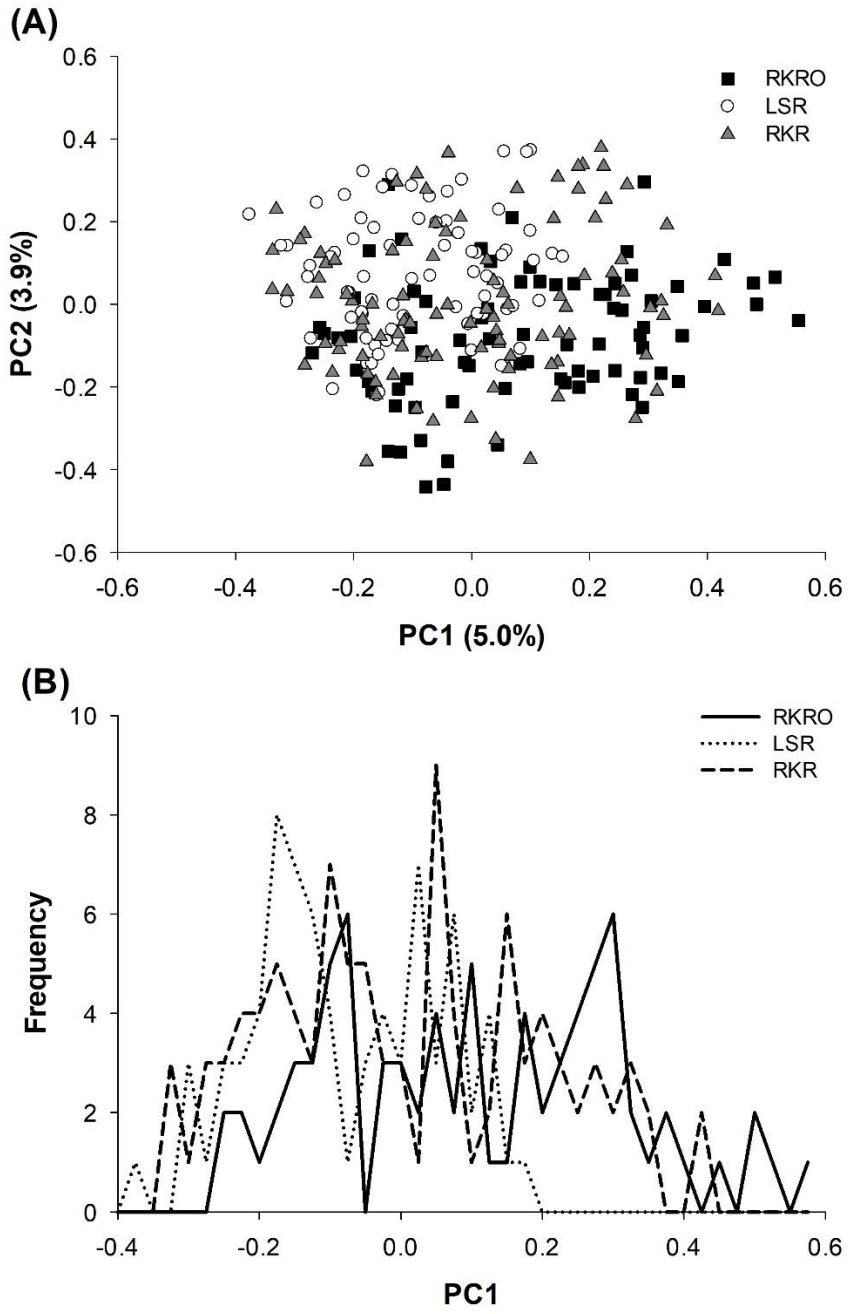

Figure 3.8: Principal coordinate analysis (PCoA) (A) using the microsatellite data, comparing RKR resident (RKRO) Atlantic salmon with the two anadromous populations (LSR, RKR). (B) Comparison of the frequency of the first principal coordinate values for all three sample groups (LSR, RKR, RKRO).

Chapter 4: Summary

Overall, the establishment of a returning Atlantic salmon population in Rocky River, Newfoundland, represents the implementation of a successful enhancement strategy (Bourgeois 1998). Our aim was to combine experimental research in the form of reciprocal transplants and in-depth genomic analysis to assess the presence of rapid evolution at a small geographical scale (< 50 km). Pure and hybrid F1 crosses were created and placed reciprocally in LSR and RKR tributaries while also monitoring crosses in a controlled laboratory setting. While there were no significant differences in growth, except for length at recapture, or survival in the transplanted fish, it appeared that purebreds in both transplant and lab settings tended to slightly outperform their hybrid counterparts, which could suggest that outbreeding depression may be developing.

We analyzed 15 microsatellite loci (175 samples), 5568 SNPs from a genome-wide SNP array (59 samples) and 8495 RAD-seq SNPs (40 samples) to assess the presence of recent genomic divergence between the source and introduced populations. The clear differentiation between source and introduced populations using two SNP datasets, and the detection of 90 loci putatively under selection supports a hypothesis of rapid evolution occurring in the Rocky River. Comparisons with resident salmon in Rocky River also revealed a potential role for introgression driving this change.

4.1 Experimental evidence of rapid evolution in Rocky River Atlantic salmon

In Chapter 2, we assessed the degree to which the introduced population at Rocky River had adapted to its new environment by conducting reciprocal crosses of F1 pure and hybrid individuals while also monitoring a subset of pure and hybrid fish in a controlled lab setting. Pure RKR offspring were slightly longer than hybrids, and survival differences were marginally non-significant between pure and hybrid crosses in the field or lab, suggests potential for a low level of outbreeding depression as a result of hybridization between the LSR and RKR populations. Moreover, more purebreds were recaptured than hybrids in both rivers, demonstrating potential adaptive differences between the crosses. The overall lack of differentiation between the crosses could results from insufficient generations having passed since the introduction event. It is possible that the introduced population has not been present in RKR for long enough in order to see distinct adaptive differences in growth and survival compared to the parent population.

It is possible that hybridization between populations created a low degree of outbreeding depression, suggesting that the introduced RKR fish have started to become locally adapted to their new environment in the 5-6 generations post-introduction. Cases of outbreeding depression in salmonid populations are not rare (Côté *et al.* 2014; Gharrett *et al.* 1999; Gilk *et al.* 2004; Lehnert *et al.* 2014; O'Toole *et al.* 2015), and some of these studies concluded that even slight differences in environments can have adverse effects on hybrids and transplants (Côté *et al.* 2014; O'Toole *et al.* 2015). In our study, offspring

were exposed to a common water source, which may have been beneficial to hybrids in their initial development, but once fish were released, environmental conditions may have favoured purebreds.

4.2 Genomic evidence of rapid evolution in Rocky River Atlantic salmon

In Chapter 3, we analyzed three genetic and genomic datasets to assess the presence of potentially adaptive divergence occurring in the Rocky River Atlantic salmon population. The presence of one pure group at the source river, Little Salmonier, and two groups with evidence of admixture at Rocky River suggests that the introduced population has evolved from the source population. Outlier tests using the SNP array revealed approximately 90 loci that are putatively under selection; 32 of which had known gene ontologies. Hybridization analysis of the second group present solely at Rocky River determined that it was likely a result of hybridization with the resident population already present; which is likely contributing to the introduced population's adaptation to their new environment. Ultimately, the results support hypotheses of rapid evolution of Atlantic salmon in RKR, both through adaptive evolution and recent introgression with resident individuals seem likely.

4.3 Importance of combining experimental and genomic analysis

Genetic and genomic studies of rapid evolution in the wild allows the determination of adaptive capacity to changing environments, and can detect evidence of selection that phenotypic studies alone cannot. It has become apparent that, while

researching rapid evolution, the use of either of the two approaches used in this study alone is not sufficient to draw informed conclusions. As such, the ever-evolving field of next-generation sequencing makes it possible to explore the molecular evidence and drivers associated with rapid evolution that may not yet be expressed phenotypically. Here, the combination of experimental reciprocal transplants and in-depth genomic analysis allowed a more complete examination of whether rapid evolution has occurred in the introduced salmon population at Rocky River ~30 years post-introduction. While the reciprocal transplant experiment yielded little significant evidence of growth or survivorship differences between populations, the genomic portion presented us with ample evidence that rapid evolution is, indeed, beginning to occur. We were able to determine that the introduced anadromous population appears to be rapidly evolving, gained insight into the underlying mechanisms of it, and what traits appear to be selected for.

Moreover, our study demonstrated the advantages of using multiple genomic approaches to assess rapid evolution in a natural setting. The microsatellite analysis, while initially showing no differentiation between the LSR and RKR populations, did support the observation that the RKR population is likely interbreeding with the resident individuals. The SNP analyses revealed the presence of a separate group at RKR, determined the degree of introgression within the population, and allowed us to pinpoint outliers, their position on the genome, as well as determining that traits are putatively being selected for.

4.4 Implications

This study provides valuable insight on how readily Atlantic salmon, a species of economic importance, can adapt to rapidly changing environments, as well as showcasing the relative success of enhancement strategies in Atlantic Canada. Rocky River represents a successful enhancement strategy with the aim of creating an anadromous run that did not previously exist in the system (Bourgeois 1998). This work also highlights the importance of several genomic approaches in analyzing and understanding rapid evolution in Newfoundland, which can be replicated in other areas of the world. It demonstrates the versatility of using SNPs when assessing rapid evolution, when arrays are annotated and mapped, and the increased resolution in identifying population differences with RAD-sequencing techniques. These genomic tools can be used to determine the degree of divergence and adaptability within many species and populations in response to changing environments, which, in turn, can influence future management protocols to maintain population health and sustainability.

4.5 Conclusions

The aim of this thesis and the work therein was to determine the presence of rapid evolution in the Rocky River Atlantic salmon population by assessing differences in growth and survivorship from reciprocal transplants, and analyzing multiple genomic datasets to detect signs of molecular adaptation. While we acknowledge that it is difficult to conclude that hybridization and potential resulting outbreeding depression in this population from a single one-generation study, our results coincide with other research in

similar species. Based on the genetic evidence, the introduced population appears to be evolving, whether by adaptive evolution or from recent introgression with resident individuals. The use of multiple genomic markers was able to provide detailed insight into the introduced population, with RAD-seq SNPs showing the highest level of differentiation between the two populations. Approximately 90 outlier loci were detected using the SNP array, which were generally widespread throughout the genome and had varying functions. Overall, our results provide evidence that rapid evolution is likely occurring in the Rocky River Atlantic salmon population, demonstrating that species can adapt over short generational time periods and at small geographical scales. Such knowledge can help guide the study and management of many anadromous and marine species, particularly those in the Northwest Atlantic.

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Appendices

Appendix S1: Supporting information for **Chapter 2:** Experimental evidence of rapid evolution in a recently introduced Atlantic salmon (*Salmo salar*) population: potential effects of outbreeding depression in Rocky River, Newfoundland

Table S1: Raw weight (g) and length (mm) measurements taken on purebred juvenile Atlantic salmon (*Salmo salar*) at three stages prior to transplant release: 50% hatch, 50% feed, prior to release.

	Hatch		**Feed**		**Release**	
Family	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
RKR1	17.03	0.0915	26.775	0.115	31.196	0.2131
	16.98	0.0856	27.563	0.127	30.629	0.2172
	17.03	0.0825	27.524	0.1074	26.439	0.0853
	16.81	0.0865	27.417	0.1138	26.435	0.0831
	17.44	0.088	26.25	0.1062	26.491	0.1148
	17.38	0.0828	27.11	0.1225	27.432	0.1167
	17.32	0.0812	27.649	0.1539	N/A	N/A
	16.61	0.0853	26.967	0.1164	N/A	N/A
	17.09	0.0878	27.04	0.102	N/A	N/A
	16.67	0.0871	27.03	0.1123	N/A	N/A
RKR3	17.42	0.1027	28.364	0.1326	27.042	0.0984
	17.95	0.1042	27.945	0.1281	26.434	0.0933
	17.58	0.1075	28.605	0.1596	27.504	0.1042
	17.42	0.1077	27.456	0.1321	26.354	0.094
	17.53	0.1037	28.133	0.1269	26.704	0.095
	17.59	0.1116	27.805	0.1266	28.214	0.1167
	18.65	0.114	28.546	0.1343	26.814	0.104
	17.53	0.106	28.569	0.1371	26.089	0.1041
	16.79	0.1004	27.378	0.1152	27.213	0.1073
	17.39	0.1041	28.16	0.1336	26.894	0.101
RKR8	18.69	0.1348	29.768	0.1602	32.163	0.2313
	18.45	0.1268	29.496	0.1402	31.301	0.2242
	18.31	0.1248	28.821	0.1458	30.97	0.2026
	18.28	0.1306	28.998	0.1439	31.383	0.2241
	18.42	0.1229	30.088	0.149	28.174	0.1055
	18.37	0.1268	28.515	0.1353	30.211	0.1876
	18.70	0.1138	28.832	0.1417	28.462	0.1236
	18.33	0.122	29.805	0.1436	29.155	0.1315
	18.80	0.1278	29.176	0.1404	29.268	0.1357
	18.72	0.1084	29.204	0.1402	28.158	0.1221
RKR10	19.66	0.1003	26.569	0.089	26.563	0.1003
	19.20	0.0927	26.663	0.0978	26.135	0.1108
	18.88	0.0899	27.937	0.1102	26.478	0.1053
	19.09	0.1026	27.381	0.1086	26.267	0.0961
	19.29	0.0978	27.836	0.1038	26.477	0.0939

	19.27	0.103	26.802	0.0905	26.563	0.0905
	19.56	0.0999	27.719	0.1068	26.786	0.1054
	18.92	0.1016	27.265	0.095	25.798	0.0937
	19.33	0.0935	27.678	0.107	26.377	0.104
	19.21	0.0966	26.63	0.0838	26.586	0.1062
RKR13	16.27	0.0882	27.365	0.1177	26.834	0.1159
	16.76	0.0862	26.06	0.1175	25.263	0.0845
	16.68	0.0739	26.43	0.1171	29.515	0.1852
	16.88	0.0831	26.367	0.1148	28.344	0.1553
	16.64	0.0921	25.977	0.1044	28.849	0.1657
	17.17	0.0895	26.99	0.1263	28.321	0.1511
	16.8	0.0888	26.699	0.1204	25.044	0.0775
	17.1	0.0904	27.306	0.1236	25.276	0.0926
	17.21	0.0796	26.538	0.1171	28.226	0.107
	16.86	0.0882	26.942	0.1184	28.017	0.1309
RKR15	16.76	0.0897	26.685	0.1135	28.166	0.1547
	16.82	0.0899	26.279	0.112	25.266	0.1009
	16.91	0.0871	26.226	0.102	29.429	0.1733
	16.57	0.0911	26.383	0.1029	28.091	0.1391
	17.03	0.0898	27.432	0.1148	26.079	0.0972
	16.54	0.0876	26.025	0.0968	27.165	0.1338
	16.64	0.0815	26.173	0.0909	28.315	0.1397
	16.72	0.0804	26.454	0.0948	29.964	0.1604
	17.72	0.0829	26.675	0.1019	26.358	0.0942
	17.09	0.0832	25.553	0.0908	27.658	0.1195
RKR16	18.78	0.0971	27.256	0.1118	27.283	0.0927
	19.13	0.0977	26.184	0.107	26.336	0.0886
	17.91	0.091	27.211	0.1228	27.32	0.094
	17.51	0.0944	27.409	0.1182	27.092	0.1036
	18.61	0.0913	26.662	0.113	26.13	0.0882
	18.41	0.0877	27.702	0.1146	27.707	0.0861
	17.91	0.0904	27.15	0.134	26.605	0.0933
	19.02	0.0977	27.062	0.1137	32.113	0.2529
	19.39	0.0903	27.049	0.1017	28.198	0.1479
	18.86	0.0912	26.802	0.1014	27.973	0.1339
RKR17	17.22	0.0904	27.101	0.1035	26.462	0.109
	16.83	0.0943	27.378	0.1121	25.491	0.0932
	17.48	0.1073	26.651	0.1131	25.226	0.0826
	17.46	0.0996	27.436	0.1007	24.386	0.0704
	16.13	0.0997	27.313	0.1064	25.07	0.0757
	17.07	0.0975	26.48	0.0991	26.72	0.1305
	16.92	0.104	27.067	0.1171	25.601	0.0775

	17.39	0.105	27.262	0.0951	25.941	0.0784
	17.33	0.0982	28.068	0.113	26.093	0.0774
	16.81	0.1008	27.416	0.1021	N/A	N/A
RKR18	18.33	0.1024	25.246	0.0951	N/A	N/A
	17.3	0.0925	25.821	0.0996	N/A	N/A
	18.1	0.1007	25.917	0.1028	N/A	N/A
	18.33	0.1037	25.444	0.0888	N/A	N/A
	17.85	0.097	26.119	0.1048	N/A	N/A
	18.21	0.1015	25.738	0.0943	N/A	N/A
	17.8	0.096	26.056	0.0923	N/A	N/A
	17.04	0.0909	25.269	0.0861	N/A	N/A
	18.6	0.106	26.308	0.0992	N/A	N/A
	18.64	0.0979	25.935	0.0929	N/A	N/A
RKR19	17.4	0.0891	N/A	N/A	N/A	N/A
	16.98	0.0938	N/A	N/A	N/A	N/A
	17.07	0.0921	N/A	N/A	N/A	N/A
	17.8	0.0882	N/A	N/A	N/A	N/A
	17.12	0.0935	N/A	N/A	N/A	N/A
	16.51	0.0892	N/A	N/A	N/A	N/A
	17.38	0.09	N/A	N/A	N/A	N/A
	16.75	0.0823	N/A	N/A	N/A	N/A
	17.07	0.0966	N/A	N/A	N/A	N/A
	17.31	0.0914	N/A	N/A	N/A	N/A
RKR21	15.04	0.0781	26.63	0.0836	26.991	0.138
	15.96	0.0815	27.413	0.0837	27.686	0.1551
	14.95	0.0769	27.468	0.0885	25.514	0.0861
	14.28	0.0818	27.445	0.0906	26.143	0.1002
	15.24	0.082	26.139	0.0856	26.592	0.1144
	14.75	0.0778	27.138	0.0961	25.143	0.0962
	15.27	0.0785	27.785	0.0873	N/A	N/A
	15.55	0.0846	26.99	0.1055	N/A	N/A
	15.29	0.0841	26.936	0.08	N/A	N/A
	14.78	0.0773	27.081	0.0917	N/A	N/A
RKR22	15.01	0.0738	25.49	0.1003	N/A	N/A
	15.01	0.0829	25.122	0.093	N/A	N/A
	15.33	0.0768	25.988	0.1044	N/A	N/A
	15.12	0.0815	26.152	0.091	N/A	N/A
	15.4	0.0841	25.693	0.1002	N/A	N/A
	14.96	0.0834	25.758	0.0948	N/A	N/A
	15.08	0.0831	25.493	0.0998	N/A	N/A
	14.55	0.0804	25.134	0.0955	N/A	N/A
	15.05	0.078	26.289	0.0938	N/A	N/A

	15.79	0.0876	25.597	0.0937	N/A	N/A

Table S2: Raw weight (g) and length (mm) measurements taken on hybrid juvenile Atlantic salmon (*Salmo salar*) at three stages prior to transplant release: 50% hatch, 50% feed, prior to release.

	Hatch		**Feed**		**Release**	
Family	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
LSR1	16.971	0.0855	26.326	0.0958	25.095	0.0779
	16.484	0.0842	26.658	0.1044	28.578	0.1712
	16.504	0.0864	27.196	0.1086	29.853	0.2042
	17.095	0.0855	26.784	0.0996	27.701	0.1498
	16.75	0.0813	26.94	0.1021	27.05	0.1462
	16.116	0.0845	25.216	0.0836	25.468	0.0772
	16.441	0.0864	26.243	0.098	27.143	0.1152
	15.895	0.0847	26.126	0.1011	27.252	0.1342
	17.413	0.0873	26.767	0.1038	26.872	0.1221
	16.403	0.0836	27.48	0.1068	27.452	0.1109
LSR2	17.143	0.0856	27.188	0.1062	N/A	N/A
	16.931	0.0785	26.435	0.1089	N/A	N/A
	16.148	0.0824	26.944	0.095	N/A	N/A
	17.184	0.0902	26.347	0.112	N/A	N/A
	16.401	0.089	26.937	0.106	N/A	N/A
	16.478	0.087	25.74	0.0954	N/A	N/A
	17.126	0.0877	25.974	0.1035	N/A	N/A
	16.693	0.0821	27.137	0.1292	N/A	N/A
	16.861	0.0801	26.215	0.0936	N/A	N/A
	16.889	0.0891	26.515	0.1112	N/A	N/A
LSR3	18.243	0.1003	28.589	0.145	27.251	0.0991
	18.264	0.1088	27.606	0.1244	26.217	0.0915
	18.47	0.113	27.01	0.105	26.462	0.0888
	18.595	0.1094	26.626	0.0932	26.434	0.0905
	18.247	0.1004	28.47	0.1322	31.418	0.2477
	17.87	0.0957	27.266	0.1115	26.543	0.0791
	18.314	0.105	27.879	0.126	29.017	0.1799
	18.254	0.1058	27.792	0.1103	27.043	0.0943
	18.489	0.1026	27.975	0.1206	30.148	0.2101
	18.44	0.1063	27.343	0.1098	27.911	0.1109
LSR4	17.353	0.1078	26.792	0.1118	N/A	N/A
	17.441	0.1101	25.98	0.1068	N/A	N/A
	17.821	0.1061	27.572	0.1191	N/A	N/A
	17.559	0.106	27.079	0.1163	N/A	N/A
	17.557	0.1006	27.487	0.1034	N/A	N/A

	17.384	0.1041	26.93	0.1061	N/A	N/A
	17.568	0.1004	27.015	0.1087	N/A	N/A
	17.345	0.1076	26.874	0.1072	N/A	N/A
	17.655	0.114	26.566	0.097	N/A	N/A
	N/A	N/A	26.715	0.1012	N/A	N/A
LSR5	18.799	0.106	28.044	0.1444	N/A	N/A
	18.253	0.1185	28.564	0.1391	N/A	N/A
	18.024	0.1045	28.128	0.1252	N/A	N/A
	18.74	0.1101	28.17	0.1262	N/A	N/A
	17.223	0.11	27.758	0.1376	N/A	N/A
	18.931	0.1174	28.088	0.1231	N/A	N/A
	18.387	0.1186	27.644	0.1316	N/A	N/A
	18.865	0.1141	27.683	0.1212	N/A	N/A
	17.911	0.1026	27.318	0.1148	N/A	N/A
	17.608	0.1101	27.703	0.1291	N/A	N/A
LSR7	17.928	0.1268	28.631	0.1442	28.503	0.1416
	18.761	0.1244	29	0.1589	28.803	0.1324
	17.583	0.1177	29.528	0.163	28.315	0.1279
	18.149	0.124	29.364	0.1563	32.368	0.2812
	18.343	0.1269	28.734	0.1634	28.863	0.1265
	17.81	0.1234	29.488	0.1632	28.685	0.1537
	18.271	0.1251	29.712	0.1599	28.441	0.1307
	17.464	0.1296	29.533	0.1642	29.765	0.1782
	17.272	0.1305	28.893	0.1576	28.336	0.1585
	17.342	0.1207	29.091	0.1545	30.137	0.2095
LSR8	18.868	0.1276	28.664	0.1284	29.919	0.1923
	18.135	0.1233	28.072	0.1418	29.734	0.1848
	18.137	0.1054	28.665	0.1376	28.955	0.1628
	18.124	0.125	28.342	0.139	28.285	0.1062
	18.15	0.127	28.667	0.1316	28.347	0.1131
	19.088	0.1271	28.723	0.146	28.311	0.1324
	18.354	0.1203	28.811	0.1366	28.245	0.1227
	18.65	0.1348	28.745	0.1293	27.82	0.126
	18.698	0.121	28.549	0.1374	29.517	0.1639
	18.591	0.1163	28.028	0.1345	26.94	0.1156
LSR9	18.558	0.0885	26.533	0.0987	27.386	0.1146
	18.494	0.1102	26.87	0.11	29.78	0.1923
	18.47	0.0882	26.882	0.1018	26.917	0.0953
	18.902	0.0909	27.158	0.1162	27.877	0.1163
	19.057	0.0898	26.33	0.0988	26.573	0.1052
	18.9	0.0943	26.267	0.1282	29.1	0.1559

	17.346	0.095	27.145	0.1083	28.214	0.1542
	18.864	0.1054	26.336	0.0963	N/A	N/A
	17.821	0.0926	27.129	0.1277	N/A	N/A
	17.956	0.096	27.029	0.0951	N/A	N/A
LSR10	17.90	0.0916	26.193	0.0951	23.871	0.0556
	17.18	0.0962	26.593	0.0962	25.682	0.0764
	18.03	0.0948	25.983	0.0918	27.379	0.079
	17.64	0.0955	26.139	0.0886	25.636	0.0953
	17.12	0.0895	26.489	0.1006	29.287	0.1737
	17.57	0.102	25.871	0.0789	26.482	0.1156
	16.61	0.0838	26.411	0.0909	26.965	0.0918
	17.70	0.0916	25.238	0.0789	26.607	0.1308
	16.95	0.0923	26.498	0.0946	N/A	N/A
	17.36	0.0929	26.244	0.1043	N/A	N/A
LSR11	18.21	0.1116	27.801	0.142	26.97	0.095
	18.44	0.1099	27.517	0.134	28.118	0.1051
	17.62	0.01117	27.538	0.1394	27.05	0.1039
	17.88	0.112	28.518	0.1417	26.939	0.0995
	17.47	0.1076	28.065	0.1428	28.074	0.1266
	18.27	0.1075	27.249	0.124	27.987	0.1202
	18.01	0.102	28.073	0.1422	29.132	0.1719
	18.07	0.108	28.167	0.147	27.628	0.1071
	17.87	0.116	26.466	0.1201	27.977	0.13
	18.06	0.1068	27.581	0.133	27.85	0.1498
LSR12	17.74	0.1107	28.435	0.1443	27.968	0.1486
	17.52	0.1113	27.477	0.1311	26.843	0.113
	17.31	0.119	25.914	0.1061	28.61	0.1404
	16.91	0.1246	27.396	0.1228	26.428	0.1032
	17.93	0.1092	27.539	0.1412	27.484	0.1297
	17.63	0.117	27.747	0.1274	27.984	0.1189
	16.87	0.101	27.169	0.1304	27.899	0.1349
	17.38	0.1113	28.193	0.1399	27.299	0.1266
	17.36	0.1139	27.238	0.1326	27.459	0.1185
	16.72	0.105	27.366	0.1152	27.577	0.1081
LSR13	16.31	0.0821	25.576	0.1301	25.419	0.0925
	16.70	0.0885	26.644	0.1156	28.499	0.1711
	17.18	0.0924	26.726	0.1178	25.764	0.0952
	17.26	0.0864	26.399	0.1218	29.245	0.1895
	17.48	0.0895	25.446	0.0988	27.908	0.1384
	17.64	0.0822	26.975	0.1238	26.209	0.1026
	17.31	0.0844	26.379	0.1106	26.245	0.0946

	17.89	0.0928	26.398	0.1085	26.03	0.0819
	17.62	0.0981	26.849	0.1158	25.796	0.0929
	17.89	0.0943	26.942	0.1195	26.277	0.0929
LSR14	17.50	0.0955	26.832	0.1137	26.111	0.0867
	17.45	0.097	27.446	0.1253	27.003	0.0968
	17.45	0.0873	26.551	0.1129	26.714	0.0955
	17.10	0.0897	26.379	0.1087	26.617	0.0907
	17.38	0.0876	26.785	0.1145	27.652	0.1271
	17.61	0.0897	26.119	0.109	26.488	0.0993
	17.44	0.0903	27.461	0.1232	26.968	0.1067
	17.64	0.0977	26.065	0.11	26.274	0.0889
	17.55	0.083	27.817	0.1216	26.769	0.1067
	17.76	0.0816	26.901	0.1227	26.728	0.0962
LSR15	17.35	0.0844	25.874	0.0968	27.205	0.1221
	16.80	0.09	25.811	0.0845	25.881	0.0975
	16.89	0.0786	26.25	0.1013	25.272	0.0767
	17.10	0.0889	26.056	0.1016	25.249	0.0649
	16.45	0.0869	26.255	0.0898	25.453	0.0735
	16.85	0.0876	25.726	0.0948	27.177	0.1157
	16.92	0.0849	25.283	0.0822	27.175	0.1378
	17.41	0.0949	25.555	0.0931	25.295	0.0712
	16.71	0.0849	26.227	0.0984	26.114	0.097
	16.75	0.0856	26.179	0.0973	26.501	0.1153
LSR19	17.017	0.0923	24.212	0.0791	N/A	N/A
	17.516	0.0991	26.173	0.0911	N/A	N/A
	17.362	0.0919	25.484	0.0881	N/A	N/A
	16.685	0.0932	26.317	0.1039	N/A	N/A
	17.309	0.0921	26.173	0.0827	N/A	N/A
	17.146	0.0988	25.27	0.0899	N/A	N/A
	17.313	0.0922	24.737	0.0755	N/A	N/A
	16.961	0.0908	25.71	0.085	N/A	N/A
	17.633	0.1001	25.411	0.0905	N/A	N/A
	17.014	0.0899	26.979	0.0969	N/A	N/A
LSR20	16.804	0.09	27.73	0.116	N/A	N/A
	16.142	0.0936	27.307	0.1536	N/A	N/A
	16.565	0.0973	27.398	0.1155	N/A	N/A
	16.243	0.096	28.365	0.1512	N/A	N/A
	16.348	0.0946	27.768	0.1443	N/A	N/A
	16.133	0.0908	27.674	0.1274	N/A	N/A
	16.412	0.099	28.072	0.1306	N/A	N/A
	16.143	0.0916	26.775	0.1098	N/A	N/A

	16.234	0.0922	27.615	0.1386	N/A	N/A
	16.725	0.0926	27.588	0.1107	N/A	N/A