MORPHOLOGICAL VARIATION AND GENETIC DIFFERENTIATION IN WILD AND DOMESTIC ATLANTIC SALMON FROM SOUTHERN NEWFOUNDLAND

by

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Abstract

Adaptive divergence is an important force structuring wild populations and directly influencing species persistence and stability. Wild Atlantic Salmon (*Salmo salar*) have declined across their native range in recent decades with genetic interactions with salmon aquaculture identified as a contributing cause. Improved understanding the nature of diversity in wild populations and the potential impact of interbreeding with domestic escapees is critical to conservation and management of wild Atlantic Salmon. Body shape of juvenile Atlantic Salmon in the wild may be considered adaptive and is often found to be associated with environmental and watershed conditions, or even with culture conditions in the case of domestic conspecifics. In this thesis I quantify the importance of body shape on population structuring in the wild, and then explore the impact of wild-domestic hybridization on shape of juvenile Atlantic Salmon. Geometric morphometrics were used to quantify and allow for statistical testing of variation in body shape. Geometric morphometric data were first combined with a large panel of sequenced microsatellite loci to understand the relationship between shape and population structure; and second used in conjunction with a SNP panel designed for wild-domestic hybrid identification to explore shape differences among cross types. My results suggest that variation in body shape is important to the structuring of wild populations, but that variation in climate was also significant in genetic structuring. Interestingly, shape differences between wild, domestic, and hybrids were minimal, likely reflecting both selection and phenotypic plasticity in the wild. My results suggest that phenotypic variation in body shape may be an important component of adaptive diversity among Atlantic Salmon populations, and that changes in body shape in the wild due to interbreeding with escaped farmed salmon may be minimal and masked by plasticity.

List of Abbreviations and Symbols Used

θ_{ST} – Weir and Cockeram's F_{ST}

β – Environmental or morphological variable coefficient

ε – Error

ANOVA – Analysis of variance

BIMr – Bayesian Inference of Migration rates (software)

COSEWIC – The Committee on the Status of Endangered Wildlife in Canada

CVA – Canonical variate analysis

$cm \bullet s^{-1}$ – Centimetre per second

df – Degrees of freedom

D – Least cost distance (km)

DNA – Deoxyribonucleic acid

EEMS – Estimation of Effective Migration Surfaces (software)

F_1 – Filial 1 hybrid

F_{ST} – Fixation index

GD – Genetic distance

IBD – Isolation by distance

IFC - Integrated Fluidic Circuits

K – Number of clusters

MCMC – Markov chain Monte Carlo

$mg \bullet l^{-1}$ – Milligrams per litre

MHC – Major histocompatibility complex

$mmol \bullet l^{-1}$ – Millimoles per litre

NB – New Brunswick

ng – Nanograms

$ng \bullet \mu l^{-1}$ – Nanograms per microlitre

PC – Principal component

PCA – Principal component analysis

PCoA – Principle coordinate analysis

PF – Pure farm

PW – Pure wild

$\hat{R}$ – Ratio of within and among chain variance

RAD sequencing – Restriction-site associated DNA sequencing

RDA – Redundancy analysis

SNP – Single nucleotide polymorphism

V – Environmental or topographic characteristic of the river

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Chapter 1 – Introduction

1.1 Introduction

Species persistence and demographic stability in the wild has been associated with increased population diversity (Maehr *et al*., 2006; Westemeier *et al*., 1998), which can allow species to respond to distrubance and new selective pressures (Barrett and Schluter, 2008). Species lacking genetic diversity are often thought to be at greater risk of extirpation, and examples exist of past extinctions having been preceeded by a loss of genetic diversity (Újvári *et al.*, 2002; Evans and Sheldon, 2008; Hellmair and Kinziger, 2014). Reduced genetic diversity in a population has also been associated with reductions in population productivity due to inbreeding depression (Frankham, 2005; Charlesworth and Willis, 2009). Therefore, both the maintainence of diversity or, increasing diversity in vulnerable populations are fundamental goals in managing and conserving wild populations (Caballero and Toro, 2002). Possible interventions aimed at managing divertsity include identifying the causes of populatioin decline, introductions, or enabling gene flow from neighbouring populations (Frankham, 2015; Jangjoo, 2016). However, before diversity can be managed or conserved in wild populations, it is necessary to first understand the distribution of genetic diversity among populations.

Gene flow can significantly alter how diversity is organized among populations. Gene flow among wild populations can increase population productivity through the addition of diversity and the rescue of small vulnerable populations, or through adaptive introgression among populations (Kremer *et al.*, 2012). However, maladaptive introgression such as often occurs through gene flow from domestic lineages can also be

detrimental to wild populations (Rhymer and Simberloff, 1996; Kidd *et al.*, 2009; Glover *et al.*, 2017) because domestic populations are either purposefully or inadvertently selected for specific traits that confer economic benefits in domestic settings but may result in negative impacts on wild populations (Gutierrez *et al*., 2016; Alberto *et al.*, 2018). Domestic lineages have also experienced relaxed selective pressures for foraging ability and predation avoidance, among other traits. Consequently, gene flow between domestic and wild can contribute to maladaptation and decreased fitness in wild populations (Gutierrez *et al*., 2016; Alberto *et al*., 2018). Understanding the effects of gene flow on adaptive diversity in the wild and among wild populations and domestic populations is therefore imperative to effective management and conservation.

Atlantic Salmon (*Salmo salar*) is a mostly anadromous species with wide ranging marine migrations (Thorstad *et al*. 2011), and extensive evidence of fine scale homing to natal rivers (Hendry *et al*., 2004). The species has been shown to be characterized by significant genetic structuring and adaptive diversity (Fraser *et al.*, 2011; Jeffery *et al*., 2018; Moore *et al*., 2014; Lehnert *et al*., 2019b). Atlantic Salmon is economically important both as a wild species that supports recreational and subsistence fisheries and as a domestic species raised in aquaculture (Carr *et al*., 1997). Across the natural range of the species a majority of populations have experienced significant declines in the last several decades (Lehnert *et al*. 2019b). For example, wild salmon in southern Newfoundland have declined subtantially since the 1980s and have been assessed as "Threatened" by COSEWIC (COSEWIC, 2011), meaning that they will face increasing risk of extirpation if current threats to populations are not managed. Although the causes of decline in wild salmon populations are often unknown, in this region there are

significant concerns regarding salmon net pen aquaculture which increased during the same period in which the populations have been declining (DFO, 2013). This concern over the impact of netpen aquaculture has also been raised in the Northeast Atlantic in part because of the effect of introgression from domestic salmon escapees into wild populations (Forseth *et al.*, 2017; Glover *et al.*, 2017). It is therefore increasingly important to assess wild population structure, adaptive diversity, and to explore causes of decline particularly the impact of introgression of aquaculture escapees on wild populations to inform conservation and management efforts.

The objective of this thesis is to explore the role of phenotypic diversity on gene flow in wild Atlantic Salmon populations. To achieve this, I first examined how morphology varies with population structure and climate in southern Newfoundland. I then explored how gene flow from domestic populations influences phenotype and possibly adaptation in wild salmon populations. The ulitmate goal of this work is to improve our understanding of the importance of morphological variation to population structuring in salmon and the potential impact of aquaculture introgression.

1.2 Thesis Overview

This thesis is comprised of two data chapters, Chapters 2 and 3, which are based on original research. Chapter 2 examines drivers of population structure in Atlantic Salmon, exploring the importance of both phenotypic and environmental variation. In this chapter, I examine the relationship between genetic structure and divergence in adaptive traits in southern Newfoundland Atlantic Salmon. Salmon with aquaculture ancestry caught in wild rivers were removed from wild salmon using a panel of 96 SNP markers

and then wild salmon genotyped using 101 microsatellites. Bayesian regression and redundancy analysis were used to quantify the relationships between temperature, precipitation, shape, and genetic divergence to better characterize factors influencing structuring Atlantic Salmon populations in southern Newfoundland. Then, in Chapter 3 the impact of hybridization between farmed and wild salmon on body shape using both laboratory reared, and wild collected individuals was explored. Geometric morphometrics were used to quantify shape differences between wild and farm stock salmon in a common garden experiment, using both tank and semi-natural conditions. These results were then compared with results from individuals sampled in rivers in southern Newfoundland with population ancestry (wild, farmed, or wild-farm hybrid) identified with a diagnostic 96 single nucleotide polymorphism (SNP) panel. This work contributes to understanding of the impacts of hybridization between wild and aquaculture salmon. Finally, in chapter 4 I summarize the findings of each chapter and discuss the implications of these findings and potential avenues for future research.

Chapter 2 – Patterns of Gene Flow, Morphological Variation, and Environmental Associations in Southern Newfoundland Atlantic Salmon (*Salmo salar*)

2.1 Abstract

Understanding the evolutionary processes that influence genetic structure in wild populations can provide insights into the scale and spatial organization of diversity and inform conservation and management efforts. Atlantic Salmon (*Salmo salar*) are generally characterized by discrete locally adapted populations at the scale of individual rivers, but the relative importance of local natural selection and gene flow from nearby rivers remains poorly understood. Here I explored the association between genetic structuring, morphological variation, and the environment in salmon populations distributed across southern Newfoundland. I used geometric morphometrics to measure differences in shape, and quantified genetic differentiation using 101 sequenced microsatellites in 2043 juvenile salmon sampled across southern Newfoundland (2015-2017). Our results suggest that salmon populations in the region are highly structured (based on F_{ST}), and surprisingly display little genetic isolation by distance. To further explore the forces structuring these populations, I used estimated migration rates and genetic distance as predicted variables in a Bayesian multiple regression with environmental variation, morphology, and geographic distance between rivers as predictors. The results suggest that climate and morphological variation both have a significant effect on genetic distance and gene flow in the region. This work highlights the potential link between selection and local adaptation on patterns of gene flow among wild Atlantic Salmon populations.

2.2 Introduction

2.2.1 Importance of Understanding Diversity in the Wild

The genetic diversity of species and populations generally influences their demographic stability and likelihood of persistence (Nowak *et al.*, 2007), because diverse species or populations are more likely to be resistant to stressors and to persist in the long term (Frankham *et al*., 2017; but see Fraser, 2017). As such, understanding the scale at which genetic diversity exists in the wild, how it is structured, and the structuring processes can be central to conservation and management efforts (Allendorf, Hohenlohe and Luikart, 2010; Manel *et al.*, 2010) and the identification of conservation units (Funk *et al*., 2012). Landscape genetic and genomic studies increasingly suggest landscape features such as habitat and environmental characteristics play a pivotal role in determining gene flow and adaptive diversity (Sexton *et al.*, 2013). Moreover, several recent studies suggest selection against immigrants can influence resultant gene flow and ultimately population structure (Baillie *et al*., 2016; Kaufmann *et al*., 2017; Mobley *et al*., 2019). Understanding this relationship between landscape features, natural selection, and gene flow in driving phenotypic and ultimately adaptive diversity is central to our ability to manage genetic diversity in the wild (Funk *et al.*, 2012). However, few studies link the full suite of available variation such as phenotype, environment, habitat, and genetic structuring into a single examination.

2.2.2 Structuring Factors and Implications for Conservation and Management

The spatial distribution of genetic diversity can provide evidence of the influence of different evolutionary processes, namely selection, migration, and drift. The two predominant spatial patterns of genetic diversity, isolation by distance or isolation by

environment, are a result of different evolutionary forces. Isolation by distance can result from the interplay of localized migration and genetic drift, such that divergence by drift increases with increasing geographic distance between populations (Wright, 1943). Isolation by environment entails a similar concept, except that gene flow is limited by adaptively important environmental differences rather than geographic. Understanding how migration and selection structure genetic diversity can help assessment of the recovery potential of disturbed populations and understanding of potential future impacts to a population resulting from climate change (Pauls *et al*., 2013).

2.2.3 Atlantic Salmon – Structuring Factors, and Conservation Challenges

In Atlantic Salmon (*Salmo salar*), both adaptive processes and geographic distance have been shown to be important in structuring populations (Dillane *et al.*, 2008; Bradbury *et al.*, 2014). Natal homing combined with low levels of straying create hierarchical population structure, with the potential for river level genetic resolution, although straying among nearby rivers contributes to isolation by distance (Hendry *et al*., 2004). Adaptations to local watershed and climate conditions have also been shown to influence structure at large spatial scales across North America (Bourret *et al.*, 2013a; Bradbury *et al.*, 2014; Sylvester *et al.*, 2018a) and Europe (Horreo *et al.*, 2011; Perrier *et al.*, 2011; Cauwelier *et al.*, 2018a); the actual scales at which these processes drive structure and to what degree is still unclear. Additionally, secondary contact between European and North American salmon has been shown to influence genetic structure in southern Newfoundland (Lehnert *et al*. 2019a). Understanding spatial structure is increasingly important with declining abundance in southern Newfoundland as it is

crucial in informing conservation and management (Bowlby *et al*., 2016; Bradbury *et al*., 2014; COSEWIC, 2011).

2.2.4 Objectives

The goal of this study was to explore the potential roles of gene flow and local adaptation as drivers of genetic structure in Atlantic Salmon populations at fine spatial scales in southern Newfoundland Atlantic Salmon. To that end, I first quantified spatial genetic structure and gene flow among 41 rivers within the southern Newfoundland region (Placentia Bay, Fortune Bay and Bay d'Espoir; Figure 1) using a previously developed microsatellite panel (Bradbury *et al.*, 2018). Next, I quantified body shape variation among rivers using geometric morphometrics (Mitteroecker and Gunz, 2009) as a measure of phenotypic variation across the study region. Shape variation was used as an indirect measure of adaptations to watershed characteristics (Garcia de Leaniz *et al.*, 2007). Our third objective was to compare the relationships between genetic structure and local environmental variation and morphological variation. Environmental variables were obtained from the Bioclim database (Fick and Hijmans, 2017). Bayesian multiple mixed regression and redundancy analysis were used to estimate significant associations between environmental, morphological and genetic differentiation while controlling for geographic distance and unmeasured river characteristics. This work builds on previous studies that show isolation by climate and hierarchical spatial structure in Atlantic Salmon (Dionne *et al.*, 2008; Bradbury *et al.*, 2014). This study examines the relationships between morphology and climate across a small geographic region of southern Newfoundland and advances our understanding of the interplay of local adaptation and gene flow and its impact on the evolution of a population.

2.3 Methods

2.3.1 Sampling and Genotyping

A total of 2175 juvenile Atlantic Salmon were sampled from 41 rivers across southern Newfoundland from July-September 2015-2017 (Figure 1a, Table 1) via electrofishing. These fish were assigned ages 0+ to 2+ based on fork length (Sethi *et al*., 2017). Fin clips were taken from each fish from the right pectoral fin and stored in 95% ethanol. DNA was extracted using DNeasy Blood and Tissue or DNeasy 96 Blood and Tissue kits (Qiagen, Toronto, ON, Canada) following manufacturer's protocol and an RNase treatment was used. Qubit dsDNA HS Assay Kit (Life Technologies, Burlington, ON, Canada) was used for DNA quantification, assays were conducted on either a Qubit v2.0 (Life Technologies), a Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) or a FLUOStar OPTIMA fluorescence plate reader (BMG Labtech, Ortenberg, Germany) and diluted to a final concentration of 10 $ng \bullet \mu l^{-1}$ in 10 $mmol \bullet l^{-1}$ Tris (Buffer EB, Qiagen). Agarose gel electrophoresis with 100 ng DNA and SYBR Safe (Life Technologies) was used to visualize DNA quality and recorded using a Gel Logic 200 (Kodak, Rochester, NY, USA). Samples collected from Fortune Bay and Bay D'Espoir were screened for the presence of aquaculture ancestry, and all hybrid and aquaculture individual were removed. This was accomplished using a 96 SNP panel developed for identifying aquaculture and hybrid individuals in the region and *parallelnewhybrid*, and hybrid and aquaculture individuals were removed (Sylvester *et al*., 2018b; Wringe *et al*., 2018). All individuals without aquaculture ancestry were then genotyped using a 101 microsatellite panel using the Illumina MiSeq using v3 chemistry (for methods see: Bradbury *et al*., 2018) and the MEGASAT pipeline was used for demultiplexing loci and scoring alleles

(Zhan *et al.*, 2017). MICRO-CHECKER was used to detect null alleles and scoring errors (van Oosterhout *et al*., 2004).

2.3.2 Quantifying Genetic Structure and Gene Flow

Genetic structure in southern Newfoundland was examined using F_{ST}, Bayesian genetic clustering, principal component analysis (PCA) and a neighbour-joining tree. Migration was estimated using BIMr (Faubet and Gaggiotti, 2008) and Estimation of Effective Migration Surfaces (EEMS) (Petkova, Novembre and Stephens, 2016). Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984) was calculated using the R (R Development Core Team, 2012) package diveRsity (Keenan *et al*., 2013). A neighbour-joining tree was constructed using the R package poppr 2.8.3 (Kamvar *et al*., 2014; Kamvar *et al*., 2015) using Nei's distance between rivers with 1 000 bootstraps. Bayesian clustering was carried out using Structure (Pritchard, Stephens and Donnelly, 2000) with 500 000 steps and a burn-in of 100 000 steps. Number of clusters from K = 1 to K = 50 were tested, and each K was run with three replicates. Evanno's method (Evanno, Regnaut & Goudet, 2005) was used to identify the most likely number of clusters using StructureHarvester (Earl & vonHoldt, 2012) and replicate runs were combined using largeGreedyK implemented in StrataG (Archer *et al*., 2017). PCA was performed on the microsatellite data using the R package adegenet 2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011) as an alternate visualization of population clustering. Migration rates were calculated with BIMr (Faubet and Gaggiotti, 2008) using 10 000 Markov Chain Monte-Carlo (MCMC) steps, and three replicates. Estimation of effective migration surfaces (EEMS) was run using 500 demes and with a burn-in of 200 000 and run for 2 000 000 steps (Petkova, Novembre and Stephens, 2016). EEMS uses demes as the number of

tessellation nodes overlayed on the study region and are then used to visualize gene flow in the region as deviations from an isolation by distance model.

2.3.3 Morphological Variation

Morphological variation was quantified using geometric morphometrics. The left side of individuals were photographed and landmarked at 19 points around the body (Figure 1b) in ImageJ (Rasband, 2018). Individuals were separated into age groups. This minimizes the risk that allometric growth would mask important morphological variation in the aligned landmarks within an age group, while having the benefit that it allows for detection of differences within age groups. To correct for dorso-ventral arching, the unbending function from tpsUtil (Rohlf, 2015) was used. Landmarks 1, 7, and 18 (Figure 1b) were specified to the function to form a line down the longitudinal axis, around which to perform the unbending. Unbent landmarks were then aligned using Generalized Procrustes Alignment implemented in the geomorph package (Adams and Otárola-Castillo, 2013). Centroid size was also calculated from these 19 landmarks to provide a measure of overall body size. A PCA was then conducted on the aligned landmarks to reduce dimensionality and remove autocorrelation between landmarks. PCA axes were retained if they explained greater than 10 percent of overall shape variation to best reflect major axes of body variation that may be biologically relevant. This resulted in retaining three shape axes per age group and then for each axis the mean value of individuals from a river for each river was taken. Aligned landmarks were also used for a Procrustes ANOVA to test for significant shape differences between rivers within age groups.

2.3.4 Environmental Data

To quantify environmental characteristics in the rivers, temperature, precipitation, and river axial length were used. Temperature and precipitation data were obtained from the Bioclim database (Fick & Hijmans, 2017) for each location. The 19 Bioclim variables were split into 11 temperature and 8 precipitation variables and a PCA was performed separately for temperature and precipitation. Axes that had eigenvalues greater than one were retained resulting in three temperature variables and two precipitation variables. Axial river length was recorded from Google Maps to use as a proxy of river size, and geographic distance was measured using a least cost distance function constrained to a maximum depth of 100 m below sea level using the package marmap in R (Pante & Simon-Bouhet, 2013). Least cost distance was used to prevent distances using paths that crossed over land.

2.3.5 Environmental and Morphological Associations with Genetic Differentiation and Gene Flow

Genetic data were combined with the morphological and environmental PCA axes in a Bayesian multiple regression and a redundancy analysis to quantify which morphological and environmental factors were associated with genetic distance measures. Geographic distance, morphological variation and environmental variables were standardized to a mean of zero and standard deviation of one and then used in a Bayesian multiple mixed linear regression with varying intercepts in Stan, a programming language to utilize Hamiltonian MCMC, through rstan (Carpenter *et al*., 2017; Stan Development Team, 2018).

$$GD_{i,j} = \sum_{k=1}^{18} c_k \left| V_{k,i} - V_{k,j} \right| + \beta_{19} D_{i,j} + \beta_{21,i} + \beta_{22,j} + \epsilon_{i,j}$$

The absolute value of the difference between each pair of rivers (i and j) environmental and morphological data (V_k) with a separate slope coefficient (β_k) for each variable, and least cost distance (D) with a slope coefficient (β_{19}) were used as predictors along with varying intercepts for each combination of rivers ($\beta_{21,i}$ and $\beta_{22,j}$). F_{ST} and migration rates ($GD_{i,j}$) between rivers were used as predicted variables between rivers in separate regressions. Rivers that were missing an age class had the morphological values imputed within the Bayesian model using a prior distribution of Normal (0,1). Prior distributions for coefficients were a Normal (0,1) distribution. The σ parameter used a prior of a Cauchy (0,1) distribution to allow for a greater likelihood of higher noise in the model than a normal distribution therefore being conservative in the model. Individual river intercepts used a hyperprior, a prior distribution for a parameter's prior distribution, of River effect to draw from with an initial Normal (0,1) distribution. MCMC were run for 2000 warm-up steps and 10 000 iterations with 3 chains. MCMC chain performance was evaluated by ensuring the ratio of within chain to between chain $\hat{R}$ statistic variance was ~1 as well as by examining the trace plots. Redundancy analysis (RDA) was performed using the package vegan (Oksanen *et al*., 2019) as an alternate method to testing the association between genetic distance and environmental and morphological variation. The RDA used principal coordinate analysis axes from microsatellite genotypes as the predicted variables and environmental and morphological variables as predictor variables.

2.4 Results

2.4.1 Sampling and Genotyping

Individuals with fewer than 60 microsatellite loci genotyped were removed from the dataset resulting in 47 individuals being removed. Ten putative brown trout (*Salmo trutta*) and brown trout hybrids were also removed based on outliers in principal component analysis and known brown trout alleles. Individuals were also filtered for severe dorso-ventral arching and other body deformations that would interfere with geometric morphometric analyses. This resulted in 473 individuals being removed leaving 652 age 0+, 593 age 1+, and 400 age 2+ with sample size per river ranging from 3 to 68 (Table 1).

2.4.2 Genetic Structure

To quantify the genetic structure of the southern Newfoundland study region various clustering approaches were used, including Bayesian clustering, θ_{ST}, PCA and a neighbour-joining trees. Bayesian clustering using Structure (Pritchard, Stephens and Donnelly, 2000) indicated that the most likely supported number of clusters was K = 22 based on Evanno's ΔK method (Figure S1) from our 41 rivers. The best K separated many rivers into their own unique cluster, along with a few multi-river and regional clusters (Figure 2a). Consistent with regional hierarchical structuring, a second smaller peak in ΔK was found at K = 3, which grouped Fortune Bay and Bay D'Espoir together, along with separate clusters for both the mouth of Placentia Bay and the head of Placentia Bay (Figure S2). The average genetic differentiation between rivers measured using θ_{ST} was 0.058 (0.007-0.167; Figure 2b). The genetic clustering of rivers was also evident in the neighbour-joining tree (Figure 2d) and PCA (Figure 2c), both of which showed three

major groupings of the same rivers as the clusters shown using Structure results for K = 3. The base of the tree has strong bootstrap support for three river clusters, and the first two axes of the PCA explain 1.8-1.6% of genetic variation and show clusters for Fortune Bay and Bay D'Espoir (red), the mouth of Placentia Bay (orange and dark blue) and the head of Placentia Bay (blue).

2.4.3 Migration Rates

Migration rates estimated by both BIMr (Faubet and Gaggiotti, 2008) and EEMS (Petkova, Novembre and Stephens, 2016) showed low migration between rivers as well as some migration between the two sides of Placentia Bay. Migration rates estimated from BIMr revealed low migration rates between rivers, with an average migration rate between rivers of $4.28*10^{-10}$, the proportion of migrants from a single population per generation, across the three replicates (Figure 3a) which means that the proportion of migrants from any river in one river is $1.71*10^{-8}$ per generation. Migration rates estimated from Estimation of Effective Migration Surfaces (EEMS) indicates that overall, there is low migration out of all rivers. However, the middle of Placentia bay has higher migration (Figure 3b) than expected from an isolation by distance model, and using a mantel test there is a weak but significant fit to an isolation by distance model ($p < 0.001$, $R^2 = 0.255$).

2.4.4 Environmental Variation

Analysis of climatic variables in the region revealed regional geographic clusters represented by locations in Fortune Bay, as well as Placentia Bay at the head and the mouth of the bay. Environmental variables across rivers clustered into broad categories I

characterized: ‘summer temperature’, ‘winter temperature’, ‘climate stability’, and ‘precipitation’ based on Euclidean distances depicted in the dendrogram at the top of the heatmap (Figure 4). PCA was used to reduce co-variation across temperature and precipitation variables. For ‘temperature’ principal components the first three axes retained each explained between 69.8% and 9.65% of the variation for a total of 94.3% for those three axes with eigenvalues of 1.01-1.66. ‘Temperature annual range’, ‘mean diurnal range’, and ‘temperature seasonality’ variables all loaded negatively onto the first temperature PC. ‘Mean temperature of the warmest quarter’, ‘annual mean temperature’, and ‘mean temperature of the driest quarter’ were found to load positively on the second axis, and ‘mean temperature of the wettest quarter’ loaded negatively on the third. The precipitation PCA resulted in two retained axes with 81.4% variation for the first and 14.8% for the second for a total of 96.2% variation explained by these two axes. These axes had eigenvalues of 1.60 and 1.04. The variables ‘annual precipitation’, ‘precipitation of the wettest quarter’, and ‘precipitation of the wettest month’ loaded positively onto the first axis of the PCA for precipitation variables. For the second PCA axis, ‘precipitation seasonality’ and ‘precipitation of the warmest quarter’ were highest loaded negatively and positively respectively.

2.4.5 Morphological Variation

Geometric morphometrics, a Procrustes ANOVA and a principal component analysis (PCA) were used to quantify morphological differences among rivers. A Procrustes ANOVA detected significant differences in morphology among rivers at all age groups (Age 0+: $F_{36, 615} = 12.015$, $p < 0.001$, Age 1+: $F_{34, 558} = 8.3348$, $p < 0.001$, Age 2+: $F_{30, 369} = 8.8694$, $p < 0.001$). In the PCA for each age group, the first axis explained

21.3-30.0% of body shape and displayed potential clustering of geographically proximate rivers along this axis. The morphology explained by this axis was primarily body depth and head size morphology, with some remaining arching artefact. The second axis explained 15.9-19.5% of the body morphology and captured caudal peduncle and head size morphology. The third axis explained 11.0-12.1% of the variation and explained remaining body depth variation.

2.4.6 Bayesian Analysis and RDA

Bayesian analysis and RDA were used to examine associations between genetic structure, environmental variation, and morphology. Climatic and morphological variation were found to be associated with migration rate and genetic distance between rivers. Bayesian multiple mixed regression using genetic distance indicated that all climate and morphological variables, except Age 2+ Shape PC1, were associated with θ_{ST}. The rivers used ($\beta_{21,i}$ and $\beta_{22,j}$) in the comparison had an effect on the intercept of the regression; on average the effect of each river of a pair on θ_{ST} was 0.011. This means that there is an average θ_{ST} of 0.022 between two adjacent rivers given identical climate, morphology, and length (Figure 6a). Among climatic variables temperature PC1 had the greatest effect on θ_{ST}; whereas, PC2, PC3 and precipitation PC1 and PC2 had a lower effect. Morphology, both size and shape, of juveniles Age 0-2+ had an effect on genetic distance. Size, measured using centroid size, was associated with θ_{ST} for Age 0+, Age 1+, and Age 2+. Shape from the first three principal component axes from aligned landmarks was also associated with θ_{ST} for Age 0 PC1-3, Age 1+ PC 1-3, and Age 2+ PC2-3. River axial lengths and least cost distances among rivers had no effect on genetic distance. The same regression was performed with migration rate estimates from BIMr used as

predicted variables. The results were similar with all environmental and morphological variables, apart from Age 1+ Size, found to be associated with migration rate estimates from BIMr. Temperature PC2 had the greatest effect on migration rate among environment and morphology and river had an average effect of 0.229 on migration rate estimates.

To further test for a relationship between genetic distance and climate and morphological variation, an RDA was performed for each age group separately. The RDA indicated that both climate and morphological variation were significantly associated with genetic distance across ages (Age 0: $F_{10, 26} = 4.2245$, $p < 0.001$, Age 1: $F_{10, 24} = 5.0418$, $p < 0.001$, Age 2: $F_{10, 20} = 3.9882$ $p < 0.001$). At Age 0+, Temperature PC1 ($p < 0.001$), Precipitation PC2 ($p = 0.023$), and River Axial Length ($p = 0.013$) were significantly associated with genetic distance. At Age 1+, Temperature PC1 ($p = 0.003$), Precipitation PC1 ($p = 0.005$), River Axial Length ($p = 0.003$) and all morphological variables (Size: $p = 0.020$, Shape PC1: $p = 0.007$, Shape PC2: $p = 0.001$, Shape PC3: $p = 0.001$) were significant, and at Age 2+, Temperature PC1 ($p = 0.010$), Precipitation PC2 ($p = 0.045$), and Shape PC2 ($p = 0.009$) were significant. Across ages the first RDA axis explains 37.0-41.4% of the overall variation, and the second RDA axis explained 20.5-30.7% of the variation. At Age 0+ most environmental variables were aligned to the first axis and Temperature PC3 was the most aligned along the second axis (Figure 7a). At Age 1+ Shape PC2 is aligned to the first RDA axis and the remaining variables are aligned to RDA2 (Figure 7b). For Age 2+, Shape PC1 and PC2, Temperature PC1 and PC2 aligned with RDA1 and the remaining variables with RDA2. The RDA agrees with the Bayesian regression that climate (i.e. Temperature and Precipitation) and morphology

(i.e. size and shape) are associated with genetic distance in southern Newfoundland rivers across ages.

2.5 Discussion

2.5.1 Main Findings and Implications

Understanding the relative importance of evolutionary forces that influence genetic structure in wild populations including selection, drift, and dispersal, can directly inform conservation and management efforts (Allendorf *et al*., 2010; Manel *et al.*, 2010). Here I found that morphological and climatic variation among populations of Atlantic Salmon (*Salmo salar*) in southern Newfoundland was associated with genetic divergence (F_{ST}) and relative levels of connectivity (migration rates) across a small geographic region. This work directly builds on other landscape genetic studies in Atlantic Salmon which have identified associations with climatic or habitat variables (Ozerov *et al.*, 2012; Bourret *et al.*, 2013a; Bradbury *et al.*, 2014; Sylvester *et al.*, 2018a), and also extends them through the identification of phenotypic associations with structure and gene flow. Understanding phenotypic associations with structure can aid in understanding the potential response to fishing pressures, aquaculture introgression, and watershed changes.

2.5.2 Spatial Population Structure

Our results suggest that hierarchical spatial structuring in this region is broadly characterized by three regional groups Fortune Bay and Bay D'Espoir, West Placentia Bay and the head of the bay, and East Placentia Bay and the tip of the Burin Peninsula across the Bay (Figure 2). Nested within these groups is finer genetic structure at the scale of individual rivers (Figure 2). A previous study of this region by Bradbury (2015) compared resolution from 15 microsatellites, 5568 SNP loci, and 8495 SNPs from RAD

sequencing after filtering and found K = 2-3 clusters. Earlier, Bradbury *et al*. (2014) found evidence of hierarchical structuring across Newfoundland and Labrador with support for K = 20 clusters based on data from 15 microsatellites although K = 3 was found most likely. In this study I detected finer structure with the most supported number of groups K = 22 within our much smaller study area; however, a break similar to the one I observed at the tip of the Burin peninsula was detected by Bradbury *et al*. (2015) with our K = 3 although this has a lower likelihood than K = 22. Our study demonstrates the utility of larger panels of microsatellites to resolve genetic structure at fine geographic scales (Bradbury *et al*. 2018).

At broader spatial scales Atlantic Salmon population structure has been examined finding similar or lower levels of structuring than found here in southern Newfoundland, though other studies typically used smaller microsatellite panels (Cauwelier *et al.*, 2018b; Dillane *et al.*, 2008; Olafsson *et al.*, 2014; Vähä *et al*., 2007; Wennevik *et al.*, 2019). Near the northern limit of salmon range, Sylvester *et al*. (2018a) found K = 2 across Labrador with the same 101 microsatellite panel showing rivers within Lake Melville to be genetically distinct from coastal populations. Vähä *et al*. (2007) found K = 3 clusters using 32 microsatellites within the Teno River in Norway and structure within these groups resulted in a total of 13 clusters. Population structure has also been examined at broader spatial scales using 9-18 microsatellites in Scotland (Cauwelier *et al.*, 2018b), Ireland (Dillane *et al.*, 2008), Iceland (Olafsson *et al.*, 2014) and Norway (Wennevik *et al.*, 2019), with observed hierarchical structuring ranging from K = 2-9 with further nested structuring. Overall, I was able to find genetic structure at finer scales using more markers than previous studies.

2.5.3. Structuring Factors

Landscape features have been shown to influence genetic population structure in many species, for example, Eucalyptus population structure is influenced by water and nutrient availability (Murray *et al.*, 2019), the Mississippi Slimy Salamander (*Plethodon mississippi*) by land use (Burgess and Garrick, 2020), a desert rodent *Dipodomys merriami* by vegetation (Flores-Manzanero *et al.*, 2019), and the American Marten (*Martes americana*) by forest and land cover and elevation (Aylward, Murdoch and Kilpatrick, 2020). Previous studies in Atlantic Salmon have shown climate associated genetic structure, but here I additionally find morphological variation associated with genetic structure while accounting for climate, geographic distance, and river length. I found that temperature had the greatest association with gene flow, while precipitation and juvenile size and shape were also associated with lower effect and least-cost geographic distance between rivers was not associated with gene flow.

Climatic variation holds the potential to drive population structuring through selection against maladapted immigrants or their offspring posing a barrier to successful migration resulting in surviving offspring. Our results suggest that climate (i.e. temperature and precipitation) explains a significant component of variation in population structuring in this region. Similar results have been found in St. Lawrence River populations (Dionne *et al.*, 2008; Bourret *et al.*, 2013b), across the North American east coast (Bradbury *et al*., 2014; Jeffery *et al*., 2017; Sylvester *et al*., 2018a), and Europe (Horreo *et al.*, 2011). Temperature-related adaptation of salmon at greater geographic scales has also been reviewed in Garcia de Leaniz *et al*. (2007) and Taylor (1991), and may involve selection on developmental stages, metabolism, and growth, or immune

functions. Of particular note, temperature has also been shown to be associated with population declines across the natural range of Atlantic Salmon, particularly warmer winter temperatures (Lehnert *et al.*, 2019b). Given the correlation between temperature and precipitation, it is feasible that these temperature-related adaptations may also underly the population structure attributed to precipitation, although adaptations to other watershed characteristics, such as water velocity, or consistency of flow, cannot be discounted particularly with the morphological associations demonstrated in this study (Garcia de Leaniz *et al.*, 2007). These adaptations indicate that differences in temperature and precipitation may pose significant barriers to gene flow through selection and thus contribute to the genetic structuring in the region. Given this, future climate change may significantly alter existing population structure and making natal rivers unideal habitats and may impact recolonization of extirpated populations.

Morphology has the potential to contribute to genetic structuring through the interaction of selection and gene flow (Garcia de Leaniz *et al.*, 2007; Tigano and Friesen, 2016). I found that there were significant associations between morphological divergence and genetic differentiation. In salmonids, intraspecific variation in morphology often reflects adaptations to the local watershed either through associations between body shape and water velocity, or head and jaw morphology and trophic adaptations (Taylor, 1991; Garcia de Leaniz *et al.*, 2007). While interactions between morphology and genetic structure in salmon have previously been uncharacterized, it has been found in another salmonid, Arctic Charr (*Salvelinus alpinus*), with divergence in morphs that correspond to trophic adaptations correlated with genetic differentiation (Gíslason *et al*., 1999). This relationship can be explained by either high gene flow homogenizing heritable

components of morphology, or maladapted morphology may be selected against and reduce effective migration. This relationship has been shown in other fish species such as Threespine Stickleback (*Gasterosteus aculeatus*) (Sharpe *et al*., 2008), European Minnows (*Phoxinus phoxinus*) (Collin and Fumagalli, 2015), perch (Bergek and Björklund, 2009), and the inland silverside (*Menidia beryllina*) (Fluker *et al*., 2011).

While morphology has not been characterized in relation to genetic structure elsewhere, juvenile habitat has been found to influence structuring in Atlantic Salmon and may relate to morphological divergence. Geological characteristics such as river substrate has been examined to correlate with genetic divergence in the Gulf of Saint Lawrence (Bourret *et al.*, 2013b) and France (Perrier *et al.*, 2011). River water characteristics have also been shown to influence population structure (Dillane *et al.*, 2008; Ozerov *et al.*, 2012; Bowlby *et al.* , 2016). River characteristics were not measured in this study but can influence juvenile morphology (Garcia de Leaniz *et al.*, 2007).

Our results show low gene flow between rivers and that the observed patterns do not follow a simple isolation by distance (IBD) model but reflect adaptive processes at this spatial scale. While our Mantel test for isolation by distance was significant, it had poor explanatory power for genetic variation in the region, and when temperature and precipitation were accounted for in the Bayesian model geographic distance was not associated with genetic distance (Figure 6). This may be a result of autocorrelation between climate differences and geographic distance resulting in climate adaptations producing the same pattern as IBD (Jenkins *et al.*, 2010). This relationship breaks down in this region with rivers at the mouth of Placentia Bay being genetically similar, and of similar temperature while being geographically distant. IBD has also been found to be

more prevalent in ectotherms than endotherms likely due to the thermal requirements of ectotherms and the correlation between distance and temperature (Jenkins *et al.*, 2010). In addition, patterns of isolation by environment are increasingly found to be common (Sexton *et al*. 2013).

2.5.4 Limitations

Although our analyses have approached the relationship from the perspective of climate adaptation and morphology driving genetic differentiation, causality may run in the other direction. In the case of morphology, greater gene flow exchange could result in the exchange of more alleles associated with body form and therefore these populations may have similar morphology. This is hard to distinguish from a scenario in which watershed adaptations represented by morphological differences present a barrier to effective migration. It is also important to highlight that the issue of non-independence of residuals in the Bayesian regression may affect the parameter estimates found (Yang, 2004); this may change the posterior probabilities of the variable coefficients, however, this is not a factor in the redundancy analysis, and both analyses show comparable results so it is unlikely to have a large effect on our results. Dorso-ventral arching could have masked morphological variation even when individuals with severe arching were filtered from the data set and a bending correction method was used (Valentin *et al*., 2008). Overall, our geometric morphometrics captured important morphological variation with known adaptive effects such as body depth and head size reflecting water velocity adaptation and trophic variation respectively (Taylor, 1991; Garcia de Leaniz *et al.*, 2007).

2.5.5 Implications for Conservation

The relationship between gene flow, climate and morphology are important to consider in future management strategies for Atlantic Salmon populations. Southern Newfoundland populations examined here are managed as a single designatable unit, and have been assessed as "Threatened" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), the nongovernmental body responsible for identification of species at risk, and managed as a single designatable unit (COSEWIC, 2011). To better aid recovery of populations, it is important to understand patterns of connectivity among populations and the factors that drive the patterns. Knowing which populations may contribute appropriately adapted immigrants, populations with similar climate and morphology, may help forecasts of possible recovery timelines. Understanding of forces that drive population structure also can help management in identifying threats to populations. Understanding the influence of climate on population structure may help predictions of these impacts and even lead to mitigation strategies.

2.5.6 Final Summary

Overall, the population structure of Atlantic Salmon in southern Newfoundland is associated with climate and morphology at a fine geographic scale. Adaptations to the watershed climate and morphology changes in response to watershed characteristics being associated with genetic structure show the importance of fine scale river-level differences between populations. Further work is needed to identify the direction of this relationship between gene flow and morphology and if there are loci under selection related to morphology that show this structuring.

Table 1: Sample counts, after filtering, of Atlantic Salmon (*Salmo salar*) by river in southern Newfoundland. Ages were determined using fork length-based aging.

River	River Code	Age 0+	Age 1+	Age 2+
Bay de l'Eau	BDL	12	37	9
Bay Du Nord	BDN	57	32	0
Big Salmonier Brook	BSB	5	35	16
Black River	BLA	10	35	15
Bottom Broom	BTB	32	5	23
Branch River	BRA	0	42	18
Cape Roger Brook	CRB	10	35	15
Come by Chance River	CBC	9	34	16
Conne River	CNR	16	0	0
Cuslett Brook	CUS	20	6	35
Dollards River	DLR	56	10	11
Fair Haven Brook	FHB	15	22	22
Garnish River	GAR	41	4	0
Grand Bank Brook	GBB	11	12	0
Great Barasway Brook	GBW	10	21	29

River	River Code	Age 0+	Age 1+	Age 2+
Lamaline River	LAM	58	3	0
Lance River	LAN	2	2	27
Lawn River	LAW	10	36	13
Little Barasway Brook	LBB	1	2	7
Little River	LTR	57	0	0
Long Harbour River	LHR	18	39	0
Malbay Brook	MAL	0	5	8
Nonsuch River	NON	10	35	14
North Harbour River	NHR	10	37	11
North West River, Fortune Bay	NWR	54	1	2
Northeast Placentia River	NEP	10	23	27
Northeast River	NEB	0	5	0
Northwest Brook, Mortier Bay	NWB	17	32	11
Old Bay Brook	OBB	31	0	16
Piercey's Brook	PIE	13	41	5
Piper's Hole	PIP	9	33	15
Red Harbour River East	RHE	9	35	14

River	River Code	Age 0+	Age 1+	Age 2+
Red Harbour River West	RHW	20	36	5
Rushoon River	RUS	10	35	15
Sandy Harbour River	SHR	51	7	2
Ship Harbour Brook	SHB	9	27	20
Simm's Brook	SIM	56	2	5
Southeast Brook	SEB	0	2	47
Southeast Placentia River	SEP	10	22	22
Tailrace Brook	TRB	30	0	0
Taylor Bay Brook, Burin Peninsula	TAY	9	13	20
Tides Brook	TID	10	37	13

Table 2: Eigenvalues and proportion of variation for principal component analysis (PCA) and redundancy analysis (RDA) performed. For PC axes, only those used in analyses and figures are presented. All RDA axes, from each RDA are presented.

Analysis	Proportion of Variation	Eigenvalue
Microsatellite PCA		
PC1	0.0176	1.9510
PC2	0.0159	1.7620
Temperature PCA		
PC1	0.6977	7.6748
PC2	0.1490	1.6385
PC3	0.0965	1.0614
Precipitation PCA		
PC1	0.8144	6.5150
PC2	0.1479	1.1829
Shape PCA Age 0+		
PC1	0.2999	0.0004
PC2	0.1948	0.0002
PC3	0.1143	0.0001

Analysis	Proportion of Variation	Eigenvalue
Shape PCA Age 1+		
PC1	0.2130	0.0002
PC2	0.1584	0.0002
PC3	0.1211	0.0001
Shape PCA Age 2+		
PC1	0.2759	0.0004
PC2	0.1879	0.0003
PC3	0.1104	0.0002
RDA Age 0+		
RD1	0.4138	0.2431
RD2	0.2052	0.1206
PC1	0.2528	0.1485
PC2	0.12814	0.07528
RDA Age 1+		
RD1	0.3700	0.1899
RD2	0.3075	0.1578
PC1	0.2359	0.1211

Analysis	Proportion of Variation	Eigenvalue
PC2	0.08658	0.04444
RDA Age 2+		
RD1	0.4139	0.2282
RD2	0.2522	0.1390
PC1	0.2598	0.1433
PC2	0.07417	0.04090

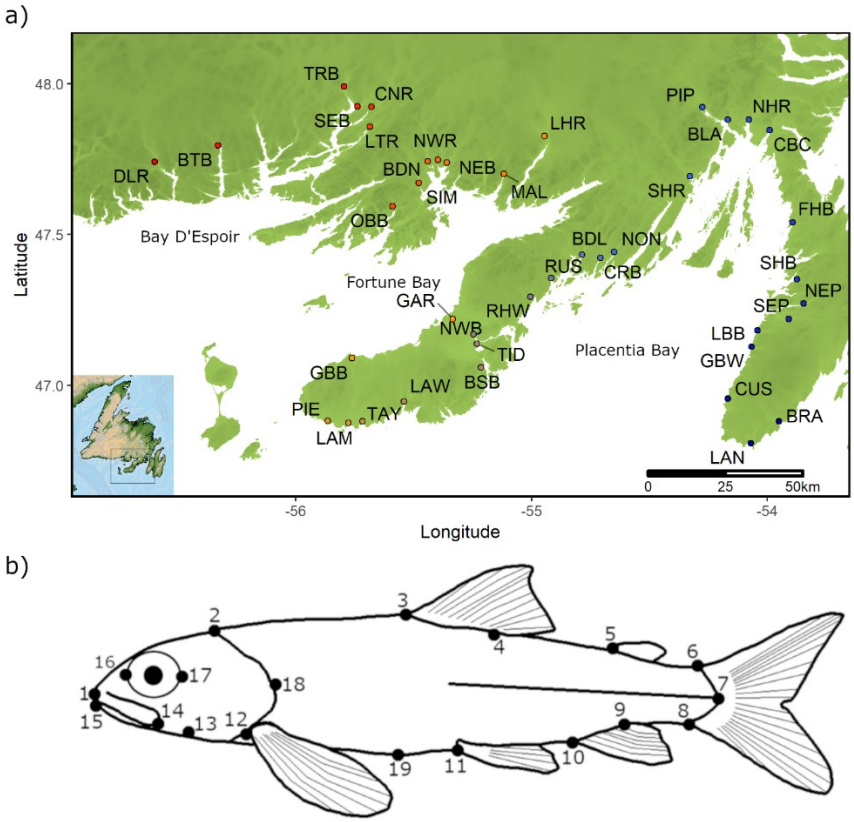

Figure 1. a) Map of sampling locations coloured Red-Blue, West-East, following the coastline for ease of showing geographic location in later figures. Juvenile Atlantic Salmon (*Salmo salar*) aged 0-2+ were collected using electrofishing. Refer to Table 1 for number of samples of each age group collected per river, and river names. b) 19 landmarks around the body used for geometric morphometrics to quantify morphological variation, see Table S1 for description of landmark locations.

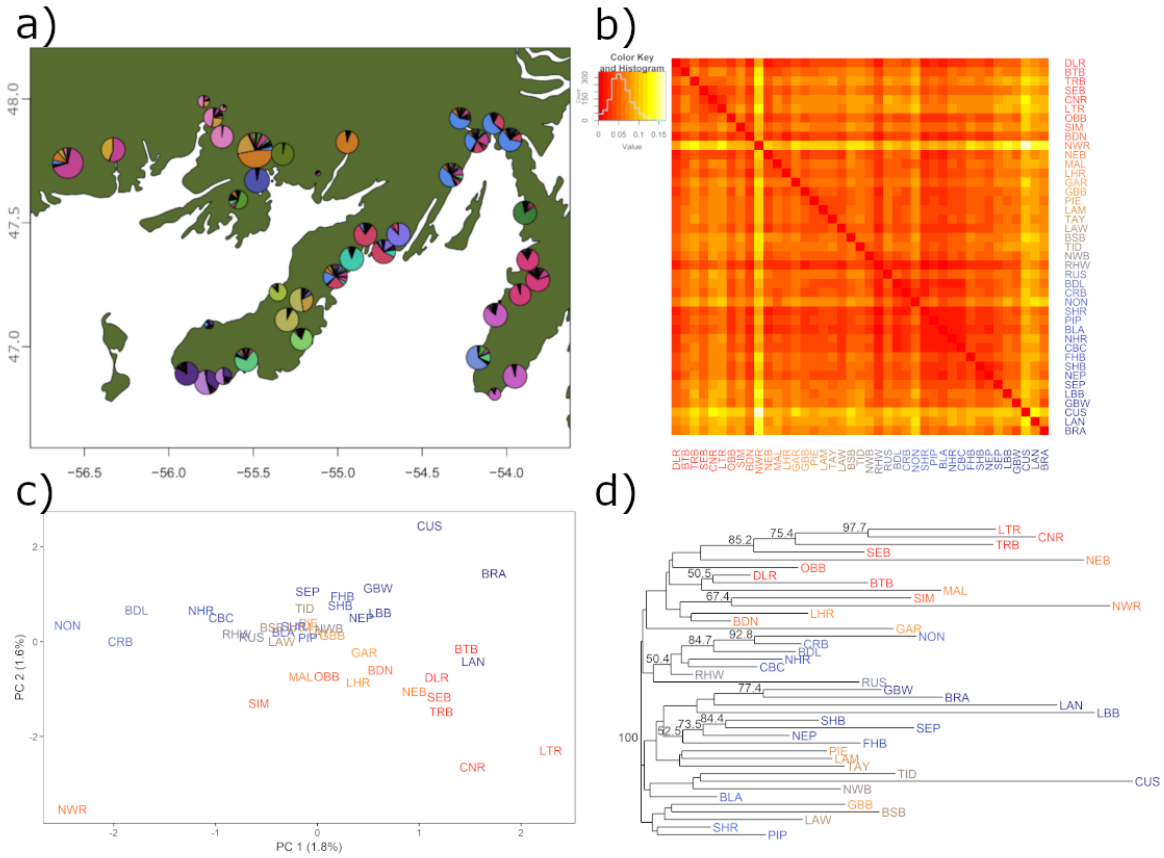

Figure 2. Genetic variation in southern Newfoundland Atlantic Salmon (*Salmo salar*). a) Proportion of genetic cluster per river from structure results with circle size proportional to sample size. Evanno's (Evanno, Regnaut & Goudet, 2005) method showed greatest support for K = 22 genetic clusters for this region. b) Heatmap of θ_{ST} matrix with rivers ordered West-East along coastline. River names have been coloured Red-Blue to denote West-East as in Figure 1a. c) Principal Components Analysis (PCA) of microsatellite genotypes with river abbreviations coloured Red-Blue, West-East along coastline. d) Neighbour-Joining tree using Nei's distance with 1000 bootstraps and >50 bootstrap support shown, tip labels coloured Red-Blue, West-East along coastline.

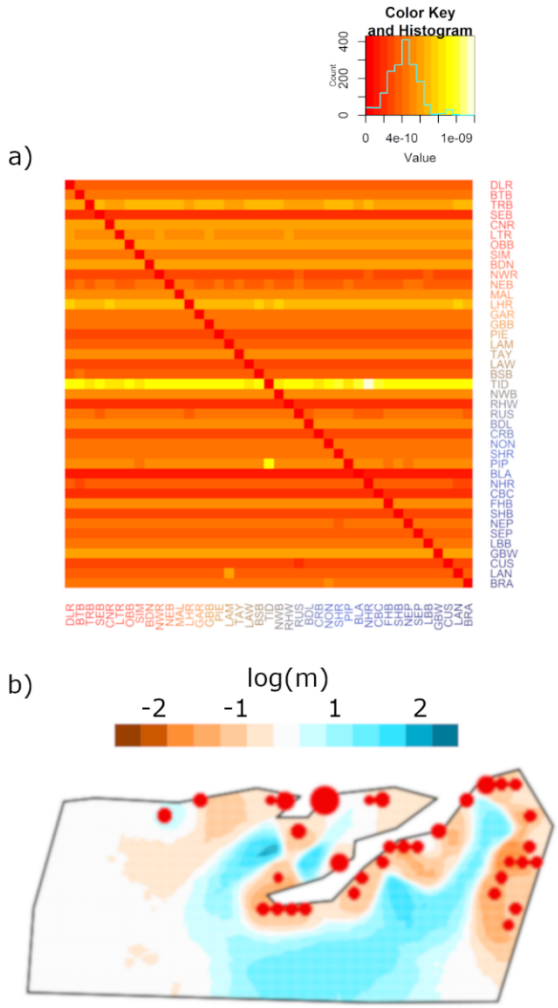

Figure 3. Estimates of Atlantic Salmon (*Salmo salar*) gene flow in southern Newfoundland. a) Heatmap of migration rate estimates from BIMr after 10 000 iterations and 3 replicates, rivers are ordered West-East along coastline. Diagonal migration rates were reduced to 0 to better show migration between rivers. b) Map of areas with migration rates that deviate from an isolation by distance model (IBD) estimated by Estimation of Effective Migration Surfaces (EEMS). Red dots indicate sample locations after placement on tresselation grid and size of samples per deme. Warm colours indicate regions of lower migration rates than expected by IBD and cold colours indicate areas of higher than expected migration rates than expected by IBD.

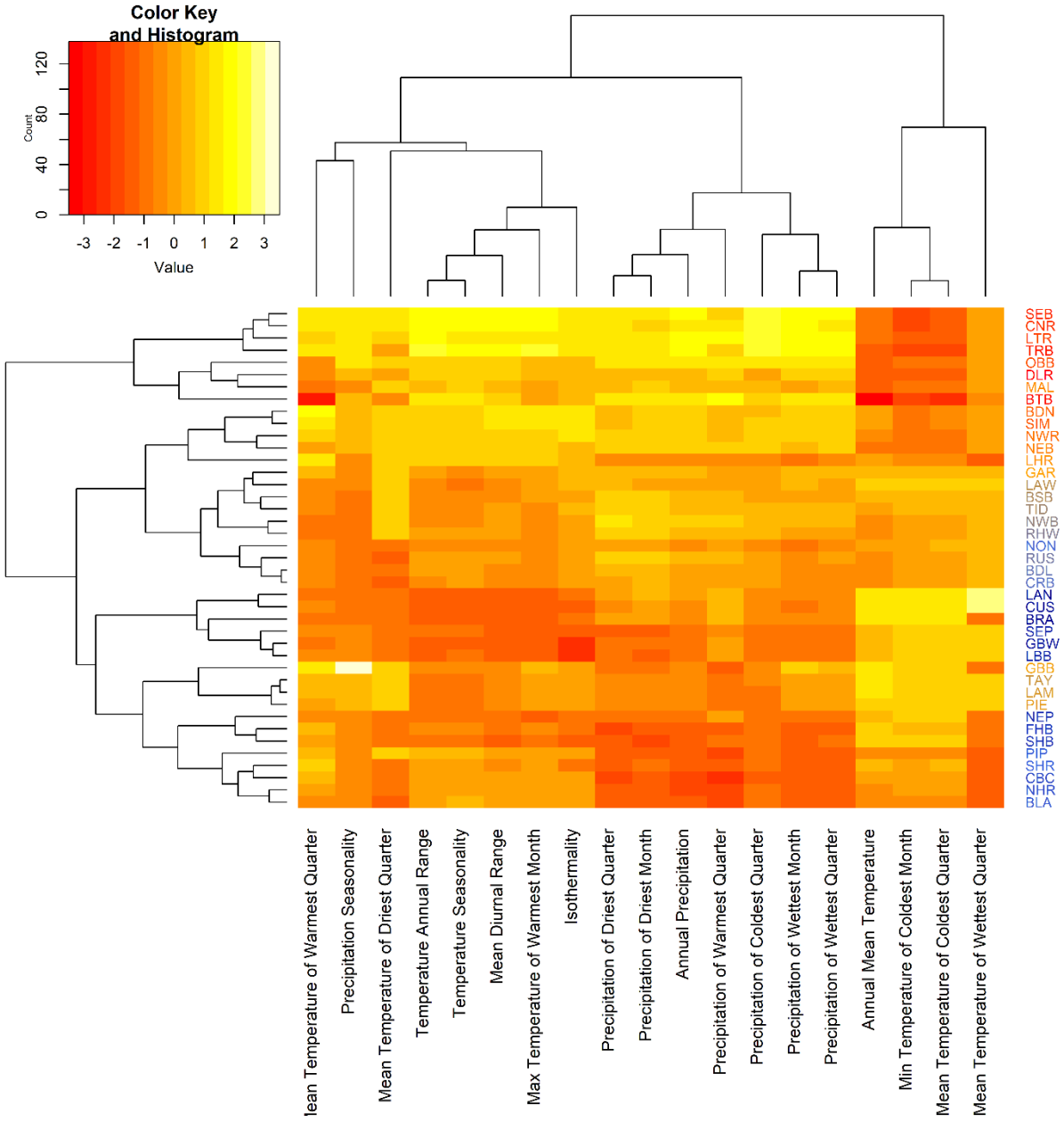

Figure 4. Heatmap of climate data with rivers and environmental variables obtained from BioClim database to obtain eleven temperature and eight precipitation variables and three axes from a principal component analysis (PCA) using the temperature variables and two axes from a PCA using precipitation variables. Dendrograms constructed using Euclidian distance for both environmental variables and rivers.

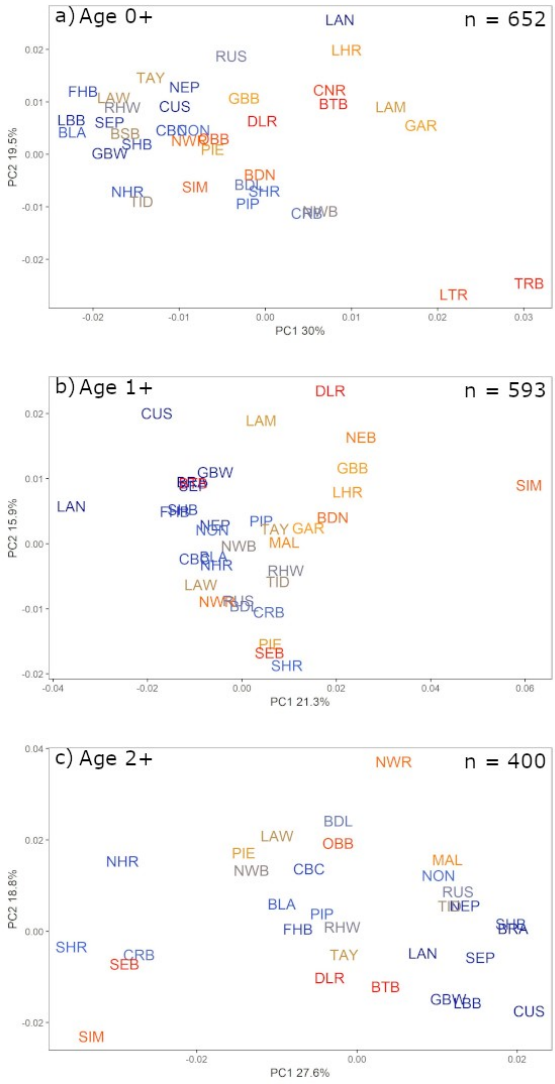

Figure 5. A Principal Component Analysis (PCA) of morphological variation in southern Newfoundland Atlantic Salmon (*Salmo salar*) based on geometric morphometrics of the 19 landmarks from Figure 1b. Rivers are coloured Red-Blue, West-East along coastline. a) Mean morphology PC per river for Age 0+ individuals based on fork-length aging. See Figure S3 for relative warps. b) Mean morphology PC per river for Age 1+ individuals based on fork-length aging. See Figure S4 for relative warps. c) Mean morphology PC per river for Age 2+ individuals based on fork-length aging. See Figure S5 for relative warps.

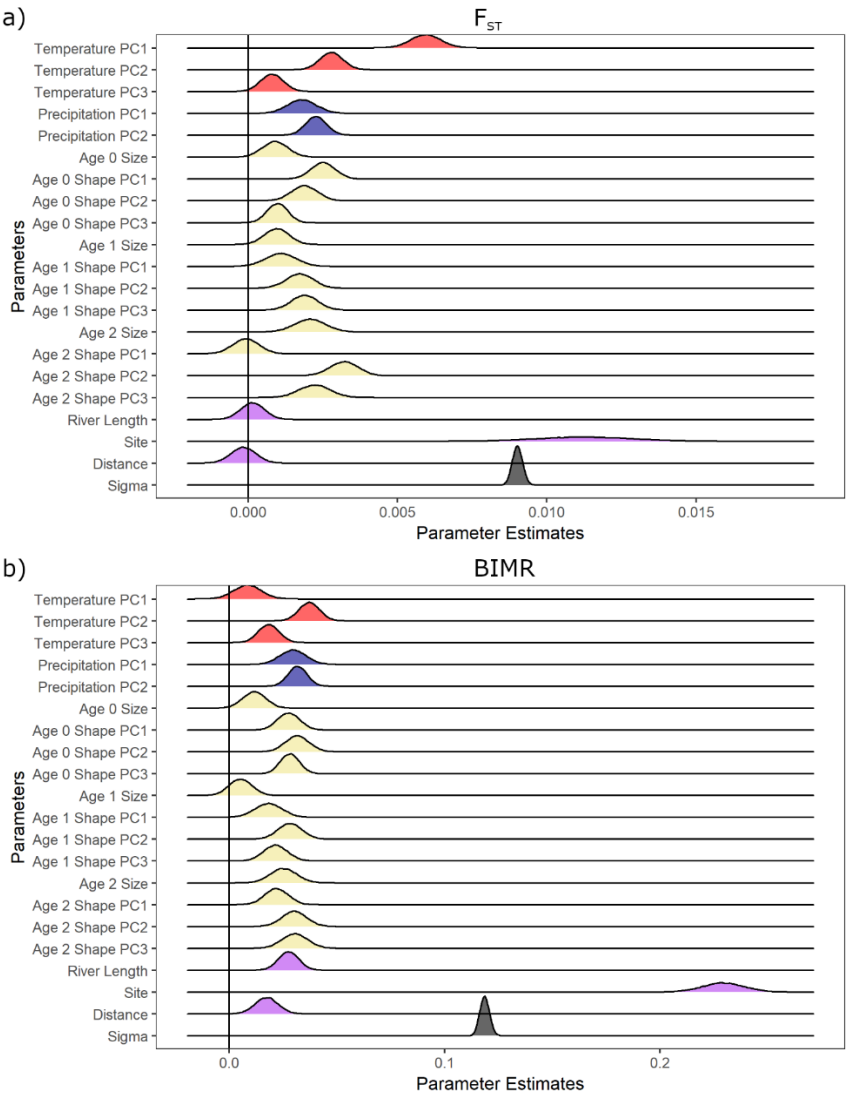

Figure 6. Posterior distributions of the slope coefficient for each variable used in a Bayesian multiple mixed regression estimating the association between morphological and environmental variation while controlling for geographic distance and site effects. Sigma is the noise around the regression. a) θ_{ST} was used as the genetic distance measure for the predicted variable in the Bayesian multiple mixed regression. b) Migration rate estimates from Bayesian Inference of Migration rates (BIMr) were used as the genetic distance measure for the predicted variable in the Bayesian multiple mixed regression.

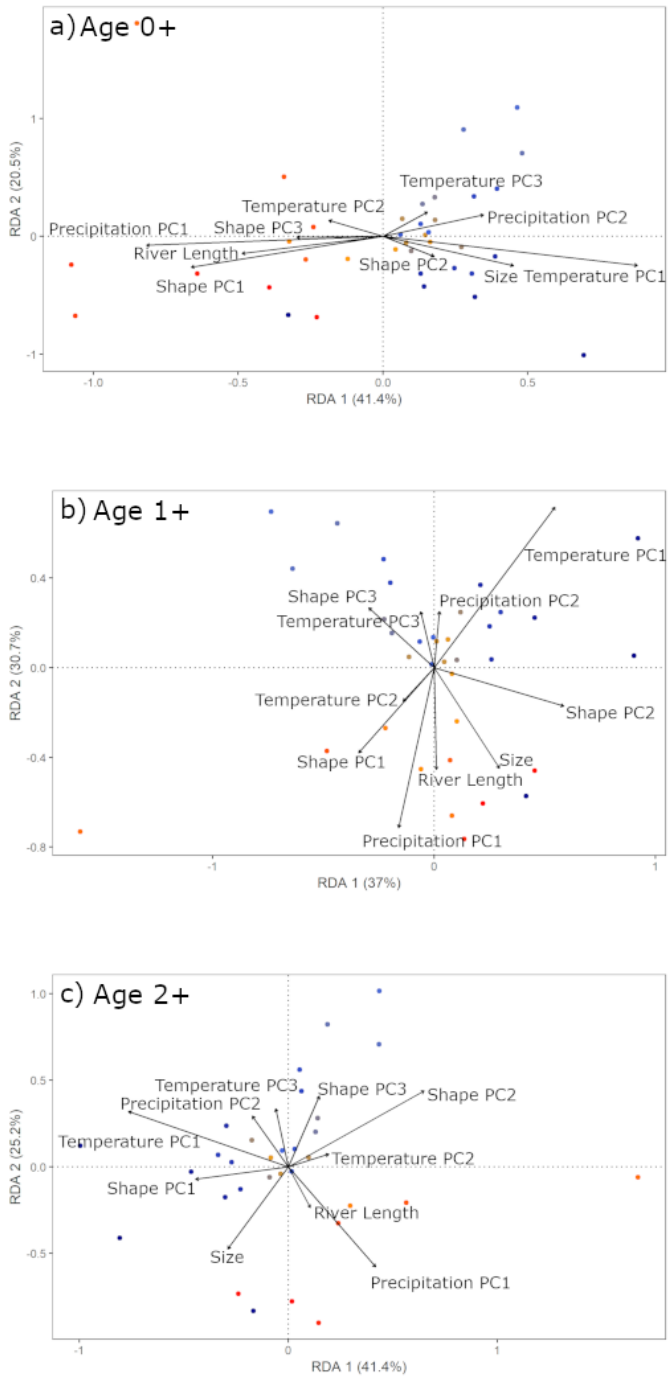

Figure 7. A redundancy analysis (RDA) using a Principal Coordinate Analysis (PCoA) of microsatellite genotypes for predicted variables and environmental and morphological variables for predictor variables separated by Atlantic Salmon juvenile morphology. a) Age 0+, b) Age 1+, c) Age 2+.

Chapter 3 – Morphological Consequences of Hybridization Between Farm and Wild Atlantic Salmon, *Salmo salar*, Under Both Wild and Experimental Conditions

3.1 Abstract

The escape of aquaculture salmon has been identified as a significant threat to the persistence and stability of wild Atlantic Salmon (*Salmo salar*), yet the magnitude of phenotypic impacts due to hybridization remain unresolved. I evaluated the phenotypic consequences of hybridization using geometric morphometrics both under natural conditions in the wild and in the laboratory using common garden experiments. Field collected juvenile Atlantic Salmon from 2015 and 2016 from 18 southern Newfoundland rivers were classified as wild, farmed, or F_1 hybrids using a diagnostic panel of 95 single nucleotide polymorphisms. Overall size and shape differences between wild and farm, and wild and F_1 hybrid individuals were small, largely size related, and present between pure farm and other crosses. Laboratory-reared pure wild, pure farm, and F_1 hybrid salmon were grown in tank and semi-natural conditions. Wild fish were significantly larger than both farmed and hybrid salmon at first feeding. While these differences in size remained at 80-days post first feeding under semi-natural conditions, they disappeared in tank conditions, and there were no differences between pure farm and hybrid individuals under either condition. Significant shape differences were present among all pairwise comparisons under tank conditions, and in semi-natural conditions pure wild individuals differed significantly from pure farm and hybrid individuals. Our results suggest phenotypic differences observed under laboratory conditions between wild and farmed-wild hybrid individuals may be less apparent in the wild and that significant genetic changes may occur in wild populations experiencing hybridization in the absence of any obvious phenotypic changes.

3.2. Introduction

3.2.1 Aquaculture Escapees and Threat of Introgression

The escape of domesticated Atlantic Salmon (*Salmo salar*) represents a significant threat to the persistence and stability of wild salmon stocks (Forseth *et al*., 2017, Glover *et al*., 2017). Domesticated salmon commonly escape aquaculture pens into the local environment (Verspoor *et al*., 2015, Keyser *et al*., 2018, Diserud *et al*., 2019, Glover *et al*., 2019) and despite the observed reduced mating success of domesticated salmon compared to wild salmon, there is widespread evidence of hybridization in the wild (Glover *et al*., 2013, Karlsson *et al*. 2016, Sylvester *et al*., 2018b, Wringe *et al*., 2018). Hybrid individuals can display reduced fitness due to outbreeding depression, thus the potential for introgression from domestic salmon into wild salmon populations is a concern if escape events continue to occur (Jonsson & Jonsson, 2006, Hutchings & Fraser, 2008, Fraser *et al*., 2010). These fitness concerns, and the magnitude of aquaculture production compared to wild salmon abundance have led to aquaculture escapees being identified as a central threat to the stability and persistence of wild salmon populations (Forseth *et al*., 2017). Although hybridization and genetic introgression between wild and escaped domestic salmon has been well documented on both sides of the North Atlantic, the resultant biological consequences remain poorly understood with few studies documenting the biological impacts in wild (see Bolstad *et al*., 2017 and Sylvester *et al*., 2018b).

3.2.2 Domestication Phenotype

The process of domestication in fish has been shown to result in convergent phenotypic divergence from their wild conspecifics; a suite of common, directional,

differences in phenotypic traits are brought about by domestication across many species (Wringe, Purchase and Fleming, 2016). In Atlantic Salmon, domesticated individuals often display smaller heads (Wringe, Purchase and Fleming, 2016), greater body depth than wild salmon (Wringe, Purchase and Fleming, 2016), as well as changes in the caudal peduncle shape (Fleming *et al*., 1994). Changes from the wild type in these traits are likely maladaptive in the wild as salmon morphology likely reflects local adaptations to water velocity and other environmental conditions (Taylor, 1991; Jonsson, 1997; Pakkasmaa & Piironen, 2001). This may be the impact of reduced survival of hybrid individuals both in fresh water and marine environments (Fleming *et al.*, 2000; Skaala *et al.*, 2012; Sylvester *et al.*, 2019), however, the degree to which hybrid phenotype is shaped by selection and plasticity remains unclear. Glover *et al.* (2018) found that the lack of growth differences between wild and domestic salmon in natural environments is explained mostly by plasticity, and not growth-dependent selection. As such, the shape differences observed under aquaculture conditions may also be masked when reared in a natural environment due to the plastic response of an individual's shape to its environment (von Cramon-Taubadel *et al*., 2005). Improved understanding of the phenotypic effects associated with hybridization between wild and domestic salmon in the wild is needed to better predict interactions, impacts, and the consequences of escape events on wild populations.

3.2.3 Newfoundland Atlantic Salmon Vulnerability

Southern Newfoundland is characterized by extensive Atlantic Salmon aquaculture activities (DFO, 2013), as well as increasingly declining wild stocks (COSEWIC, 2011). Aquaculture salmon in the region originally derive from Saint John

River salmon, which differ genetically from southern Newfoundland wild salmon (Jeffery *et al.*, 2018) and as such escapees and hybrids have been identified using genetic markers (Keyser *et al*., 2018; Wringe *et al*., 2019). Surveys and monitoring for aquaculture escapees regularly detect escapees in the wild both in the presence and absence of reported escape events (Keyser *et al*., 2018; Sylvester *et al*., 2018b; Wringe *et al*., 2018). Recent work has explored the impact, in terms of the numbers of hybridization events, of a large escape event which released approximately 20 000 salmon; an amount equal to estimated abundance of wild salmon in the area (Keyser *et al*., 2018; Wringe *et al*., 2018). While the distribution and number of hybrids produced following this escape event has been investigated, the impact of hybridization on phenotype, growth, and other fitness-related traits remains unstudied.

3.2.4 Objectives

The overall objective of this study was to explore the magnitude and nature of phenotypic differences resulting from hybridization between pure wild and pure farm individuals using geometric morphometrics. Specifically, I first examine shape differences among genetically identified wild, farmed, and hybrid individuals produced in the wild collected in southern Newfoundland. Second, I used a common garden experiment to compare pure wild, pure farm, and hybrid salmon in aquaculture-like tank conditions and semi-natural (i.e. stream channel) conditions. The comparison of individuals from field collections, semi-natural, and tank experimental conditions is an exceptional opportunity and provides an exemplary gradient across which phenotypic effects may be examined. This study builds directly on previous studies exploring the prevalence of hybridization between pure wild and pure farm salmon in southern

Newfoundland (Sylvester *et al*. 2018b; Wringe *et al*. 2018) and differences in survival between wild, feral and hybrid individuals in the region (Sylvester *et al*., 2019). Moreover, I complement a recent study exploring phenotypic differences due to hybridization in the Northeast Atlantic (Glover *et al.*, 2018).

3.3. Methods

3.3.1. Field Sampling and Hybrid Screening

Juvenile Atlantic Salmon (*Salmo salar*) parr (n = 3823) were caught by electrofishing in 18 rivers in Fortune Bay and Bay D'Espoir, Newfoundland in 2015 and 2016 (Figure 8, Table S3). The juvenile salmon caught in southern Newfoundland should be genetically like their respective common garden cross type (see below) because the aquaculture salmon used for the common garden cross is from the same Saint John River-derived strain that escaped into the rivers. Parr were aged based on fork length (Sethi *et al*., 2017), and only 0+ or 1+ individuals were analyzed due to the low number of age 2+ pure farm and hybrid individuals caught. Fin clips were taken from the right pectoral fin and stored in 95% ethanol for genotyping. Individuals were genotyped at 95 SNP loci using a custom Fluidigm EPI array (see Wringe *et al*., 2019 for SNP selection) following the manufacturer's protocol using 96.96 genotyping Integrated Fluidic Circuits (IFC) and read on an EP1 (Fluidigm) then analyzed using SNP Genotyping Analysis software (Fluidigm). Each 96 well plate contained 10 redundant samples and positive controls. Hybrid class of individuals were estimated using *parallelnewhybrid* with the s parameter and the package hybriddetective (Wringe *et al*., 2017; Wringe *et al*., 2018). *Parallelnewhybrid* executes the program newhybrids (Anderson and Thompson, 2002) in parallel utilizing multicore processors to increase the speed of analysis.

Parallelnewhybrid was run with a burn-in of 50 000 with 100 000 sweeps. A cut-off posterior probability of >0.8 in a single hybrid class was used to assign individuals (Wringe *et al*., 2019).

3.3.2. Common Garden Experiment

Wild Atlantic Salmon individuals were collected from Northeast Placentia River and farmed salmon were sourced from the West Atlantic principle aquaculture strain derived from the Saint John River. Salmon crosses generated 44 families of three cross types: 20 pure Saint John River farm families, 13 one direction F_1 hybrid families (female farm x male wild), and 11 pure Northeast Placentia wild families. Before being placed in their rearing environment, each fry was anaesthetized using MS-222, and tagged with elastomer for individual identification. Individuals were raised in common garden experiments with pure wild, pure farm and F_1 hybrid crosses raised in semi-natural (i.e. stream) and tank conditions. For each group (wild, farmed, hybrid) there were 10 individuals with 12 replicates per environment. After absorption of the yolk-sac (Day 0), fry were randomly selected from rearing tanks to be reared in either tank or semi-natural conditions. Tanks were made of plexiglass and measured 0.32 m x 0.24 m x 0.16 m, while the semi-natural conditions consisted of stream tanks measuring 1.2 m x 0.22 m x 0.15 m with a constant flow rate of 10-15 $cm \bullet s^{-1}$ and a gravel substrate. Salmon in tanks were fed salmonid starter dry feed (EWOS-Cargill, BC, Canada) four times daily, and salmon in semi-natural conditions were fed live *Artemia* four times daily. Both tank and semi-natural conditions received 12hrs of light daily and had a dissolved oxygen level of 8.03 $mg \bullet l^{-1}$.

3.3.3. Shape Analysis

Geometric morphometrics was used to quantify shape differences among wild, farm and hybrid salmon using 19 landmarks, from photographs of individuals on their left side (Figure 8). Individuals in the common garden experiment were photographed on Day 0 and 80 post yolk sac absorption and all field sampled individuals were photographed after capture. A total of 205 Day 0 and 179 Day 80 individuals were analyzed after filtering photos for image quality, arching, and any other deformations (Table S2). Of the field samples genotyped, after filtering for image quality, arching and deformations, 1328 were landmarked for geometric morphometrics. All individuals assigned to second generation hybrid class (F_2, or backcross) were removed from the analysis because the low number of individuals caught prevented meaningful statistical analysis. This left 964 samples for geometric morphometric analysis from 18 rivers aged 0+ or 1+, and assigned to Pure Wild, Pure Farm, or F_1 hybrid genotype frequency classes (Anderson and Thompson, 2002) (Table S3).

Landmarking was done using ImageJ (Rasband, 2018) and landmarks were corrected for body arching using tpsUtil unbend function along landmarks 1, 7, and 18 (refer to Figure 8) (Rohlf, 2015). A Generalized Procrustes Analysis was performed using the geomorph package in R (Adams and Otárola-Castillo, 2013), and a principal component analysis (PCA) to look at the greatest shape variation and a canonical variate analysis (CVA) to identify between group variation using the package Morpho (Schalger, 2017). The first two principal components were plotted to identify areas of shape variation. To determine if overall shape differences were present, a Procrustes ANOVA was performed on the aligned shape coordinates between cross type using the package

geomorph (Adams and Otárola-Castillo, 2013). Centroid size was calculated as a measure of overall body size of individuals. Body depth was measured as the linear distance between landmarks 3 and 17. An ANOVA was performed on both body depth and centroid size to identify significant differences between wild, farm and F_1 cross types (Goodall, 1991) using Tukey's multiple comparison test for comparisons among types. To identify differences in allometric growth a comparison of the slopes between cross types from a shape-size regression was performed using aligned landmarks for shape and centroid size for size.

3.4. Results

Sample sizes per river for wild caught Atlantic Salmon (*Salmo salar*) collected individuals ranged from 7 to 171 across ages with an overall missing genotype of 1.3 %. Using a cut-off probability of 0.8 for hybrid assignment, 168 individuals could not be assigned to a genotype frequency class and were removed from the analysis. For image analysis, images were filtered for jaw deformations, damage to landmark locations, and severe dorso-ventral arching. This resulted in 47 individuals being removed from tank conditions, 77 individuals being removed from semi-natural conditions, and 647 individuals removed from field samples.

3.4.1. Geometric Morphometric Shape Differences

To determine overall differences in shape, Procrustes ANOVA was used. Where significant differences were detected, pairwise differences in shape were tested between all combinations of pure wild and farm and F_1 hybrids, and false discovery was accounted for using Bonferroni correction for experimental comparisons. Only age 1+ field

collected samples were analyzed for differences in shape because of low sample size for age 0+ individuals classified as pure farm. Age 1+ field samples showed overall shape differences between groups ($df = 2$, $p = 0.002$). Pairwise comparisons revealed pure wild individuals had significant shape differences from F_1 hybrid individuals, but F_1 did not differ significantly from pure farm (Bonferroni correction $\alpha = 0.05/3 = 0.017$, Pure Farm - F_1 $p = 0.040$, Pure Wild - F_1 $p = 0.001$, Pure Wild – Pure Farm $p = 0.028$).

In the common garden experiment, significant shape differences between groups were found at the end of both time periods in tank conditions (Day 0: $df = 2$, $p = 0.001$, Day 80: $df = 2$, $p = 0.001$). For both Day 0 and 80, pairwise comparisons revealed significant differences in shape between all groups (Bonferroni correction $\alpha = 0.05/3 = 0.017$, all $p < 0.01$). In the semi-natural conditions, overall differences in shape between groups were also detected at both measurement times (Day 0: $df = 2$, $p = 0.001$, Day 80: $df = 2$, $p = 0.001$). Pairwise comparisons at Day 0 found pure wild to differ significantly in shape from both pure farm and F_1 hybrid, but no significant difference in shape between F_1 hybrid and pure farm (Bonferroni correction $\alpha = 0.05/3 = 0.017$, Pure Farm - F_1 $p = 0.332$, Pure Wild - F_1 $p = 0.001$, Pure Wild – Pure Farm $p = 0.001$). When measured at Day 80, pairwise comparison revealed significant differences between all combinations (Bonferroni correction $\alpha = 0.05/3 = 0.017$, Pure Farm - F_1 $p = 0.001$, Pure Wild - F_1 $p = 0.001$, Pure Wild – Pure Farm $p = 0.004$).

3.4.2. Principal Component Analysis

To identify major sources of shape variation a principal component analysis was performed on the aligned shape coordinates. The first axis for common garden and field

samples explained 17.4% and 32.5% variance respectively (Figure 10). This axis largely corresponded with residual arching and body depth variance based on relative warps. The second axis explained 10.8% to 20.4% of the shape variance (Figure 10). Based on relative warps this axis explained body depth, and head size variation. While these axes explain a large proportion of the variance, the eigenvalues of these axes are less than one and there is a large degree of overlap in 95% confidence intervals between cross types.

3.4.3. Canonical Variate Analysis

In order to characterize between-group shape variation across rearing environments, a canonical variate analysis was performed between pure wild, pure farm and F_1 cross types. The first axis across common garden and field conditions appears to characterize shape differences between pure wild and pure farm or F_1 hybrid. This accounts for 61.9 % to 81.9 % of the between group variation in shape (Figure 11). The second axis characterizes shape differences between pure farm and F_1 hybrid individuals across conditions and accounts for 38.1 % to 18.1 % variation between groups (Figure 11). Using a leave-one-out method for cross-validation of assigning individuals to group (i.e., wild, pure farmed, pure wild) based on shape resulted in 65.8 % accuracy identifying field age 1+ individuals, and 96.6 % accuracy in field age 0+ individuals. In the common garden conditions, the cross-validation accuracy ranged from 74.1 % to 79.6 %. The majority of the misassignment to group occurred between F_1 and pure farm except in field samples age 0+ where majority of individuals were assigned as pure wild.

3.4.4. Body Depth ANOVA

The mean shape comparison and relative warps along PC1 in the PCA analysis both suggested differences in body depth were one of the major contributors to overall shape differences between groups. ANOVA were performed on the inter-landmark distance between landmarks 3 and 19 (Figure 8), separately by age and rearing condition. When reared in tank conditions, significant differences in body depth were detected at Day 80, but not Day 0 (Day 0: $F_{2, 105} = 0.795$, $p = 0.454$, Day 80: $F_{2, 68} = 8.162$, $p < 0.001$). Pure farm and F_1 individuals were found to have a significantly deeper body depth than pure wild (Pure Farm - F_1 $p = 0.619$, Pure Wild - F_1 $p = 0.022$, Pure Wild – Pure Farm $p = 0.001$), the mean body depth of F_1 hybrids was intermediate to pure wild and pure farm (Pure Farm = 0.127732, F_1 = 0.1261624, Pure Wild = 0.1217055). In both semi-natural (Day 0: $F_{2, 94} = 0.267$, $p = 0.766$, Day 80: $F_{2, 105} = 2.034$, $p = 0.136$) and field conditions (Age 1: $F_{2, 257} = 0.057$, $p = 0.945$) there were no significant differences between the body depth of cross types (Figure 12).

3.4.5. Size ANOVA

To compare overall size differences between groups ANOVA were performed between cross types using centroid size as a measure of overall individual size. Centroid size is the square root of the sum of squared distances from the centroid to each landmark. In field samples pure wild individuals were significantly larger than F_1 individuals at age 0+, but there is insufficient sample size to compare to pure farm individuals (Figure 9). By age 1+ there were significant differences in size between cross types ($F_{2, 257} = 3.168$, $p = 0.044$) with pure farm being significantly larger than F_1 (Pure

Farm - F_1 p = 0.033, Pure Wild - F_1 p = 0.490, Pure Wild – Pure Farm p = 0.136). At day 0 in the common garden experiment pure wild in both tank ($F_{2, 105}$ = 28.12, p = $1.65*10^{-10}$, Pure Farm - F_1 p = 0.647, Pure Wild - F_1 p < 0.001, Pure Wild – Pure Farm p < 0.001) and semi-natural conditions ($F_{2, 94}$ = 28.56, p = $2.04*10^{-10}$, Pure Farm - F_1 p = 0.804, Pure Wild - F_1 p < 0.001, Pure Wild – Pure Farm p < 0.001) had a greater centroid size than F_1 hybrid and pure farm. After 80 days, there were no differences between groups under tank conditions ($F_{2, 68}$ = 0.695, p = 0.503). Under semi-natural conditions, pure wild were still significantly larger than pure farm and F_1 hybrid ($F_{2, 105}$ = 24.05, p = $2.51*10^{-9}$, Pure Farm - F_1 p = 0.003, Pure Wild - F_1 p = 0.001, Pure Wild – Pure Farm p < 0.001), and pure farm were significantly larger than F_1 hybrid.

3.4.6. Shape ~ Size Regression Slopes Comparison

To determine if differences in shape were related to differences in allometry between groups, a regression of centroid size on shape was performed. In the common garden tank conditions, there were no significant differences in the slope of shape and size between cross types at both day 0 (p = 0.276) and day 80 (p = 0.147). In both the common garden semi-natural conditions (Day 0: p = 0.006, Day 80: p = 0.042) and in field samples (p = 0.045) there were significant differences in the shape and size slopes between cross types. This indicates that the shape differences present are not solely the result of allometric growth.

3.5. Discussion

The escape of aquaculture Atlantic Salmon (*Salmo salar*) represents a significant threat to the persistence and stability of wild populations (Forseth *et al.*, 2017), yet the

magnitude of phenotypic impacts due to hybridization remains largely unresolved (but see Bolstad *et al*. 2017). Here I evaluated the phenotypic consequences of hybridization using geometric morphometrics in both common garden experiments and following field escape events. Our results suggest significant variation across settings and that the phenotype (i.e., shape and size) of pure farm and wild-farm hybrid Atlantic Salmon in the wild has a strong plastic component. As the environmental conditions in this study approached a wild setting (i.e., from aquaculture-like tank conditions, to a semi-natural environment, to natural rivers) differences between cross types (e.g., wild, hybrid, farmed) decreased and the size and shape converged. These results build on recent work documenting a lack of morphological phenotypic differences among wild and farm crosses, supporting a significant role for phenotypic plasticity (Glover *et al.*, 2018) and highlighting the role that plasticity may play in masking genetic changes associated with introgression between wild and farm escaped Atlantic Salmon.

3.5.1. Differences in Size

In general, differences in size between wild and pure farm offspring decreased over time under both experimental and field conditions, potentially reflecting differing influences of maternal contributions, phenotypic plasticity, and selection in the wild. Interestingly, initial sizes differed significantly between wild and farm or F_1 hybrid individuals in the common garden experiments (tank and semi-natural), but by day 80 differences were absent under tank conditions. The initial differences are likely due to the maternal effects of egg size (Einum and Fleming, 2000), and this is subsequently overcome by the increased growth of aquaculture offspring under tank conditions as shown elsewhere (Solberg *et al*., 2013), and if the experiment was carried further then it

is likely that the farm individuals would grow to a larger size than wild in tank conditions. Similarly, the difference in size between wild and pure farm offspring decreased over time under fully natural conditions and this convergence of size among field collected individuals could be a result of either phenotypic plasticity and/or size-selective mortality (e.g., Glover *et al*. 2019).

Farmed Atlantic Salmon have been found to outgrow wild salmon raised in hatchery conditions while hybrid individuals are often intermediate (Glover *et al*., 2009; Harvey *et al*., 2016a-c). This difference in growth is maintained under different feeding regimes (Harvey *et al*., 2016b), feed type (Harvey *et al*., 2016c), and salmon density (Harvey *et al*., 2016a). The increase in farm salmon growth rate in hatchery conditions is a result of increased growth hormone production (Fleming *et al*., 2002). In Harvey *et al*., (2016a) farm individuals outgrew wild individuals in semi-natural conditions contrasting the results found here. However, in that study, fry were fed commercial feed ad libitum as opposed to live feed done in our study. A previous study has shown limiting commercial feed resulted in reduced growth rate differences between farm and wild salmon (Solberg *et al.*, 2013), and using live feed in semi-natural conditions resulted in no growth differences after six weeks (Solberg *et al*., 2020). Our results reinforce previous conclusions that differences in farm salmon growth rates compared to wild individuals are a result of domestication (Fleming *et al*. 2002), but also that the response is still plastic, and as the environment approaches a natural river the differences in size may be reduced.

3.5.2. Shape

The differences observed in shape between wild, farm and hybrid Atlantic Salmon were subtle, most pronounced under artificial conditions and reduced or absent among the wild collected samples. Previous studies have shown salmon phenotype to be plastic, with shape differences in the head and fin lengths reported between wild salmon reared in a hatchery compared to those reared in a river (Blanchet *et al*., 2008). Similarly, differences in head shape have been observed between wild Atlantic Salmon caught in a river compared to hatchery reared salmon (Fleming *et al*., 1994; Solem *et al*., 2006). Phenotypic differences between wild and hatchery individuals has also been observed in Coho Salmon (*Oncorhynchus kisutch*) (Swain *et al*., 1991; Hard *et al*., 2000), and Chinook Salmon (*Oncorhynchus tshawytscha*) (Busack *et al*., 2007; Tiffan & Connor, 2011), and Rainbow Trout (*Oncorhynchus mykiss*) (Pulcini *et al.*, 2012), as well as a suite of other fish species (Wringe, Purchase and Fleming, 2016). However, while phenotypic differences between individuals reared in artificial and wild conditions are often detected, when farmed individuals are reared in natural rivers longer term these phenotypic differences have been shown to become reduced or absent over time (Fleming, Jonsson and Gross, 1994) supporting conclusions made here that Atlantic Salmon phenotypic variation is subject to significant phenotypic plasticity and heavily influenced by environmental conditions experienced.

3.5.3. Plasticity

Our results demonstrate that both size and shape of wild and pure farm Atlantic Salmon may display significant divergence under artificial (i.e. aquaculture) settings, but

in more natural conditions their size and shape may converge. This plasticity in phenotypic response matches the results of Glover *et al*. (2018) studying the growth potential of Atlantic Salmon in Norway. Glover *et al*. (2018) found that in aquaculture-like tank conditions farmed salmon outgrew wild salmon at a ratio of ~1:1.8 and have been shown to outgrow even further in standard hatchery by a ratio of ~1:4.91-5.15 (Solberg *et al.*, 2013), but there were no differences in the river (Glover *et al.*, 2018). This is consistent with our results that revealed different phenotypic outcomes in tank and semi-natural conditions at day 80. The plasticity of juvenile salmon phenotype allows for rapid phenotypic changes over short time periods (Pakkasmaa and Piironen, 2001). This can result in wild salmon shifting towards the hatchery phenotype when reared in hatchery conditions and domestic salmon shifting towards the wild phenotype in more complex environments (von Cramon-Taubadel *et al.*, 2005), a phenomenon also observed in domestic Brown Trout raised in the wild (Sánchez-González and Nicieza, 2017). However, there remains a genetic component to shape and size that can manifest in significant phenotypic variation among wild salmon from different populations when raised in simple hatchery conditions (Sheehan *et al.*, 2005), even though fish from the same populations may converge in phenotype when raised in more complex environments (von Cramon-Taubadel *et al*., 2005; Glover *et al*., 2018).

3.5.4. Limitations

Attempts to control for dorso-ventral arching may have biased estimates of significant shape variation in regions of the body where these differences are present (Valentin *et al*., 2008). However, individuals with severe arching were removed prior to analysis, there were no changes in significance of the results with or without the

utilization of the arching correction, and it is unlikely either method would be biased in the severity affecting one group (Figure S5-S7 to see results without bending correction). After Procrustes superimposition, any error in the position landmarks due to this arching would be distributed across all the landmarks making identification of single landmarks with higher measurement error difficult (Fruciano, 2016). The combination of measurement error with small differences in highly variable traits could have contributed to inferred convergence in shape within experiments. However, measurement error of this type is unlikely to influence our conclusions of convergence in size across cross types.

It is also possible in the field samples there was size and/or shape biased natural selection whereby any farm or hybrid individuals that diverged from the optimal phenotype suffered reduced survivorship or were assigned to different ages due to significantly increased or decreased growth. As such, selection may be contributing to convergence observed in the wild. Nonetheless, the consistent trends I detected in the common garden experiments and the results of Glover *et al*. (2018) clearly support a strong role for phenotypic plasticity in the patterns they observed.

3.5.5. Final Summary

This study utilizes a combination of a common garden experiment and field sampling to evaluate genetic and environmental contributions to juvenile salmon shape in wild Atlantic Salmon, aquaculture escaped and hybrid individuals. Our results indicate the presence of significant phenotypic plasticity of shape and size in salmon with evidence of convergence under natural river environments while the typical phenotypic differences between wild, farm and hybrid fish occurred in an aquaculture setting. Our

results are consistent with Glover *et al*. (2018) where the faster growth of farm salmon compared to wild was reduced when reared in a natural river, but when then transferred to a tank environment the increased growth trait characteristic of farm salmon reappeared. The results of our semi-natural experiment and those of the Glover *et al*. (2018) study support the hypothesis that not all the size and shape convergence between farm and wild salmon is due to size or shape biased selection (genetics) but that there is a plastic component. Characterizing shape consequences resulting from wild and farm salmon interactions is increasingly important as the industry increases in size and there are more opportunities of contact between wild and aquaculture salmon (Keyser *et al*., 2018; Sylvester *et al*., 2018b).

Table 3. Summary of statistical analyses performed comparing size, shape and body depth between pure wild, pure farm, and F_1 in tank, semi-natural, and field conditions. Age is counted as days post yolk sac absorption. * indicates significance after Bonferroni correction ($p < \alpha = 0.05/3 = 0.017$).

Location	Age	Comparison	Size	Shape	Body Depth
Tank	0	PW-PF	PW>PF	PW≠PF*	PW=PF
Tank	0	PW-F_1	PW>F_1	PW≠F_1*	PW=F_1
Tank	0	PF-F_1	PF=F_1	PF≠F_1*	PF=F_1
Tank	80	PW-PF	PW=PF	PW≠PF*	PW<PF
Tank	80	PW-F_1	PW=F_1	PW≠F_1*	PW<F_1
Tank	80	PF-F_1	PF=F_1	PF≠F_1*	PF=F_1
Semi-Natural	0	PW-PF	PW>PF	PW≠PF*	PW=PF
Semi-Natural	0	PW-F_1	PW>F_1	PW≠F_1*	PW=F_1
Semi-Natural	0	PF-F_1	PF=F_1	PF=F_1	PF=F_1
Semi-Natural	80	PW-PF	PW>PF	PW≠PF*	PW=PF
Semi-Natural	80	PW-F_1	PW>F_1	PW≠F_1*	PW=F_1
Semi-Natural	80	PF-F_1	PF>F_1	PF=F_1	PF=F_1
Field	0	PW-PF	N/A	N/A	N/A

Location	Age	Comparison	Size	Shape	Body Depth
Field	0	PW-F_1	PW>F_1	N/A	PW=F_1
Field	0	PF-F_1	N/A	N/A	N/A
Field	1	PW-PF	PW=PF	PW=PF	PW=PF
Field	1	PW-F_1	PW=F_1	PW≠F_1*	PW=F_1
Field	1	PF-F_1	PF=F_1	PF=F_1	PF=F_1

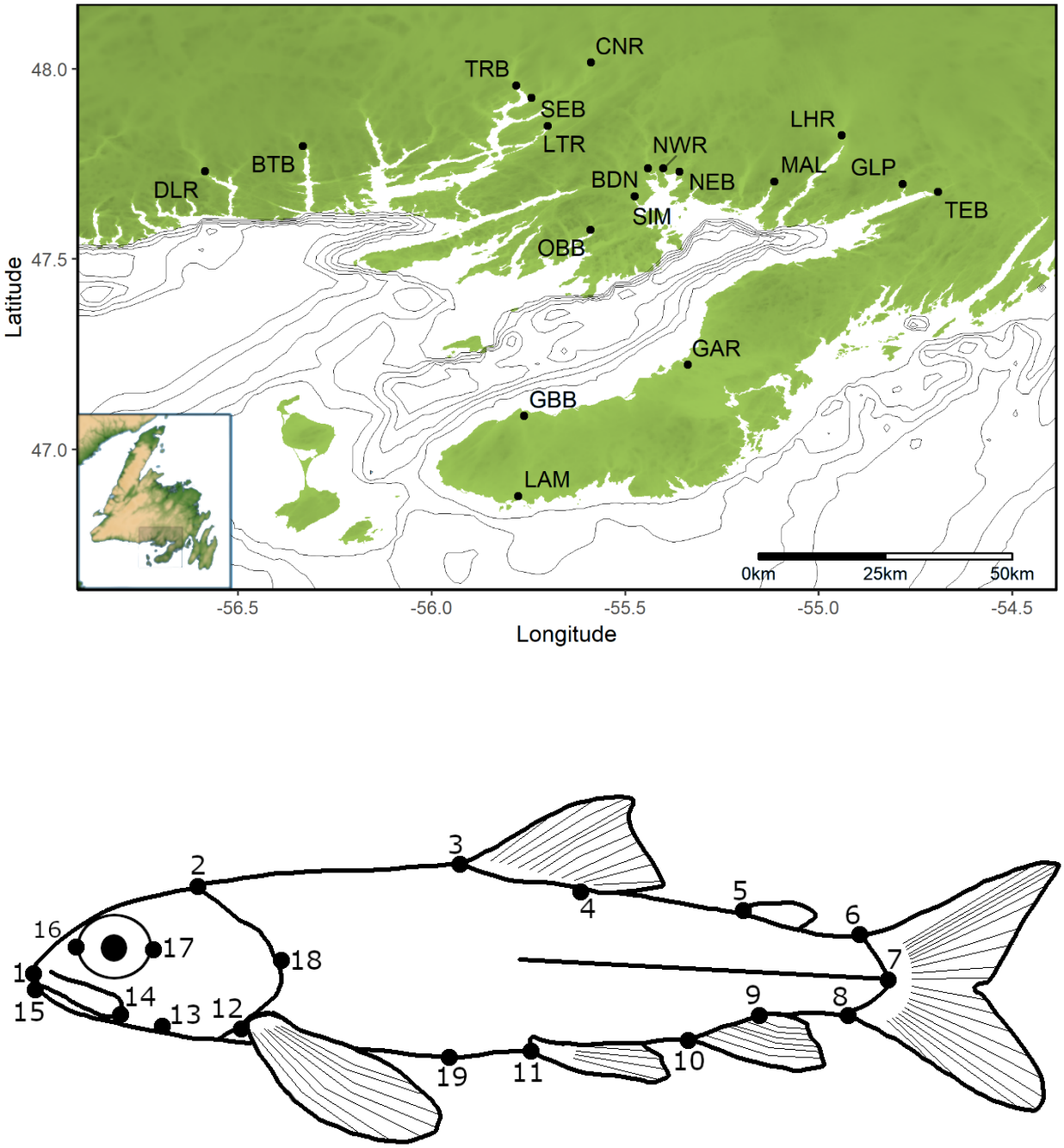

Figure 8. (a) Map of Newfoundland river sample locations for 2015 and 2016. (b) Location of 19 landmarks used for geometric morphometric analysis (see Table S1 for description of landmark locations).

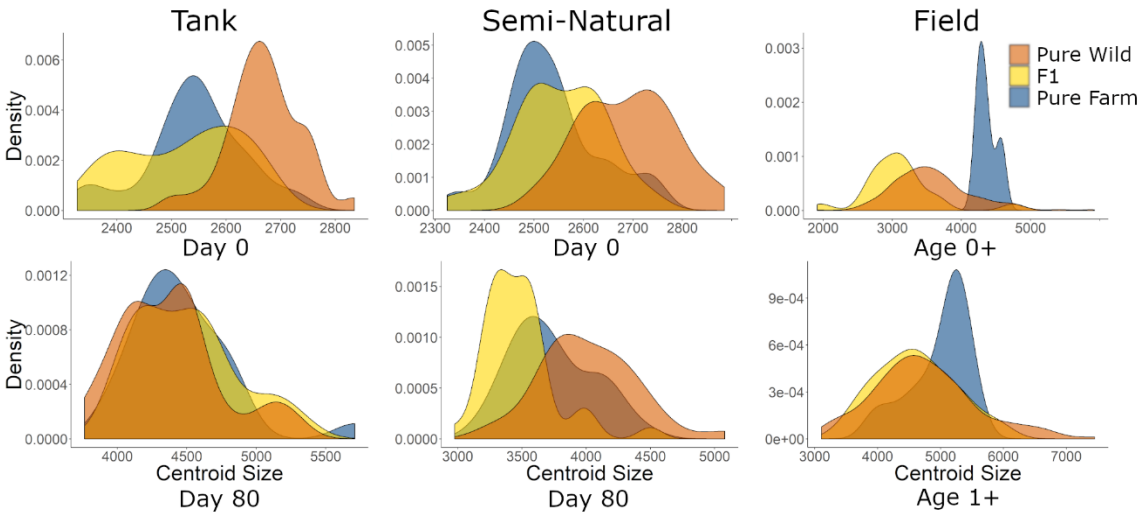

Figure 9. Centroid Sizes of juvenile salmon calculated using 19 landmarks for each sample. The y-axis represents the distribution of individuals for each group of that size. Measurements for the common garden experiment were taken at Day 0 (Start) and Day 80 (End). Juveniles aged 0+ and 1+ were sampled from southern Newfoundland rivers (Figure 1, Table S3). Experimental conditions are organized in columns and time periods and ages are in rows. Field-captured/collected pure farm salmon aged 0+ have a small sample size and were not evaluated statistically but are presented for completeness. Determination of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.

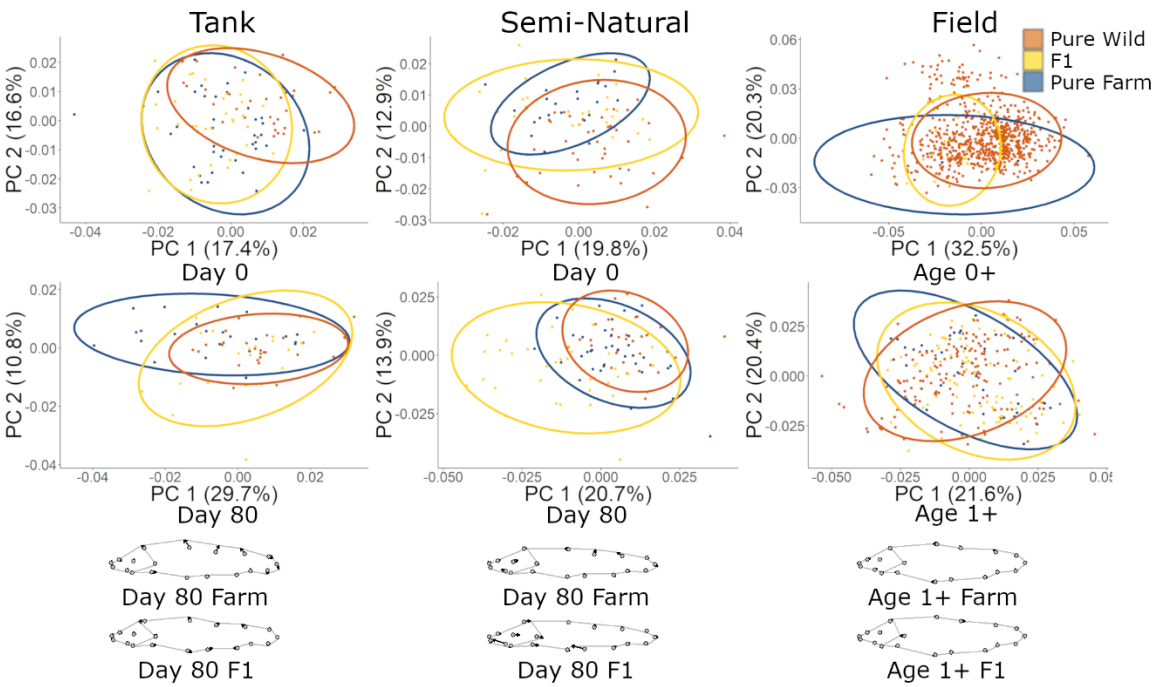

Figure 10. Principal component analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. For the common garden experiment shape is shown at Day 0 (Start) and Day 80 (End). Juveniles aged 0+ (young of year) and 1+ were sampled from field locations (18 southern Newfoundland rivers; Figure 1, Table S3). Relative differences in mean shape are also shown for each experiment end comparing mean shape of farm or F_1 hybrid to wild with 4x magnification of shape differences for visualization (see Figures S8-S13 for relative warps for principal component axes). Experimental conditions are organized in columns and time periods and ages are in rows. Age 0+ field collected shape differences are not shown due to low sample size for pure farm individuals and pure wild-F_1 comparison is the same as age 1+. Determination of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.

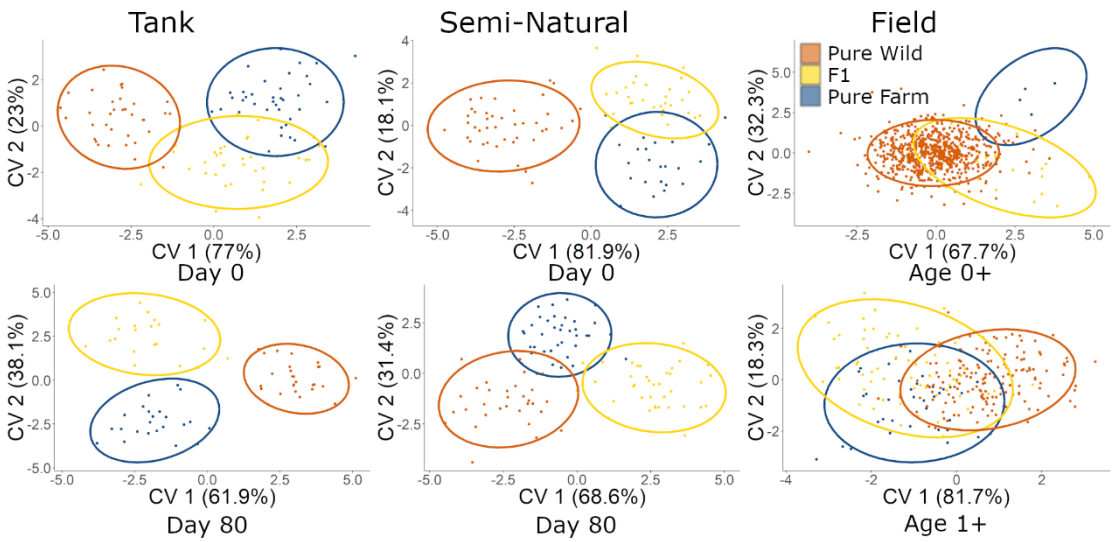

Figure 11. Canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. For the common garden experiment shape is shown at Day 0 (Start) and Day 80 (End). Juveniles aged 0+ (young of year) and 1+ were sampled from field locations (18 southern Newfoundland rivers; Figure 1). See Figures S8-S13 for mean shapes for each group. Experimental conditions are organized in columns and time periods and ages are in rows. Determination of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.

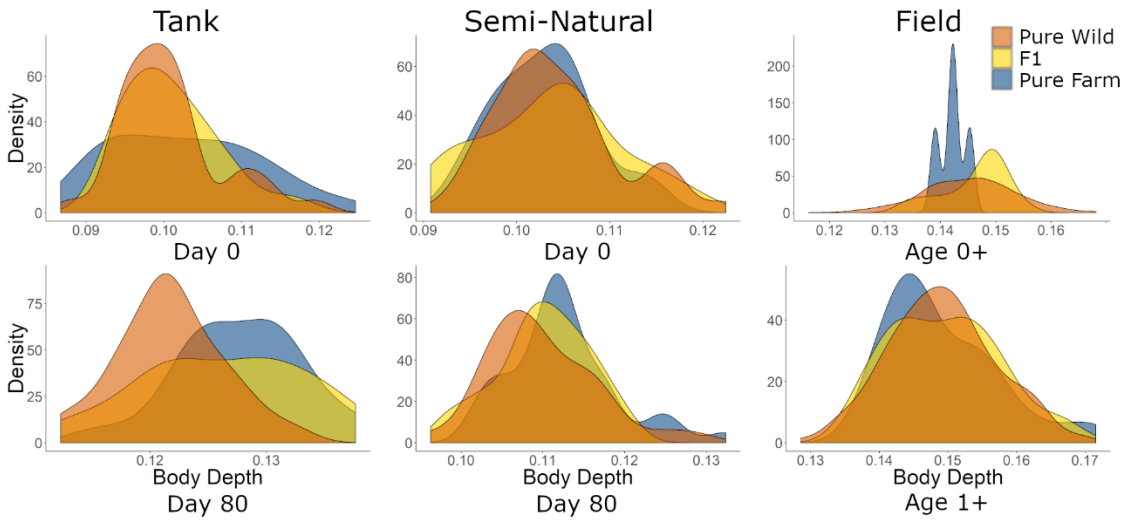

Figure 12. Body depth measurements using inter-landmark distance between landmarks 3 and 17. Landmarks are as illustrated in Figure 2. Individuals were measured at Day 0 (Start) and Day 80 (End) for the common garden experiment. Field salmon aged 0+ and 1+ were sampled from 18 rivers in southern Newfoundland as shown in Figure 1. Experimental conditions are organized in columns and time periods and ages are in rows. Determination of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.

Chapter 4 – Conclusion

4.1 Summary

Genetic diversity of species and populations directly influence their demographic stability with populations lacking diversity expected to be at greater risk of extirpation, (Újvári *et al.*, 2002; Evans and Sheldon, 2008; Hellmair and Kinziger, 2014). Understanding the distribution of genetic diversity in the wild, and forces influencing it are therfore central to wildlife management and conservation. Atlantic Salmon populations in southern Newfoundland have been declining over the last few decades to the point that they have been assessed as "Threatened" by COSEWIC (COSEWIC, 2011). Here I took advantage of new panels of sequenced microsatellite markers which allow for greater resolution of genetic structure in the region, and for improved resolution of the forces influencing structure at fine geographic scales. Southern Newfoundland is also of interest as there is significant Atlantic Salmon net pen aquaculture and plans for expansion into the previously unutilized Placentia Bay. The plans for aquaculture expansion raise concerns of introgression from escaped aquaculture salmon in a region previously unharmed by them. An improved understanding of how introgression from aquaculture escapees could affect wild populations phenotypically could improve our understanding of the direct genetic impact of hybridization. Ultimately, understanding the spatial scale and the forces influencing structure are central to developing conservation and management efforts for all depleted and at-risk stocks, including these salmon populations.

In Chapter 2, I examined population structuring in the wild, and the results show that phenotypic differences are associated with genetic distances among rivers. I resolve

genetic structure in this region at a much finer scale than has previously been done and also identify climate and phenotype as important to the spatial organization of genetic diversity. Phenotypic associations with genetic distance suggest the importance of juvenile habitat characteristics in population structure. Previous studies have shown climate associations with genetic distance in southern Newfoundland, but the addition of phenotype in characterization is novel and allows for a greater understanding of the scale of hierarchical population structuring. In the face of declining stocks of many wild species, understanding current patterns of genetic diversity and forces influencing this diversity can directly inform management and recovery efforts (Funk *et al.*, 2012; Lehnert *et al.*, 2019b). The observation that phenotypic variation was associated with genetic structure highlights the importance fine scale structure and local watershed conditions to Atlantic Salmon. As such, changes to watershed characteristics such as water flow and food availability may have severe impacts on genetic structure and gene flow. The genomic vulnerability of southern Newfoundland populations will influence how drastic these changes will be to each population. Moreover, climate change may also severely impact wild populations either driving range further north as water temperatures rise or extirpate southern populations.

In Chapter 3 I quantified morphological differences among wild, farm and hybrid salmon both in the laboratory and the field. Farm (feral), and hybrid salmon caught in rivers displayed very similar morphology to wild salmon across ages, possibly indicating plasticity in morphology despite known differences in aquaculture reared salmon. In common garden environment these aquaculture typical traits of increased growth were present in tank conditions but were reduced as the environment approached natural

conditions. As such, phenotypic plasticity may mask genetic introgression as aquaculture escapes continue into wild populations and hybridize.

4.2 Implications

The fine scale resolution of genetic structure is important in aiding conservation efforts. With declining wild stocks, understanding current patterns of genetic diversity and forces influencing this diversity can aid in managing populations and recovery efforts (Funk *et al.*, 2012; Lehnert *et al.*, 2019b). The finer scale structure found here shows population connections between individual rivers as well as the region in a broader context of three groups of the mouth of Placentia Bay, head of Placentia Bay and Fortune Bay/ Bay D'Espoir. This subdivision of the current single management unit may improve monitoring and population recovery efforts.

The phenotypic variation associated with genetic structure highlights the importance of maintaining (and restoring) the local watershed in properly managing salmon populations. Changes to watershed characteristics such as water flow and food availability may have severe impacts on genetic structure and gene flow in the area. This can be seen in the importance of habitat for structure in Arctic Charr (Gíslason *et al*., 1999), and Threespine Stickleback (Sharpe *et al*., 2008), as well as the association between habitat and Atlantic Salmon introgression (Sylvester *et al*., 2019).

Climate change may also severely impact wild populations either driving range expansion further north as water temperatures rise or extirpate southern populations (Lehnert *et al*., 2019b). Higher temperatures due to climate change may drive salmon populations to seek more northern rivers to match current river temperatures if adaptation

to rising temperatures is not attained (Hedger *et al*., 2013). This is only a limited solution as there is only so many northern freshwater rivers needed for rearing juveniles.

My observation that the morphological impact of wild-aquaculture hybridization on juvenile morphology was subtle and largely plastic in the wild, suggests the fitness impact (Sylvester *et al*., 2019) may be cryptic and not readily observable in phenotype. There is little phenotypic difference in feral and hybrid salmon reared in natural habitats from their wild counterparts despite the known growth and morphology differences found in aquaculture. This plasticity highlights the importance of habitat on morphology and may further explain patterns of introgression found in the region being related to landscape features such as river size and elevation (Sylvester *et al*., 2018b).

Nonetheless, genetic changes caused by introgression from domestic lineages may be detrimental over the long-term causing population decline and potentially extirpation without showing clear shifts in morphological traits towards that characteristic of domestic populations (Sylvester *et al*., 2019). Ultimately, preventing aquaculture escapes and continued monitoring for impacts will be important for the stability of wild populations near aquaculture developments (Glover *et al.*, 2017). The one-way flow of genes from a larger source of domestic individuals into wild populations can reduce genetic diversity and reduce the potential of wild populations to adapt to a changing environment (Glover *et al*., 2012). This will only become increasingly important as aquaculture production increases, other anthropogenic influences continue, and climate change progresses.

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Appendix A: Supplementary Tables

Table S1. Description of landmark locations used for geometric morphometric analyses. These landmarks are visualized in Figure 8.

Landmark	Description
1	Anteriormost point of the snout.
2	Dorsalmost point of the structure that demarcates the skull and body.
3	Origin of the dorsal fin.
4	Insertion of the dorsal fin.
5	Origin of the adipose fin.
6	Dorsal origin of the caudal fin.
7	Posteriormost point of the hypural plate.
8	Ventral origin of the caudal fin.
9	Insertion of the anal fin.
10	Origin of the anal fin.
11	Origin of the pelvic fin.
12	Origin of the pectoral fin.
13	Posteriormost point of the lower jaw.
14	Posteriormost point of the upper jaw.

Landmark	Description
15	Anteriormost point of the lower jaw.
16	Anteriormost point of the orbit of the eye.
17	Posteriormost point of the orbit of the eye drawn horizontal to the anterior-posterior axis from point 16.
18	Posteriormost point on the bony process that sticks out of the operculum.
19	Ventralmost point of the belly of the fish on a line drawn perpendicular to the anterior-posterior axis from point 3.

Table S2. Sample counts for each common garden experiment at each time point after filtering. Counts in parentheses indicate breakdown between cross types.

Environment	Day 0 (Wild, Farm, F_1)	Day 80 (Wild, Farm, F_1)
Tank	108 (35, 39, 34)	71 (27, 24, 20)
Semi-Natural	97 (42, 23, 32)	108 (33, 37, 38)

Table S3. Sample counts from 2015 and 2016 for each river for ages 0+ and 1+. Counts in parentheses indicate breakdown between cross types.

River	Latitude	Longitude	2015 0+ (Wild, Farm, F_1)	2015 1+ (Wild, Farm, F_1)	2016 0+ (Wild, Farm, F_1)	2016 1+ (Wild, Farm, F_1)
Bay Du Nord River (BDN)	47.742	-55.432	32 (32, 0, 0)	32 (32, 0, 0)	58 (58, 0, 0)	2 (2, 0, 0)
Bottom Brook (BTB)	47.795	-56.329	0 (0, 0, 0)	5 (2, 2, 1)	20 (20, 0, 0)	3 (3, 0, 0)
Conne River (CNR)	47.924	-55.678	9 (9, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)	17 (17, 0, 0)
Dollards River (DLR)	47.741	-56.598	15 (15, 0, 0)	12 (11, 1, 0)	34 (34, 0, 0)	0 (0, 0, 0)
Garnish River (GAR)	47.220	-55.334	40 (40, 0, 0)	9 (8, 0, 1)	0 (0, 0, 0)	0 (0, 0, 0)
Grand Bank Brook (GBB)	47.090	-55.762	13 (13, 0, 0)	14 (11, 0, 3)	3 (3, 0, 0)	0 (0, 0, 0)
Grand le Pierre River (GLP)	47.697	-54.783	7 (0, 0, 7)	10 (0, 1, 9)	4 (0, 0, 4)	27 (0, 5, 22)
Lamaline River (LAM)	46.876	-55.776	38 (38, 0, 0)	5 (5, 0, 0)	47 (47, 0, 0)	0 (0, 0, 0)
Long Harbour River (LHR)	47.826	-54.944	31 (18, 4, 9)	73 (37, 5, 13)	0 (0, 0, 0)	18 (17, 0, 1)

River	Latitude	Longitude	2015 0+ (Wild, Farm, F_1)	2015 1+ (Wild, Farm, F_1)	2016 0+ (Wild, Farm, F_1)	2016 1+ (Wild, Farm, F_1)
Little River (LTR)	47.857	-55.686	0 (0, 0, 0)	0 (0, 0, 0)	165 (165, 0, 0)	0 (0, 0, 0)
Malbay Brook (MAL)	47.701	-55.117	0 (0, 0, 0)	22 (0, 15, 7)	0 (0, 0, 0)	13 (1, 6, 6)
North east River (NEB)	47.738	-55.358	0 (0, 0, 0)	7 (5, 0, 2)	0 (0, 0, 0)	0 (0, 0, 0)
North west River (NWR)	47.747	-55.396	0 (0, 0, 0)	0 (0, 0, 0)	74 (74, 0, 0)	3 (1, 0, 2)
Old Bay Brook (OBB)	47.593	-55.589	0 (0, 0, 0)	0 (0, 0, 0)	26 (26, 0, 0)	0 (0, 0, 0)
South east Brook (SEB)	47.925	-55.737	0 (0, 0, 0)	3 (3, 0, 0)	0 (0, 0, 0)	1 (1, 0, 0)
Simm's Brook (SIM)	47.672	-55.477	12 (12, 0, 0)	0 (0, 0, 0)	56 (56, 0, 0)	2 (2, 0, 0)
Tailrace Brook (TRB)	47.990	-55.794	0 (0, 0, 0)	0 (0, 0, 0)	20 (20, 0, 0)	0 (0, 0, 0)

Appendix B: Supplementary Figures

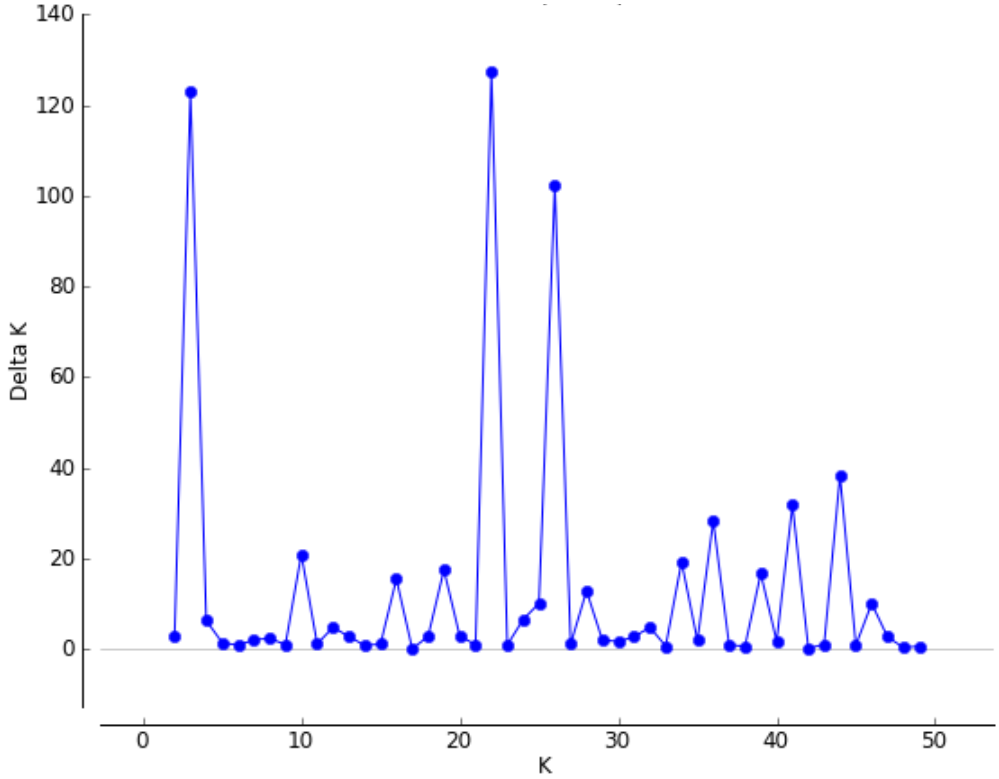

Figure S1. Evanno's delta K results from Structure for southern Newfoundland Atlantic Salmon (*Salmo salar*) showing most likely number of clusters to be K = 22, with other small, less supported, peaks at K = 3 and K = 26.

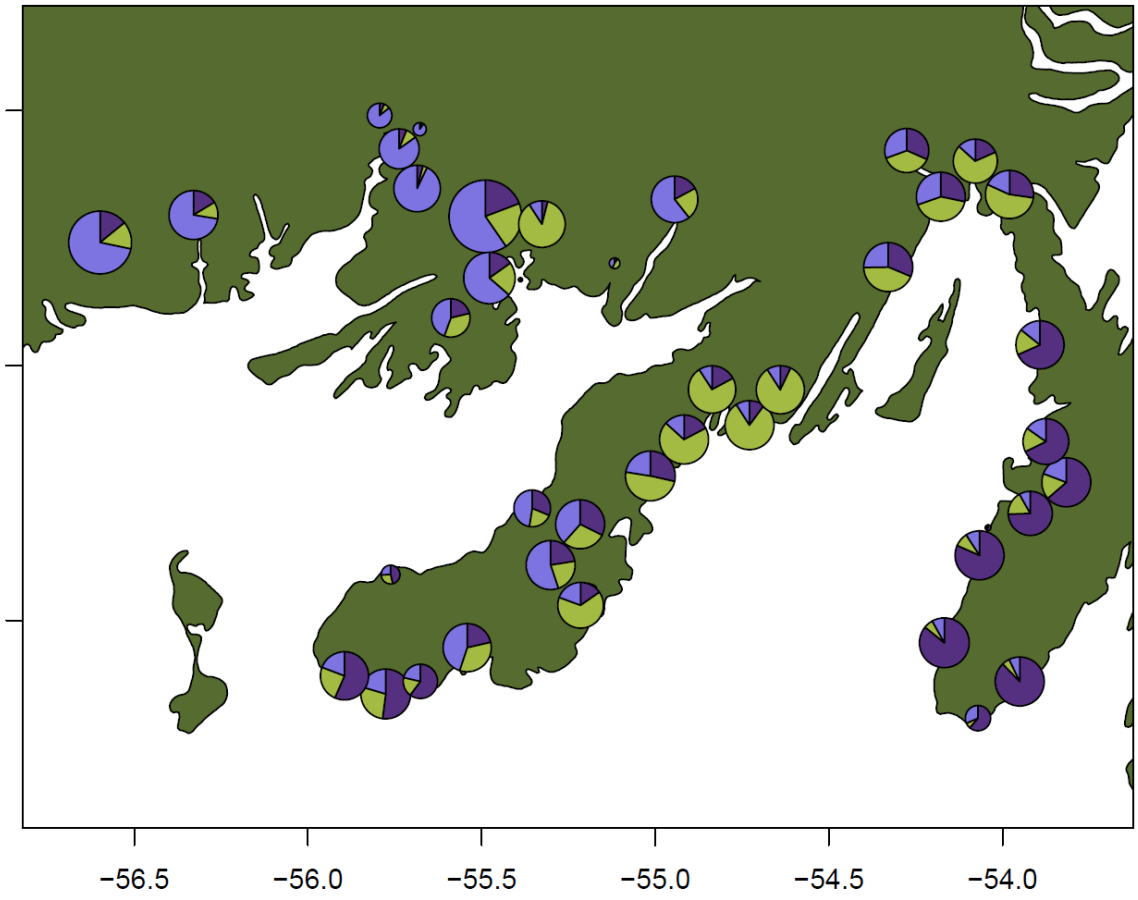

Figure S2. Structure results for K = 3 for southern Newfoundland showing proportion of assignment to each cluster in a river. Assignments are noted by colour, and size of the circle is proportional to sample size. Note the general large-scale groupings of Eastern Placentia Bay-Avalon Peninsula (purple), Western Placentia Bau-Burin Peninsula (green), and Fortune Bay-Bay d'Espoir (lilac).

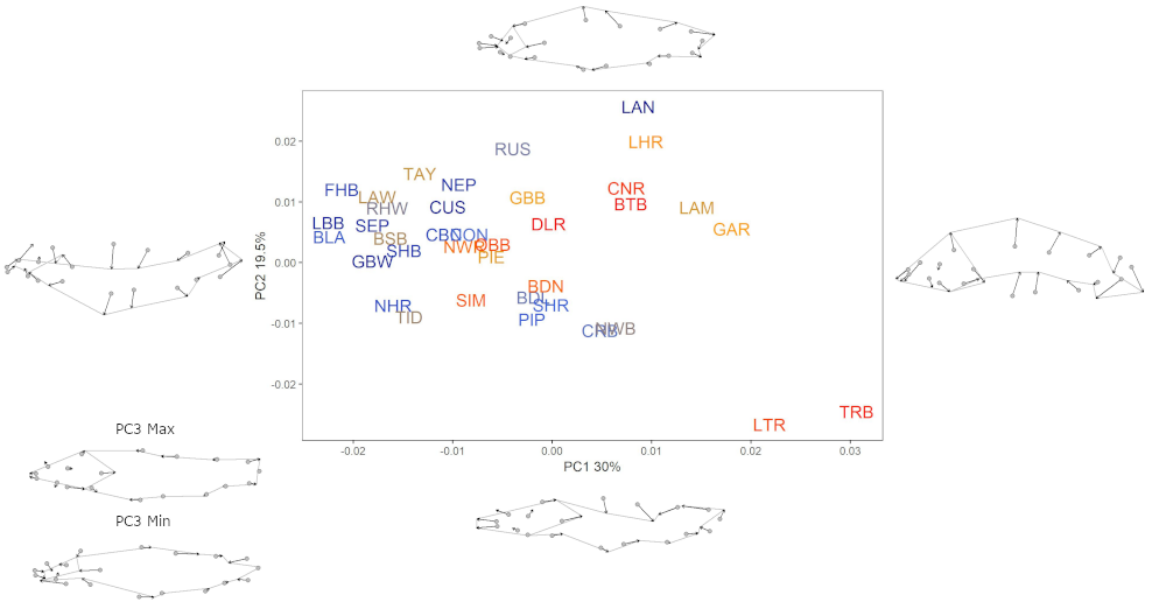

Figure S3. A Principal Component Analysis (PCA) of morphological variation in southern Newfoundland Atlantic Salmon (*Salmo salar*) based on geometric morphometrics of the 19 landmarks from Figure 1b. Rivers are coloured Red-Blue, West-East along coastline. Mean morphology PC per river for Age 0+ individuals based on fork-length aging. With relative warps corresponding to the first three axes at 4x magnification of differences.

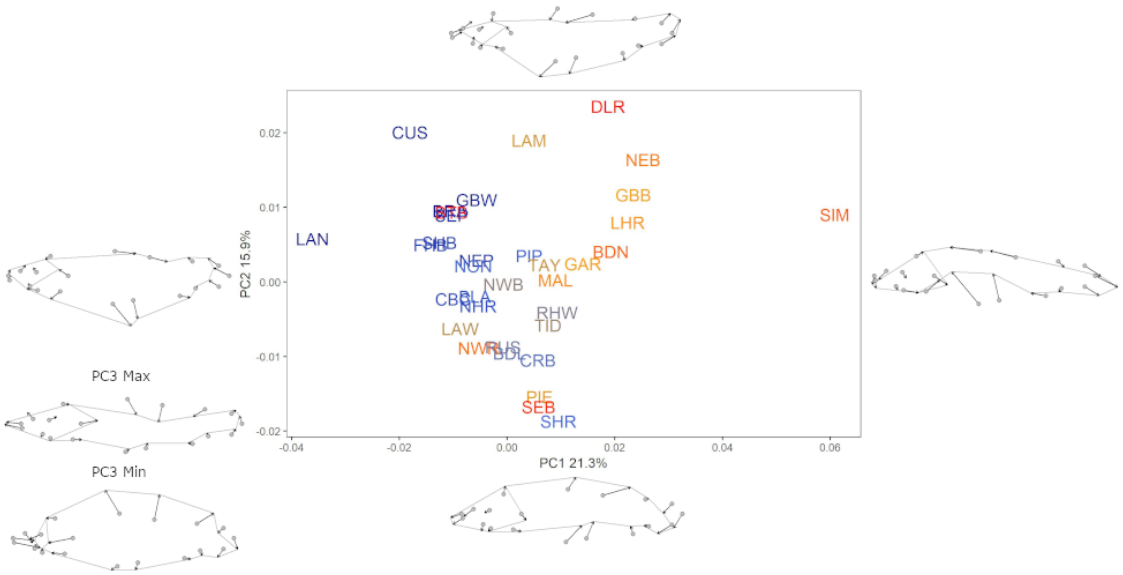

Figure S4. A Principal Component Analysis (PCA) of morphological variation in southern Newfoundland Atlantic Salmon (*Salmo salar*) based on geometric morphometrics of the 19 landmarks from Figure 1b. Rivers are coloured Red-Blue, West-East along coastline. Mean morphology PC per river for Age 1+ individuals based on fork-length aging. With relative warps corresponding to the first three axes at 4x magnification of differences.

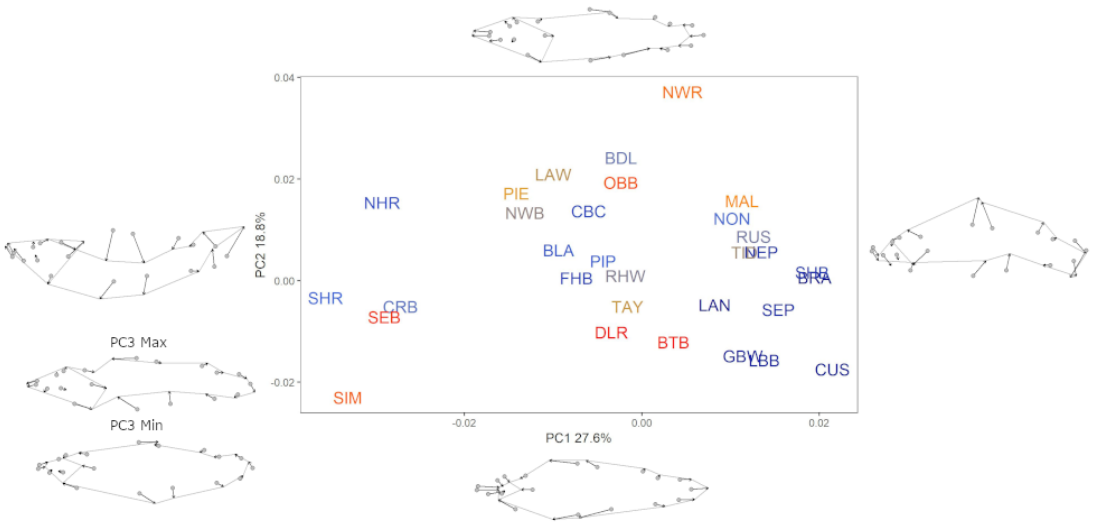

Figure S5. A Principal Component Analysis (PCA) of morphological variation in southern Newfoundland Atlantic Salmon (*Salmo salar*) based on geometric morphometrics of the 19 landmarks from Figure 1b. Rivers are coloured Red-Blue, West-East along coastline. Mean morphology PC per river for Age 2+ individuals based on fork-length aging. With relative warps corresponding to the first three axes at 4x magnification of differences.

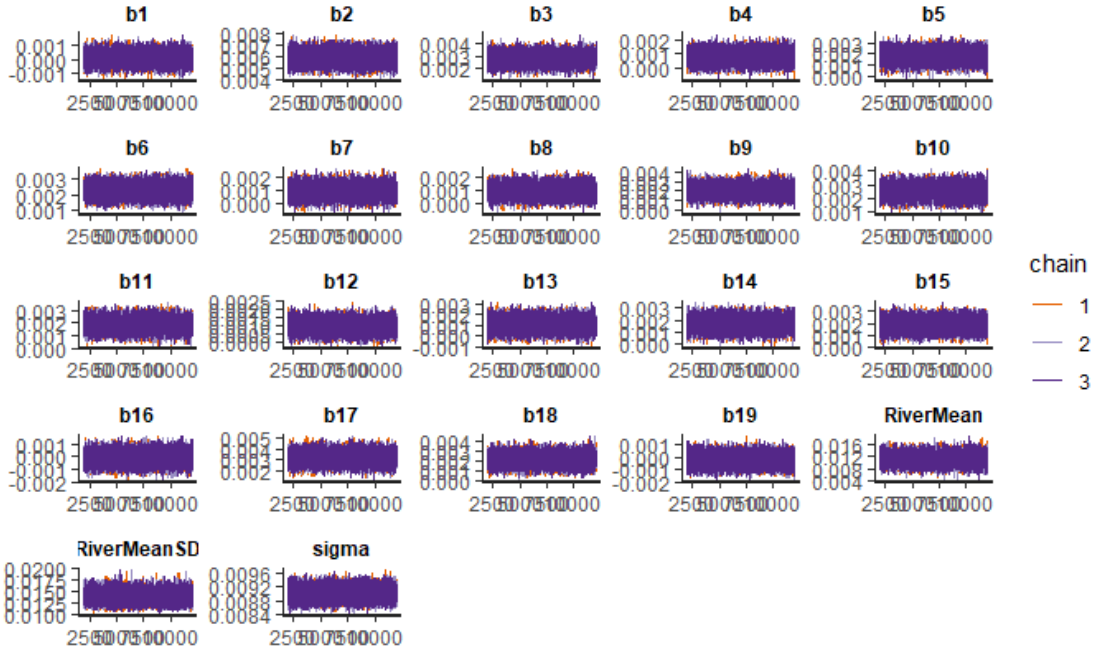

Figure S6. Trace plots for Bayesian regression using F_{ST} as the predicted variable. Large overlap in chains shows the chains converged at the same value for the coefficient.

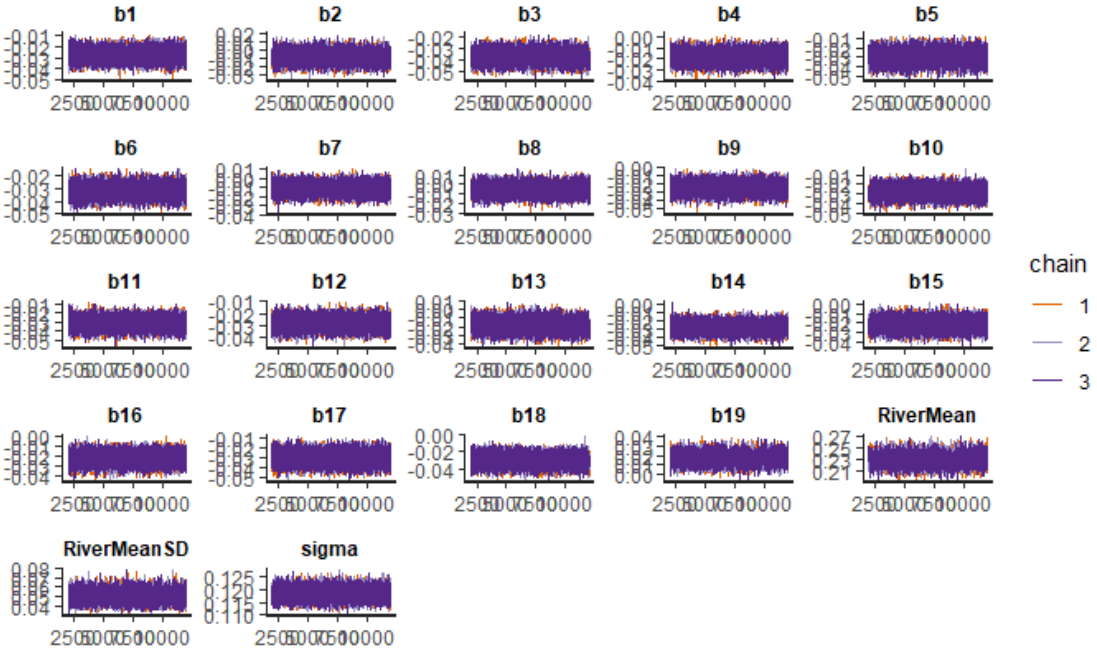

Figure S7. Trace plots for Bayesian regression using BIMr migration rates as the predicted variable. Large overlap in chains shows the chains converged at the same value for the coefficient.

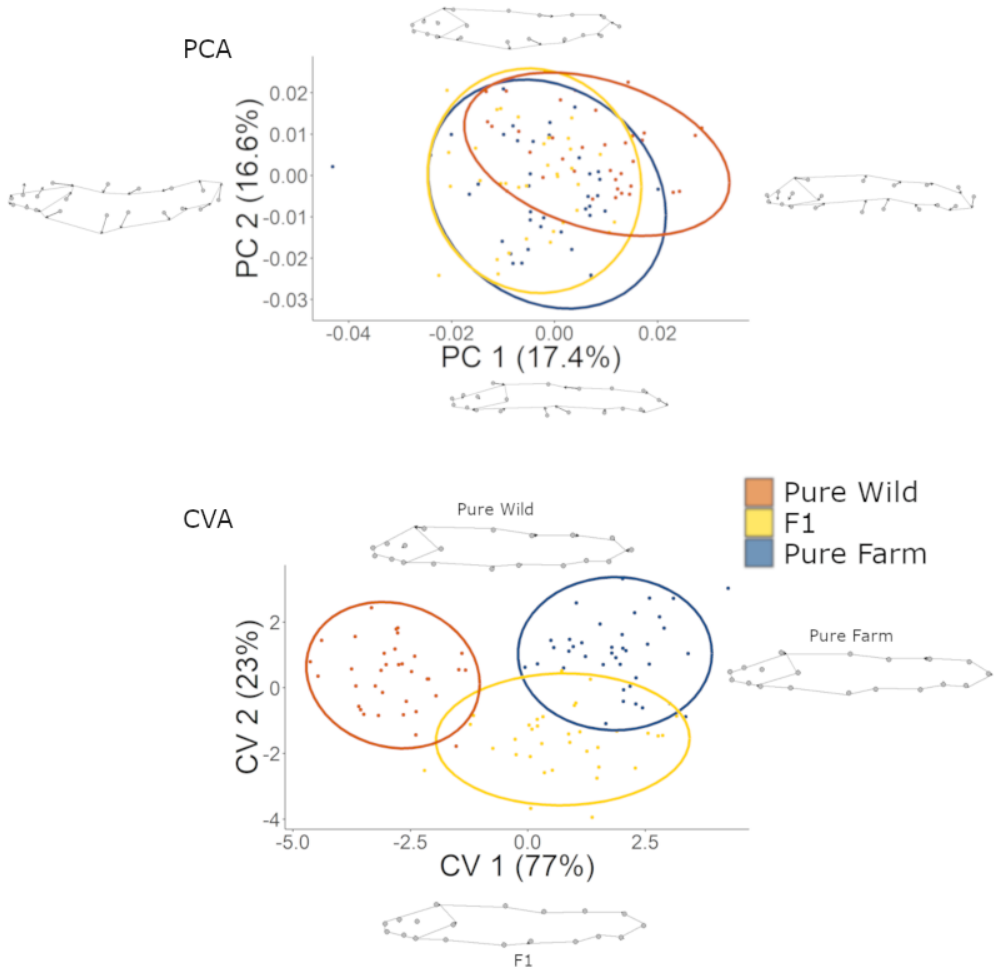

Figure S8. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Individuals are from Day 0 post yolk-sac absorption reared in tank conditions. Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

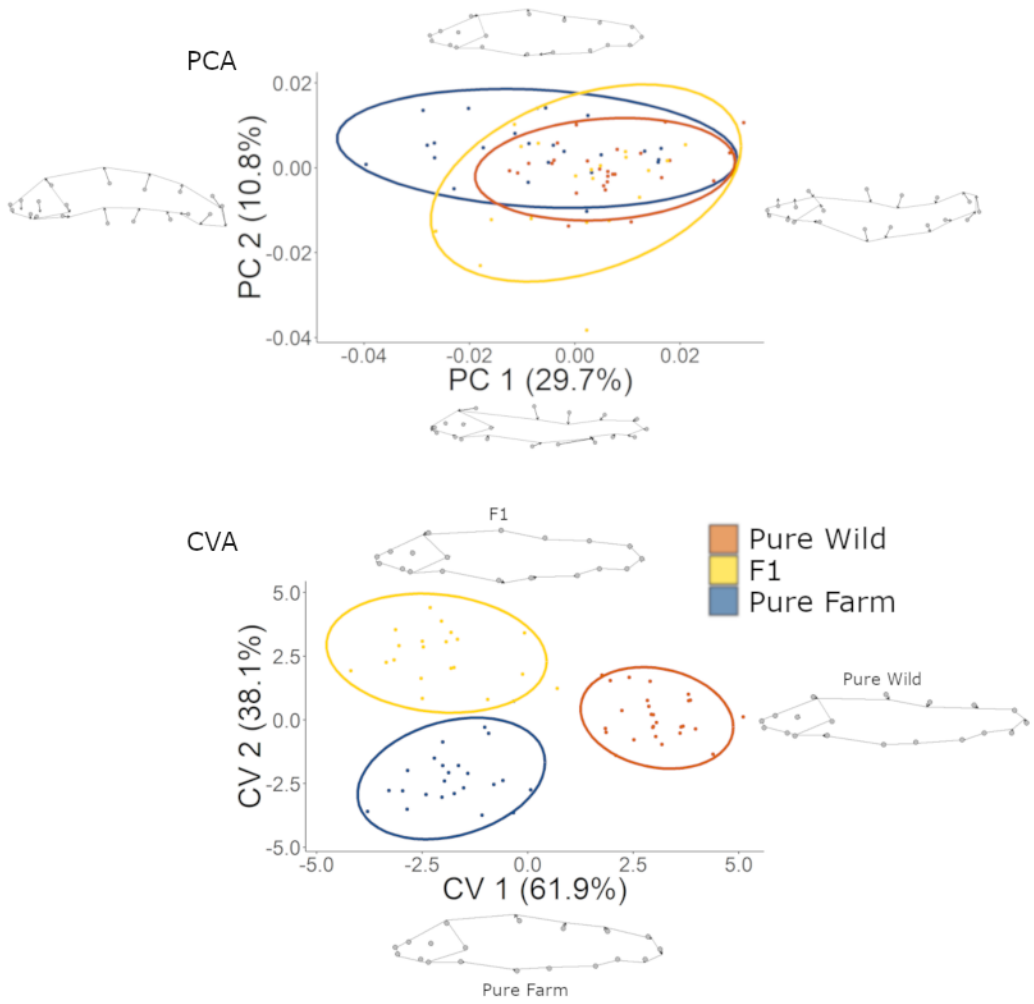

Figure S9. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Individuals are from Day 80 post yolk-sac absorption reared in tank conditions. Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

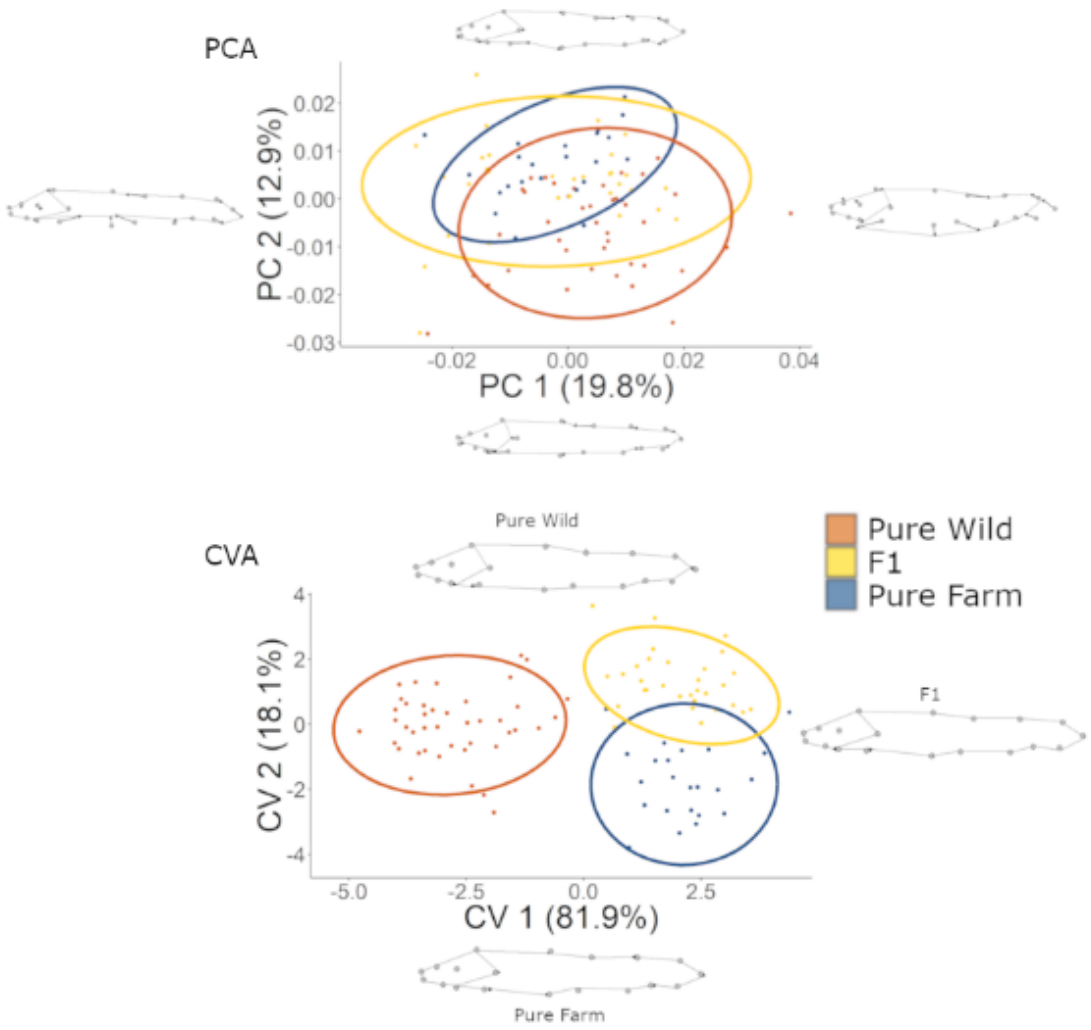

Figure S10. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Individuals are from Day 0 post yolk-sac absorption reared in semi-natural conditions. Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

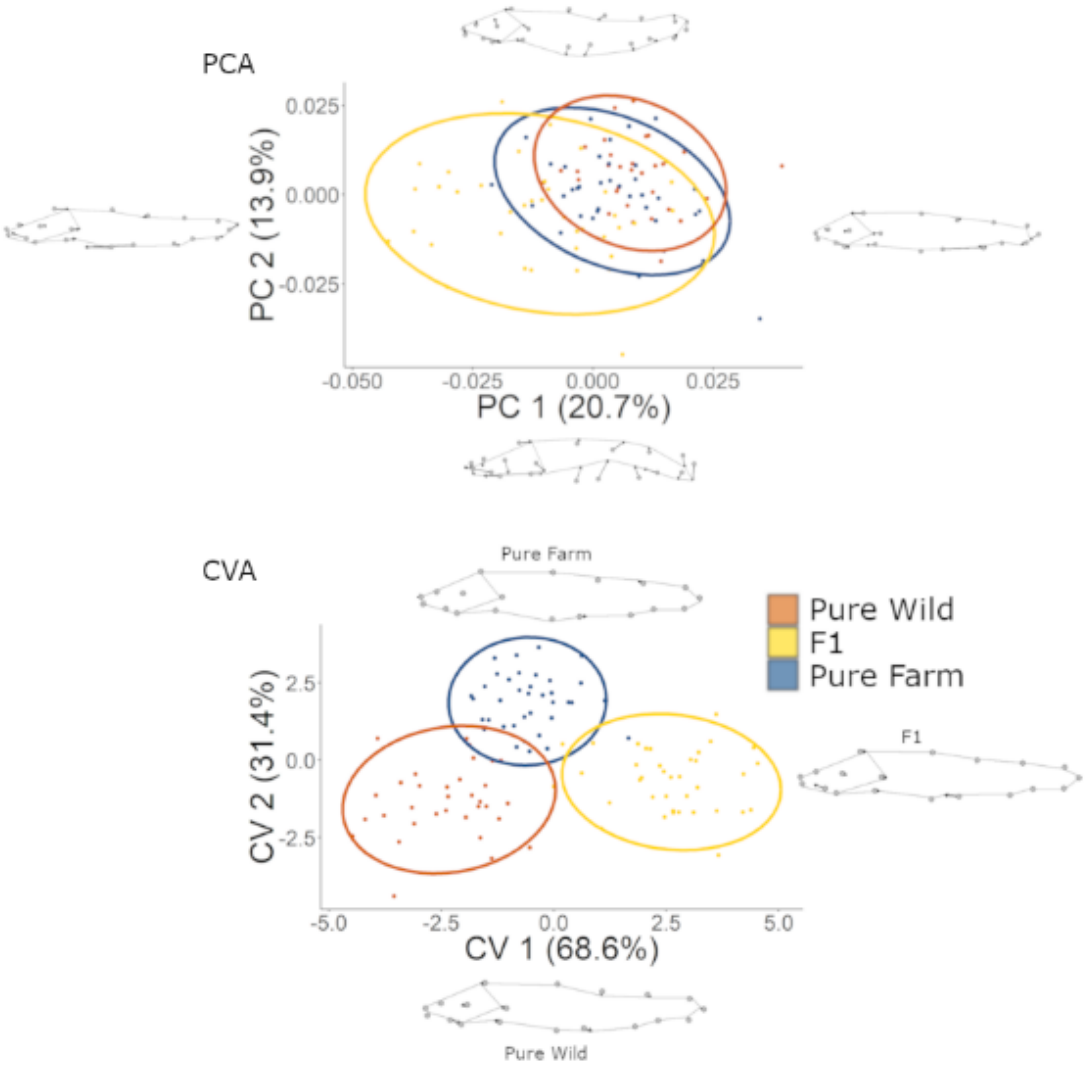

Figure S11. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Individuals are from Day 80 post yolk-sac absorption reared in semi-natural conditions. Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

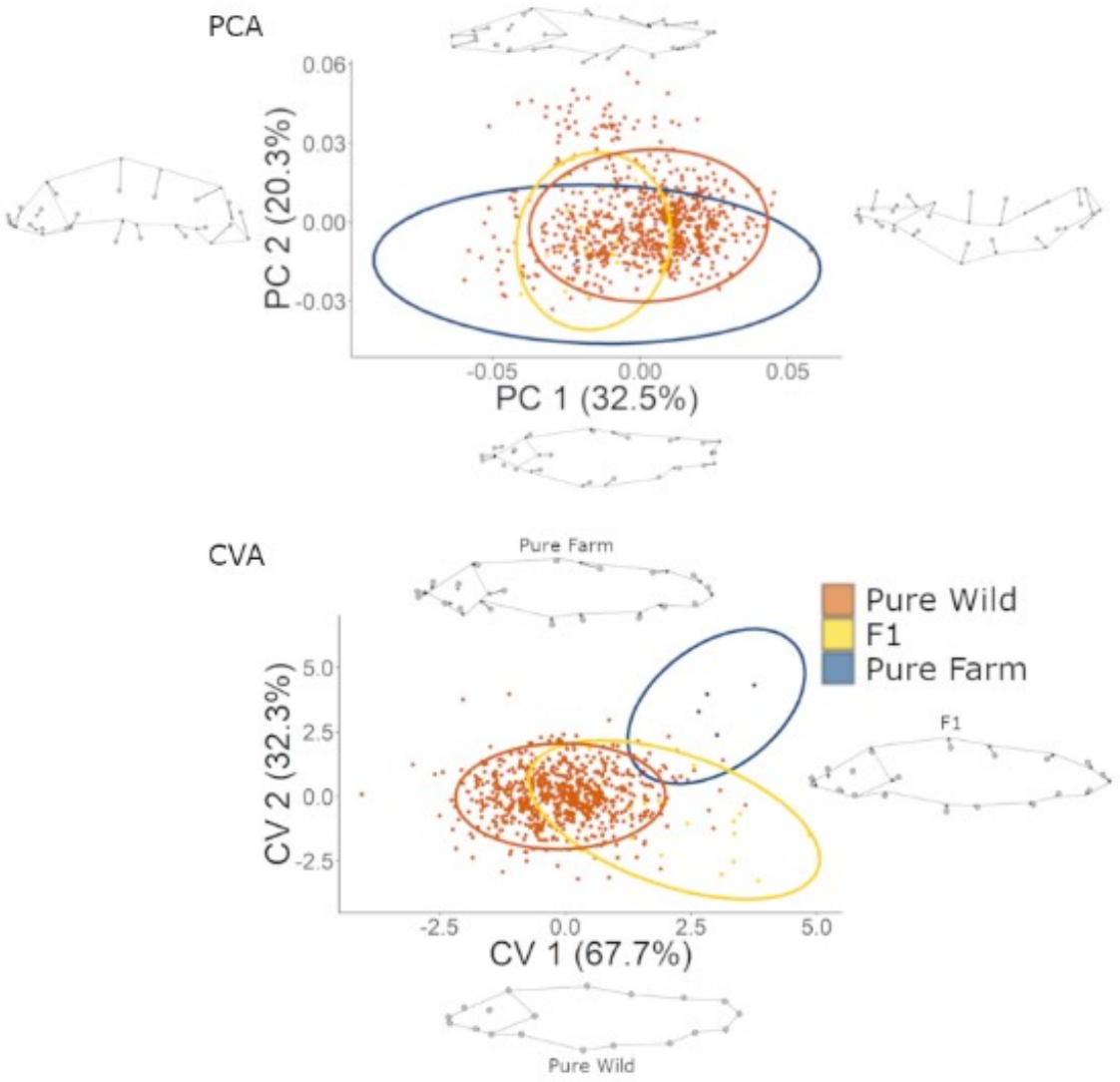

Figure S12. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Juveniles aged 0+ (young of year) were sampled from field locations (18 southern Newfoundland rivers; Figure 1, Table S2). Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

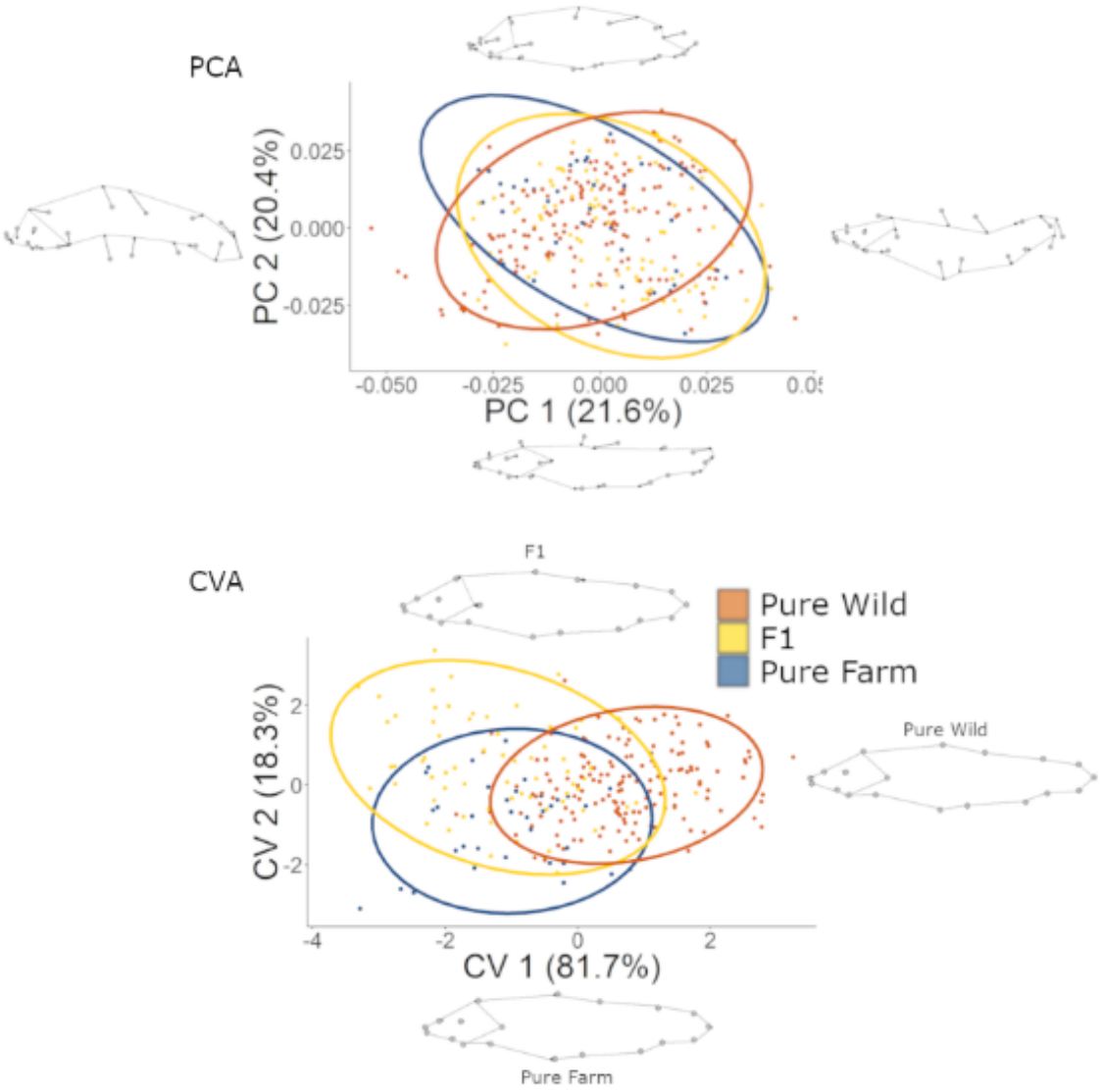

Figure S13. Principal component analysis and canonical variate analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each hybrid class. Juveniles aged 1+ were sampled from field locations (18 southern Newfoundland rivers; Figure 1, Table S2). Relative warps are shown for the first two principal components in the PCA and mean shape for each group are shown for the canonical variate analysis with 4x magnification of shape differences.

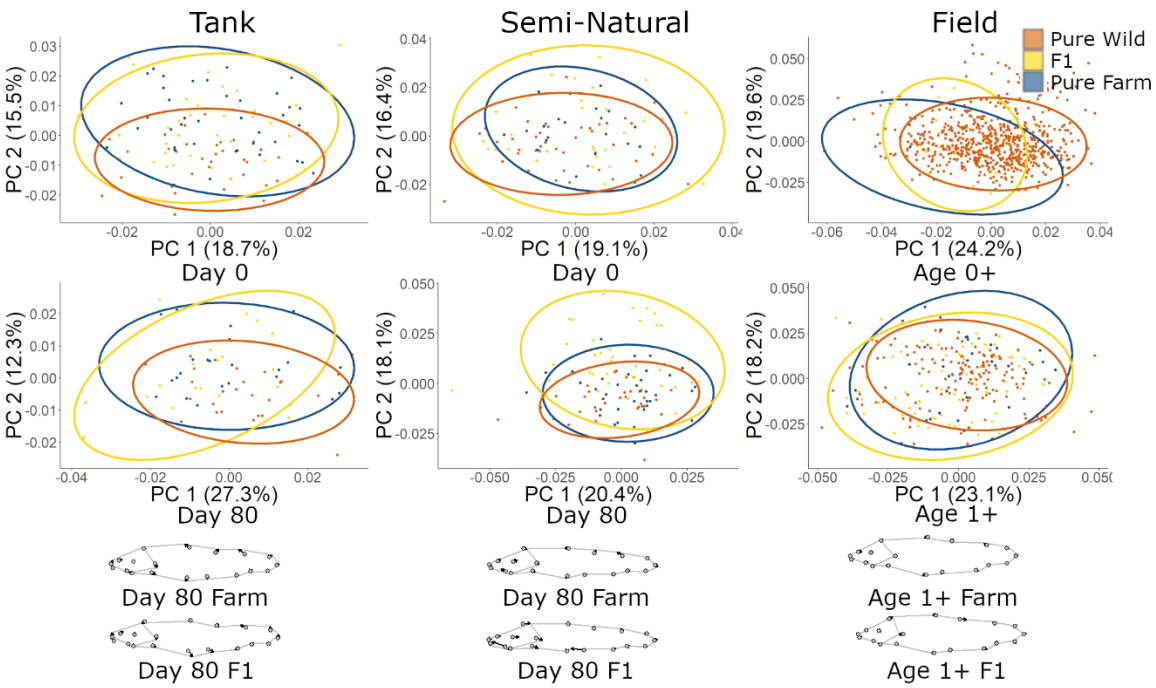

Figure S14. Principal component analysis of landmarks after Procrustes alignment without bending correction (see Figure 10 for with bending correction applied) with 95% confidence ellipsis for each hybrid class. For the common garden experiment shape is shown at Day 0 (Start) and Day 80 (End). Juveniles aged 0+ (young of year) and 1+ were sampled from field locations (18 southern Newfoundland rivers; Figure 1, Table S2). Relative differences in mean shape are also shown for each end time comparing mean shape of farm or F_1 hybrid to wild with 4x magnification of shape differences. Age 0+ field collected shape differences are not shown due to low sample size for pure farm individuals and pure wild-F_1 comparison is the same as age 1+.

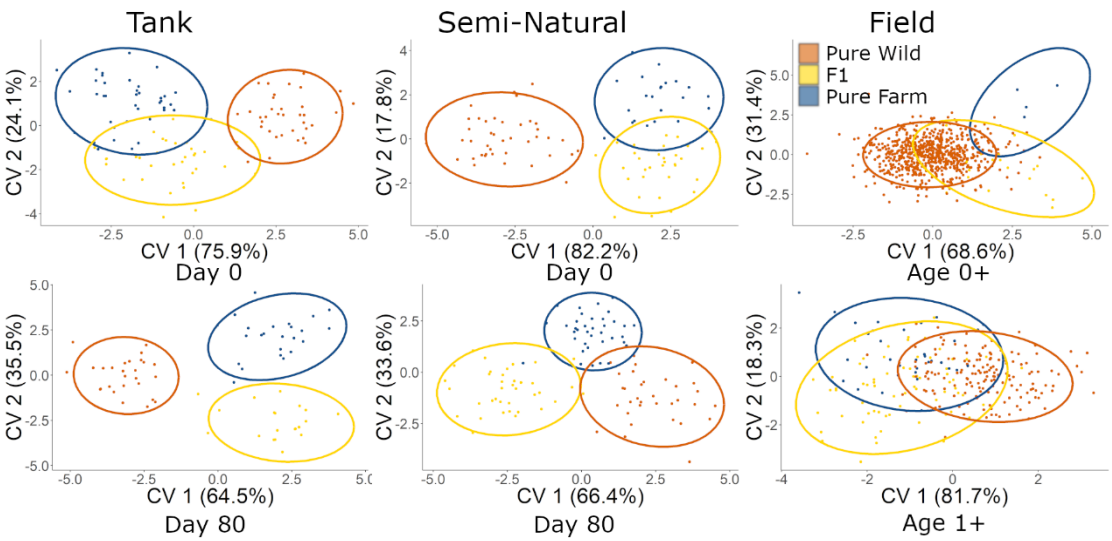

Figure S15. Canonical variate analysis of landmarks after Procrustes alignment without bending correction (see Figure 11 for with bending correction applied) with 95% confidence ellipsis for each hybrid class. For the common garden experiment shape is shown at Day 0 (Start) and Day 80 (End). Juveniles aged 0+ (young of year) and 1+ were sampled from field locations (18 southern Newfoundland rivers; Figure 1). Identification of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.

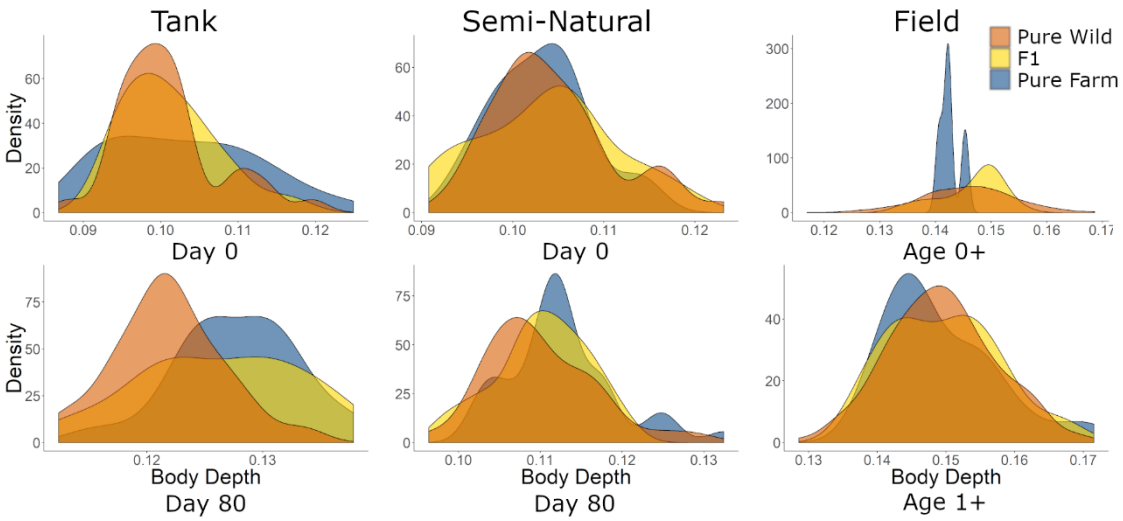

Figure S16. Body depth measurements using inter-landmark distance without bending correction (see Figure 12 for with bending correction applied) between landmarks 3 and 17. Landmarks are as defined in Figure 2. Individuals were measured at Day 0 (Start) and Day 80 (End) for the common garden experiment. Field salmon aged 0+ and 1+ were sampled from 18 rivers in southern Newfoundland as shown in Figure 1. Identification of hybrid status of field sampled individuals was accomplished using a 96 SNP panel and the program NewHybrids and is described in the Materials and Methods.