Assessing reproductive success of Atlantic salmon in two restoration programs

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General Abstract

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Restoration programs, including reintroductions and supplementations are often used to offset the continued decline of freshwater fish populations. While frequently implemented, few succeed in establishing self-sustaining populations. Some limitations that are thought to impede the success of these programs often stem from genetic and ecological issues due to carry over effects from a captive setting. These risks often influence the donor populations ability to reproduce in the wild. In this study, we evaluated the reproductive success of Atlantic salmon through genetic parentage analyses from a reintroduction program in two major tributaries of Lake Champlain, USA (Boquet, and Winooski Rivers, NY, VT respectively); as well as four different adult rearing groups (with varying duration of time spent in captivity) from a smolt-to-adult supplementation program and live gene-banking program in the Upper Salmon River, Fundy National Park, NB. We determined that adults which returned to both Lake Champlain tributaries, successfully reproduced in the wild. However, few adults produced the low numbers of surviving offspring sampled in this system. On the other hand, in Fundy National Park, we determined that all adult rearing groups were successful in reproducing in the wild. However, the relative reproductive success of the rearing group that spent the shortest time in captivity was comparable to other groups which spent longer periods of time in captivity. While our results suggested that fish from these restoration programs can reproduce in the wild, adults in both systems had low reproductive success. If this continues over subsequent years, the establishment and long-term persistence of these populations may be difficult to achieve. These studies not only contribute to a greater understanding of the reproductive ability of fish from both programs in the wild, but demonstrate the complexity of these restoration programs and the various factors involved in influencing restoration success. Therefore, these studies emphasize the importance of combining continued monitoring and an adaptive management to promote re-establishment and long-term persistence in restoration programs.

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Analysis and interpretation of data: Prevost

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General Introduction

Worldwide freshwater biodiversity is consistently declining due to the alteration of habitats, namely degradation and fragmentation, modification of flow regimes, introduction of invasive species, overexploitation of fishes and pollution (Dudgeon et al. 2006; Jelks et al. 2008). In North America alone, approximately 39% of fish species are imperiled, which includes 280 endangered taxa and 61 others that are either extinct or extirpated (Jelks et al. 2008). With these substantial declines, restoration programs are often implemented as a conservation tool to mitigate species losses and extinctions in many regions. These programs typically focus on habitat restorations and stocking programs (Armstrong and Seddon 2008; Dunham et al. 2011).

Stocking programs including reintroductions and supplementations, may provide an immediate demographic boost to imperiled or extirpated populations by releasing captively reared fish into the wild. Reintroductions are defined as the intentional movement and release of an organism within its indigenous range with an effort to establish a viable population (IUCN, 2013). Conversely, supplementation programs are defined as the intentional demographic integration of hatchery and natural fish into the wild, with the goal of improving the status of the existing natural population (Waples et al. 2007; Fraser 2008). Both types of programs involve a certain degree of captive breeding and/or rearing; normally fish are raised to a given life stage or from one to several generations in captivity until individuals or their progeny are released into the wild respectively (Fraser 2008). While these programs are frequently implemented and are successful in increasing some populations, few lead to self-sustaining populations in the wild and need to be continuously propagated with hatchery fish (Fischer and Lindenmayer 2000; Cochran-Biederman et al. 2015). Though there are several factors that impede the successful re-establishment of a population (Waples et al. 2007; Fraser 2008; Dunham et al 2011; Cochran-Biederman et al. 2015; Galloway et al, 2016), a key factor is quality of the donor population itself (Dunham et al. 2011).

Issues with the donor population can be due to genetic and ecological risks involved with captive rearing. Genetic risks associated with captive rearing can be due to domestication selection and a reduction or loss of genetic diversity (Frankham 2008; Fraser 2008). Domestication arises due to an extended period of time spent in captivity and can result in maladaptive traits (e.g. behavioral and morphological) due to unintentional selection and relaxed selective pressures in a hatchery (Frankham 2008; Araki et al. 2008; Fraser 2008). Additionally, because many fish

populations exhibit local adaptations (Fraser et al. 2011), maladapted individuals from a non-local population are often released into the wild, especially when populations are extirpated or severely depleted (Araki et al. 2008; Fraser 2008). Furthermore, the loss or reduction of genetic diversity often occurs when low numbers of adults are used in captivity to generate juveniles for releases in reintroduction/supplementation programs, which can lead to inbreeding depression (Fraser 2008). While the genetic risks are prominent, ecological risks often result in low breeding fitness as captive individuals are often not as reproductively fit as their wild counterparts (Araki et al. 2009; Theriault et al. 2011; Milot et al. 2013; Christie et al. 2014; Evans et al. 2014). Therefore, carry over effects, which is a nonlethal influence on an individual's performance of a previous (environmental) event in life (Harrison et al. 2011; Milot et al. 2013) – in this case, due to the captive setting – often lead to a decrease in reproductive success, a measure of reproductive fitness among released individuals in the wild (Fleming et al. 1997; Araki et al. 2009; Theriault et al. 2011; Christie et al. 2014; Evans et al. 2014; Clarke et al. 2016).

Reproductive success, defined in these studies as the number of offspring produced by individuals in the population may be hindered due to these carry over effects. Therefore, the evaluation of the reproductive capabilities of released individuals in the wild is warranted during reintroductions/supplementations. Programs often evaluate reproductive success by combining molecular analysis with river surveys; which is often achieved by sampling adults and their naturally emergent progeny in the wild and linking them through a parentage analysis. By quantifying reproductive success of released individuals, fisheries managers may not only acquire information on the reproductive capabilities of fish but also understand the challenges faced during reintroduction/supplementation programs.

In this thesis we assessed the reproductive success of the donor population during a stocking program with a molecular parentage analysis. Specifically, we quantified the number of offspring produced by reintroduced and supplemented Atlantic salmon in Lake Champlain basin, USA basin and in the inner Bay of Fundy, NB respectively. Lake Champlain and the Inner Bay of Fundy (hereafter Bay of Fundy) once had a thriving landlocked and anadromous Atlantic salmon population respectively. The Lake Champlain population was extirpated in the 1800s due to overfishing and damming of the tributaries (Marsden and Langdon, 2012), while the Bay of Fundy population began to decline in 1930s (Dadswell 1968) due to severe marine mortality and the alteration of freshwater systems (Department of Fisheries and Oceans (DFO), 2010). As a result,

reintroduction/supplementation programs were implemented in both systems, however self-sustaining populations have not yet been achieved. The lack of success may stem from genetic and ecological risks, as individuals from these populations all experience various degrees of exposure to a captive environment. Furthermore, poor juvenile survival and adult reproduction in Lake Champlain and the smolt to adult rearing phase in Bay of Fundy may be hindering restoration success.

In chapter one, we assessed the reproductive success of the Lake Champlain population to determine if individuals are capable of reproducing in the wild and, if so the genetic makeup of progeny. Traditional stocking programs are underway in Lake Champlain that include releasing thousands of juveniles of varying life stages into several of its tributaries. Previous studies have demonstrated several challenges for reintroduction success, including the quality of juvenile habitat (Brunsdon et al. Submitted) and the quantity and quality of spawning habitat (Hill et al. Submitted), the homing abilities of adults (Andrew Harbicht, Concordia University, PhD Dissertation 2018) and invasive species (Harbicht et al. 2018; Nicole Hill, Concordia University, Master's Thesis 2018). Successful natural reproduction has not been observed in Lake Champlain in over a century. Therefore, our goals were to: quantify the reproductive success of returning adults; determine the traits involved in influencing reproductive success; and, describe the genetic makeup of the juveniles.

In chapter two, we assessed the relative reproductive success of four adult rearing groups in the Bay of Fundy system and determined if duration of captive exposure influenced relative reproductive success. Unlike Lake Champlain, the limiting factor in the Bay of Fundy is the severe marine mortality during adult life stages in the ocean (DFO 2010). To mitigate marine mortality whilst minimizing the carry over effects from captive rearing, a smolt to adult supplementation (SAS) program was implemented. SAS programs, aim to capture surviving smolts (previously stocked as fry), rear them to the adult life stage in sea pens (captivity) and then later release these adults in the wild to spawn naturally (Fraser 2016). In doing so, the degree of environmental captivity during earlier life stages is minimized, marine mortality is avoided and free mate choice in the river can occur. Furthermore, other initiatives such as a live gene-banking programs are conducted to conserve the genetic stocks of fish that previously resided in this system. Collectively, groups of adult fish from each program differ in their duration of captive exposure due to various rearing treatments and are then released to spawn in the Upper Salmon River, Fundy

National Park, NB. While these programs are conducted annually, little is known about the reproductive success of each group and the influence of the duration of captive exposure on their reproductive performance. Therefore, we quantified reproductive success of each group and tested the prediction that relative reproductive success would increase as the duration of exposure to captivity decreases.

In both chapters, we contributed to the growing body of literature on reintroduction/supplementation and learned about complexity of restoration success in both systems. These studies will help inform effective adaptive management decision-making for not only these restoration programs but for other programs on related species.

Chapter 1.

Patterns of reproductive success among reintroduced Atlantic salmon in two Lake Champlain tributaries

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Abstract

Reintroduction programs are increasingly implemented to regenerate self-sustaining salmon populations. The extent to which returning adults successfully produce surviving offspring is useful for informing reintroduction efforts but often unknown, as is knowledge of the genetic makeup of those offspring. We investigated the patterns of reproductive success among returning Atlantic salmon reintroduced as juveniles in two tributaries of Lake Champlain, by combining redd surveys (n=120) and DNA parentage analysis. Tissue samples collected from returning adults and their offspring produced in the wild (young-of-the-year) were genotyped using microsatellite loci. Our results suggest that a low proportion of returning adults produced surviving offspring in either tributary (range 2-9%, or 11-15 of 124 and 3-4 of 81 returning adults, respectively) and that reproductive success was not variable among the sexes. These results demonstrate that adults can naturally and successfully reproduce in both of the studied tributaries (Winooski River, Boquet River). Nevertheless, a larger number of breeders would need to produce offspring in subsequent years to demographically augment the reintroduced population whilst avoiding short-term issues associated with low genetic diversity and inbreeding. Our results also point to underlying ecological problems which may have reduced reproductive success and potential genetic issues within the hatchery-reared population being used for supplementation, indicating further adaptive management will be useful to ensure self-sustaining populations in this system.

Introduction

Species extinctions and population declines are occurring at unprecedented rates in freshwater ecosystems due to anthropogenic activities (Dudgeon et al. 2006). As a result, reintroduction programs are frequently implemented in many regions as an applied management approach to promote species recovery (Cochran-Biederman et al. 2015). These programs often aim to reestablish an extirpated or declining population by moving and releasing captive-reared or wild individuals to their native habitat ranges (Seddon et al. 2007). While such programs can succeed in attaining these goals, few successfully establish a self-sustaining population (Fischer and Lindenmayer 2000; Cochran-Biederman et al. 2015).

Reintroduction failure can occur for a multitude of reasons, with one of the main predictors being whether the initial cause of extirpation has been remedied (Fischer and Lindenmayer 2000, Cochran-Biederman et al. 2015). While guidelines and suggestions have been put forward to ensure consideration of these issues during initial stages of reintroduction programs (Dunham et al. 2011; IUCN 2013; Anderson et al. 2014; Cochran-Biederman et al. 2015), released individuals still may not be able to establish themselves in the wild. This could be due to reduced reproductive success (Rs), a measure of reproductive output which is defined in this study as the number of surviving offspring produced by an individual within a population. Considerable Rs achieved by a large number of individuals within a population can maintain genetic diversity and increase the likelihood of long-term persistence through divergence of life history traits and local adaptation (Taylor 1991). Therefore, if released individuals are not achieving high Rs, either due to issues with the individuals themselves or the receiving habitat, establishment and long-term persistence which requires positive population growth (i.e. $\lambda>1$) may be difficult to achieve.

Challenges with released individuals and the receiving habitat are of particular concern for reintroduced migratory salmonids. Salmonid populations, namely Atlantic and Pacific salmon, are often supplemented with hatchery reared individuals that are stocked at juvenile life stages. While this provides an immediate boost to the populations, few may reproduce in the wild as hatchery fish tend to have lower Rs than their wild counterparts (Araki et al. 2007a; 2008; Williamson et al. 2010; Theriault et al. 2011; Milot et al. 2013). This is often influenced by several mechanisms, one of which can stem from captive breeding and domestication selection. Therefore, maladaptive

traits due to relaxed selective pressures (Fraser 2008), inbreeding and outbreeding depression (Houde et al. 2011), and loss or reduction of local adaptation (Araki et al. 2008) must be properly considered and preferably avoided before reintroduction attempts are made. Additionally, the receiving habitat can impede salmon recovery at various life stages. For instance, barriers such as dams or stream blockages prevent surviving adults from returning to natal spawning streams (Anderson et al. 2013). This often forces spawning in suboptimal habitats, which may have a negative influence on Rs (Castro Santos and Haro 2003; Burnett et al. 2014; Harbicht et al. 2018). Barrier removal and translocations are important mitigation options, as they promote connectivity to spawning habitats and providing opportunity for reestablishment (Anderson et al. 2010; Pess et al. 2012; Sard et al. 2015).

In addition to issues with released individuals and receiving habitat, traits such as body size, arrival date and life history strategies can affect Rs of reintroduced salmonids. In both sexes, there is a positive relationship between body size and Rs (Fleming 1996; Dickerson et al. 2002; Anderson et al. 2010). Larger females produce larger, more numerous eggs (Fleming 1996; Dickerson et al. 2002) and can better defend nests (Foote 1990; Dickerson et al. 2005), whereas larger, males thrive in intrasexual competition for mating opportunities (Fleming 1996). Arrival date can also influence Rs, previous studies have showed that earlier (Williamson et al. 2010; Anderson et al. 2013), later (Ford et al. 2008; Anderson et al. 2013), and intermediate arrival time can all favor higher Rs (Anderson et al. 2013). Differences in Rs can also be variable between years and sexes (Dickerson et al. 2005) and be influenced by local environmental conditions. Lastly, some male salmonids have an alternative life history tactic known as mature parr or jacks. While these mature parr or jacks are small and cannot physically outcompete larger males, they can significantly contribute to several cohorts due to their sneaker mating tactic (Fleming 1998). As a result of these alternative life history tactics and intense sexual selection, Rs is commonly more variable among males than females (Quinn and Foote 1994; Weir et al. 2010). Overall, by collectively understanding how different traits affect RS, crucial information for enhanced reintroduction design may be acquired (Hendry et al. 2003; Anderson et al. 2013).

Here, we evaluated the Rs of hatchery reared Atlantic salmon (*Salmo salar*) being reintroduced into Lake Champlain, USA. Lake Champlain once had a thriving landlocked salmon

population, which became extirpated in the 1800s due to overfishing, the logging industry and damming of the tributaries (Marden and Langdon 2012). To restore the population, its tributaries have been stocked annually since the 1970s with hatchery reared salmon of varying life stages (Marsden and Langdon 2012; Brunsdon et al. 2017). Two of these major tributaries are the Winooski River, Vermont and Boquet River, New York. Complete and partial barriers such as dams are still established in both systems that prevent surviving adult salmon from returning to their natal streams to spawn. As a result, translocations and dam removal have been initiated to facilitate upstream migration during the spawning run. In the Boquet River, a run of the river dam and accompanying fishway were thought to be a partial barrier (Harbicht et al. 2018) to upstream migration. After removal of the dam and fishway in summer 2015, the historic cascades (hereafter the Willsboro Cascades) immediately downstream of the dam were thought to be a partial barrier to movement (Harbicht et al. 2018). Hence, adult fish were captured below the Cascades and translocated to historic spawning grounds upstream in subsequent years. In the Winooski River, several dams serve as complete barriers to migration, therefore translocations take place annually.

To evaluate returning adult Rs, river surveys including adult and young of the year (YOY) sampling were combined with a molecular parentage analysis using several highly polymorphic microsatellite loci that linked putative parents with their progeny. Adults were sampled as they were intercepted at the first migration barrier encountered and were translocated upstream during fall 2015 in the Winooski and 2016 in both rivers. Their naturally spawned YOY were then located and sampled the following summers (2016, 2017). Our study had three specific objectives. First, we determined the number of surviving offspring produced in both rivers in all years. Since there were no previous reports highlighting natural reproduction throughout the past century, finding YOY was essential to understanding if salmon could re-establish in each river. Second, we quantified the mean and variance in Rs and determined the numbers of individuals contributing the next generation in each river. Finally, we determined if body size and arrival date influenced adult Rs. This work allowed us to evaluate the Rs of surviving hatchery-reared salmon in the wild and gain important insight on the ability of a reintroduced population to become self-sustaining.

Materials and methods

Study sites

The Lake Champlain watershed, approximately 21,326 km², is bounded by New York, Vermont and southern Quebec (Marsden and Langdon, 2012). This study was conducted in two large tributaries, the Winooski River, Vermont and the Boquet River, New York (Figure 1.1).

The Winooski River is the largest tributary of Lake Champlain spanning 142 km from the Green Mountains until emptying into the Lake Champlain. It is composed of sedimentary rock and surrounded by forested, urban and agricultural land (McDowell et al. 2001). Several dams on the river are complete barriers for Atlantic salmon migration (See Nyqvist et al. 2017). Therefore, to facilitate upstream migrations trap and truck programs are conducted annually (Lake Champlain Technical Report 2015, see below).

The Boquet River, one of the steepest rivers in New York, flows 126 km from the Adirondack Mountains until emptying into Lake Champlain (Brunsdon et al. 2017). The watershed is mainly composed of limestone/dolostone rock, surrounded by a secondary growth forest (Marsden and Langdon, 2012; Wu and Kalma, 2013). Wilsboro Dam is located 4 km from the mouth of the river, and is the first dam to be encountered by returning salmon. While the dam had a fish lift to promote salmon migration, previous studies indicated that few fish were passing above the dam (Lake Champlain Technical Report 2009-2014; Harbicht et al. 2018). Therefore, in summer 2015, the Willsboro Dam was removed to facilitate upstream migration. Monitoring efforts that included fishing and redd surveys were conducted during the spawning run of 2015 to determine if fish were able to traverse the Wilsboro cascades. Although there were several fish caught, no redds were detected within the surveyed sites upstream of the cascades (Lake Champlain Technical Report 2015).

Stocking

Both the Winooski and Boquet Rivers are stocked annually with Lake Sebago strain Atlantic salmon (Maine, USA) (Table S1.4). All stocked fish originated from the captive broodstock at Bald Hill Fish Culture Station (FCS) (Vermont, USA). Embryos were transferred from Bald Hill FCS to Dwight D. Eisenhower National Fish Hatchery (Vermont, USA)

immediately after fertilization where they were reared until stocking as; unfed fry (stocked in May at age 0), fall fingerlings (stocked in October at age 0+) and yearling smolts (stocked March – May at age 1+).

Adult and juvenile sampling

Adult salmon returning to the Winooski River in fall 2015, 2016 and the Boquet River in 2016 were intercepted at the first barrier encountered, then processed and translocated upstream of the cascades from September to November. Fish returning to Winooski River were caught at the Winooski One Dam, which is roughly 16.5 km from Lake Champlain, by a fish lift. Fish returning to the Boquet River were caught by quick set gill netting (<3min in the nets) in two large pools below the Willsboro Cascades. Gill nets were 15 meters long, 3 meters deep and had mesh spacing of 16 cm^2 (#139 mono netting, Duluth Fish Nets). Nets were deployed throughout the pools by either wading or by canoe. Once a fish was caught it was immediately removed from the net. After removal, fish were sexed, measured for fork and total length and weight, and their time of arrival was noted. Once measured, a tissue sample was taken from the caudal fin and preserved in 95% ethanol. After processing was completed, fish were placed in a 946L aerated tank on a truck and translocated to the release site upstream of barriers.

Translocations in the Winooski took place 3-5 times per week (more often when more fish were caught). Fish were driven by a truck to bypass two dams upstream of Winooski One Dam (Gorge #18, 2.8km from Winooski One; and Essex #19, ~13.2 km from Winooski One). Fish were released in the mainstem of the Winooski at the first available drop-off point after the third dam to allow them to have access to the available spawning habitat between the Essex #19 and Bolton dams (~33 km from Winooski One). Boquet fish were translocated twice a week to the mainstem of the North Branch River, a historic spawning site in which salmon fishing is prohibited.

Redd surveys were conducted in both rivers by walking along the banks of previously known spawning sites in the Winooski or historical spawning sites in the Boquet (Figure 1.1); approximately 25km and 16 km were surveyed for redds respectively. The GPS coordinates of each redd were taken with a Garmin eTrex 20x. These redd sites were then sampled for YOY the following spring and summer by a snorkeler with a dipnet. To catch YOY, a snorkeler would start

100-250 m downstream of the most downstream redd within the given site and swim upstream in a zig-zag formation (Brunsdon et al. 2017). Once a fish was captured it was placed in a bucket with fresh river water. A caudal fin tissue sample was collected and preserved as described above for adults. Juveniles were then released back to the exact location where they were caught. Sites that had YOY were sampled up to 4-5 times, while sites without YOY were sampled 1-2 times throughout the summer (in both systems). To avoid clipping the same fish twice, sampling surveys occurred within 2 weeks of the first attempt to insure fin regrowth had not occurred.

While YOY surveys took place in both rivers in summer 2016 and 2017, YOY were only detected in the Winooski in 2016 and the Boquet in 2017. The former was due to high water levels that made sampling dangerous and difficult in 2017, while the latter was due to no YOY being detected below the Willsboro Cascades in 2016 (no redds were detected above the cascades).

Genotyping

DNA from all adult and juvenile tissue samples was extracted using a modified chelex protocol (adapted from Hua and Orban 2005). A 5% chelex solution (Chelex 100, Sigma) containing 125 µg/L of 25 mg/ml proteinase K and a ~2mm cutting of individual fin clips were incubated at 45℃ for one to six hours. Once incubated, samples were then boiled at 98℃ for 5 minutes to lyse cells. Samples were subsequently genotyped at nine highly polymorphic microsatellite loci; *SsaD144*, *SsaD157*, *SsaD48*, *SsaD71* (King et al. 2005), *SSsp2215*, *SSsp2216*, *SSsp 2201*,*SSspG7* (Paterson et al. 2004) and Ssa407UOS (Cairney et al. 2000) and amplified using seven polymerase chain (PCR) reactions. Detailed reactions and thermocycler profiles for the 9 loci can be found in the supplementary material (Table S1.4). Amplified PCR products were then visualized using the ABI 3500 sequencer and resulting alleles were manually scored on Genemapper 3.2 (Applied Biosystems Inc.).

Samples that were not successfully genotyped on the first attempt were re-genotyped. Genotyping error and allelic dropout rate were calculated on per-locus basis using the nine loci panel and included samples with complete genotypes. The per locus analysis was conducted by comparing alleles for a given locus to their original genotype and the proportion of which fully or partially matched as well as those that completely mismatched were then tallied (Table S1.1).

Furthermore, null allele rate and error rate was assessed by MICROCHECKER (v.2.2.3; van Oosterhout et al. 2004). Finally, GeneALEx (v.6.502; Peakall and Smouse, 2012) was used to obtain number of alleles, effective number of alleles, expected heterozygosity and observed heterozygosity for each locus.

Exclusionary power analysis

A series of simulations were performed on P-LOCI (Matson et al. 2008) to assess the ability of the microsatellite loci panel to correctly assign parent-offspring triplets. This software simulates offspring based on a mating matrix and per-locus error rates and then re-assigns the simulated offspring with all putative parents using Mendelian principles (Matson et al. 2008). Genotyping error rates, null allele frequencies as well as the genotypes of all parental pairs were used to generate three simulations of 100 offspring per parental pair. Similar simulations were also performed using error rates ranging from 0-2% (Table S1.2). The simulated offspring genotypes were then reassigned back to the parental pairs (among all putative parents).

Parentage Analysis

To determine which adults were successful in producing YOY, parentage analyses were conducted in SOLOMON (v.1.01; Christie et al. 2013) using exclusionary principles (Jones and Ardren 2003; Jones et al. 2010). Separate analysis was conducted for each both the Boquet and Winooski Rivers, which included juveniles found and putative parents that returned to their respective river. Exclusionary principles follow the logic of Mendelian inheritance; if a putative parent does not share at least one allele per locus with the given offspring, they are excluded as the true parent of the given offspring (Jones and Ardren 2003; Jones et al. 2010). All genotypes spanning across the nine loci from the adults and juveniles in the Winooski and Boquet Rivers were included in two separate analyses (separate analyses per river). Parent offspring triplet matches were made when matches occurred at eight of nine loci (see Exclusionary power analysis, Appendix). Pedigree Viewer (v.6.5f; Kinghorn and Kinghorn, 2015) was used to visualize the parent offspring triplet match outputs (Figure 1.2). Finally, to consolidate parental assignments, an estimate of the effective number of breeders (N_b) for each of the two juvenile cohorts was independently generated using the linkage disequilibrium approach implemented in LDNe (Waples and Do 2008); we used an allele exclusion criterion Pcrit=0.02 for our samples that were

between 25 and 100 (Waples and Do 2010). Confidence intervals were generated through the parametric method (Waples 2006).

Statistical Analysis

One-way ANOVAs were used to compare the arrival time, body mass and length between males and females that returned to the Winooski and Boquet River in 2015 and 2016 respectively. A Levene's test was used to compare the variance in Rs between males and females. Two fish were not sexed in the Winooski River and were therefore taken out of the analysis regarding the comparisons between sexes. Pearson correlations were conducted on the known males and females separately to determine if body mass and arrival date were associated with RS. Body mass, number of offspring and mates were log transformed due to violation of normality. Arrival date was transformed to Julian days (1-365) to ensure feasibility of statistical testing. Pearson correlations were also used to explore the association between number of adult pairings and the number of offspring produced. A power test was used to determine the statistical power of our Pearson correlation tests.

Results:

Returning adults, redd surveys, YOY surveys

Winooski River - A total of 124 adult Atlantic salmon returned in 2015 (60 females, 62 males, 2 unsexed). The number of fish caught in the lift and translocated per day ranged between 2-18 individuals. Twenty-one fish arrived in September, 80 in October, and 23 in November. Female, and males did not differ significantly in arrival date (one-way ANOVA, $F_{1,120}$=0.026, p=0.86). Mean body length and mass were 556mm (range=469-668mm) and 1.75kg (range=0.76-2.88kg) respectively. Female and male length and mass did not differ significantly (one-way ANOVA; $F_{1,120}$=3.086, p=0.08, $F_{1,120}$=2.297, p=0.132, respectively), females were slightly heavier than males (mean = 1.81kg vs. 1.70kg) while males were slightly longer than females (mean = 561 mm vs. 549 mm). Thirty-seven redds were detected along the mainstem of the Winooski and in the Huntington River (one of its major tributaries, Figure 1.1b). A total of 38 YOY were captured on the mainstem of the Winooski in 2016 and could have originated from up to 15 redds from one site (Figure 1.1b).

Boquet River - A total of 81 adult salmon returned in fall 2016 (32 females, 49 males): 48 in October; 9 in September and 24 in November. There was a significant difference between male and female arrival date (one-way ANOVA, $F_{1,79}$=8.49, p=0.004). Males arrived earlier than females: most males (n=34) arrived from September 27th to October 21st while most females (n=21) arrived from October 24th to November 11th. Overall mean body length was 577mm (range=359-750) and mass was 2.18kg (range=0.87-4.57kg). There were no significant differences between female and male body length and weight (one-way ANOVA, $F_{1,79}$=0.176, p=0.676, $F_{1,79}$=1.699, p=0.196, respectively). Females were on average slightly longer (581mm vs. 574mm) and heavier (2.33kg vs. 2.03kg) than males. Eighty-three redds were detected in the North Branch of the Boquet River (tributary of the Boquet), 79 of which were sampled for YOY in 2017. These redd sites were scattered in five distinct locations (Figure 1.1a). Four redds out of 83 were not sampled due to the fact that they were single redds (not found in clusters) and extreme sampling conditions. A total of 85 YOY were found and collected from two of the locations. One YOY was found at the adult release site; while the other 84 were collected in a location further upstream. These YOY could have originated from a total of eight possible redds established in that particular location (Figure 1.1a).

Genotyping

Genotyping repeatability was confirmed at 98.5% concordance (1.5% error mismatch rate) as only 7 of the 478 alleles implemented in the per-locus error rate analysis conflicted with each other. Allelic dropout occurred once in *SSaD48, SSaD71* and *SSsp2216.* Per-locus error rate ranged from 0-2.4% (Table S1.1). There was also no evidence of null alleles or scoring error as per MICROCHECKER. Simulations conducted on P-LOCI indicated a 100% assignment rate with our loci panel as there were no instances of incorrect assignments (Table S1.2).

Adults returning to the Winooski and Boquet Rivers had an average of 21.9 alleles per locus (range 10-38); average heterozygosity across loci for both the Boquet and Winooski River was 0.82 (range:0.815-0.984). Compared to adult samples, YOY samples had fewer alleles per locus [12 (range=8-16; Winooski); 8 (range=5-12; Boquet)] and average heterozygosity [0.94 (range 0.90-0.97; Winooski); 0.75 (range 0.58-0.98; Boquet)] (Table 1.2).

Parentage assignments

Winooski River - Of the 38 YOY offspring, 31 (81.5%) were successfully assigned back to single parental pairs; the remaining seven offspring (18.4 %) were assigned to a female but no male. A total of 6 known females and males produced the 31 offspring found (Table 1.1). There were also seven occasions when four females mated with unknown males; based on comparisons of offspring and females genotyped, there could have been up to 2-3 unknown males that could have sired these offspring. A total of 30 and 37 offspring were assigned back to a father and mother with no mismatching loci respectively, while one offspring mismatched at one locus with potential mother and fathers. Finally, all offspring that did not assign back to a father mismatched at 3 or more loci with putative fathers.

The variance in Rs between male and females did not significantly differ (Levene's test, $F_{1,10}$=1.421, p=0.26). However, Rs between males was highly variable: one male produced 47.3% of the offspring (18 offspring) while the other males produced 1-7 offspring (two or three unknown males produced 7 offspring). Female Rs somewhat varied as three each produced 9 offspring, while the remaining three produced 1, 4 and 6 offspring. Of the parents that produced surviving YOY, females each paired with 2-4 partners (with the exception of one female), while only 3-4

males paired with 2-3 females. Point estimates of N_b using LDNe suggested that 13 (95%, CI: 11-15) spawning adults in 2015 contributed genetically to the YOY cohort sampled in the spring of 2016.

Boquet River-A single offspring out of 85 was successfully assigned back to two parents, while the remaining 84 offspring were unassigned. These results suggested that unknown adults were potentially able to overcome the partial barrier. The unassigned YOY were mismatched at an average 3.74 loci with the putative parents. The single YOY that was successfully assigned parentage was captured at the adult release site; all others were found in the higher reaches of the North Branch River; these sites were geographically separated by ~5 km. Point estimates of N_b using LDNe suggested that a very low number of adults (4, 95% CI=3-4) produced the remaining 84 offspring at the North Branch site.

Adult features and Rs – A series of Pearson correlations were performed to determine if body size (length and mass) as well as arrival date associated with Rs and number of adult pairings. This analysis was performed on the adults returning to the Winooski only (Boquet Adults were all unknown). Given the low number of parents in the Winooski a two tailed power analysis was performed on all correlations. Body length and mass were log transformed due to the violations of normality and were highly correlated with each other for both sexes (R^2=0.97, p=<0.001). Therefore, to determine if body size associated with Rs, body mass was used. The number of offspring and parings were log transformed due to violations from normality. Female and male body mass did not significantly correlate with the number of offspring an individual produced (R^2=0.15, p=0.76, β=0.06, R^2=0.54, p=0.26, β=0.21, respectively) and the number of pairings for females and males (R^2=0.50, p=0.30, β=0.18, R^2=0.72, p=0.10, β=0.40, respectively, Figure S1.1 and S1.2). Furthermore, arrival date did not significantly correlate with the number of offspring produced for both females and males (R^2=0.62, p=0.18, β=0.28, R^2=0.33, p=0.52, β=0.09, respectively) or the number of adult pairings (R^2=0.35, p=0.49, β=0.10, R^2=0.22, p=0.66, β=0.07, respectively, Figure S1.1 and S1.2). Finally, for males, the number of pairings significantly correlated with the number of offspring produced (R^2=0.96, p=0.002, β=0.94), while there was no significant relationship for females (R^2=0.68, p=0.13, β=0.34, Figure 1.3).

Discussion

Our study investigated the Rs of surviving hatchery reared salmon that were reintroduced into the wild. Overall, there were few surviving offspring detected in both rivers assessed, as well as few successful adults that they assigned to. In the Winooski River, the variance in Rs between sexes did not differ significantly. However, Rs was extremely variable among males. In the Boquet, results suggest that unknown adults overcame the barrier and parented the majority of the offspring sampled. Therefore, in this river, it is not possible to know the number of each sex that contributed offspring and we cannot make any distinctions about their Rs or variation in Rs. Finally, although there was no correlation between body size, arrival date and Rs or number of adult parings for both sexes in the Winooski due to power issues, we did find that the number of pairings and Rs positively correlated for males. While we demonstrated that hatchery reared fish were able to reproduce in the wild, our parentage and N_b estimates indicated that there were few successful parents. With few founders producing offspring over subsequent generations, problems with reduced genetic diversity and inbreeding depression could hinder future re-establishment of a self-sustaining population in the study system.

While several more adults may have successfully reproduced in both rivers, few were represented in the sampled offspring. There were 12 known and ~3 unknown fish and 2 known and 4 unknown fish that produced surviving offspring in the Winooski and Boquet Rivers, respectively. These low numbers of successful adults represented in the offspring could be due to reduced egg to fry survival. Low offspring survival could be indicative of poor spawning habitat or issues with invasive species. Salmon construct their redds in habitats with substrate that is small enough to excavate but large enough to promote aeration of eggs (Louhi et al. 2008; Beechie et al. 2008). While adults may have selected habitats with these specific substrate types, high siltation rates could have inevitably suffocated the eggs by preventing interstitial velocities (Lapointe et al. 2004). Qualitative observations and measured siltation rates in the Boquet and Winooski spawning sites (respectively) have indicated that there are areas with large amounts of silt deposits (Nicole Hill, Concordia University, 2017, pers. Comm.), which could have profoundly influenced egg to fry emergence. Another issue is early mortality syndrome (EMS), which stems from a non-native forage fish introduced into Lake Champlain in 2003 (Marsden and Langdon, 2012). EMS is caused by a Vitamin B (thiamine) deficiency in eggs of females that forage on non-native alewife in Lake

Champlain (Fisher et al. 1995; Harder et al. In Press). If there were multiple returning females with low thiamine levels many offspring likely did not survive to emergence.

While the variance in Rs between male and females did not significantly differ in the Winooski (adults in the Boquet were unknown), Rs was skewed between successful males (Table 1.1). This could be due to a male-biased sex ratio and body size. A male-biased sex ratio, could have led to intense competition leading to the formation of a dominance hierarchical structures (Fleming 1996; Weir et al. 2004), and may have promoted higher Rs in larger more dominant individuals (Fleming and Gross 1994; Fleming 1998; Garant et al. 2001). While we did not find a correlation between body size, Rs, and the number of pairings due to low statistical power, the male with the highest Rs and number of pairings was indeed the largest among the known fathers. Finally, life history strategies could have influenced the variation of Rs (Weir et al. 2010). Since salmon cannot reach the spawning ground unless translocated, it is likely that the unknown males were mature parr. In 2014, roughly 8860 fall fingerlings were stocked in the site where we found YOY in 2016 (Lake Champlain Technical report 2014) and could have matured as parr. Mature parr can significantly contribute to a cohort of the offspring (Thomaz, et al.1997; Taggart et al. 2001; Weir et al. 2010) due to their sneaker mating tactic (Fleming 1998). In the present study, 2-3 mature parr produced 18% of the total amount of offspring in the Winooski River and mated with four different females.

Female Rs was consistent between successful mothers. There were three females that produced 9 offspring while the three others produced 1-6 (Table 1.1). Female reproductive success has been previously shown to correlate with larger body size (Fleming 1996). We did not find this relationship in our study, which may be due to the fact that the majority of successful mothers were quite large. Furthermore, females could have invested less time in nest defense due to lower breeding densities or established several redds (Fleming 1996), which could have allowed for pairings with several males (Garant et al. 2005), leading to increased paternity and variance per redd (Weir et al. 2010) and overall greater fitness (Garant et al. 2005). Here we showed that females paired with multiple males and produced roughly the same amount of offspring each. While these multiple pairings between successful males and females indicate a polyandrous mating system, we cannot generalize about Atlantic salmon mating systems as our sample sizes were low.

Surprisingly, there was no correlation between Rs or number of pairing and body size and arrival date in either sex. This could be due to the fact that there were few successful parents that produced offspring, which led to statistical power issues in our dataset. However, for males, we did find a significant positive relationship between the number of mating and Rs, which can be indicative of the Bateman's Principle (Bateman 1948; Janicke et al. 2016). Male Rs is limited to the number of females they can mate with and females are choosier as sexual selection for males is often intense (Janicke et al. 2016). In the present study, we showed that males who paired with more females had greater Rs.

While heterozygosity remained relatively high in the Boquet and Winooski Rivers, there was an overall reduction of number of alleles in YOY samples from both rivers relative to adult samples (Table 1.2). A decrease in the number of alleles tends to occur before changes in heterozygosity (Allendorf 1986; Frankham 1995; Greenbaum et al. 2014) and is indicative of few adult producing progeny (Luikart et al. 1998), which is consistent with our findings. A decrease in the number of alleles may signal issues for the long-term response to selection, survival of a population and evolutionary potential (Allendorf 1986; Waples 1990; Frankham 1995; but see Wood et al. 2016). Therefore in order to avoid short- term loss of genetic diversity (through inbreeding) a minimum effective number of breeders (N_b) of 100 (Waples 1990) and effective population size (N_e) of 50 (Franklin 1980) is often recommended. In the present study, our estimates of N_b indicated that 11-15, 3-4 adults could have produced the offspring sampled in the Winooski and Boquet Rivers, respectively. Estimates of N_e for our populations given the generation time of salmon in our system, were 33-45, 9-12 adults in the Winooski and Boquet respectively (see Waples 1990) and are much lower than an N_b=100 (Waples 1990) and N_e=50 (Franklin 1980) respectively. Given these low estimates, a larger number of adults exceeding 800 individuals in the Winooski and over 2000 in the Boquet would have needed to return to achieve a N_b of 100.

In terms of the restoration efforts, our low number of surviving offspring and successful adults would underestimate adult census (N_c) size (Bacles et al. 2018). While this may be so, the relationship between N_b / N_c is often difficult to discern for Atlantic salmon, which is due to their different life history strategies (Yates et al. 2017). Male Atlantic salmon often have two extremely

different reproductive phenotypes that include the early maturing and the later maturing anadromous phenotype (in Lake Champlain the latter includes individuals that migrate to the lake while the former do not) (Yates et al. 2017). Nonetheless, N_b / N_c relationships in salmonids with similar life histories are commonly on the order of 0.05-0.2; the most empirical ratios (0.23, range=0.10-0.31) are reported in Palstra and Fraser (2012). In the present study, the estimated N_b / N_c fall within these ranges (0.11 for the Winooski and 0.05 for the Boquet Rivers). However, to promote persistence of this population, genetic diversity needs to be maintained with an increased number of adults returning to spawn and achieving high Rs over subsequent generations in both rivers.

Implication for species reintroduction endeavors

Our results indicated that the hatchery reared salmon stocked in Lake Champlain can reproduce in the wild. It is encouraging to note that these events of natural reproduction are the first documented in over a century. Through this study we showed that fish recolonized a historical spawning site (the Boquet), there was contribution from adults of multiple life history strategies and potential natural selection favoring larger males (the Winooski). Although these findings are positive, the low number of founders and reproductive contribution over subsequent generations may prove to be an issue. With the reduced number of parents and surviving offspring, issues with genetic diversity and inbreeding likely will become inevitable, thereby reducing the chance of generating a self-sustaining population over the long-term. As a result, care must be taken to avoid or minimize underlying issues with the reintroduced individuals and the receiving habitat to improve the chance of reintroduction success. Our study on Lake Champlain reintroduction illustrates that although a program has been in place for several years and has had success in various aspects of it, reintroduction success is complex and challenging to attain. This study ultimately contributes to the growing body of literature on reintroductions and demonstrated that continued planning, monitoring and adaptive management needs to be a main priority to promote the re-establishment of a given population.

Tables

Table 1.1: All known parents of YOY offspring sampled in the Winooski and Boquet Rivers. The table indicates the number of offspring adults produced and the number of pairings per adult.

Parent	River	ID	Number of Mates	Number of Offspring
Mothers	Winooski	W-008F	3 or 4	9
	Winooski	W-016F	2	9
	Winooski	W-036F	2	6
	Winooski	W-075F	3	4
	Winooski	W-080F	3	9
	Winooski	W-096F	1	1
	Boquet	B-035F	1	1
Fathers	Winooski	W-017M	3	18
	Winooski	W-037M	1	2
	Winooski	W-054M	1	1
	Winooski	W-063M	1	2
	Winooski	W-101M	2	7
	Winooski	W-111M	1	1
	Winooski	Unknown	4	6
	Boquet	B-056-M	1	1

Table 1.2: Genetic descriptive statistics for nine microsatellite loci, showing number of individuals (N), number of alleles (Na), effective number of alleles (Ae), observed heterozygosity (Ho) and expected heterozygosity (He) for the study's salmon adults and juveniles.

Name		SsaD144	SsaD157	SsaD48	SsaD71	SSsp2215	SSsp2216	SSspG7	Ssa407UOS	SS2201
Winooski Adults	N	124	124	124	124	124	124	124	124	124
	Na	19	22	34	23	10	38	15	17	22
	As	10.079	13.313	19.513	14.33	5.446	18.338	8.875	8.276	12.713
	Ho	0.96	0.919	0.976	0.976	0.815	0.984	0.911	0.895	0.895
	He	0.901	0.925	0.949	0.93	0.816	0.945	0.887	0.879	0.921
Winooski YOY	N	38	38	38	38	38	38	38	38	38
	Na	13	13	16	11	8	14	11	11	11
	Ae	8.843	8.403	9.108	7.511	6.584	7.511	8.691	8.112	7.84
	Ho	0.949	0.974	0.974	0.897	0.974	0.923	0.974	0.897	0.949
	He	0.888	0.883	0.892	0.868	0.847	0.866	0.885	0.875	0.874
Boquet Adults	N	81	81	81	81	81	81	81	81	81
	Na	20	23	33	22	12	29	15	19	22
	Ae	8.933	14.388	19.44	15.81	6.306	18	8.994	9.481	13.281
	Ho	0.877	0.889	0.951	0.963	0.827	0.889	0.901	0.926	0.914
	He	0.888	0.93	0.949	0.937	0.841	0.944	0.889	0.895	0.925
Boquet YOY	N	85	85	85	85	85	85	85	85	85
	NE	9	8	8	8	6	12	5	7	10
	Ae	5.055	3.071	2.32	3.316	2.26	5.164	2.222	2.586	2.678
	Ho	0.988	0.826	0.581	0.837	0.884	0.988	0.616	0.616	0.581
	He	0.802	0.674	0.569	0.698	0.557	0.806	0.55	0.613	0.627

Figures

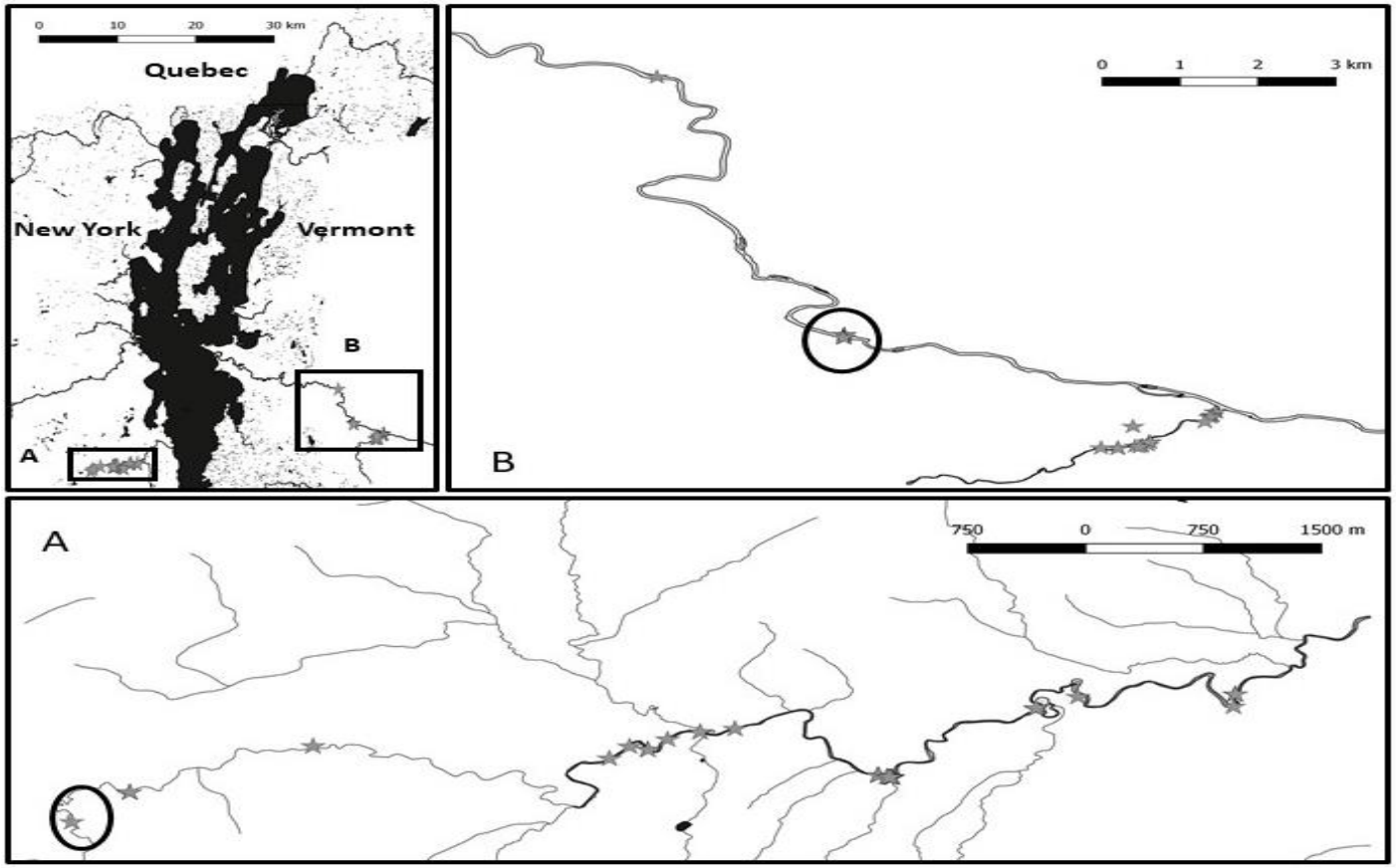

Figure 1.1. Lake Champlain, USA, showing the Boquet River, New York (Box A) and the Winooski River, Vermont (Box B), the grey stars along each river are the salmon redds established in 2015 (Winooski) and 2016 (Boquet). The circled areas are location where YOY were found.

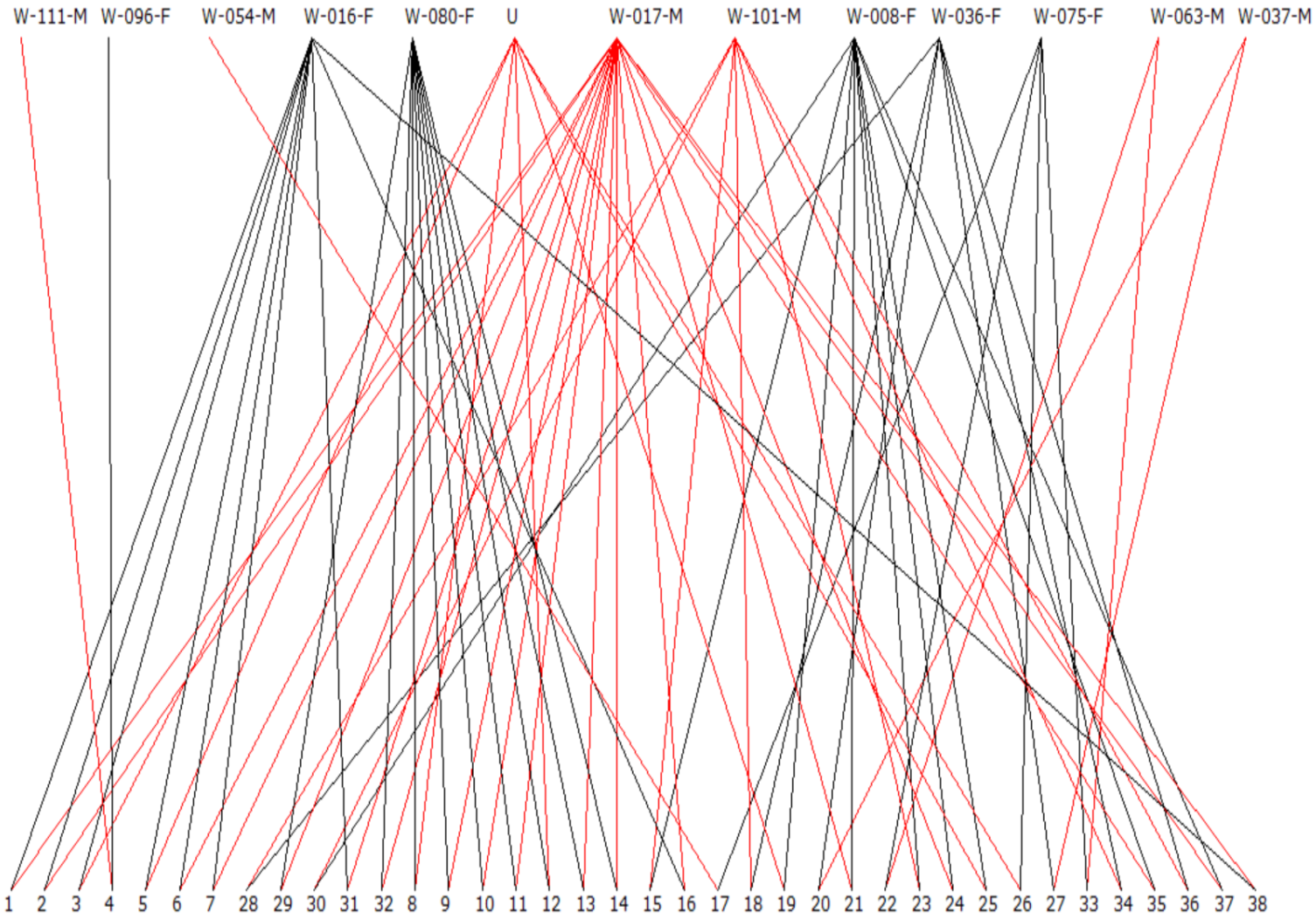

Figure 1.2: Pedigree Viewer (v.6.5f; Kinghorn and Kinghorn, 2015), demonstrating the Winooski River mating structure. Male (red) and female (black) IDs are listed at the top and offspring numbers are listed at the bottom (1-38).

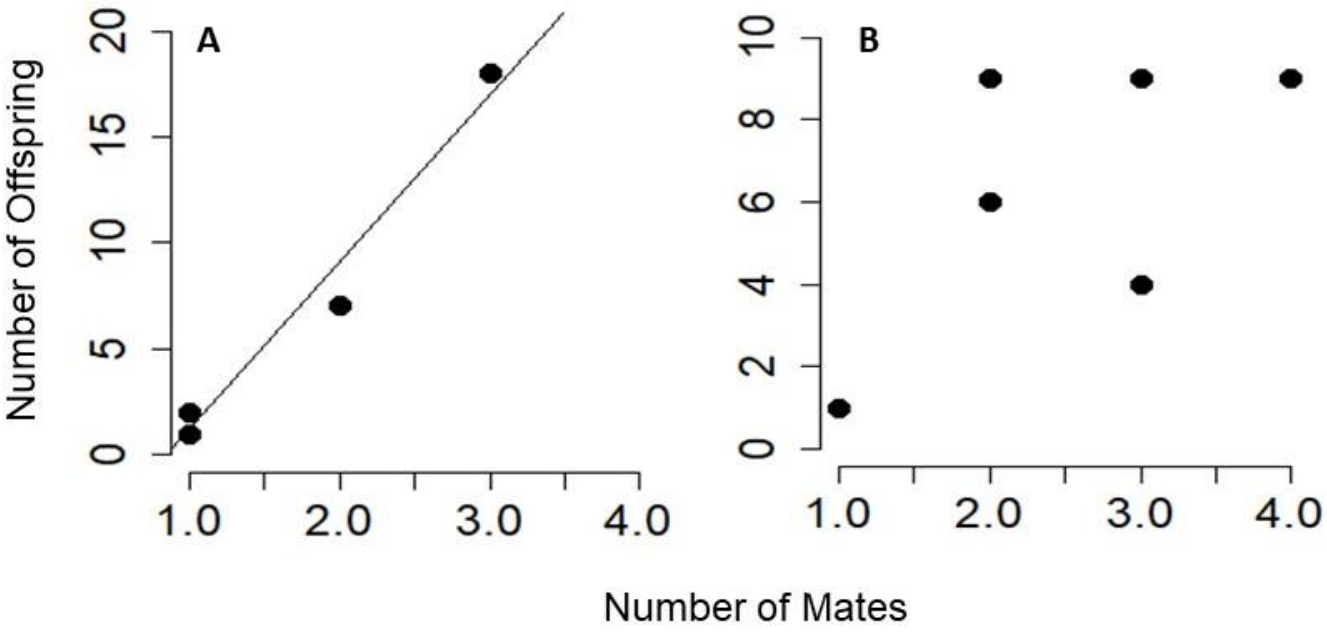

Figure 1.3: The relationship between male (A) and female (B) reproductive success (the number of offspring produced) and the number of matings between adults. Note that there were instances of overlapping data points displayed on the graph.

Supplementary Material

Table S1.1: Calculated per-locus genotyping error rates spanning across the Winooski and Boquet adults and their YOY of offspring. The full, partial and mismatch columns indicate when re-genotyped alleles fully matched, one of the two alleles matched and both alleles did not match with original genotypes respectively.

Locus	Reruns	Full matches	Partial Matches	Mismatches	Error rates
SSSp144	2	1.000	0	0	0
SSsp2215	2	1.000	0	0	0
SSsp2216	56	0.983	0.017	0	0.017
SSspG7	50	1.000	0	0	0
SSsp2201	46	0.978	0.022	0	0.022
SSaD157	68	0.985	0	0.015	0.015
SSaD48	42	0.976	0.024	0	0.024
SSaD71	102	0.981	0.019	0	0.019
Ssa407UOS	110	0.991	0.018	0	0.018

Table S1.2: P-Loci results for the exclusionary power of the 9 microsatellites to assign the Winooski YOY to their potential parents. The values displayed in the table are average values are the averages of three simulations with varying error rates.

	Loci used for exclusion								
	1	2	3	4	5	6	7	8	9
0% error rate									
Correctly assigned	21.42	88.58	95.83	97.58	97.14	98.31	100	100	100
Incorrectly assigned	0	0	0	0	0	0	0	0	0
Unassigned	79.17	7.5	6.67	2.42	2.86	1.69	0	0	0
1% error rate									
Correctly assigned	20.5	88.44	94.19	97.22	97.06	98.08	100	100	100
Incorrectly assigned	0	0	0	0	0	0	0	0	0
Unassigned	79.5	11.56	5.81	2.78	2.94	1.917	0	0	0
2% error rate									
Correctly assigned	20.69	88.25	93.75	96.92	96.72	98.05	98.62	100	100
Incorrectly assigned	0	0	0	0	0	0	0	0	0
Unassigned	79.31	11.75	6.25	3.08	3.28	2	0	0	0
Calculated genotyping error									
Correctly assigned	20.75	79.69	87.83	96.66	96.99	98.08	98.5	100	100
Incorrectly assigned	0	0	0	0	0	0	0	0	0
Unassigned	79.25	20.31	12.17	3.338	3.01	1.918	1.5	0	0

Table S1.3. Polymerase chain reaction (PCR) condition for 9 loci examined in Atlantic salmon sampled from the Winooski and Boquet Rivers in Vermont and New York, USA.

PCR Reaction	Locus	PCR Primer Dye label	Concentration for PCR (μM)	Reference
1	*SSsp2215*	NED	0.36	Paterson et al. 2004
	SsaD157	VIC	0.70	King et al. 2005
	SsaD71	6FAM	0.40	King et al. 2005
2	*SsaD48*	NED	0.50	King et al. 2005
3	*SSspG7*	6FAM	0.50	Paterson et al. 2004
4	*SsaD144*	VIC	0.40	King et al. 2005
5	*SSsp2216*	PET	0.60	Paterson et al. 2004
6	*SSsp 2201*	NED	0.54	Paterson et al. 2004
7	Ssa407UOS	6FAM	0.50	Cairney et al. 2000

The final volume of each PCR reaction was 10ul and contained: 1μL TAQ buffer, 1 μL dNTP, 0.875-1 μL MgSO4, 0.2-0.35 μL of each primer, 0.20 μL of BSA, 0.12 μL of TAQ polymerase and 2 μL of the template DNA from the cell lysate. The final primer concentration is listed in the table; annealing temperatures for each reaction was 60°C (reaction 1 and 4) and 58°C (reaction 2-3,5-7) for 35 cycles. Conditions for all sets excluding 7 were as follows: initial denaturation at 95°C for 4 min; then 35 cycles of 95°C for 20s, 20s at the reaction specific annealing temperature, 15s of primer extension at 72°C and a final extension at 72°C for 5min. For reaction seven the initial denaturation was at 94°C for 5 min; then 35 cycles of at 94°C for 35s, 30s at the reaction specific annealing temperature, 1min of primer extension at 72°C and a final extension and final extension at 72°C for 7min.

Table S1.4: The stocking history of the Boquet and Winooski Rivers from 2013-15.

Year	Location	Life stage	Number stocked
2013	Boquet	Fry	34983
2013	Boquet	Yearling	48037
2013	Winooski	Fry	47500
2013	Winooski	smolt	29577
2014	Boquet	Fry	183000
2014	Boquet	Yearling	47680
2014	Winooski	Fry	62054
2014	Winooski	Fingerlings	8860
2014	Winooski	smolt	36417
2015	Boquet	Fry	200000
2015	Boquet	Yearling	50143
2015	Winooski	Fry	57148
2015	Winooski	smolt	31388

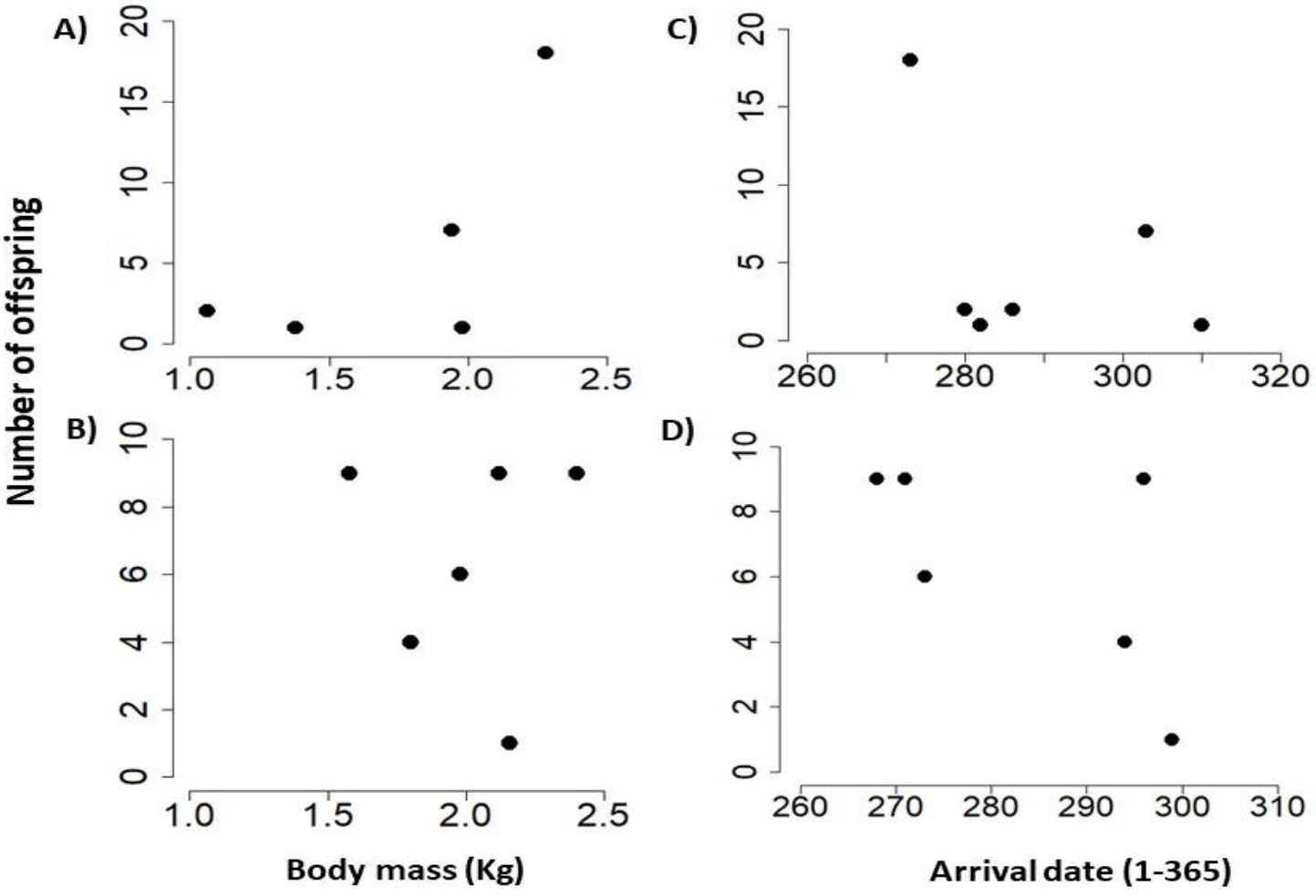

Figure S1.1: The relationship between male (A) and female (B) body mass (Kg) of and the number of offspring produced and between male (C) and female (D) arrival date and number of offspring produced.

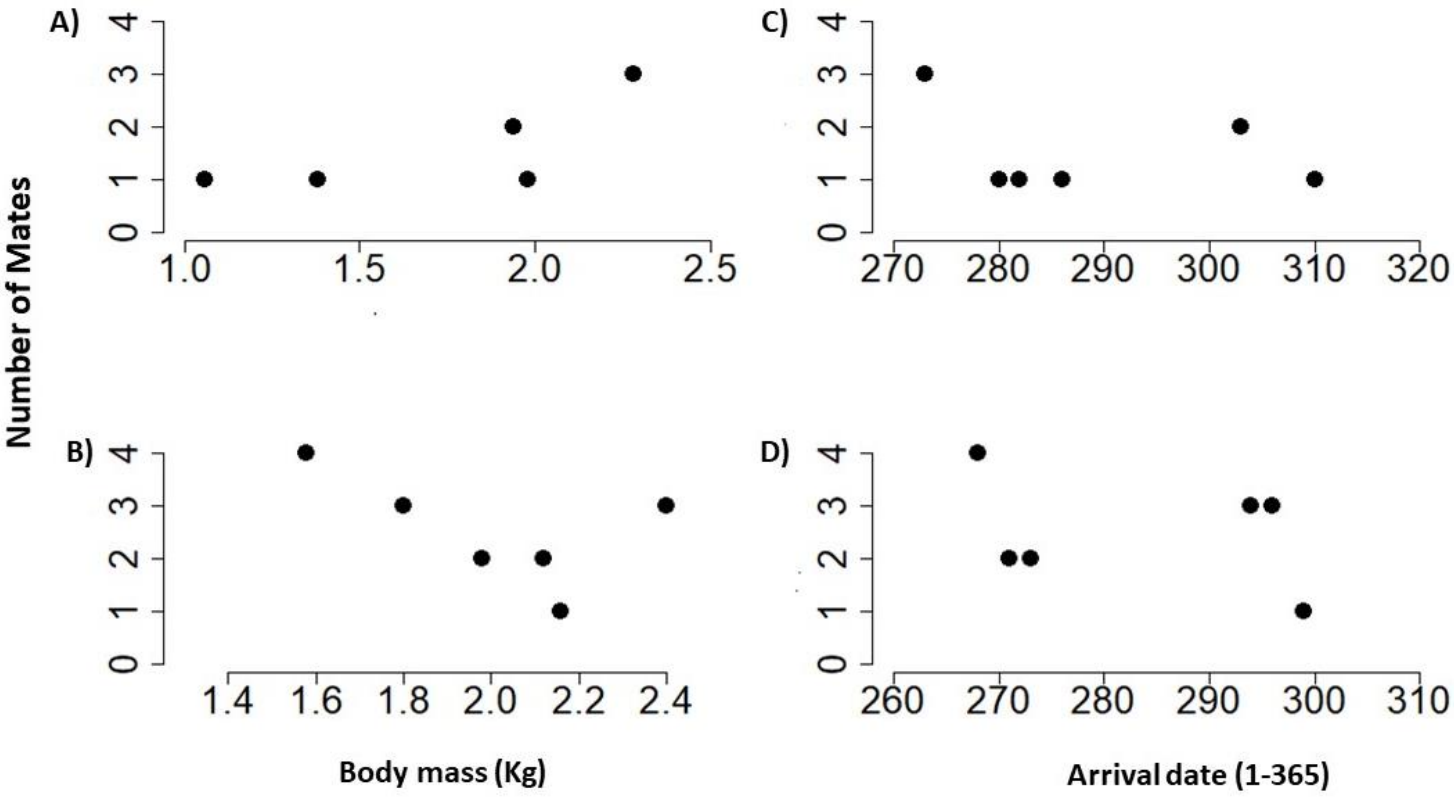

Figure S1.2: The relationship between male (A) and female (B) body mass (Kg) of and the number of mates they spawned with and between male (C) and female (D) arrival date and the number of mates they spawned with.

Chapter 2.

The effect of wild exposure on relative reproductive success of Atlantic salmon during smolt-to-adult supplementations in Fundy National Park

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Abstract:

Smolt-to-adult (SAS) supplementation programs are used to increase extirpated or declining Atlantic salmon populations. SAS programs collect surviving out-migrating smolts (stocked as unfed fry or other early life stages), which are then reared in captivity to maturity (often in sea pens) and released as adults to spawn naturally. SAS is a promising conservation approach that can increase juvenile wild exposure whilst mitigating marine mortality of anadromous populations where this is severe. However, to date, little is known about the ability of SAS fish to reproduce in the wild and the effect of wild exposure on their reproductive success. Here, we used a genetic parentage analysis to evaluate the relative reproductive success of four adult rearing groups in the Upper Salmon River, Fundy National Park, NB, and tested the hypothesis that relative reproductive success would be highest in groups with increased durations of wild exposure. The groups implemented in the study included two SAS groups, a live gene-banking group and a small number of adults that returned to spawn. The two SAS groups were wild exposed as juveniles but were either reared to maturity in sea pens (SAS-SEA, n=323) or in a hatchery (SAS-HAT, n=321). The live gene-banking group were wild exposed as adults but not as juveniles and were reared to maturity in sea pens (F1-HAT, n=195). The last group were previously released and returned to the study river as repeat spawners (AD-15, n=8). Our results demonstrated that adults from the SAS program successfully reproduced in the wild, however, reproductive success was low among parents (range=1-17 offspring per parent). Furthermore, the group with an intermediate duration of wild exposure (SAS-HAT) had the highest relative reproductive success, while the groups with the most and least duration of wild exposure (SAS-SEA, F1-HAT respectively) did not differ in their relative reproductive success. Finally, although a low sample size, the AD-15 fish proportionally had the highest number of successful parents as 3 of the 8 adults reproduced. This study contributes to a growing body of information that can be applied to future fisheries management directions for other SAS and traditional supplementation programs.

Introduction:

Freshwater and marine fish species are steadily declining throughout much of their native ranges due to anthropogenic and environmental factors (Dungeon et al. 2006). As a result, supplementation and captive-breeding programs are implemented to offset these declines by releasing hatchery reared fish into the wild (Waples et al 2007; Fraser 2008). These programs serve as important tools for conservation and management of populations as they can prevent extinction of critically endangered species. Currently, more than 300 fish species are captive-reared for supplementation and released in the billions annually (Brown and Day 2003; Evans 2014). Salmonids species in particular are some of the most highly propagated fishes (Heard 1995; Araki et al. 2008; Christie et al. 2014). While these programs may provide an immediate demographic boost to a given population, many do not lead to self-sustaining populations in the wild and are continuously supplemented (Wolf et al. 1996; Fischer and Lindenmayer 2000; Anderson et al. 2013).

Although many issues contribute to the lack of long-term success of salmonid supplementation programs (Dunham et al. 2011; Cochran-Biederman et al. 2015), domestication selection is thought to be a key factor (Frankham, 2008; Fraser, 2008). Domestication can result from relaxed selective pressures and unintentional selection in a captive environment (Fraser 2016). It can have negative consequences on reproductive fitness in the wild due to; genetic issues (Araki et al. 2007b; 2009; Thierault et al. 2011), plastic changes to phenotypic traits (Fraser 2016); and carry over effects from the hatchery environment (Fleming et al. 1997; Thierault et al. 2011). Carry over effects are defined as non-lethal influences on an individual's performance due to a previous (environmental) event in life (Harrison et al. 2011; Milot et al. 2013). These effects can reduce maternal provisioning, hinder fry development in the wild (Fraser 2016) and lead to transgenerational fitness reductions (Araki et al. 2009; Evans et al. 2014; Clarke et al. 2016). Furthermore, genetic diversity in a captive setting can be challenging to maintain as few adults may be artificially selected to produce offspring (Fraser 2008). Therefore, to reduce the effect of domestication and promote natural reproduction, an extended period of wild exposure (reduced time in captivity) of released fish is often recommended to fisheries managers (Theriault et al. 2011; Milot et al. 2013; Evans et al. 2014; Clarke et al. 2016).

One method adopted by traditional supplementation programs to mitigate domestication selection, is increasing juvenile wild exposure by stocking fish at earlier life stages. Stocking fish at earlier rather than later juvenile life stages has been demonstrated to play a key role on reproductive success (hereafter Rs) (Theriault 2010; 2011; Milot et al 2013; Christie et al. 2014; Evans et al. 2014). Therefore, relative Rs, defined as the number of surviving offspring produced by one group in relation to another, is a parameter often measured during supplementations (Christie et al. 2014). While juvenile supplementation is a popular strategy, it may not be sufficient to satisfy program goals, particularly if severe marine mortality at later life stages is the limiting factor. Marine mortality occurs at high rates in anadromous Atlantic salmon populations (Chaput 2012) and is variable for Pacific salmon populations (Holt and Bradford 2011). Therefore, there is also a need to address these particular challenges during supplementation programs whilst minimizing the genetic and ecological risks associated with captive rearing (Fraser 2016).

Smolt-to-adult supplementation (hereafter SAS) is an attractive conservation tool for anadromous populations that experience high marine mortality (reviewed in Fraser 2016). In short, SAS programs collect surviving out-migrating smolts (stocked as unfed fry or other early life stages), rear them in captivity to maturity (often in sea pens) and release them as adults into the river to spawn naturally. In doing so, the time spent in captivity at juvenile life stages is reduced; released juveniles and adults that are reared in sea pens are subjected to life-stage specific natural environments; and free mate choice can occur. Currently, SAS is the only method that avoids risks associated with juvenile captive rearing whilst minimizing marine mortality for anadromous salmon (Fraser 2016). SAS programs are now adopted in areas where wild populations have drastically declined bellow conservation targets (Fraser 2016; Kozfkay et al. 2017; Department of Fisheries and Oceans (DFO), 2018), including endangered Atlantic salmon populations in the Inner (Corey Clarke, Parks Canada, unpublished data) and Outer Bay of Fundy (Jones et al. 2014), Maine, and previously for Pacific salmon populations (Stark et al. 2014; Fraser 2016). While SAS is of growing interest as a restoration tool for endangered salmonids, questions remain about the reproductive success of released adults in the wild.

Here, we investigated the relative Rs of four Atlantic salmon rearing groups in Fundy National Park. The Inner Bay of Fundy (hereafter Bay of Fundy) salmon population once inhabited

32 rivers (Amiro 2003; Gibson et al. 2008) but declined due to human related impacts on freshwater systems and drastic marine mortality (DFO, 2010). As a result, several restoration initiatives including SAS (Corey Clarke, Parks Canada, pers. comm.) and a live gene-banking program (DFO, 2016) were implemented to restore the population. Two SAS groups were stocked as unfed fry, collected as large parr about 1.5 years later, and were either reared to maturity in sea pens or in a hatchery. Of these two SAS groups, the sea pen fish were subjected to greater environmental variability as they were reared in the open ocean. The third group from the live gene-banking program was reared in captivity during their juvenile life stages and transferred to sea pens as smolts. These fish experienced natural environmental conditions in the sea pens during their adult life stages. All three groups were released into the wild as adults in fall 2016 to spawn naturally. Finally, a fourth group of adults that were previously released, returned to the spawning ground. This group had the longet duration of wild exposure as they originated from the SAS program (stocked in the wild as fry and then reared in sea pens) and spent a year in the open ocean after being released in 2015. Collectively, the four adult groups with different rearing histories in the study were; the SAS sea pen (hereafter SAS-SEA) and SAS hatchery (hereafter SAS-HAT) reared groups, the live gene-banking hatchery fish (hereafter F1-HAT) and repeat spawners from previous years (hereafter AD-15).

With these differing adult groups, we hypothesized that relative Rs would increase with an increasing duration of wild exposure (Frankham 2008; Fraser 2008; Theriault 2010; 2011; Milot et al 2013; Christie et al. 2014; Evans et al. 2014). We therefore tested the prediction that relative Rs and the proportion of successfully reproducing adults would be highest in the AD-15 group, followed by the SAS-SEA, SAS-HAT and F1-HAT groups. To test this prediction, we quantified Rs by performing a parentage analysis using seven highly polymorphic microsatellite markers which assigned emergent offspring sampled to putative parents from all adult groups. Finally, to acquire information on the mating system, such as sexual selection and competition between groups we explored the variance in Rs among groups and sexes. This work allowed us to determine the effect of wild exposure on Rs in a SAS program and hence provides relevant information for salmonid recovery programs.

Materials and methods:

Study site

SAS supplementation programs are currently conducted in two rivers in Fundy National Park that drain into the Bay of Fundy, known as the Upper Salmon and the Point Wolfe Rivers. The Upper Salmon River, with a drainage area of 174km^2 (Fraser et al. 2007), is a steep mountainous tributary with swift water velocities and large pools (Dadwell 1968). The Point Wolf River, with a drainage area of 130 km^2 (Fraser et al. 2007), has 11 pools with large gravel beds in between (Dadswell 1968). Both rivers once had a native population of salmon, which were extirpated due to the construction of logging dams (both rivers) and collapse of a fishway (in the Upper Salmon River) in the 1930s (Dadswell 1968; Fraser et al. 2007). As a result, stocking initiatives in the Point Wolf River and dam removal in the Upper Salmon River were conducted to promote recolonization of salmon (Dadswell 1968). However, natural recolonization was slow (Dadwell 1968) and marine mortality (DFO 2010) became a prominent issue. Therefore, the SAS program was implemented to overcome these challenges and promote a self-sustaining population in each river.

Adult rearing groups:

The adults used in our study, originated from four rearing groups that varied in their duration of wild exposure. Two of these groups originated from the SAS program and were treated similarly during their earlier life stages but differed in treatment during their adult life stages. Unfed fry from both groups were stocked in late May to early June into Dickson Brook, a tributary of the Point Wolf River, where they resided for 1.5 years until collected through electrofishing surveys as parr in the fall. All wild exposed parr were then overwintered at the Mactaquac Biodiversity Centre, and either transferred as smolts to sea pens (SAS-SEA) in Dark Harbour, Gran Manan, NB or remained at the hatchery (SAS-HAT). Typically, sea pens were 12x6x6m whereas the hatchery had 8m concrete ponds.

The third group, F1-HAT, originated from the live gene-banking program and did not have wild exposure as juveniles. Instead, they spent about 1-2 years in a hatchery until later transported to the sea pens as smolts. In the sea pens, fish experienced the open ocean environment.

Finally, the fourth group, AD-15, comprised of eight fish that were released in 2015 as SAS-SEA fish and were detected entering the Upper Salmon River via pit tag readers in fall 2016 when they returned to the spawn. The AD-15 group experienced the longest duration of wild exposure, followed by the SAS-SEA, SAS-HAT and F1-HAT groups.

Adult releases:

Salmon originating from the three main adult rearing groups (SAS-SEA, F1-HAT, SAS-HAT) were then released into pools within three distinct sections of the Upper Salmon River to spawn naturally (Table 2.1, Figure 2.1). The pools were Pumphouse, section 1 (3km from the mouth of the river); Black Hole and Jiggers in section 2 (8km from the mouth of the river); and Forks in section 3 (9 km from the mouth of the river, Figure 2.1). Since Black Hole and Jiggers were less than 300m apart they were combined and hereafter known as Black Hole.

Fish from the SAS-SEA and F1-HAT groups were released via helicopter in Black Hole (SAS-SEA=157, F1-HAT=97) and in Forks (SAS-SEA=166, F1-HAT=98) on October 10th, 2016 (Table 2.1). The day before release, fish were loaded into 5 cubic meter aerated holding tanks. On the day of release, an average of 50 fish (range: 31-77) were transferred from the holding tanks to D332 insulated bins (1.22 x 1.07 x 0.96 m) at a time and helicoptered to their destination pool.

Conversely, the SAS-HAT group were either released early into Black Hole (n=38) on September 12th and 19th, 2016 (n=22 and 16 respectively) or later into Pumphouse on October 19th, 2016 (n=274) or November 25th, 2016 (n=9, Table 2.1). All fish from this group were transported to the release site via truck and hand released. Fifteen (39.5%) and 28 (10.2%) of the 38 and 274 fish released into Black Hole and Pumphouse were not genetically tagged. Furthermore, 287 (89.4%) of the 321 fish from this group were not sexed before release.

Fry sampling:

Emergent young of the year (YOY) salmon were then sampled from July 31st to August 25th, 2017 by electrofishing surveys. Twenty-one electrofishing sites (mean= 200m^{2,} range=168.5-258m^2) along the Upper Salmon River were sampled. All sites were sampled once during the summer, while three were sampled for a second time to acquire more fry. YOY densities in the

electrofishing sites were low with an average density of 4 fish/100m^2, range=1.19-11.27. Once a YOY was collected a tissue sample from their adipose fin was removed and preserved in 95% ethanol. All salmon were released back into their site of capture.

Genotyping

DNA from the caudal fin clips of 804 adult salmon (323 SAS-SEA, 278 SAS-HAT, 195 F1-HAT, 8 AD-15) and adipose fin clips of 269 YOY was extracted using a modified Chelex protocol (adapted from Hua and Orban 2005). Eight of the adult fin clips (6 from SAS-HAT, 1 from SAS-SEA, 1 from F1-HAT group) could not be genotyped after 2 attempts of re-extraction and polymerase chain reactions (PCR) due to degraded tissue (Figure 2.1). Furthermore, since there were low densities of YOY per electrofishing site, few were acquired in the river.

To extract DNA, a 5% Chelex solution (Chelex 100, Sigma) containing 125 μg/L of 25 mg/ml proteinase K and a ~2mm cutting of individual fin clips were incubated at 45℃ for a minimum of 1 hour and a maximum of 5 hours. Once incubated, samples were then boiled at 98℃ for 5 minutes to insure cell lysis. Samples were then subsequently genotyped at 7 highly polymorphic microsatellite loci, which included *SsaD144*, *SsaD157*, *SsaD48*, *SsaD71* (King et al. 2005), *SSsp2215*, *SSsp2216*, *SSspG7* (Paterson et al. 2004). Detailed reactions and thermocycler profiles for the 7 loci can be found in the supplementary material (Table S2.4). The amplified PCR products were then visualized using the ABI 3500 sequencer and resulting alleles were manually scored on Genemapper 3.2 (Applied Biosystems Inc.).

Parentage analysis

To determine which adult rearing groups were successful in reproducing, a parentage analysis was conducted on SOLOMON (v.1.01; Christie et al. 2013) using exclusionary principles (Jones and Ardren 2003; Jones et al. 2010). Exclusionary principles follow the logic of Mendelian inheritance; if a putative parent does not share at-least one allele per locus with the given offspring, they are excluded as the true parent of the given offspring (Jones and Ardren 2003; Jones et al. 2010). All seven loci were used to assign the 269 offspring to the 804 adults. Based on simulations on P-Loci (see supplementary material), we accepted parental pairs whose genotypes matched at 6 or 7 loci with a given offspring. In the case where full exclusion did not occur and several parents

were assigning back to a given offspring, all putative parents and offspring were genotyped at an additional 3 loci (*SSsp 2201* (Paterson et al. 2004), *SSa407UOS* and *SSa408UOS* (Cairney et al. 2000).

Finally, an estimate of the effective number of breeders (N_b) that could have produced the 269 offspring, was performed using a linkage disequilibrium approach on LDNe (v.1.31; Waples & Do, 2008). We used an exclusion criterion of (P_{crit}) of 0.01 since our offspring sample size exceeded 100 individuals and to avoid upwards bias due to presence of rare alleles (Waples and Do, 2008). Confidence intervals were generated using the parametric approach (Waples 2006).

Statistical analysis:

Reproductive success-A series of Chi-squared tests of independence were used to explore the relative Rs (the number of offspring produced) among groups with varying durations of wild exposure. Due to differences in release sites (section 1 and 2), times of release (earlier or later) and in handling method (e.g. truck release) of the SAS-HAT fish, they were not compared to the other groups (SAS-SEA, F1-HAT) in these tests. Therefore, in section 2 and 3 only, the frequencies of successful and unsuccessful adults from the SAS-SEA and F1-HAT groups and their relative Rs (number of offspring produced) were tallied (Table S2.3) and compared in two different 2x2 contingency tables (Table S2.3). Similar tests were used to compare the sexes within a given group. Specifically, the frequency of successful and unsuccessful females and males within a group and their relative Rs (number of offspring produced) were compared in four different 2x2 contingency tables (Table S2.3). The expected values for all tests were the initial numbers of adults from each group and sexes released in sections 2 and 3. A false discovery rate correction was used to account for performing multiple tests and adjusted p values were therefore reported (Benjamini and Hochberg 1995).

Variance of reproductive success-To explore the mating system, such as sexual selection and competition between groups, the variance in Rs between all groups and sexes within the group was compared using Levene's Tests.

Overall predictors of Rs-To investigate the influence of predictors on Rs of the successful parents only (adults which produced offspring), a series of generalized linear models with a Poisson family distribution were constructed using MASS in R 3.4.3. A Poisson family distribution was used because the response variable (number of offspring) was count data. An overall model encompassing pooled data from all sites was constructed with the following predictors: adult group type (SAS-HAT, SAS-SEA, F1-HAT), sex, timing of release, release site, section where juveniles were found and release type (helicopter or hand) were included in the models as fixed effects. The interaction between group type and release date, group type and site, and group type and sex were also included in the models as separate fixed factors. Release site and the section where juveniles were found were separate predictors due to adults not necessarily spawning in sites where they were released. Furthermore, these predictors were designated as fixed rather than random factors because we were interested in understanding their effect in predicting Rs. The Akaike Information criterion (AIC) was then used to compare and select the most parsimonious models (Akaike 1987) among all these predictors. Upon selecting the most parsimonious model, Wald's Tests were used to assess the significance of predictor variables (Breslow 1990) and goodness of fit was assessed using the residual deviance (http://qcbs.ca/wiki/r_workshop7).

Due to the large presence of SAS-HAT in each section (see below), the SAS-SEA and F1-HAT were compared in subdivided models that were broken down by section. Release type and timing were not included as predictors in the models because fish in these groups were released in the same manner and time. Section was also not included in the models due to the subdivision by section. Therefore, group type (SAS-SEA and F1-HAT), release site, sex and the interaction between group type and release site, group type and sex, and release site and sex were included in the models.

Results:

Parentage analysis

Overall, 183 known parents produced the 269 offspring sampled in Upper Salmon River (Figure 2.2). Of the 269 offspring, 68.4% (184) were assigned to two parents, 25.3% (68) to a single known and unknown parent(s), 3.3% (9) could not be assigned due to fish not being sexed and 3% (8) were unassigned at three or more mismatching loci (Table 2.3). All eight of these unassigned offspring had at least two private or uncommon alleles, suggesting that they did not originate from the known released adults.

Reproductive Success

A total of 81, 40, and 148 offspring were collected from sections 1, 2 and 3 respectively (Table 2.2). The SAS-HAT fish produced the majority of offspring in sections 1 and 2, while the SAS-SEA fish produced the majority in section 3; the SAS-HAT produced 83.7% (67 of the 81 offspring) and 80% (30 of the 40 offspring) of the offspring respectively; the SAS-SEA produced 49.8% (83 of the 148 offspring) of the offspring. The number of parents from each group is displayed in Figure S2.1.

Since the SAS-SEA and F1-HAT groups were released in the same sites, times and handled in the same manner, these groups were compared in Chi-squared tests of independence in section 2 and 3. Overall there were no differences between groups or their relative Rs as similar proportions of adults were assigned to offspring (section 2: χ^2=0.04, df=1, p=0.84, section 3: χ^2=1.35e-30, df=1, p=1) and produced the same numbers of offspring (section 2: χ^2=1.78, df=1, p=0.19, section 3: χ^2=0, df=1, p=1, see Table S2.3, for expected and observed frequencies). There were also no overall differences between sexes within groups, as the proportion of females and males that produced offspring did not differ significantly in both sections [(section 2: SAS-SEA, χ^2=1.65, df=1, p=0.198; F1-HAT, χ^2=0.01, df=1, p=0.9); (section 3: SAS-SEA, χ^2=1.29, df=1 p=0.26, F1-HAT, χ^2=0.66, df=1, p=0.42, Table S2.3)]. Furthermore, the number of offspring produced between sexes within groups and between sexes in the F1-HAT group in section 2 and 3 (respectively) did not differ significantly [(section 2: SAS-SEA, χ^2=2.62, df=1 p=0.11, F1-HAT: χ^2<0.001, df=1, p=1); (section 3: F1-HAT, χ^2=1.41, df=1, p=0.24, Table S2.3)]. However, males

of the SAS-SEA group in section 3, produced more offspring in comparison to females (χ^2=21.81, df=1, p<0.001, Figure 2.3d).

Variance of reproductive success

The variance in Rs between all groups differed significantly in section 1 (Levene's test, all other stats reported are from the Levine's test, $F_{2,61}$=5.618, p=0.005) but not in section 2 ($F_{2,35}$=1.56, p=0.224, Figure 2.3c) or 3 ($F_{2,109}$=1.445, p=0.233). In section 1, adults from the SAS-HAT group had more variable Rs then the other groups (Figure 2.3b).

The variance in Rs between sexes within the SAS-HAT and SAS-SEA groups; the SAS-SEA and F1-HAT; and the SAS-HAT groups in sections 1, 2 and 3 respectively did not differ significantly [(section 1: SAS-HAT, $F_{1,41}$=0.06, p=0.805, SAS-SEA, $F_{1,14}$=0, p=1); (section 2: SAS-SEA, $F_{1,10}$=0.23 p=0.644, F1-HAT $F_{1,7}$=2.60, p=0.14); (section 3: SAS-HAT, $F_{1,34}$=1.12, p=0.29, Figure 2.3)]. However, in section 1, females of the F1-HAT group had more variable Rs than males ($F_{1,3}$=9.6, p=0.05, Figure 2.3). In section 2, males of the SAS-HAT group had more variable Rs than females ($F_{1,14}$=4.39, p=0.05, Figure 2.3). One male produced 12 offspring while females produced 1-3. In section 3, males of both the SAS-SEA and F1-HAT group had more variable Rs than females (SAS-SEA, $F_{1,46}$=5.98, p=0.01, F1-HAT, $F_{1,27}$=6.24, p=0.01, Figure 2.3). One male in the SAS-SEA and F1-HAT produced 9 offspring while the majority of females produced 1-4.

Effective number of breeders:

Point estimates of N_b on LDNe were taken on a per section basis, these estimates suggested that 29 (95% CI=25-35), 31 (95% CI=27-38) and 37 (95% CI=33-40) parents could have produced the 81, 40 and 148 offspring in sections 1, 2 and 3 respectively. An overall estimate of N_b for all combined sections indicated that 56 (95% CI=52-60) fish could have produced the 269 offspring sampled in the Upper Salmon River.

Predictors of reproductive success

An overall model encompassing the pooled data from successful adults in all sections are displayed in Table 2.4. While several competing models had similar AIC values, the most

parsimonious model included adult rearing type and release date as predictors of Rs in the Upper Salmon River (Table 2.4).

Surprisingly, the SAS-HAT group had the highest Rs in comparison to all other rearing groups (Wald's test, $\chi^2 = 17.8$, df = 208, p = 0.0001, all other stats reported are from the Wald's test) as they produced 82.1% of the offspring sampled. Additionally, fish that were released later in the run had higher Rs than those released earlier ($\chi^2 = 14.6$, df = 209, p = 0.0001). This model explained 9% of the variance suggesting that there were other unmeasured variables that could have predicted Rs (Figure 2.4). Since there was an overwhelming presence of the SAS-HAT group that assigned to offspring throughout the river, separate models were established for each section. These models included the SAS-SEA and F1-HAT groups only as well as sex and release site as predictors of Rs.

In section 1 and 2, there were no significant predictors of Rs as group type, sex, and release site were non-significant [(Section 1: Group type, $\chi^2 = 0.0187$, df = 20, p = 0.8911, AIC=50.39, Sex, $\chi^2= 0.03$, df = 20, p = 0.861, AIC=50.38, release site, $\chi^2= 0.004$, df = 20, p = 0.94, AIC=50.41), (Section 2: Group type, $\chi^2= 0.579$, df = 20, p =0.446, AIC=57.71, Sex, $\chi^2 = 0.03$, df = 20, p =0.861, AIC=57.89, release site, $\chi^2= 0.268$, df = 20, p =0.604, AIC=58.02, Table 2.4)]. Moreover, the models established explained little variance in Rs [(Section 1: 0.9% -for group type, 1.6%- for sex and 0.2% for release site); (Section 2: 6%-for group type, 4% for sex and 3% for release site)] suggesting that unmeasured variables were influencing Rs.

In section 3, sex was included as the only significant predictor of Rs (Table 2.4). Males from both rearing groups (SAS-SEA, F1-HAT) had higher Rs in comparison to females ($\chi^2 = 6.59$, df = 74, p = 0.01, AIC=245.19). This model explained 9% of the variance of the variance suggesting that there were other unmeasured variables that could have predicted Rs.

Discussion:

Our study demonstrated that fish from the Fundy National Park SAS program successfully reproduced in the wild. By quantifying Rs, we compared the performance of four adult groups and tested the prediction that relative Rs and the proportion of successfully reproducing adults would be highest in the AD-15 followed by the SAS-SEA, SAS-HAT and F1-HAT groups. Despite the low sample size, the AD-15 fish had the highest proportion of successfully reproducing adults but not relative Rs. Furthermore, among release groups, the SAS-SEA fish, the group with the longest duration of wild exposure, had similar relative Rs and proportion of successfully reproducing adults to the F1-HAT fish, the group with the shortest duration of wild exposure. Additionally, the SAS-HAT fish, the group with an intermediate duration of wild exposure, had the highest relative Rs and the proportion of successfully reproducing adults among released groups. An overall model showed that group type and release date were predictors of Rs, however it explained little variance of the data, suggesting unmeasured variables could have influenced Rs. Finally, the differences in handling of the SAS-HAT group, specifically with release dates, type and locations, mean that a number of confounding variables could be influencing their performance, these are discussed in detail below as this information is useful for future use of SAS as a conservation tool.

Contrary to our initial prediction, the SAS-SEA fish did not have the highest relative Rs or proportion of successfully reproducing adults among the released groups. Despite being wild exposed at both their juvenile and adult life stages, the SAS-SEA fish had similar relative Rs and proportion of parents to the F1-HAT fish. We contend that Rs of the SAS-SEA fish could have been influenced by several factors including the method of handling and physiological state upon release. For example, the SAS-SEA group (as well as the F1-HAT group) transitioned from salt to freshwater in a short period of time. While there was a transitioning protocol in place, the timing may not have been sufficient for freshwater acclimation, which could have caused acclimation and transport stress (Barton and Iwama 1991; Harmon 2009). Natural migrations to the spawning grounds depend on various abiotic and biotic factors (Høgåsen 1998) and can take several weeks to achieve (Heggberget et al. 1988). The helicopter transport may have also resulted in further stress, as upwards of 50 individuals were placed in bins and lifted at a given time. Furthermore, the timing of release may have been suboptimal, which could have played a role on spawning preparedness (Fraser 2016). All SAS-SEA adults were mature upon release; however, the majority

were grilse (held in the sea pens for one winter) and may have had lower Rs in comparison to older larger released fish (Garant et al. 2003). Finally, despite the low relative Rs among SAS-SEA fish, it is encouraging to note that natural reproduction took place. These findings will allow fisheries managers to refine SAS practices and improve their programs to promote Rs.

Unexpectedly, parents from the SAS-HAT group were highly represented among the surviving offspring found in each section. While we cannot draw firm conclusions about why these fish were so successful, the overall model suggested that timing of release (later release) may have been an important predictor of their Rs. These later released fish may have had greater relative Rs as they have spent less time and energy searching for optimal habitat and mates (Fleming 1996). Furthermore, the type of release may have played a role on relative Rs, as these fish were driven and hand released rather than helicopter transported. Finally, these fish were older than the other release groups, which may have resulted in improved release state and preparedness to spawn (Fraser 2016). While this group was represented in each section, their Rs may have been overestimated since 89.4% of these fish were not sexed upon release.

In addition to handling differences, timing of release and physiological state upon release, we speculated that carry over effects from captive-rearing may have played a key role on Rs of all groups. Carry over effects can influence female and male Rs in different ways. Female Rs may be affected by changes in maternal provisioning, egg quality, spawning time (Dempson et al. 1999; Stark et al. 2014; Fraser 2016), retention of eggs and not constructing or covering nests (Weir and Grant 2005; Jonsson and Jonsson 2006; Berejikian et al. 2001; Fraser 2016). Moreover, SAS (Berejikian et al. 2001) and captive-reared males have been demonstrated to be inferior in courtship, competition for females and spawning behavior (Jonsson and Jonsson 2006; Fraser 2016). Overall, in the present study, both male and female relative Rs was similar in all groups. However, we did detect a difference in relative Rs between sexes of the SAS-SEA and F1-HAT fish in section 3 only. Furthermore, there were slight differences in the variance of male Rs in section 2 and 3 in the SAS-HAT and SAS-SEA groups respectively, however these trends did not occur in all sections. Overall, all sexes in each group had low relative Rs, which may indirectly indicate that carry over effects could be influencing their Rs.

As predicted, despite low sample sizes, the AD-15 fish had the highest proportion of adults that produced offspring; 3 (37.5%) of the 8 detected fish produced 4 (1.5%) of the offspring sampled. These fish had the greatest degree of wild exposure as they were previously released as SAS-SEA fish in 2015, and returned as repeat spawners in 2016. Although not formally tested, we speculated that the proportion of successful adults from this group could have been due to increased wild exposure.

Surprisingly, there were several unknown adults that produced 68 (25.3%) of the 269 offspring. This can be due to the 43 released adults that were not genetically tagged. All of these adults originated from the SAS-HAT group. Given the large representation of this group in the offspring, many of these unknown parents could have been among these 43 fish. Furthermore, given the high marine mortality of wild fish, it is unlikely that many of these unknown parents were wild.

Our overall point estimate of N_b (56, 95% CI=52-60) did not coincide with the number of parents (183) that produced offspring. These results could have been due to the presence of several related adults in each group or to the inflation of assigned parents from the unsexed fish from the SAS-HAT group. Despite this discrepancy, it is encouraging to note that heterozygosity and number of alleles in offspring remained relatively high (Table S2.1). To avoid short term loss of genetic diversity through inbreeding a N_b of 100 is often recommended (Waples 1990). Our N_b of 183 is above this recommended value and hence the offspring genetic diversity remained high with many adults contributing to the next generation. Additionally, while the N_b / N_c relationship is often difficult to discern for Atlantic salmon due to their alternative male life history strategies (Yates et al. 2017), our estimates of N_b / N_c for the AD-15, SAS-SEA, SAS-HAT and F1-HAT were 0.38, 0.19, 0.24, 0.20 (respectively), which are either greater than or coinciding with empirical ratios (range: 0.10-0.22) reported in Palstra and Fraser (2012). These overall values are encouraging for the restoration efforts, and would need to be maintained through higher Rs in the wild over subsequent years to promote the re-establishment and long-term persistence of this population.

Finally, our estimates of Rs among parents within a given group are relative rather than absolute measures of Rs. Given the low number of offspring sampled, overall conclusions about groups and their absolute Rs could not be made (Anderson et al. 2011). Therefore, according to simulations in Anderson et al. 2011, our power in comparing Rs among groups was low due to small sample sizes of offspring sampled. Furthermore, type 1 assignment errors for a single sex can occur at higher probabilities as the number of potential parents increases (Anderson et al. 2011). In the present study, 804 and 269 potential parents and offspring were genotyped, respectively. Upon comparing to simulations in Anderson et al. 2011, over 1600 YOY would need to have been sampled to reduce type 1 assignment error to 10%. While we did not conduct a formal simulation analysis, our adult to juvenile ratios suggest a potential high probability of type 1 assignment error rates due to offspring sample size.

Our results demonstrated that the adults originating from the SAS and live gene-banking program reproduced in the wild. It is encouraging to note that this is the first instance of natural reproduction in the Upper Salmon River from these groups and our N_b / N_c ratios were similar to wild populations. While these findings are positive, the relative Rs of each group was low and may have been influenced by release timing, physiological condition, handling and the genetic/ecological carry over effects associated with captive- rearing. While we made overall generalizations about these carry over effects, they are mainly speculative and would need to be further explored over subsequent years. Issues with carry over effects often arise with traditional supplementation and captive rearing programs, but have not been readily addressed with SAS programs (Fraser 2016). Therefore, our study suggests that future work which combines long term assessment, continued monitoring and alteration of management strategies is merited to determine the influence of the aforementioned factors toward improving Rs of captive SAS fish.

Recommendations for adaptive management

While it is encouraging that released SAS fish reproduced in Upper Salmon River, the influence of group type (with differing wild exposure) on Rs is still relatively unknown. Therefore, to understand the effect of group type on Rs, controlling for confounding variables with new management designs is recommended. These changes in conjunction with longer-term assessments, continued monitoring and increased fry collection can aid in determining the extent of the supplementation needs in Fundy National Park and further our understanding of Rs for other SAS programs.

Adult releases: There were a number of confounding variables that could have influenced adult Rs, including timing, location, and type of release. To reduce these confounding effects, adults from each group are recommended to be released at the same time and proportions in each location. In doing so, one group would not be overly represented in the river during spawning. An even sex ratio within groups should also be considered during releases. Maintaining an even sex ratio in each section can allow for differences between sexes to be explored as well as the influences of carry over effects on Rs. By inferring the difference between sexes, changes can be made to promote Rs of released fish of a given sex. Additionally, the timing of release could play a large role in Rs, due to trade-offs during earlier or later periods of the run (Fleming 1998). The F1-HAT and SAS-SEA groups were released on the same day, while the SAS-HAT group was either released earlier or later during the run. This difference in timing could have allowed for earlier individuals to have longer period of time seeking optimal habitats while later individuals could have destroyed redds that were already established from the main groups. Lastly, the type of release (helicopter or truck) could have played a role on Rs. Both the F1-HAT and SAS-SEA groups were released via helicopter while the SAS-HAT group was released via truck. Both these release methods could cause different levels of stress, which can influence Rs. Therefore, releasing fish in the same manner is recommended to control for this handling type effect.

YOY sampling: If the number of released adults is substantially high, the number of juvenile tissue samples collected would need to exceed that of adults by two-fold. With a large number of juveniles collected, the power to detect differences of each group will be greater. In the current study there were several adults that produced only one offspring while few produced more than

five. With this low Rs among adults, overall patterns may be difficult to establish without acquiring more juveniles. Finally, comparisons to a simulation study indicated that the probability of a type 1 assignment error could have been high.

Genetic standpoint: We showed that adults from different groups spawned with one another. While it is encouraging that both SAS groups produced offspring, both mixed with the F1-HAT group. The F1-HAT group, spent their entire juvenile life stage in captivity and were subjected to the open ocean environments in sea pens as adults. From a genetic standpoint, fish originating from a captive brood stock tend to have reduced fitness in comparison to wild fish (Araki et al. 2007a). While these fish originated from a local brood stock, the relaxed selective pressures in the hatchery may favor maladaptive traits and the effect of domestication selection could occur in one to two generations (Araki et al. 2007b). While we did not find any difference between the SAS-SEA and F1-HAT groups in terms of Rs, the fact that they were mixing may reduce fitness of their progeny. Therefore, in order to promote the success of the SAS program, it may be beneficial to either refrain from stocking the F1-HAT group or release them into the wild as juveniles with the other groups.

General suggestions:

- If more parentage analyses are conducted over subsequent years, all released fish should be sexed to ensure that there are no issues or ambiguities with any parental assignments.
- Consider releasing fewer adults from each group. With the large numbers of fish released at once, there may be competition for habitat and mates that may skew Rs to only a few individuals.
- Use SAS programs as a short term means of restoration. Fish from supplementations and captive rearing programs often have lower fitness then wild fish. If mixture between groups continues to occur, the long-term persistence of the population may be difficult to achieve due to fitness reductions and to the aforementioned carry over effects.

Tables

Table 2.1: Summary of the 2016 adult releases in the Upper Salmon River. The number of fish per adult rearing type, their locations of release, date of release and release type are indicated.

Adult rearing group	Number released	Location released	Section	Release type	Date
SAS-SEA	157*	Black Hole Pool	2	Helicopter	10/12/2016
	166	Forks Pool	3	Helicopter	10/12/2016
SAS-HAT	274[+]*	Pumphouse Pool	1	Truck	10/19/2016
	9	Pumphouse Pool	1	Truck	11/25/2016
	38[+]	Black Hole Pool	2	Truck	9/12/2016
F1-HAT	97*	Black Hole Pool	2	Helicopter	10/12/2016
	98	Forks Pool	3	Helicopter	10/12/2016
AD-15	8	-	-	-	10/14/2015

[+] 15 and 28 of the released fish were not genetically tagged

*6, 2 adults (6 from SAS-HAT and 1 from each group) were not genetically tagged after several genotyping attempts

Table 2.2: Summary of the number of offspring and electrofishing sites in each section and the adult group types released in the sections.

Section	Number of Offspring	Electrofishing Sites	Adult Release Sites	Group Types
1	81	4	Pumphouse	SAS-HAT
2	40	9	Black Hole	SAS-SEA, SAS-HAT, F1-HAT
3	148	9	Forks	SAS-SEA, F1-HAT

Table 2.3: Breakdown of the parentage analysis per section in the Upper Salmon River. There were a total of 81, 40, 148 offspring sampled in section 1, 2, and 3 respectively. The parent type are the adult rearing groups that produced offspring in the given section. The type of mating indicates whether parents were from one group (pure), from different groups (mixed, which includes unknown (UN) and multi-matched adults (MM)) or if offspring were not unassigned (UN) and the total type is the total number of adults from that category (e.g. pure, mixed or unknown) that produced offspring.

Section	Parent type	Total type	Type of mating	Number of parents
1	SAS-HAT	50	pure	48
	SAS-SEA	50	pure	2
	F1-HAT	50	pure	0
	SAS-HAT/SAS-SEA	17	mixed	9
	SAS-HAT/F1-HAT	17	mixed	2
	SAS-SEA/F1-HAT	17	mixed	1
	SAS-SEA/AD-15	17	mixed	0
	SAS-HAT/MM	17	mixed	2
	SAS-SEA/MM	17	mixed	2
	F1-HAT/MM	17	mixed	1
	SAS-HAT/UN	9	mixed	6
	SAS-SEA/UN	9	mixed	2
	F1-HAT/UN	9	mixed	1
	AD-15/UN	9	mixed	0
	UN	5	UN	5
2	SAS-HAT	3	pure	1
	SAS-SEA	3	pure	1
	F1-HAT	3	pure	1
	SAS-HAT/SAS-SEA	22	mixed	9
	SAS-HAT/F1-HAT	22	mixed	10
	SAS-SEA/F1-HAT	22	mixed	2
	SAS-SEA/AD-15	22	mixed	0
	SAS-HAT/MM	22	mixed	1
	SAS-SEA/MM	22	mixed	0
	F1-HAT/MM	22	mixed	0
	SAS-HAT/UN	12	mixed	10

	SAS-SEA/UN	12	mixed	1
	F1-HAT/UN	12	mixed	0
	AD-15/UN	12	mixed	1
	UN	3	UN	3
	SAS-HAT	14	pure	1
	SAS-SEA	14	pure	10
	F1-HAT	14	pure	3
	SAS-HAT/SAS-SEA	78	mixed	40
	SAS-HAT/F1-HAT	78	mixed	15
	SAS-SEA/F1-HAT	78	mixed	12
3	SAS-SEA/AD-15	78	mixed	2
	SAS-HAT/MM	78	mixed	6
	SAS-SEA/MM	78	mixed	2
	F1-HAT/MM	78	mixed	1
	SAS-HAT/UN	47	mixed	23
	SAS-SEA/UN	47	mixed	15
	F1-HAT/UN	47	mixed	9
	AD-15/UN	47	mixed	0
	UN	9	UN	9

Table 2.4: The results for the competing general linearized models, which demonstrates the predictors of adult reproductive success (number of offspring produced) in a pooled over all model and subdivided models in section 1, 2, and 3 in the Upper Salmon River, NB. The abbreviations in the table are 1) type=adult rearing type, 2) RDJ= release date (days are from 1-365), 3) RSC= release site, 4) RT= release type (helicopter, truck, wild). The table includes models that are <6 AIC values from the most parsimonious model.

Section	Residual df	AIC	ΔAIC	Model	Estimate	Odds Ratio	SE	Z-value	Pr(>|z|
Overall	208	773.24	0	Overall: Type+ RDJ					
				(intercept)	5.19	267.9	1.23	4.20	<0.0001
				TypeF1-HAT	0.40	0.67	0.137	2.91	0.003
				TypeSAS-SEA	-0.032	0.65	0.152	-0.21	0.83
				RDJ	-0.016	0.98	0.004	-3.83	0.0001
	208	773.24	0	Overall: Type* RSC					
				(intercept)	0.286	1.31	0.663	0.41	0.69
				TypeSAS-HAT	-0.215	0.81	0.694	-0.31	0.76
				TypeSAS-SEA	0.749	2.11	0.828	0.90	0.37
				RSC	0.07	1.08	0.248	0.32	0.75
				TypeSAS-HAT: RSC	0.598	1.82	0.294	2.03	0.04
				TypeSAS-SEA:RSC	-0.299	0.74	0.312	-0.96	0.34
	207	774.01	0.77	Overall: Type + RDJ +Sex					
				(intercept)	5.117	166.8	1.241	4.12	<0.0001
				TypeSAS-HAT	0.391	1.48	0.138	2.84	0.004
				TypeSAS-SEA	-0.048	0.95	0.153	-0.32	0.75
				SexM	0.101	1.11	0.099	1.02	0.31
				RDJ	-0.016	0.98	0.004	-3.80	0.00015
	207	775.34	1.81	Overall: Type +RDJ + RSC					
				(intercept)	5.153	173.0	1.862	2.77	0.005
				TypeSAS-HAT	0.405	1.50	0.245	1.66	0.10
				TypeSAS-SEA	-0.032	0.97	0.152	-0.21	0.83
				RDJ	-0.016	0.98	0.005	-2.94	0.003
				RSC	0.003	1.003	0.144	0.03	0.98
1	19	50.38	0	Overall: Sex					
				(intercept)	0.167	1.18	0.27735	0.60	0.55
				SexM	-0.071	0.93	0.4096	-0.18	0.86
	19	50.39	0.01	Overall: Type					

				(intercept)	0.1823	1.2	0.4082	0.45	0.66
				TypeSAS-SEA	-0.065	0.94	0.4714	-0.14	0.89
	19	50.41	0.03	Overall: RSC					
				(intercept)	0.2121	1.23	1.233	0.17	0.86
				Section 3	-0.0289	0.97	0.449	-0.07	0.95
	18	53.11	2.73	Overall:Sex+RSC					
				(intercept)	0.311	1.36	1.3307	0.23	0.82
				SexM	-0.083	0.92	0.4224	-0.20	0.84
				RSC	-0.051	0.95	0.4631	0.11	0.91
	18	53.13	2.75	Overall: Type+RSC					
				(intercept)	0.2829	1.32	1.326	0.21	0.83
				TypeSAS-SEA	-0.068	0.93	0.474	-0.15	0.89
				RSC	-0.035	0.96	0.451	-0.08	0.94
	17	56.11	5.7	Overall: Type*RSC					
				(intercept)	-0.4463	0.64	3.13	-0.14	0.89
				TypeSAS-SEA	0.819	2.268	3.41	0.24	0.81
				RSC	0.223	1.25	1.09	0.20	0.84
				TypeSAS-SEA:RSC	-0.318	0.73	1.2	-0.26	0.79
	17	56.11	5.7	Overall: Type*Sex					
				(intercept)	0.287	1.33	0.5	0.58	0.57
				TypeSAS-SEA	-0.169	0.84	0.601	-0.28	0.78
				SexM	-0.287	0.75	0.866	-0.33	0.74
				TypeSAS-SEA:SexM	0.287	1.33	0.986	0.29	0.77
2	19	57.713	0	Overall: Type					
				(intercept)	0.4418	1.55	0.267	1.65	0.10
				TypeSAS-SEA	-0.287	0.75	0.378	-0.76	0.45
	19	57.89	0.177	Overall: Sex					
				(intercept)	0.4055	1.50	0.258	1.57	0.12
				SexM	-0.2384	0.79	0.3789	-0.63	0.53
	19	58.02	0.307	Overall: RSC					
				(intercept)	0.8011	2.22	1	0.80	0.42
				Section 3	-0.1978	0.82	0.3819	-0.52	0.60
	18	60.49	2.777	Overall: Sex+RSC					
				(intercept)	0.775	2.17	1.001	0.77	0.44
				SexM	-0.204	0.82	0.389	-0.52	0.60
				RSC	-0.149	0.86	0.393	-0.38	0.71
	17	62.49	4.777	Overall: Type*RSC					
				(intercept)	1.71	5.55	1.34	1.28	0.20
				TypeSAS-SEA	-2.16	0.12	2.11	-1.03	0.31
				RSC	-0.511	0.60	0.54	-0.95	0.34

				TypeSAS-SEA:RSC	0.734	2.08	0.801	0.92	0.36
	17	63.09	5.377	Overall: Type*Sex					
				(intercept)	0.587	1.80	0.333	1.76	0.07
				TypeSAS-SEA	-0.405	0.67	0.527	-0.77	0.44
				SexM	-0.365	0.69	0.558	-0.65	0.51
				TypeSAS-SEA:SexM	0.315	1.37	0.776	0.41	0.68
3	73	245.19	0	Overall: Sex					
				(intercept)	0.313	1.36	0.138	2.63	0.02
				Male	0.457	1.58	0.178	2.57	0.01
	72	247.27	2.08	Overall:Sex+RSC					
				(intercept)	0.543	1.72	0.473	1.15	0.25
				SexM	0.461	1.58	0.178	2.59	0.009
				RSC	-0.08	0.92	0.176	-0.51	0.61
	71	249.26	4.07	Overall: Type*Sex					
				(intercept)	0.245	1.27	0.208	1.18	0.239
				TypeSAS-SEA	0.126	1.13	0.279	0.44	0.65
				SexM	0.615	1.84	0.286	2.15	0.04
				TypeSAS-SEA:SexM	-0.256	0.77	0.367	-0.70	0.49
	71	249.49	4.3	Overall: Type+RSC+Sex					
				(intercept)	0.558	1.74	0.486	1.15	0.251
				TypeSAS-SEA	-0.023	0.98	0.183	-0.13	0.90
				RSC	-0.09	0.91	0.176	-0.51	0.61
				SexM	0.466	1.59	0.181	2.56	0.01

Figures

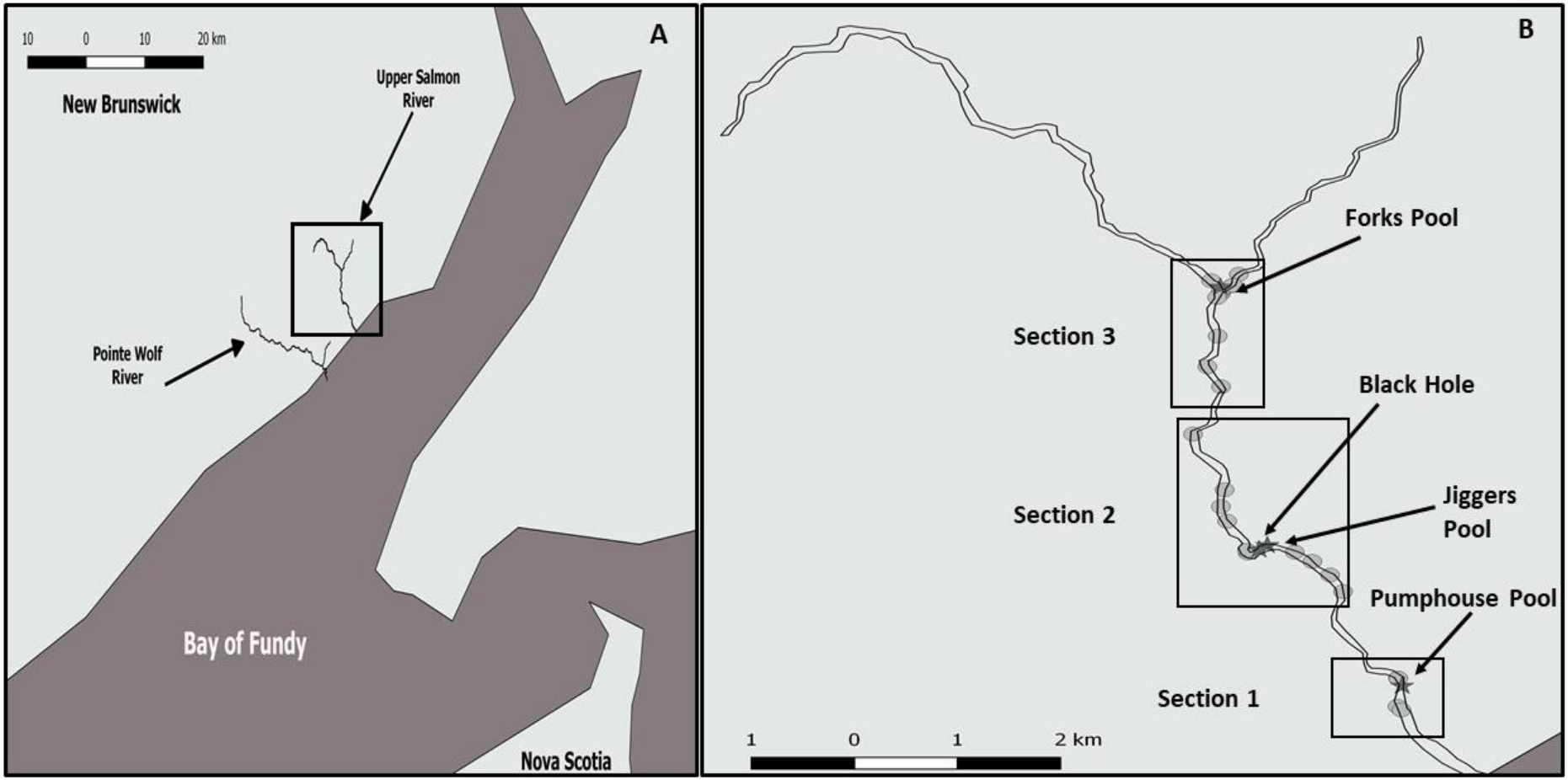

Figure 2.1. The inner Bay of Fundy, Fundy National Park, New Brunswick, showing the Pointe Wolf and Upper Salmon Rivers in box A and each section encompassing the electrofishing sites (grey circles) and adult release sites (grey stars) in Box B.

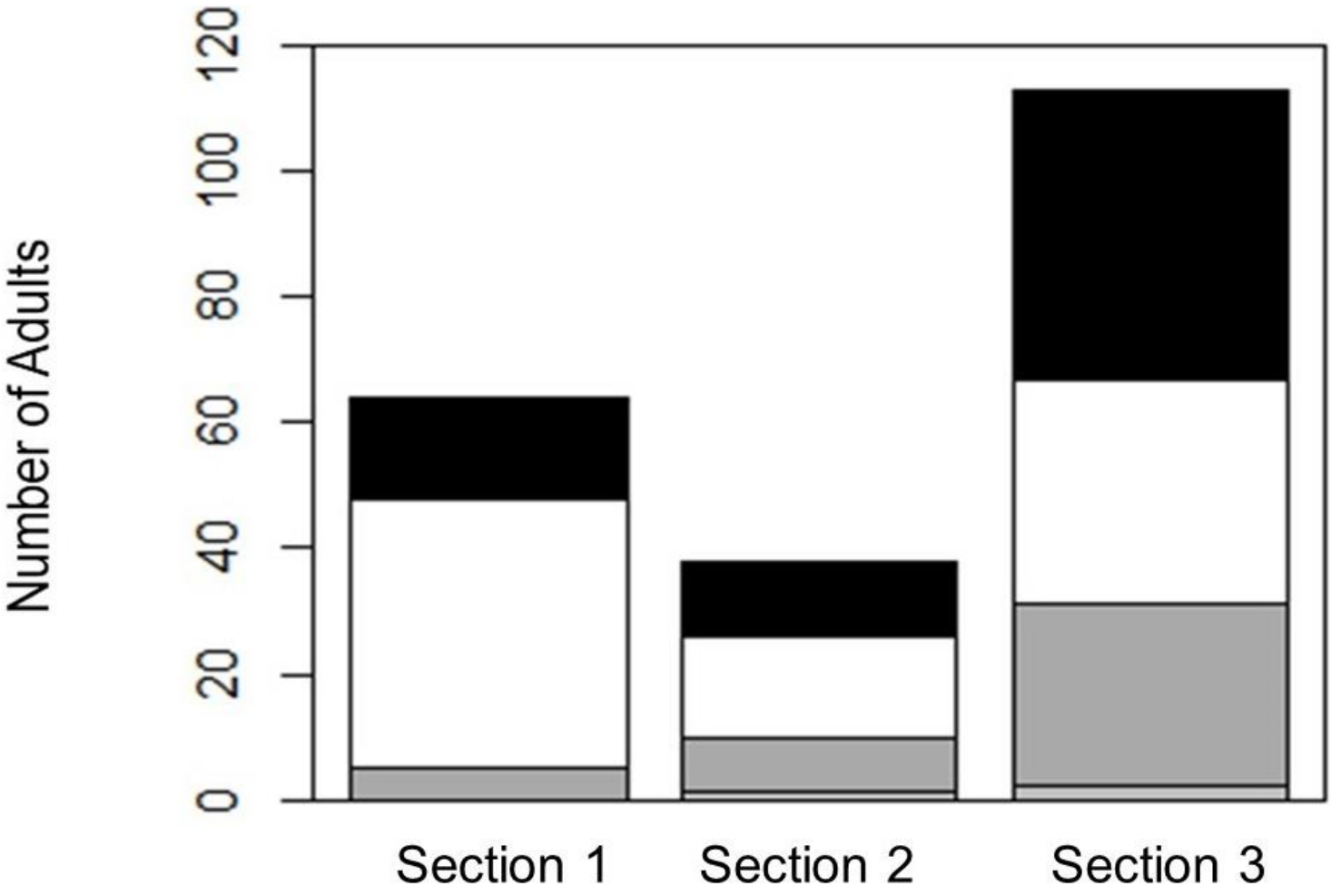

Figure 2.2: The breakdown of the total number of adults that produced offspring in section 1, 2 and 3. The white boxes are the parents originating from the SAS-HAT group, the black boxes are the SAS-SEA group, the dark grey boxes are the F1-HAT group and light grey boxes are the AD-15 group.

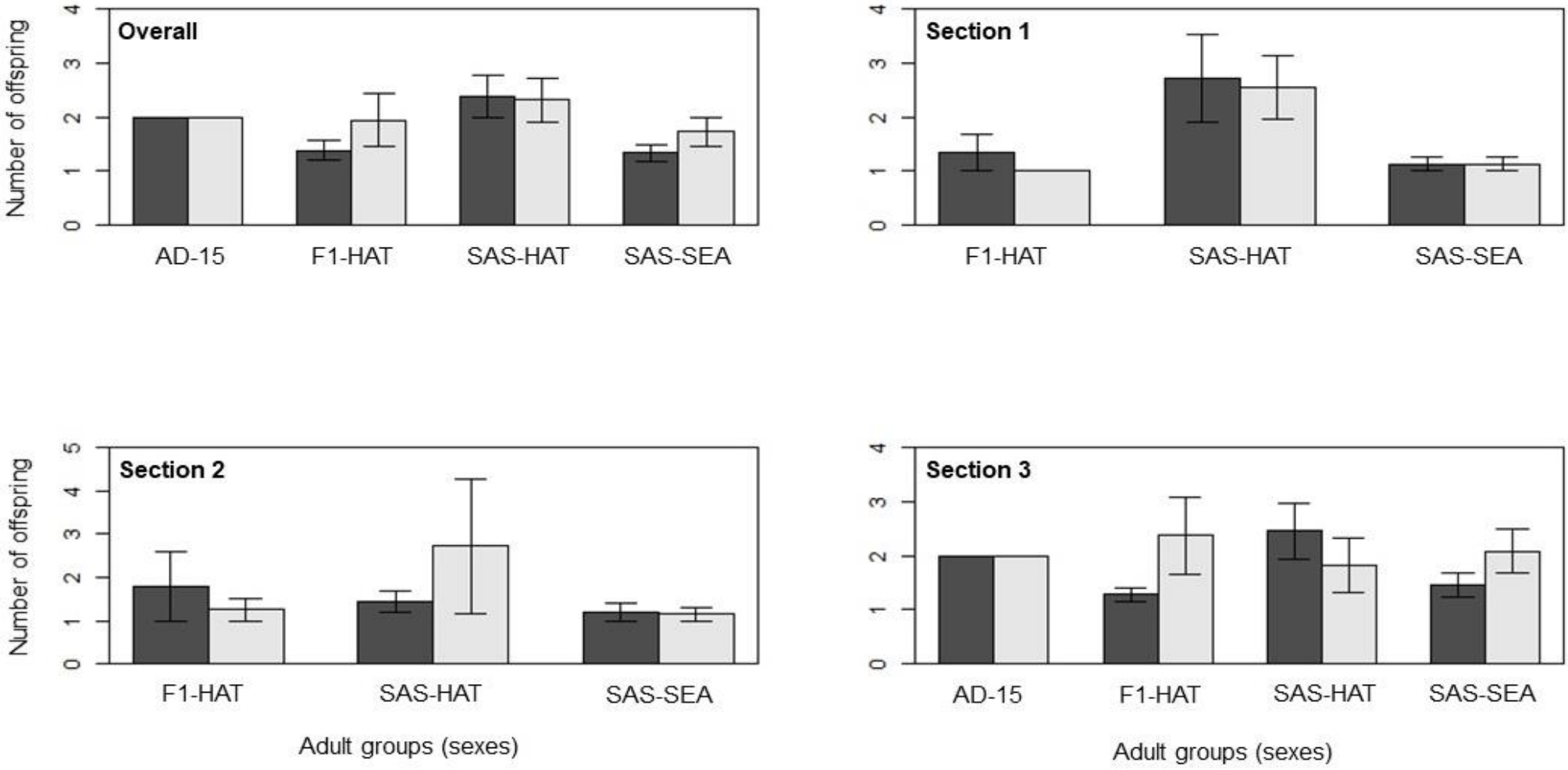

Figure 2.3: Bar graphs displaying the average number of offspring produced by each sex within the adult groups and the standard error of Rs in the Upper Salmon River and in each section. Dark grey bars represent females while light grey bars represent males within the given group.

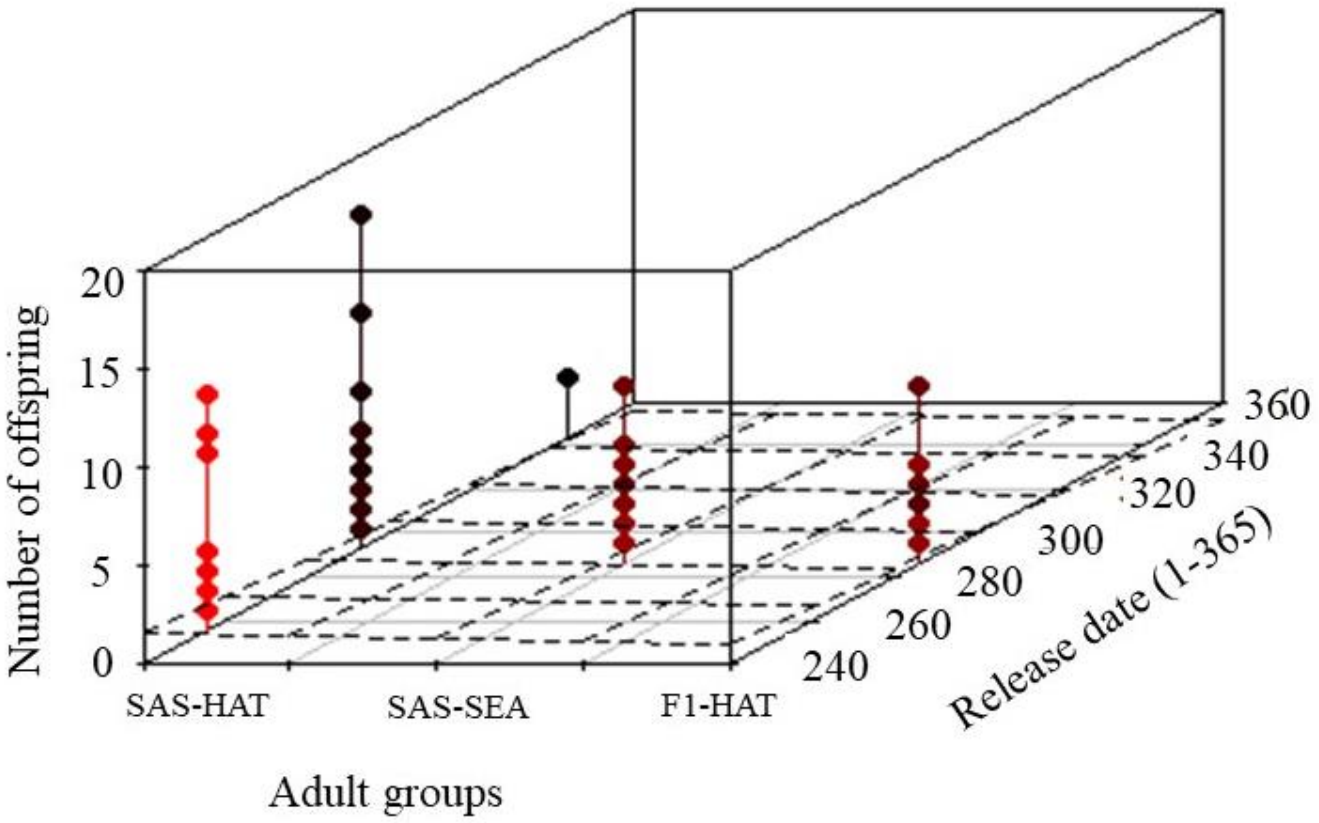

Figure 2.4: The overall fitted model depicting the relationship between adult rearing type and release date as predictors of Rs in the Upper Salmon River. The release date is in Julian days from 1-365.

Supplementary material

Genetic Statistics

All plates contained one negative and two positive controls to identify any possible contamination. A subset of 32 samples were re-genotyped to obtain measurements of genotyping error and allelic dropout rate. With this subset, there was a 99.5% genotyping concordance (0.5% genotyping error rate) and one instance of allelic dropout observed in *SSaD48* and *SSaD71*. Additionally, MICROCHECKER (v.2.2.3; van Oosterhout et al. 2004) was used to identify null alleles, large scale allelic dropout and scoring errors in the dataset. Which did not occur upon verification of the dataset. Finally, GeneALEx (v.6.502; Peakall and Smouse,, 2012) was used to obtain the number of alleles, effective number of alleles, expected and observed heterozygosity for each locus. All loci were highly polymorphic, averaging at 22.6 alleles per locus (range: 13-42) with an average heterozygosity of 0.856 (range: 0.775-0.940, Table S2.1).

Exclusionary power analysis

Once all samples were genotyped, a series of simulations were performed on P-LOCI (Matson et al. 2008) to assess the ability of the microsatellite loci panel to correctly assign parent-offspring triplets. This software simulates offspring based on a mating matrix, error rates and then re-assigns the simulated offspring with all putative parents using Mendelian principles (Matson et al. 2008). Genotyping error rates as well as the random parental pairing matrices were used to simulate 100 offspring per parental pair. These simulated offspring genotypes were then reassigned back to the parental pairs using Mendelian exclusion methods. Exclusionary power of the loci panel was high with a 98.63% confidence for all 7 loci (Table S2.2). With this simulation we would expect more than 98% of offspring to be correctly assigned to the adults in our dataset.

Table S2.1: Descriptive statistics for the seven loci across all parent groups and offspring showing the number of alleles per locus (Na), the effective number of alleles (Ae), the observed heterozygosity (Ho), and the expected heterozygosity (He).

Name		***SsaD144***	***SsaD157***	***SsaD48***	***SsaD71***	***SSsp2215***	***SSsp2216***	***SSspG7***
Adults	N	800	800	800	800	800	800	800
	Na	25	18	36	21	12	19	13
	Ae	11.11	12.48	10.38	9.01	4.49	4.75	6.4
	Ho	0.948	0.859	0.833	0.908	0.771	0.765	0.897
	He	0.91	0.92	0.904	0.889	0.777	0.79	0.844
Juveniles	N	269	269	269	269	269	269	269
	Na	22	18	39	23	11	17	13
	Ae	11.96	11.64	13.41	8.41	4.98	4.08	2.04
	Ho	0.9	0.861	0.88	0.888	0.837	0.689	0.865
	He	0.916	0.914	0.925	0.881	0.797	0.755	0.84

Table S2.2: Results of the P-Loci simulation assessing exclusionary power of the seven loci to assign potential adults from all groups to their offspring. Two parallel analyses were conducted using varying error rates.

Error	Number loci	1	2	3	4	5	6	7
0%	Correctly assigned	6.13	46.04	86.04	96.26	97.54	98.63	98.63
	Incorrectly assigned	0	0	0	0	0	0	0
	Unassigned	93.87	53.96	13.96	3.74	2.46	1.367	1.367
1%	Correctly assigned	5.17	45.633	85.527	96.245	97.3	98.65	98.65
	Incorrectly assigned	0	0	0	0	0	0	0
	Unassigned	94.83	54.36	14.47	3.755	2.7	1.35	1.35

Table S2.3: The Chi-Squared tests of independence that were performed in section 2 and 3 between the SAS-SEA and F1-HAT parents only. The given test either compared the frequency of the number of successful and unsuccessful adults (displayed as number of adults in the table) that assigned back to offspring or to the Rs (displayed as Rs in the table) of these adults within the given group. The same test was conducted for the different sexes within each group. A False discovery rate correction was performed to account for multiple testing and the adjusted p-values are displayed.

Section	Group Type	Successful Adults	Unsuccessful Adults	Adults released	χ^2	p	Test
2	SAS-SEA and F1-HAT	-	-	-	0.04	0.84	Number of adults
	SAS-SEA	12	145	157	-	-	-
	F1-HAT	9	88	97	-	-	-
	SAS-SEA and F1-HAT		-	-	1.71	0.19	Rs
	SAS-SEA	13	144	157	-	-	-
	F1-HAT	14	83	97	-	-	-
	SAS-SEA	-	-	-	1.65	0.19	Number of adults (Sex)
	SAS-SEA Females	5	94	99	-	-	-
	SAS-SEA males	7	51	58	-	-	-
	F1-HAT	-	-	-	<0.01	1	Number of adults (Sex)
	F1-HAT Females	5	53	58	-	-	-
	F1-HAT males	4	35	39	-	-	-
	SAS-SEA	-	-	-	2.62	0.11	Rs (Sex)
	SAS-SEA Females	5	94	99	-	-	-

	SAS-SEA males	8	50	58	-	-	-
	F1-HAT	-	-	-	0.01	0.9	Rs (Sex)
	F1-HAT Females	9	49	58	-	-	-
	F1-HAT males	5	35	40	-	-	-
	SAS-SEA and F1-HAT	-	-	-	0	1	Number of adults
	SAS-SEA	48	118	166	-	-	-
	F1-HAT	29	69	98	-	-	-
	SAS-SEA and F1-HAT	-	-	-	0.10	0.75	Rs
	SAS-SEA	83	83	166	-	-	-
	F1-HAT	49	51	98	-	-	-
3							
	SAS-SEA	-	-	-	1.29	0.26	Number of adults (Sex)
	SAS-SEA Females	21	68	89	-	-	-
	SAS-SEA Males	27	50	77	-	-	-
	F1-HAT	-	-	-	0.66	0.416	Number of adults (Sex)
	F1-HAT Females	18	34	52	-	-	-
	F1-HAT Males	11	35	46	-	-	-
	SAS-SEA	-	-	-	21.81	<0.001	Rs (Sex)
	SAS-SEA Females	29	60	89	-	-	-
	SAS-SEA Males	54	23	77	-	-	-
	F1-HAT	-	-	-	1.41	0.24	Rs (Sex)
	F1-HAT Females	23	29	52	-	-	-
	F1-HAT Males	26	20	46	-	-	-

Table S2.4. Polymerase chain reaction (PCR) condition for the primary seven and secondary three loci examined in Atlantic salmon sampled from the Upper Salmon River, NB.

PCR Reaction	Locus	PCR Primer Dye label	Concentration for PCR (μM)	Reference
1	*SSsp2215*	NED	0.36	Paterson et al. 2004
	SsaD157	VIC	0.7	King et al. 2005
	SsaD71	6FAM	0.4	King et al. 2005
2	*SsaD48*	NED	0.5	King et al. 2005
3	*SSspG7*	6FAM	0.5	Paterson et al. 2004
4	*SsaD144*	VIC	0.4	King et al. 2005
5	*SSsp2216*	PET	0.6	Paterson et al.2004
6	*SSsp 2201*	NED	0.54	Paterson et al. 2004
7	*Ssa407UOS*	6FAM	0.5	Cairney et al. 2000
	Ssa408UOS	6FAM	0.6	Cairney et al. 2000

The final volume of each PCR reaction was 10ul and contained: 1μL TAQ buffer, 1 μL dNTP, 0.875-1 μL MgSO4, 0.2-0.35 μL of each primer, 0.20 μL of BSA, 0.12 μL of TAQ polymerase and 2 μL of the template DNA from the cell lysate. The final primer concentration is listed in the table; annealing temperatures for each reaction was 60°C (reaction 1 and 4) and 58°C (reaction 2-3,5-7) for 35 cycles. Conditions for all sets excluding 7 were as follows: initial denaturation at 95°C for 4 min; then 35 cycles of 95°C for 20s, 20s at the reaction specific annealing temperature, 15s of primer extension at 72°C and a final extension at 72°C for 5min. For reaction seven the initial denaturation was at 94°C for 5 min; then 35 cycles of at 94°C for 35s, 30s at the reaction specific annealing temperature, 1min of primer extension at 72°C and a final extension and final extension at 72°C for 7min.

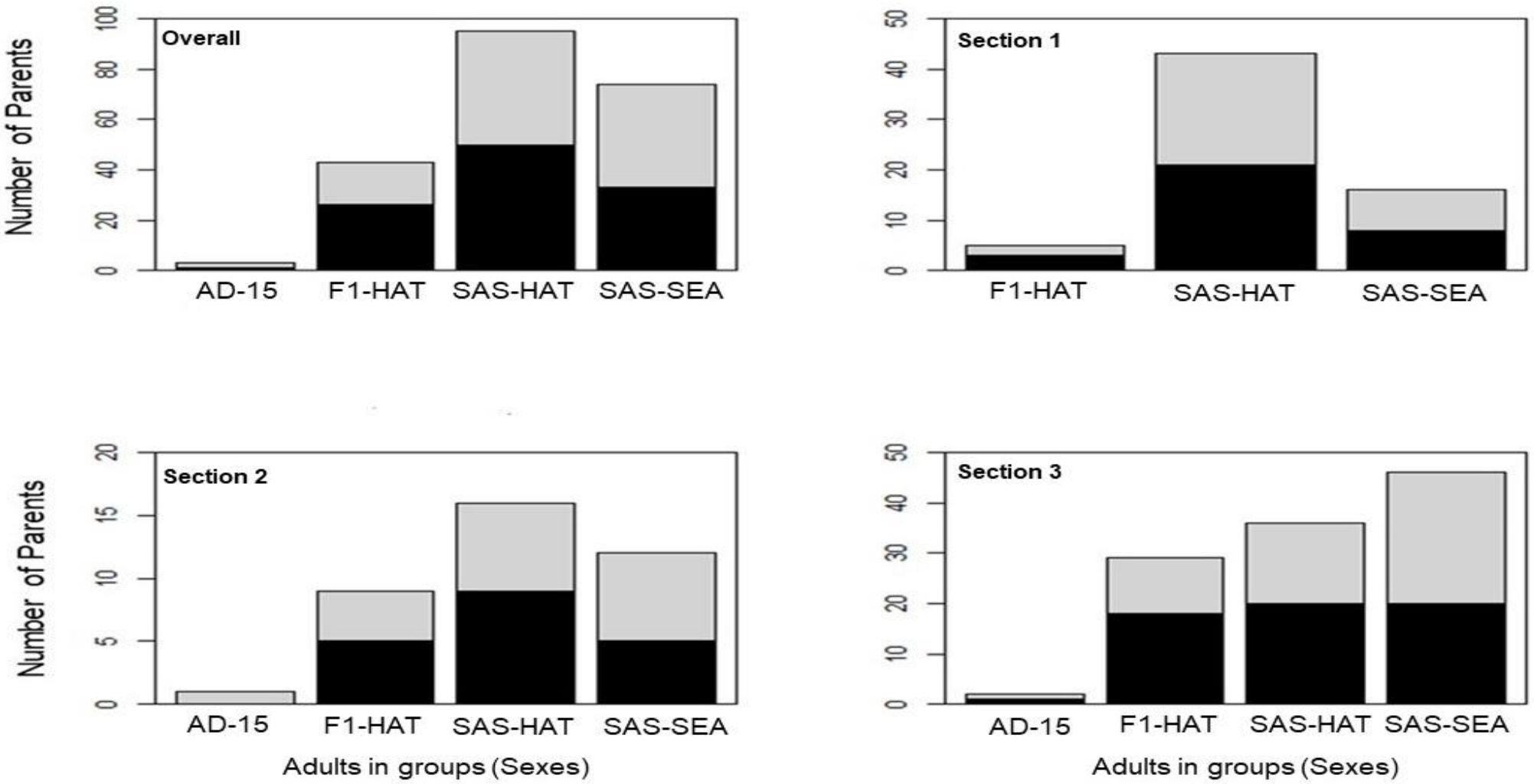

Figure S2.1: Bar graphs displaying the total number of adults of a given group that produced offspring in the Upper Salmon River and all sections. The dark black bars are females and light grey are males. Note that the same adults could have produced offspring in more than one section (particularly for males).

General Conclusions

While it is encouraging that there was natural reproduction of Atlantic salmon in both systems, Rs among adults in each population was extremely low. If this continues, a self-sustaining population may take several years to achieve due to the myriad of challenges associated with restoration efforts. The challenges experienced in both Lake Champlain and the Inner Bay of Fundy are not entirely unique to these systems and occur widely in other restoration projects. There are many factors that limit the success of these projects; the donor population; the receiving habitat; and if the initial cause of decline or extirpation has not been remedied (Armstrong and Seddon 2008; Dunham et al. 2011). It is for these reasons that monitoring and adaptively managing the reintroduced/supplemented population are of the utmost importance to understanding the limitations of reestablishment (Armstrong and Seddon 2008; Dunham et al. 2011, Anderson et al. 2014). Adaptive management not only involves conducting studies in one's own system but through sharing information between projects. Valuable information on the success, failures, mistakes, discoveries and methodologies from different programs should be communicated to ensure that others can succeed in achieving their restoration goals. Through publishing data, organizing meetings and reaching out to other fisheries managers, we would be able to adapt our own programs to promote the restoration success in the future. Furthermore, by adopting a multidisciplinary approach, exploring every step of the program and life cycle of the species, may allow for new techniques and methods to arise.

As with many programs, in the present study, there were several presumed limitations which could have influenced adult Rs and juvenile survival in Lake Champlain. Many of these issues stem from the donor population and the recipient habitat. While the population has been continuously managed for several years and a series of studies have been conducted, natural population growth has been extremely slow. With the few surviving offspring sampled in both rivers, problems with genetic diversity as well as inbreeding may occur, ultimately hindering future reintroduction success. On the other hand, in the Inner Bay of Fundy, potential issues with the transport, differences in release timing, condition and handling and the captive rearing may have influenced Rs of adults which would need to be further explored over subsequent years. While we showed that the SAS-SEA fish were not performing as well as the other SAS group or achieving high Rs, many unmeasured factors could have influenced not only adult Rs but offspring survival

in the system. Since this was the first year of quantifying adult Rs and juvenile survival of this population, continued monitoring needs to occur. Therefore, to achieve a greater understanding of this population's ability to establish itself, future work which combines long term assessment, continued monitoring and alteration of management strategies and of experimental protocol is recommended.

Through these studies we demonstrated that restoration success (reintroductions and supplementations) is complex and can be influenced by many factors. These theoretically simple programs tend to fail more often than succeed. However, it is only with the continued monitoring of a given population, adaptive management at every step and life-cycle, and through sharing our knowledge, that we can gain further insight into the limitations impeding our own programs success and ultimately aid in bettering restoration efforts around the world.

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