

An Assessment of Inner Bay of Fundy Atlantic Salmon (*Salmo salar*) Spawning Success in Fundy National Park

by

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Abstract

Atlantic salmon (*Salmo salar*) have declined precipitously throughout most of their North American range. As a result, many strategies have been implemented to try and restore Atlantic salmon (*Salmo salar*). One such restoration strategy, implemented by the Fundy Salmon Recovery project, involves the collection of endangered inner Bay of Fundy salmon smolts from their natal rivers to be reared at the world's first marine conservation farm to maturity. This strategy includes both an open net ocean pen and freshwater hatchery components. Upon maturity, these salmon are returned to their natal rivers as adults. This release may be done by hand, by carrying the sexually mature salmon to the water or by carefully lowering them into pools using a helicopter, so they can naturally spawn. In my study, I aim to determine whether these differences in rearing and release strategies led to significant changes in offspring production in the adults of two Fundy National Park rivers. Single nucleotide polymorphisms (SNPs) at 185 loci were used to match parents with the next year's offspring using Colony, a parentage analysis software. Using a fixed effects linear model, I found that there was no significant effect on offspring production caused by release strategy in both rivers. On the Point Wolfe River, rearing strategy was found to have a significant effect on offspring production – with marine-reared adults out-performing freshwater reared adults. This suggests that the marine-rearing strategy, implemented by the Fundy Salmon Recovery project can outperform, in some cases the more traditional freshwater rearing strategy in terms of releasing high-fitness adult Atlantic salmon.

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List of Symbols, Nomenclature, or Abbreviations

DFO – Fisheries and Oceans Canada

FNP – Fundy National Park

FSR – Fundy Salmon Recovery

HDX – Half-Duplex

iBoF – inner Bay of Fundy

MBF – Mactaquac Biodiversity Facility

PIT – Passive Integrated Transponder

PWR – Point Wolfe River

SNP – Single Nucleotide Polymorphism

SARA – Species at Risk Act

SAS – Smolt to Adult Supplementation

USR – Upper Salmon River

Introduction

Atlantic salmon (*Salmo salar*) is among the most recognizable species in Atlantic Canada due to their profound cultural, ecological, and economical importance to the region. Atlantic Canada is home to 16 populations of Atlantic salmon (COSEWIC, 2010). Among these, the inner Bay of Fundy (iBoF) population has a unique life history. While all other populations of Atlantic salmon migrate from their natal rivers to the North Atlantic Ocean to grow and mature before returning to spawn, the iBoF population embarks on a much shorter journey, staying within the Bay of Fundy and Gulf of Maine (DFO, 2010). This makes the iBoF population genetically and behaviourally distinct from all other Atlantic salmon in Atlantic Canada (Vandersteen Tymchuk et al., 2010). Their isolation makes them particularly vulnerable, as changes to a comparatively small ocean range can affect their entire marine feeding habitat. Their limited range and precipitous population decline (only ~225 wild individuals remain (DFO, 2020)) resulted in the population being listed as endangered under the Species at Risk Act (SARA) in 2003 (DFO, 2010). The SARA listing prompted necessary human intervention by Fisheries and Oceans Canada (DFO) and Parks Canada (DFO, 2010).

Atlantic salmon spawn polygamously in FNP rivers during the Fall (mid-October to December), where their eggs remain in the gravel until they hatch into alevins in spring (Figure 1). These alevins will typically emerge from the riverbed in late May/early June as fry (Figure 1), which are now considered viable offspring for the purposes of this project. One of the greatest challenges to iBoF Atlantic salmon survival is during their first venture into the marine environment as smolts (Figure 1), with less than 1% of these individuals returning (DFO, 2010). Fundy National Park (FNP), New Brunswick (NB) is

home to two rivers listed as critical habitat for the iBoF Atlantic salmon, the Upper Salmon River (USR) and the Point Wolfe River (PWR) (SARA, 2002). In 2015, the Fundy Salmon Recovery (FSR) project began, with a particular focus on these two crucial rivers, along with the Petitcodiac River, NB. The FSR project aims to overcome the marine phase bottleneck by capturing outward-migrating smolts, rearing them at the world's first Marine Conservation Farm located in Dark Harbour on the island of Grand Manan, NB, then releasing the mature adults back to their natal rivers (Robinson, 2023).

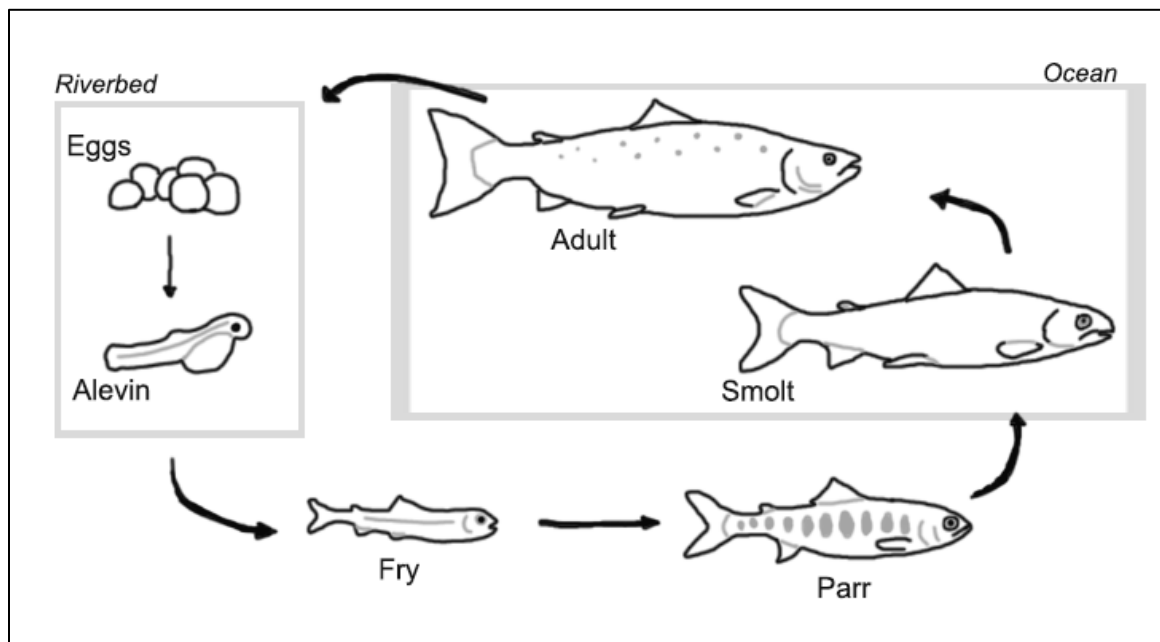


Figure 1. The Atlantic salmon (*Salmo salar*) life cycle. Juvenile salmon grow in the river, enter the marine environment as smolts, where they grow into mature adults. Upon maturity, salmon return to their natal river to spawn.

In addition to wild hatched smolts, fall parr (pre-smolts destined to smoltify the following spring) are collected from the isolated river Dickson Brook in FNP, which adults cannot reach naturally. Fish in this brook are part of the closed loop Live Gene Bank program developed by DFO and FNP to preserve the remaining genetic material of

the iBoF population and prevent the population from going extinct (DFO, 2010). These parr are taken to the DFO-operated Mactaquac Biodiversity Facility (MBF), where they are raised to maturity and spawned. Their eggs are raised to the unfed fry stage and released back to Dickson Brook.

A critical aspect of the FSR program is the successful spawning as a result of natural mate selection through the release of mature adults. The subsequent increase in naturally produced offspring (juveniles reared in the wild) are free of captive exposure during this early life stage. This strategy is predicated on the work demonstrating that juveniles with reduced captive exposure during early life stages have a greater fitness than captive bred juveniles (Clarke et al., 2016). It is therefore vital to the success of the FSR project to ensure that the implemented methods of adult rearing and release are leading to successful spawning in river systems.

Inherent to the FSR program, iBoF adults undergo two different release strategies. Due to limited vehicle access to the rivers, fish are loaded into large plastic exactic holding tanks filled with water, and slung to the upper reaches of the rivers by helicopter, where they are received by a river crew and released. This is repeated many times over the course of one day, allowing hundreds of salmon to be transported to otherwise inaccessible sections of the river quickly. The other method of release was implemented on a weekly basis from August until the end of September to gradually release fish to the lower sections of the river, simulating the natural return of adults. This involved carrying salmon one at a time in a large rubber tube filled with water to the river's edge, releasing the salmon into a suitable pool.

To measure spawning success in the rivers, the number of viable offspring produced by each adult must be assessed. The parentage of fry can be inferred using DNA sequencing, by matching a parent candidate genotype to offspring genotypes. If this process is done for each adult entering the system and each fry produced, the number of offspring produced by each adult can be determined.

Sequencing the entire genome of each individual would be extremely costly and time-consuming, so genetic markers like Single Nucleotide Polymorphisms (SNPs) can be measured throughout the individual genomes, providing a much more cost-effective method of genotyping (Morin et al., 2004). Measuring microsatellites (or simple sequence repeats) is another common method of genotyping individuals, which relies on measuring the number of repeating motifs in intergenic DNA (Bagshaw, 2017). Although microsatellites offer more information per locus (due to their higher number of alleles per locus compared to bi-allelic SNPs), the variable mutation patterns (Morin et al., 2004) and possibly subjective interpretation of these markers (Fernández et al., 2013) can lead to results that are difficult to compare among labs. SNPs were chosen to avoid these ambiguities, allowing for the comparison of this SNPs dataset with SNPs of future years – which would be much more difficult with microsatellites.

This study aims to assess the efficacy of the FSR smolt to adult supplementation (SAS) program to determine which rearing and/or release strategy results in the greatest spawning success as measured by fry production. This will be done using SNPs and parentage analysis software to screen all adult Atlantic salmon that were present in both the USR and PWR systems in 2020 and match them with the 2021 fry cohort. Inferring parents and progeny will provide insight into optimal methods for maximizing spawning

success. This information can be used to adaptively manage the FSR program and methodologies going forward to improve the efficacy of their restoration strategy.

Methods

Study Design

As part of the ongoing FSR project, salmon are collected from the USR and PWR to be reared to adulthood, to help them overcome the high marine mortality phase. Salmon are either collected as wild-hatched smolts in the spring as they exit the river (April-June), or as fall parr/pre-smolt in the fall (Oct-Nov) of their preceding smolt year. Upon collection, wild smolts are taken to Dark Harbour and reared to mature adults in the marine environment. Currently 30 - 50% of the smolts will mature after one year (Parks Canada, 2022), with the remaining fish spending an additional year in the marine environment before being returned to their natal river to spawn. Parr have not yet undergone smoltification and are therefore not yet capable of surviving in salt-water, so they are taken to MBF to be reared overwinter until they are smolts the following spring. After smoltifying, the MBF salmon are split, with approximately half being sent to Dark Harbour to mature in the marine environment, while the other half are retained at MBF to mature in freshwater to be broodstock as part of the Live Gene Bank. All mature adult salmon not selected for spawning in the Live Gene Bank are returned to their natal rivers in the fall to spawn. The adults that are spawned for the Live Gene Bank are held overwinter to recondition, and released the following year.

This study focuses on adult salmon released into the PWR and USR in 2020. In total, 630 adults were released that year: 304 to the PWR and 326 to the USR (Figure 2). A portion of the salmon were slowly re-introduced by hand, using a water-filled rubber tube to transport them to the river, and released into a suitable pool. This was done for 60 PWR-bound salmon (40 from Dark Harbour, 20 from MBF), and 60 USR-bound salmon

(40 from Dark Harbour, 20 from MBF) between September 9th – October 1st, 2020 (Figure 2, Table 1). The remaining mature adults (USR: 246; PWR: 244) were transported to the release sites in a large water-filled, oxygenated exactic tank via helicopter on October 6, 2020 (Table 1). Upon reaching the site, the exactic tank was gently lowered into a deep pool, where a river crew would slowly release the fish. Additionally, 20 MBF adults were released by hand to the USR on October 6th, 2020.

In addition to the adults released into the river system, naturally returning adults were also considered as candidate parents. All FSR adults released into iBoF river systems are tagged with a 28mm half-duplex (HDX) passive integrated transponder (PIT) tag, allowing each individual to be identified. Returning salmon in 2020 were identified using a large HDX PIT antenna system at the mouth of both rivers (Parks Canada, 2022), which they must pass through as they enter the river. In total, 3 adults returned to the PWR, and 35 adults returned to the USR. All identified returning adults were released into the USR in 2019 through the FSR project.

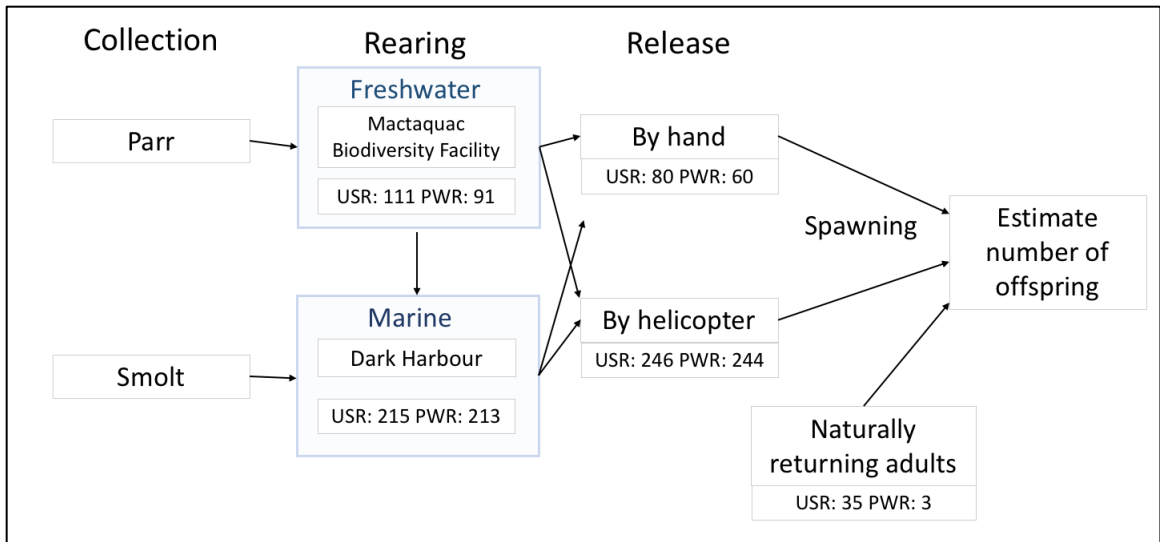


Figure 2. The design of this study, laid out in a flow-chart. Relevant numbers are indicated below each step in the adult release process.

Table 1. Number of adults released to each river in 2020, using each method of release, and rearing strategy.

USR			PWR		
Rearing	Release	Adults	Rearing	Release	Adults
MBF	Helicopter	71	MBF	Helicopter	71
	By Hand	40		By Hand	20
Dark Harbour	Helicopter	175	Dark Harbour	Helicopter	173
	By Hand	40		By Hand	40

Tissue samples were taken from the upper caudal fin of all adults prior to their release in 2020. The adults were anaesthetized using 80 ppm tricaine methanesulfonate (MS 222), and a 1cm^2 section from the tip of the upper caudal fin was taken. These samples were promptly placed in vials containing 95% ethanol and stored at 4 °C until analysis. Tissue samples from naturally returning adults were previously collected from adults released in 2019 using the same method. A total of 634 adult samples from 2020

(PWR: 307; USR: 327) and 38 adult samples from 2019 were used in this study (Figure 2).

In the summer of 2021, both the PWR and the USR were surveyed from head of tide to the waterfall barriers for salmon fry by electrofishing available fry habitat. The electrofishing unit was set at a standard power output of 80W (Jones et al., 2020) to ensure minimal stress/harm to the fish. Fry collected at a given site were sampled quickly to reduce stress, using clove oil (80 ppm) to anaesthetize fry, where a small (<5mm²) clip was taken from the tip of their upper caudal fin. These tissue samples were stored in 95% ethanol at 4 °C. A total of 415 fry tissue samples from the PWR and 66 fry tissue samples from the USR were used in this study.

SNP Sequencing

The best genotyping strategy based on previous simulations by the Pavey Lab was employed (see Pavey, 2016). It was determined that a 185 SNP panel (Kyle Wellband, 2022) would provide sufficient power for this project. Fry and adult tissue samples were sent to the Pavey Lab for inventory and packaging. A total of 1153 (672 adult, 481 fry) tissue samples were cut and packaged using LGLs BioArk sampling kits. Samples were then shipped to the LGL lab in England, which performed the SNP genotyping using their KASP sequencing (Alvarez-Fernandez et al., 2021) (which is a similar method to TaqMan SNP genotyping (Shen et al., 2009)). SNPs were acquired for 185 loci in the Atlantic salmon genome.

Parentage Analysis

Individuals with less than an 80% complete SNPs dataset (as a result of loci being uncallable, bad, and/or not measured) were removed from the study. As a result, a total of 617 adult samples and 443 fry samples were used in the parentage analysis. To further refine the SNPs dataset, the data were scanned for any individuals with identical genotypes. No individuals were found to have identical genotypes among the adults and fry.

Parents were assigned to each fry using two parentage analysis programs, Colony (Jones & Wang, 2010). The mating system was set to male and female polygamy with no inbreeding or cloning, the analysis method was set to Full-Likelihood, with medium likelihood precision, and the species was set to be dioecious and diploid. The run was set to update allele frequencies, with sibship scaling, and a random number seed of 1234 (the default). Sibship prior was set to weak, with maternal and paternal sibship sizes both set at 1. The run-time was set to long, and one run was done in both programs. Following the parent-assignment, parent-offspring pairs assigned with less than a 95% confidence were removed.

Data Analysis

To assess which rearing and release strategy of iBoF adults led to the greatest spawning success, a generalized fixed effects linear model (Equation 1) was used. This model included the fixed effect caused by the rearing strategy of the adult, the fixed effect caused by release type, the fixed effect caused by the life history of the adult, and the fixed effect caused by the stock origin. This model was used for the USR and the PWR separately due to the large discrepancy in collected offspring for the two rivers. An

analysis of variance (ANOVA) was done in R (version 3.6.2) (R Core Team, 2021) using the factors the model (Equation 1).

$$Y_{ijkl} = \mu + L_i + R_j + H_k + S_l + \varepsilon_{ijkl}$$

Equation 1. Generalized fixed effects linear model, where:

Y_{ijk} = The measured number of offspring produced by an adult under treatment ijk .

μ = The mean parameter (average number of offspring any iBoF salmon would produce).

L_i = The fixed effect caused by release type, i (by hand, helicopter, or a natural return).

R_j = The fixed effect caused by rearing strategy, j (MBF, or Dark Harbour).

H_k = The fixed effect caused by life history, k (reared for 1 year, 2 years, or 2 years and reconditioned).

S_l = The fixed effect caused by the stock origin of the adult, l (wild smolt, or wild exposed parr).

ε_{ijkl} = The error term.

The model (Equation 1) was used again to analyze the effects among only spawning adults on the USR. This was done in an attempt to overcome the tremendous bias toward zero offspring. Additionally, a Welch two sample t-test (Welch, 1947) was used to determine whether there was a significant difference in offspring production between reconditioned (adults who were artificially spawned at MBF) and non-reconditioned adults on the PWR (where sample size was large).

A Shapiro-Wilk test was done to assess the normality of the offspring count data (Shapiro & Wilk, 1965). The test found that the count data for both rivers were

significantly non-normal ($p < 0.001$). No transformations were used to manipulate the data of the ANOVAs, this was under the assumption that our datasets (USR: 66, PWR: 415) would be large enough to avoid any loss of power. This was done in accordance with advice on ANOVA use published by Stevens, 2013, who recommends having more than 20-50 samples for non-normal datasets.

Results

Parentage Analysis

Of the 443 offspring that were used in the parentage analysis, 222 were successfully matched with adult parent candidates in Colony, with a minimum probability of 95%. In total, the average number of offspring produced by a spawning individual was 3.46. A total of 103 offspring were matched with both mother and father candidates. The average full-sibling family size (not including half-siblings) was 2.82 offspring. 115 offspring were matched with female parent candidates, but not fathers. These 115 mother-only offspring were matched with 30 individuals. In comparison, only 4 offspring were contributed from fathers, whose female mating partner could not be identified (each producing one offspring).

The parentage analysis found that 120 adults contributed to spawning in 2020, out of the 617 that were sampled and analysed. On the USR, only 50 out of the 340 (14.7%) sampled adults spawned successfully. On the PWR, 70 out of the 277 (25.3%) sampled adults spawned successfully.

Upper Salmon River

The fixed effects linear model (Equation 1) ANOVA found no significant effects caused by release type, rearing strategy, life history, or stock origin in USR adults (Figure 3). This test included all adults released into the USR that year, and all naturally returning individuals. This was done to account for differences in sample sizes for the different factors, since the majority of adults contributed zero offspring.

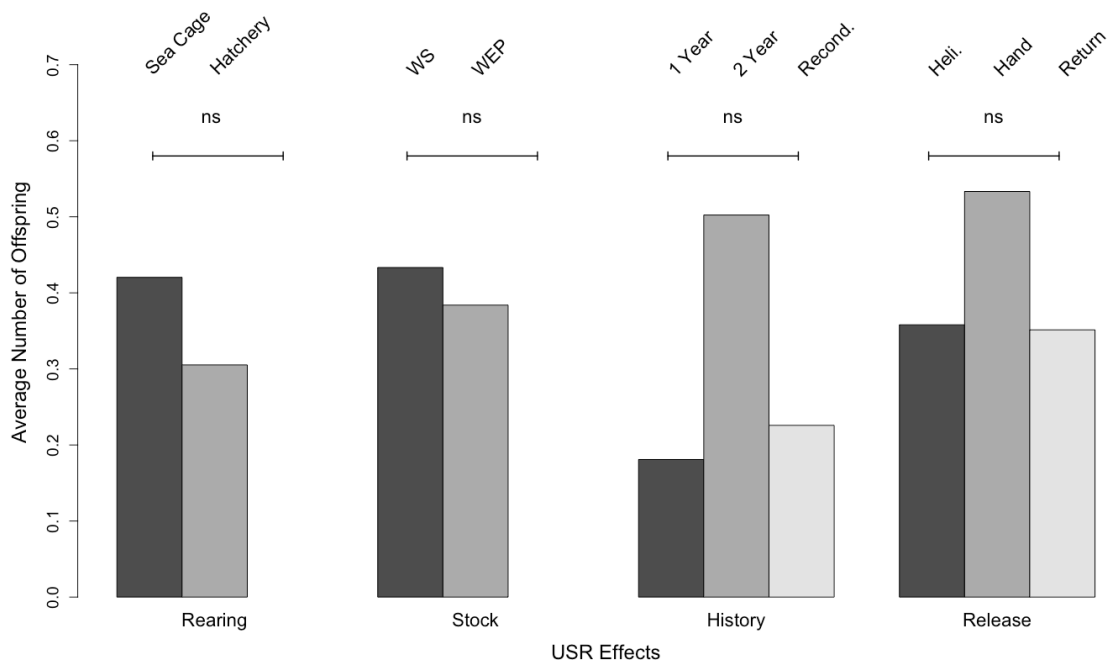


Figure 3. The average number of offspring produced by adults under various treatments (effects) on the Upper Salmon River (USR). Different levels within the four labelled factors are denoted above (WS: wild smolt, WEP: wild exposed parr, Recond: reconditioned, Heli: helicopter). No significant effects were found using a fixed effects linear model, though high variations can be seen between life-history groups and release groups. “ns” indicates no significant effect.

Using the same linear model (Equation 1), an ANOVA including only the spawning adults from the USR found that there was a significant effect caused by the rearing of the adults (F value: 6.476, p-value: 0.0146), and no significant effect caused by release type, life history, or stock origin. The average number of offspring produced by a Dark Harbour spawning adult was 3.323, while the average number of offspring produced by an MBF spawning adult was 1.526.

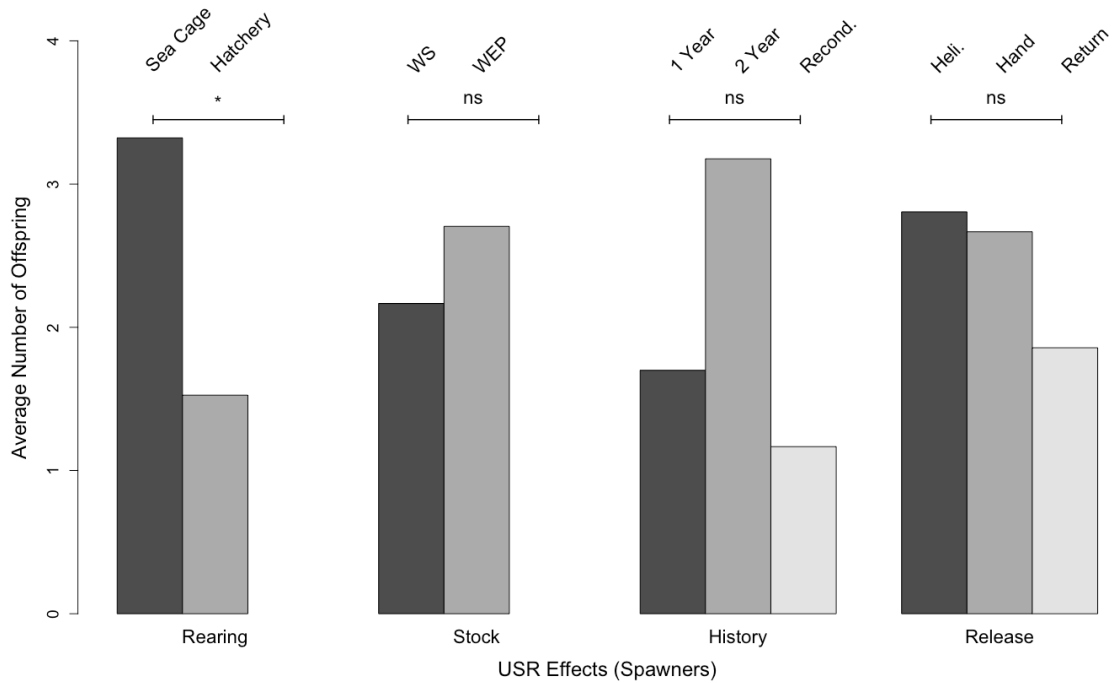


Figure 4. The average number of offspring produced by adults under various treatments (effects), excluding non-spawners, on the Upper Salmon River (USR). Different levels within the four labelled factors are denoted above (WS: wild smolt, WEP: wild exposed parr, Recond: reconditioned, Heli: helicopter). Rearing strategy was found to be cause significant effect on average number of offspring using a fixed effects linear model, and high variation can be seen between the life-history groups – though this was not found to be significant using the linear model. “ns” indicates no significant effect, “*” indicates a p-value < 0.05.

Due to the observed variance in reconditioned versus non-reconditioned adults, a further test was used to compare the average number of offspring produced by these two groups. Including all adults, using a Welch two sample t-test (Welch, 1947), no significant difference was observed between reconditioned and non-reconditioned adults ($t = 1.435$ p-value: 0.154). Including only spawning adults, aimed to mitigate the effects

of having few offspring samples, a t-test was used to compare the average number of offspring produce by reconditioned and non-reconditioned adults. This t-test found a significant difference ($t = 3.379$, p -value: 0.00145) between the two groups in spawning adults, where reconditioned spawners produced an average of 1.167 offspring and non-reconditioned spawners produced an average of 2.841 offspring.

Point Wolfe River

All Point Wolfe River (PWR) adults were subjected to the fixed effects linear model (Equation 1). The ANOVA found a significant effect on offspring production caused by adult rearing strategy (F value: 7.616, p -value: 0.00618), but no significant effects caused by release type, life history, or stock origin (Figure 5). On average, Dark Harbour adults produced 1.296 offspring while MBF adults produced 0.321 offspring on average. Among spawning adults only, Dark Harbour adults produced an average of 4.448 offspring while MBF adults produced an average of 2.083 offspring.

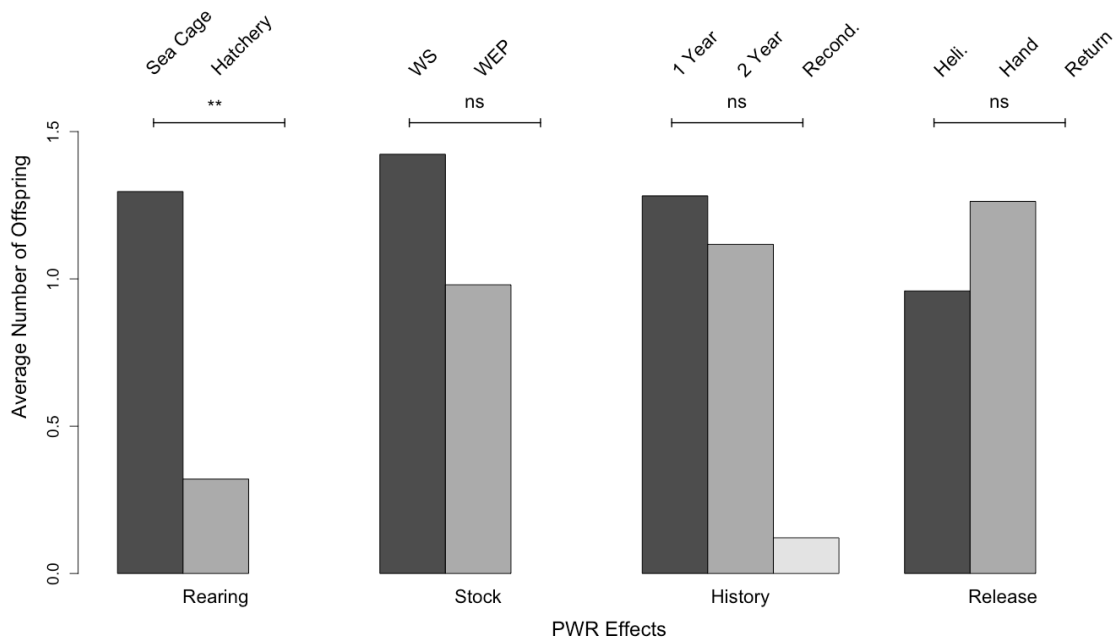


Figure 5. The average number of offspring produced by adults under various treatments (effects) on the Point Wolfe River (PWR). Different groups within the four labelled effects are denoted above (WS: wild smolt, WEP: wild exposed parr, Recond: reconditioned, Heli: helicopter). Rearing strategy was found to be a significant effect using a fixed effects linear model, and high variation can be seen between the life-history groups – though this was not found to be significant using the linear model. Of the three returning adults in the system, none were found to have spawned, so no data is present. “ns” indicates no significant effect, “**” indicates a p-value less than 0.01.

Due to the observed discrepancy in offspring production between reconditioned and non-reconditioned adults in the PWR, a Welch two sample t-test was done to compare the two groups. The average number of offspring produced by a non-reconditioned adult in the PWR (1.143), was significantly greater than the average number produced by a reconditioned adult (0.121) ($t = 5.155$, $p\text{-value} < 0.001$).

Discussion

This study measured the effects of rearing, release, and origin on the spawning success of Fundy National Park (FNP) Atlantic salmon (*Salmo salar*) adults in 2020, by using genetic markers (SNPs) to match parent candidates to offspring from the following year. Based on their measured spawning success, with on average higher numbers of offspring produced, it can be concluded that Dark Harbour adults spawn at a significantly higher rate than MBF adults (Figures 4, 5). Conversely, other factors considered in the fixed effects linear model (Equation 1) were shown to have no significant effect on spawning success. Despite this, reconditioned adults were shown to be less successful spawners in the river environment than their non-reconditioned counterparts.

Rearing Strategy

Evidence of higher spawning success from Dark Harbour adults is particularly convincing from the PWR, where more offspring were captured, giving more insight into which adults were truly contributing the most. On the PWR, when non-spawning adults were included in the analysis to account for sample-size differences, Dark Harbour adults contributed more than four-times the average amount of offspring produced by hatchery adults.

The USR analysis also showed a tendency toward higher spawning from Dark Harbour adults, however this effect was not found to be significant while including all parent candidates (Figure 3). This can likely be attributed to the minimal number of offspring captured (66 total USR samples), as this led to a large number of non-spawners in the analysis – skewing the results. When considering the successful spawners alone, rearing strategy was found to have a significant effect on offspring amounts (Figure 4).

On average, Dark Harbour adults produced more than twice the number of offspring that MBF adults produced. This is further evidence that marine-reared adults out-perform freshwater-reared adults in spawning.

These findings support the results of Lacroix (2008) who demonstrated that rearing strategy, and origin were important factors in the survival rate of Bay of Fundy smolts during their migration from the river. Lacroix found that smolts of hatchery reared smolts had a lower survival rate than wild smolts. This study builds on these previous findings, suggesting that rearing strategy is an important factor in the maturation process of the iBoF salmon as well, where hatchery rearing of smolts has been shown to have an adverse effect on spawning success.

Additionally, this is supported by studies (Clarke et al., 2016; Evans et al., 2014) that show that captivity can lead to reduced survival and success in riverine environments. Clarke et al, found that FNP females produced smaller eggs after increased juvenile hatchery exposure. The increased hatchery exposure adults were also shown to produce significantly less successful embryos (Clarke et al., 2016). Overall, this is compelling evidence that the more wild-like marine rearing strategy is less detrimental to the well-being of maturing iBoF salmon than their freshwater-reared counterparts, as the marine-reared adults dominated spawning in the FNP river systems in 2020.

Release Type, Stock Origin, and Life History

Of the other factors included in the fixed effects linear model (Equation 1), stock origin, release type, and life history had no significant effect on spawning success in adults on either river (Figures 3, 4, 5). This does not imply that these are unimportant factors in the overall health of the released adults, but these factors have been shown in

this study to have no significant effect on spawning success during their first adult year in their natal rivers, and for most, their first year of spawning.

This indicates that the various methods of release and life history are not having a measurable effect on spawning in the FNP rivers. This is in contrast to previous findings, which have shown negative impacts of captivity on juveniles (Clarke et al., 2016; Evans et al., 2014). In this study, no significant effect was caused by stock origin, which can relate to time in captivity (as parr will be reared for longer than smolts). No difference was observed between 1-year and 2-years reared adults, which could indicate a lower dependency on rearing time, compared to rearing type.

Reconditioned Adults

The adults who had been spawned in captivity at MBF, then reconditioned and released the subsequent year were shown to spawn significantly less on average than non-reconditioned adults on the PWR. This is possibly due to their captive spawning, as they were subjected to drastically different conditions than what they would be experiencing in the river. It has been shown previously how broad of an effect captive rearing can have on the behaviour of Atlantic salmon (Clarke et al., 2016; De Mestral et al., 2013). I speculate that this captive-imprinting applies to the spawning tendencies of reconditioned adults, due to their evident spawning failure in the wild during their fall after release.

This major discrepancy in spawning success between reconditioned and non-reconditioned adults was not seen on the USR while including all adults, however a significant difference was observed when only including successful spawners. This is further evidence that reconditioned adults are less fit for spawning, although I acknowledge that this test is more debatable than the definitive results from the PWR.

Single Parents

There are several reasons that explain why one parent candidate would not be paired with another in the assignment of offspring in this study. The first, and most obvious explanation is that the parentage analysis software (Colony) was unable to match a pair with higher confidence than 95% - leading to only one of the parents being accounted for. Similarly, samples were removed prior to analysis based on their poor SNPs availability, which could have led to the removal of a potential parent.

Another possible explanation for the un-paired adults, in particular the tremendous amount of un-paired female spawners, is spawning with precocious parr – male salmon parr that are sexually mature. Although there is no way of showing this definitively with this study, I speculate that this could be a contributing factor to the huge amount of un-matched female spawners. Additionally, the iBoF population has a particularly high rate of precocity among male parr (Bartlett, unpublished data, 2023).

Significance

This study has shown that the marine-rearing strategy, used by the Fundy Salmon Recovery project, produces adult iBoF Atlantic salmon that spawn more effectively than the traditional hatchery rearing method. It is critical to continue adapting the methods being used to restore the iBoF population in such a way that maximizes the fitness of the released individuals. Releasing adults that are more likely to spawn, and produce more offspring is a clear way to achieve this. Additionally, this study has shown the potentially harmful consequences of human handling in reconditioned adults, who spawned at a drastically lower rate than non-reconditioned adults. These findings can be used to continue improving efforts in restoration strategies for Atlantic salmon.

Bibliography

Alvarez-Fernandez, A., Bernal, M. J., Fradejas, I., Martin Ramírez, A., Md Yusuf, N. A.,

Lanza, M., Hisam, S., Pérez de Ayala, A., & Rubio, J. M. (2021). KASP: A genotyping method to rapid identification of resistance in *Plasmodium falciparum*. *Malaria Journal*, 20(1), 16. <https://doi.org/10.1186/s12936-020-03544-7>

Bagshaw, A. T. M. (2017). Functional Mechanisms of Microsatellite DNA in Eukaryotic Genomes. *Genome Biology and Evolution*, 9(9), 2428–2443.

<https://doi.org/10.1093/gbe/evx164>

Bartlett, A. (2023). Unpublished data, University of New Brunswick.

Clarke, C. N., Fraser, D. J., & Purchase, C. F. (2016). Lifelong and carry-over effects of early captive exposure in a recovery program for Atlantic salmon (*Salmo salar*). *Animal Conservation*, 19(4), 350–359. <https://doi.org/10.1111/acv.12251>

COSEWIC. (2010). COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy

population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xlvii + 136 pp.

de Mestral, L. G., O'Reilly, P. T., Jones, R., Flanagan, J., & Herbinger, C. M. (2013). Preliminary assessment of the environmental and selective effects of a captive breeding and rearing programme for endangered Atlantic salmon, *Salmo Salar*. *Fisheries Management and Ecology*, 20(1), 75–89.
<https://doi.org/10.1111/fme.12022>

Department of Fisheries and Oceans Canada. (2010). Recovery Strategy for the Atlantic salmon (*Salmo salar*), inner Bay of Fundy populations [Final]. *In Species at Risk Act Recovery Strategy Series*. Ottawa: Fisheries and Oceans Canada. xiii + 58 pp + Appendices.

Department of Fisheries and Oceans Canada. (2020). Inner Bay of Fundy (iBoF) Returning Adult Atlantic Salmon Population Abundance Estimate. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2020/027.

Evans, M. L., Wilke, N. F., O'Reilly, P. T., & Fleming, I. A. (2014). Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. *Conservation Letters*, 7(4), 371–379.
<https://doi.org/10.1111/conl.12092>

Fernández, M. E., Goszczyński, D. E., Lirón, J. P., Villegas-Castagnasso, E. E., Carino, M. H., Ripoli, M. V., Rogberg-Muñoz, A., Posik, D. M., Peral-García, P., & Giovambattista, G. (2013). Comparison of the effectiveness of microsatellites and

SNP panels for genetic identification, traceability and assessment of parentage in an inbred Angus herd. *Genetics and Molecular Biology*, 36(2), 185–191.

<https://doi.org/10.1590/S1415-47572013000200008>

Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10(3), 551–555. <https://doi.org/10.1111/j.1755-0998.2009.02787.x>

Jones, R.A., Ratelle, S.M., Tuziak, S.M., Harvie, C., Lenentine, B., and O'Reilly, P.T. (2020). Review of the Inner Bay of Fundy Atlantic Salmon (*Salmo salar*) Monitoring Activities Associated with the Live Gene Bank. DFO Can. Sci. Advis. Sec. Res. Doc. 2018/043. vii + 128 p.

Lacroix, G. L. (2008). Influence of origin on migration and survival of Atlantic salmon (*Salmo salar*) in the Bay of Fundy, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 65(9), 2063–2079. <https://doi.org/10.1139/f08-119>

Morin, P. A., Luikart, G., Wayne, R. K., & the SNP workshop group. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution*, 19(4), 208–216. <https://doi.org/10.1016/j.tree.2004.01.009>

Parks Canada (2022). Unpublished Data Report, Fundy National Park.

Pavey, S.A. (2016). Molecular techniques for parentage analysis to assess Supplementation effectiveness for Atlantic Salmon (*Salmo salar*) on the Miramichi River. DFO Can. Sci. Advis. Sec. Res. Doc. 2016/031. v + 8 p.

R Core Team (2021). R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria.

URL <https://www.R-project.org/>.

Robinson, J.W. (2023). Unpublished Data Report, Fundy National Park.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4), 591–611.

<https://doi.org/10.1093/biomet/52.3-4.591>

Shen, G.-Q., Abdullah, K. G., & Wang, Q. K. (2009). The TaqMan Method for SNP Genotyping. In A. A. Komar (Ed.), *Single Nucleotide Polymorphisms* (Vol. 578, pp. 293–306). Humana Press. https://doi.org/10.1007/978-1-60327-411-1_19

Species at Risk Act. (n.d.). (S.C. 2002, c. 29). <https://laws.justice.gc.ca/eng/acts/s-15.3/page-4.html#docCont>

Stevens, J. P. (2013). *Intermediate statistics a modern approach, third edition*. Taylor and Francis.

Vandersteen Tymchuk, W., O'Reilly, P., Bittman, J., Macdonald, D., & Schulte, P. (2010). Conservation genomics of Atlantic salmon: Variation in gene expression between and within regions of the Bay of Fundy. *Molecular Ecology*, 19(9), 1842–1859. <https://doi.org/10.1111/j.1365-294X.2010.04596.x>

Welch, B. L. (1947). The generalization of 'student's' problem when several different population variances are involved. *Biometrika*, 34(1-2), 28–35.

<https://doi.org/10.1093/biomet/34.1-2.28>

Wellband, K. (2022). Unpublished Data, University of New Brunswick.