

**Reproductive success of inner Bay of Fundy Atlantic salmon
(*Salmo salar*) exposed to modified feeding regimes**

by

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Abstract

Atlantic salmon (*Salmo salar*) are widely considered to be at-risk across much of their natural distribution. Reproductive success was investigated for inner Bay of Fundy (iBoF) Atlantic salmon reared from smolt to one sea winter maturity under various feeding regimes. The experiment measured fecundity, egg weight and diameter, and survival to the eyed-stage of development from 13 females and across 39 unique crosses. Fecundity and egg metrics (*i.e.*, egg weight and diameter) did not vary significantly across feeding treatments, however egg mortality was significantly higher in the commercial-style feeding control group (SS4) than any experimental, food-limiting treatment group. Females of treatment group SS5, which experienced the second-most food limitation, produced the greatest number of eggs per centimeter fork length, as well as the lowest mortality rates. The results of this study suggest feeding limitation influences reproductive success in iBoF salmon and inform conservation strategies for the recovery of this species.

Acknowledgements

I would like to first and foremost acknowledge my supervisor, Dr. Kurt M. Samways, for his guidance, patience, and enthusiasm for this world of science that has and continues to inspire my own passion in this field. I would also like to extend my gratitude to Josh LeBlanc for consistently being the first man on the scene to help, and his guidance and insight through all aspects of this study, as well as the rest of my lab mates, especially Hannah, Kate, Jack, Lauren, and Ben for their reassurances, unwavering selflessness, and contributions to my project. Thank you to my friends Cam and Matt: Cam for the unparalleled support and being a study-long motivating force, and Matt for his encouragement and willingness to step into unfamiliar territory.

I would also like to acknowledge the partners and collaborators to the Fundy Salmon Recovery project, without whom this project could not have been accomplished: Parks Canada, Fort Folly Habitat Recovery (FFHR), Cooke Aquaculture, and Atlantic Canada Fish Farmers Association (ACFFA). I would like to specifically thank John Whitelaw and John Robinson of Parks Canada for their guidance and expertise throughout the spawning and egg-rearing processes. Special acknowledgement to Tim Robinson, Alanah Bartlett, and the rest of the FFHR team for the donation of equipment for egg rearing and their assistance with adult releases. Thank you to Stanley Fleet, Sean Neary, and other Cooke Aquaculture staff members for their contributions to the rearing, holding, and transportation from Dark Harbour. I would also like to thank the University of New Brunswick Saint John (UNBSJ) Department of Biological Sciences and members of the Canadian Rivers Institute for their help with fish holding on campus, especially Dr. Ben Speers-Roesch, Dr. Jim Kieffer, and Animal Care Technician Kelly Cummings-

Martell for the facilities and equipment to hold and spawn the adults, and rear the eggs and subsequent fry.

Statement of Research Contributions

My research contributions consist of tagging and assessing adult salmon at the conservation farm on Grand Manan, the care and spawning of study adults, the rearing of subsequent eggs to eyed stage, as well as the collection and analysis of project data, and the care of resulting fry until their release.

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Introduction

Atlantic salmon (*Salmo salar*) are a species of anadromous fish native to the east coast of North America, with a range extending across the North Atlantic Ocean through to Northern Europe. Regarded as a species of considerable socio-economic importance, the Atlantic salmon is widely considered to be at-risk across much of its natural distribution (WWF, 2001; Lennox, et. al., 2021). Critically endangered is the inner Bay of Fundy (iBoF) Atlantic salmon population. Considered genetically distinct from the rest of its conspecifics, the iBoF population has a migration typically confined to the Bay of Fundy/Gulf of Maine in Atlantic Canada, unlike other Atlantic salmon populations in Canada which generally migrate to the North Atlantic (Vandersteen Tymchuk, et. al., 2010). Many factors are attributed to the decline of Atlantic salmon, including the development of mills, damming, logging, overfishing, and the pollution of rivers (Lennox et. al., 2021). Despite conservation efforts, the iBoF population has experienced continued declines, with population estimates remaining below 250 returning adults (DFO, 2020).

This severe decline resulted in the iBoF population becoming the only Atlantic salmon population listed as endangered on the Species at Risk Act (SARA) (DFO, 2020). The listing prompted the development of various conservation efforts. One such effort was the formation of the Live Gene Bank (LGB) program by DFO, in which federal biodiversity facilities (hatcheries) in Nova Scotia and New Brunswick conserve the unique genetic diversity of the population (DFO, 2020). While the LGB has prevented the population from going extinct, recovery has remained elusive. Another key conservation and restoration strategy that has been implemented is the Fundy Salmon

Recovery (FSR) program, which aims to employ a smolt-to-adult supplementation strategy to minimize the high marine mortality largely believed to contribute to the continued decline of the population (Amiro, 2003).

Established in 2015, the FSR strategy is designed to limit captive exposure of iBoF salmon to only the post-smolt/adult stages, while promoting natural mate selection and spawning, and wild juvenile development to the smolt stage. This is due to the negative impact captivity has been shown to have on fitness, especially when captive exposure occurs at early life stages (Clarke, et. al., 2016). The primary strategy of the FSR program is to collect out-migrating smolts which are then transported to the world's first Marine Conservation Farm located in Dark Harbour on the island of Grand Manan, New Brunswick (Figure 1), where they are reared in modified sea-cages to maturity and subsequently released back to their natal rivers to spawn naturally. While this restoration strategy is new, the feeding strategy employed for rearing follows the traditional production model which is to produce large fish. This production-style feeding has remained largely unchanged for the century that salmon aquaculture has existed. The less-streamlined, deep body morphology resulting from a commercial feeding regime has been shown to lead to reduced fitness in a wild setting (Crowley, et. al., 2023). As such, it would be advantageous to develop a feeding regime that better mimicked how salmon feed in the wild to minimize effects the current commercial feeding strategy may have on the FSR reared salmon.

Interest in integrating a more natural captive feeding regime into current conservation strategy led to the development of a project conducted by partners of the FSR to experimentally rear smolts of the FSR program under different feeding regimes. These

experimental feeding regimes varied in daily frequency and weekly occurrence, to assess how feeding affected the growth and maturation of fish. This feeding experiment was conducted for one year from smolt to one sea-winter maturation, concluding in September 2023. A subset of these mature experimental fish were selected for the purposes of this study.

The goal of this study was to assess the effect of feeding trials on the reproductive success of inner Bay of Fundy Atlantic salmon adults. To address this, a spawning experiment was conducted with mature adults from the aforementioned feeding study. Specifically, the objectives of this study were to 1) assess how feeding trials affect egg characteristics (*e.g.*, egg diameter, weight, survival, and fecundity), and 2) create a female length-fecundity relationship as exists for other populations. I hypothesized that egg production and survival would vary with food restriction. Specifically, I predicted that females receiving less feed (*i.e.*, more food restriction) would produce fewer, but larger eggs with greater survival. This is because wild females (*i.e.*, females experiencing food limitation) have been shown to produce fewer but larger eggs compared to females in captive environments (Jonsson et al. 1996). The second objective serves as an assessment tool for iBoF salmon as previous relationships were established on wild individuals, while the remaining population (both in captivity and in the wild) are the

result of generations of conservation efforts and may no longer retain the historical, wild biological characteristics (*i.e.*, length-fecundity relationships).

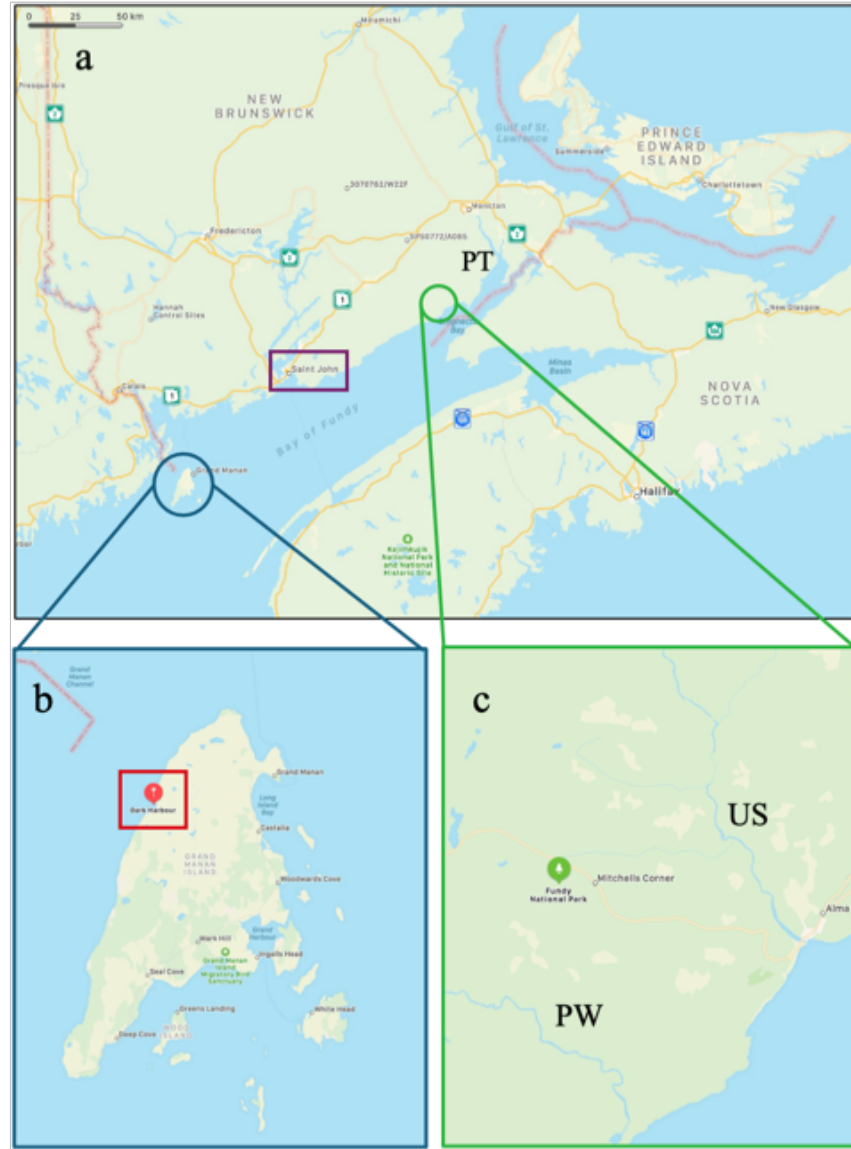


Figure 1. Maps displaying locations of origin, holding, and release of adults and eggs. Panel a) the maritime provinces with the relative locations of Grand Manan island (blue circle), the city of Saint John where the University of New Brunswick is (location of holding, spawning, and rearing) (purple box), and Fundy National Park (green circle) and the Petitcodiac River (“PT”) where releases occurred; panel b) Dark Harbour (red box) on Grand Manan island, where the adults originated; and panel c) Fundy National Park, where the Point Wolfe (“PW”) and Upper Salmon (“US”) rivers are located.

Materials and methods

Adults

Origins of fish for captive spawning

Inner Bay of Fundy (iBoF) Atlantic salmon were captively reared from smolt to maturity under the Fundy Salmon Recovery's (FSR) conservation program, at the wild salmon marine conservation farm in Dark Harbour, Grand Manan, New Brunswick (Figure 1). Some fish on site were included in a feeding experiment which assessed growth and maturation across different feeding strategies. Four different regimes were implemented, which varied by weekly occurrence and daily frequency. Fish were fed by one of two occurrence schedules: 1) Monday, Wednesday, Friday; or 2) an irregular feeding schedule, which consisted of a two-week rotation between Week 1: Monday, Wednesday, Thursday, Friday and Week 2: Tuesday, Wednesday, Thursday. Furthermore, fish were fed at different daily frequencies, either once or twice a day, within the occurrence schedule. Fish in each sea cage were assigned to one feeding regime for the entirety of the experiment. There was also a control treatment which consisted of the current commercial-style feeding regime at the conservation farm, where fish were fed Monday to Friday, twice a day. The control treatment was the regime which received the greatest total amount of food. Each individual sea cage corresponded to a single feeding regime, and were identified as SS8, SS7, SS6, SS5, and SS4 (Figure 2). All experimental fish were collected by FSR partners from the Pollett and Little rivers of the Petitcodiac watershed, and the Upper Salmon and Point Wolfe rivers of Fundy National Park (FNP) (Figure 1). All fish are from the iBoF population which is

genetically distinct from all other Atlantic salmon populations (Vandersteen Tymchuk, et. al., 2010). Approximately 400 fish were held in each sea cage for the duration of the feeding experiment.

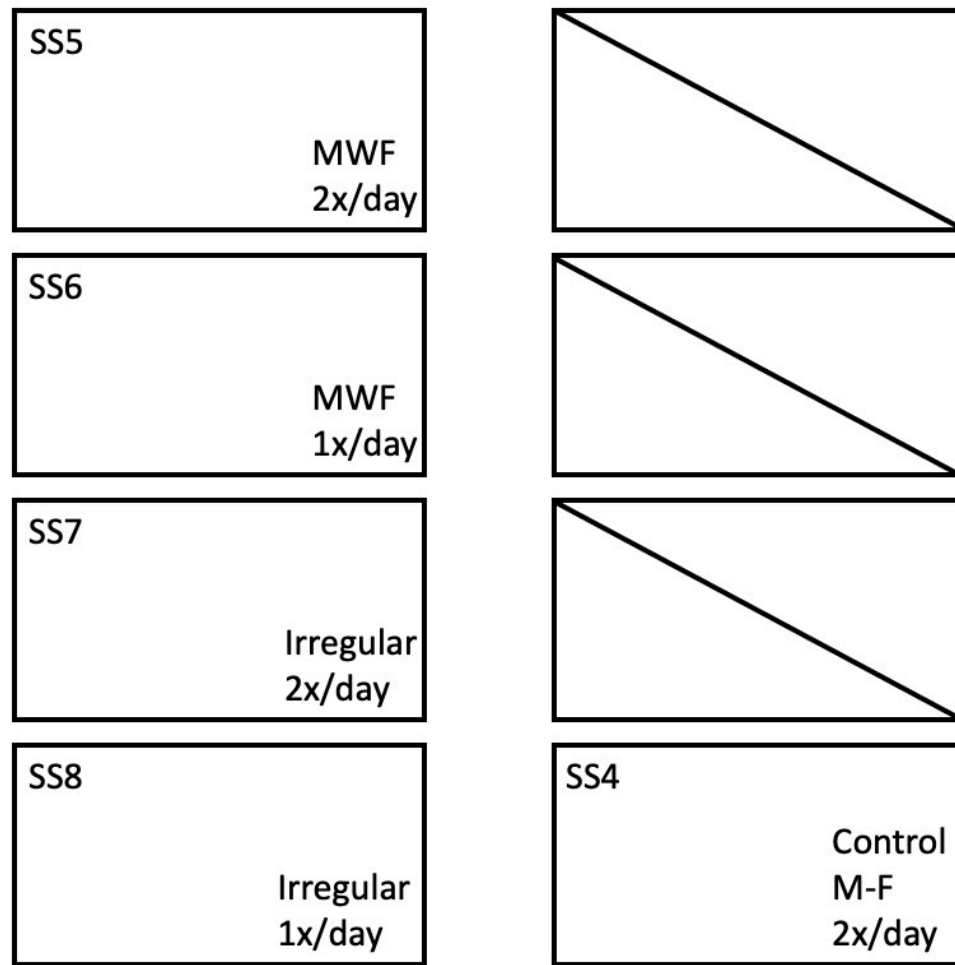


Figure 2. Layout of sea cages in Dark Harbour, with corresponding feeding regime conditions. Rectangles (i.e., sea cages) with diagonal lines represent sea cages on-site that were not associated with the feeding experiment. Feeding regimes are as follows: SS5) fed Monday, Wednesday, and Friday, twice daily, SS6) fed Monday, Wednesday, and Friday, once daily, SS7) fed following the rotating irregular schedule, twice daily, SS8) fed on the irregular schedule, once daily, and SS4) the control group, which was fed everyday Monday through Friday, twice daily.

Selection and Transfer

A total of 30 adults were selected in September 2023 during the Fundy Salmon Recovery's annual maturity sorts for release from the marine conservation farm. Three mature males and three mature females from each of the five feeding trials were chosen based on physical cues of maturity. Individuals deemed "strongly mature" (*i.e.*, displaying obvious signs of maturity, such as kypes, protruding vents, and/or softening of the underbelly) were sorted into separate holding pens for later transfer. Upon selection, the fish were sampled for fork length (cm), mass (kg), total lipid content (g/kg) using Bioelectrical Impedance Analysis, and photographed. Selected fish were held on site until October 25, 2023, when they were transported to the University of New Brunswick, Saint John (UNBSJ) and held until they were ready to spawn. Transportation of salmon was approved by Fisheries and Oceans Canada (DFO) Introduction and Transfers under permit NB 23-111.

Housing

Adults were housed at UNBSJ in holding tanks receiving dechlorinated freshwater. A chiller system was set up to provide additional cold water to the holding tanks, while the main flow was supplied directly from the dechlorinated water lines. Two 5 cm air stones were placed in the head-tank, as well as a single 5 cm air stone in each holding tank, to ensure adequate oxygen levels were maintained. Temperatures during holding ranged from 16°C to 9°C, getting cooler with seasonal temperatures, and dissolved oxygen (DO) levels were kept above 85%. Lighting in the holding facilities were adjusted throughout holding to crudely mimic natural photoperiod. Holding tanks were organized by feeding trial (SS4, SS5, SS6, SS7, and SS8), with males and females

held separately. Tanks were monitored daily to measure temperature and DO, and adults checked for signs of stress, development of fungus, or injury. Additional monitoring was performed to assess maturation progression of females. Progress checks were completed upon arrival, and subsequently performed at least once weekly leading up to spawning, with increasing frequency as the females approached ovulation. During these checks, females were physically assessed for cues of ovulation which included a soft underbelly, pulsating vent with gentle pressure, and pocketing of the skin around the vent following pressure release.

During holding, some individuals were infected by *Saprolegnia sp.*, a common captive-setting fungus. Salt treatments were employed to mitigate the development and spread of the infection. Affected fish were treated with a 2% salt solution bath for two hours, as needed.

Spawning and eggs

Spawning procedures

Spawn-ready females were anesthetized using tricaine methanesulfonate (also known as TMS or MS-222) with a sodium bicarbonate buffer. Prior to stripping the eggs from the female, the anesthetized fish was dipped in clean water to rinse off remaining anesthetic, and then gently cloth-dried, along with the handler's hands, to avoid water from prematurely hardening the eggs. A female's total egg clutch was then stripped into a large bowl by applying gentle pressure along the length of her abdomen. The eggs and accompanying ovarian fluid were subsequently divided equally into three separate containers. Each of these three containers were then fertilized by a single male (also anesthetized, rinsed, and dried), for a total of three crosses per female. Crosses were

conducted within feeding trial, such that each female within a given trial was crossed with each of the three males in her trial. However, pre-spawn mortalities, an infertile male, as well as the need to release some males pre-spawn due to developing fungus, led to the necessity for inter-regime crosses and a reduction in the total planned crosses. In total, 13 females and 12 males were spawned, resulting in a total of 39 unique crosses (Table 1; Appendix A).

Table 1. Male and female adults used in spawning which successfully contributed to crosses analyzed for this study. Unique passive integrated transponder (PIT) identification number and cage (i.e., feeding regime) are listed for each individual. Twelve males and thirteen females were successfully spawned, with 1-3 fish per feeding regime.

Female PIT	Female cage	Male PIT	Male cage
699382	SS4	699383	SS4
699442	SS4	699384	SS4
921933	SS5	699390	SS4
921976	SS5	921921	SS5
922677	SS5	921969	SS5
921894	SS6	921975	SS5
922314	SS6	922324	SS6
922344	SS6	922351	SS6
922044	SS7	922373	SS6
922070	SS7	922022	SS7
921612	SS8	921603	SS8
921657	SS8	921663	SS8
921666	SS8		

Spent fish (*i.e.*, kelts) were placed back into their holding tanks and held in the same conditions under daily monitoring until transport off-site. Kelts were released by partners of the Fundy Salmon Recovery (FSR) into their natal rivers, being the Upper Salmon or Point Wolfe Rivers of Fundy National Park (FNP), or tributaries off the Petitcodiac River, under the permitted permission by Fisheries and Oceans Canada (DFO) Introduction and Transfers permit NB 23-112.

Fertilization and preparation for incubation

Once a male's milt was manually stripped onto the eggs, the cross container (*i.e.*, container containing the eggs and milt) was gently swirled to ensure full distribution of milt across all eggs. The sealed cross container was then transferred to a cold-water bath (approximately 9°C) to hold the eggs at a stable temperature during the two-minute fertilization period, followed by gently adding water to the cross container to a level of 5 cm above the eggs and letting the eggs sit for an additional two minutes. Once this second 2-minute period had concluded, eggs were rinsed to remove milt and ovarian fluid by gently adding water to the cross container and subsequently pouring the water out (while retaining the eggs in the cross container) until the water poured out clean (*i.e.*, without the cloudy appearance the milt produces); this typically took 4-6 rinses. Once the eggs were rinsed clear, the cross containers were filled with water, sealed, and returned to the water bath for one hour. This allowed the eggs to water harden so they could be handled for transfer to incubators and allow for sampling.

Egg baskets (15 cm diameter x 8 cm tall PVC pipe with fine mesh on the bottom to allow water flow) were weighed and labelled with their weight (g), cross ID, and the parent information (female and male's PIT numbers and feeding trials). Egg baskets were

submerged in a tray of water at the same temperature as for the water hardening process. Once water hardening had concluded, the cross containers were removed from the water bath and their contents were transferred to their respective egg baskets by gentle pouring. Having the egg baskets in water was to allow for gentle transfer to the egg baskets to avoid egg damage. Once eggs were in their respective egg baskets, the full egg basket was weighed, and this total weight (eggs and egg basket) was recorded. Once weighed, the full egg baskets were transferred to another tray for a 10-minute disinfection bath in an Ovadine® solution. During this Ovadine® bath, 10 individual eggs were haphazardly sampled from each egg basket (*i.e.*, each cross) and placed in a labelled vial for later measurements. When the 10 minutes had concluded, egg baskets were rinsed by submerging them in the clean, cold-water tray, and then carefully placed in the incubator.

Incubation conditions

Two vertical Heath stack incubators were used to house all crosses. Two water chiller systems cooled the dechlorinated water in a head-tank before it entered the incubators, and as before, two 5 cm air stones were placed in the head-tank to provide adequate oxygen and prevent nitrogen supersaturation of the water. Water temperatures ranged from approximately 9°C to 5°C throughout the rearing process, getting cooler over time, and DO was maintained above 100%. Egg baskets were placed in all trays except the very top and very bottom trays to avoid negative impacts of water flow into the top of the incubator (*i.e.*, turbulence and/or air bubbles) and oxygen depletion toward the bottom. Lights remained off in the incubation room, and incubators were also covered with black plastic to minimize any remaining natural light from a nearby window. Water was monitored for temperature and DO, and eggs were monitored for mortalities and the

development of fungus. It should be noted that no fungus was recorded throughout the study's egg-rearing period (*i.e.*, to the eyed stage). Mortalities were removed and kept in vials of Stockard's solution, separated by cross and collection date for later observation of developmental stage at mortality as part of another ongoing study. Eggs were reared to the eyed-stage of development, after which final survival counts were gathered. Although this project considers data up to and including the eyed-stage of egg development, rearing was continued to hatch and the subsequent release of the fry (scheduled for May) to the rivers corresponding to their parents.

Sample collection and measurements

The 10-egg samples collected during egg disinfection were assessed within 24 hours of collection, where each individual egg was measured for diameter and weight. This allowed for an estimate of average egg diameter and weight per female to be calculated from a 30-egg subsample. Once measured, sampled eggs were returned to their vial and stored at -80°C for later analysis in another ongoing study. Female fecundity was estimated using the total weights of all eggs in the cross divided by the weight of the 30-egg sample. Survival was assessed by the total egg loss over the rearing period (*i.e.*, the total number of mortalities). Mortalities were counted from water hardening to eyed-stage per cross, and summed per female, to get an estimate of survival per cross and female.

Statistical analysis

Fecundity

Fecundity estimates were converted into a "fecundity ratio" (eggs/cm) by dividing a female's fecundity estimate by her fork length in centimeters, and these fecundity ratios were used to standardize for length across the feeding regimes in the cross-treatment

analysis. A one-way ANOVA was used to compare the fecundity ratios across the feeding trials (*i.e.*, cages).

A linear regression was used to analyze the length-fecundity relationship, which compared the total fecundity estimate of a female to her fork length (cm). The fecundity estimates were also compared to estimates of the length-fecundity equation established by Amiro & McNeill (1986) for Fisheries and Oceans Canada (DFO). Fork lengths of study females were input into the DFO equation to retrieve a fecundity estimate based on the relationship between female fork length and fecundity at the time of population decline. The estimates resulting from the DFO equation were compared to the fecundity estimates observed in this study and analyzed using a paired t-test.

Egg metrics

Preliminary generalized linear model analyses first compared the egg metrics of weight and diameter across females, which established that an effect of individual/female existed for both metrics. As such, female was included as a random factor in the subsequent linear mixed model analyses which compared egg weight and egg diameter across the fixed factor of feeding trial. Analyses were separate for each of the egg weight and diameter metrics. A linear model was used to investigate the relationship between egg weight and female fecundity, as well as a generalized linear model to investigate the relationship between egg diameter and female fecundity.

Egg mortality

Percent mortality was used to compare egg mortality across feeding treatments. It was calculated per cross, as the total count of mortalities to the eyed stage of development divided by the total number of eggs fertilized in the cross. Preliminary

generalized linear model analyses were again used to compare the percent mortalities across females, as well as males. The data was transformed by adding 1×10^{-6} to percent mortality values, to allow the linear model to be analyzed using a gamma distribution. These preliminary models established that there was an effect of female on percent mortality ($t=2.334$, $p=0.0276$), however males did not have a significant effect ($t=1.077$, $p=0.291$). As such, only female was included as a random factor in the subsequent linear mixed model analysis which compared cross percent mortality across the fixed factor of feeding trial.

An additional linear model analysis was used to analyze the comparison of egg mortalities with total egg abundance to assess the relationship between the total count of egg mortalities in a cross and the total number of eggs fertilized in that cross.

Permitting

All experimental procedures for this study were approved by the Species at Risk Act (SARA) under permit DFO-MAR-2021-20(b), as well as University of New Brunswick Animal Care approval under permit 2023-2S-02.

Results

Fecundity

Fecundity comparison across feeding treatment

Female fecundity ratios were not significantly different when compared across feeding treatment ($F=0.623$, $p = 0.658$). Though not statistically significant, there were observable differences across the feeding treatments (Figure 3). Cages SS5 and SS6 consistently had high and low fecundity ratios, respectively, and the control cage SS4 displayed the greatest variability with both the highest and two of the lowest ratios.

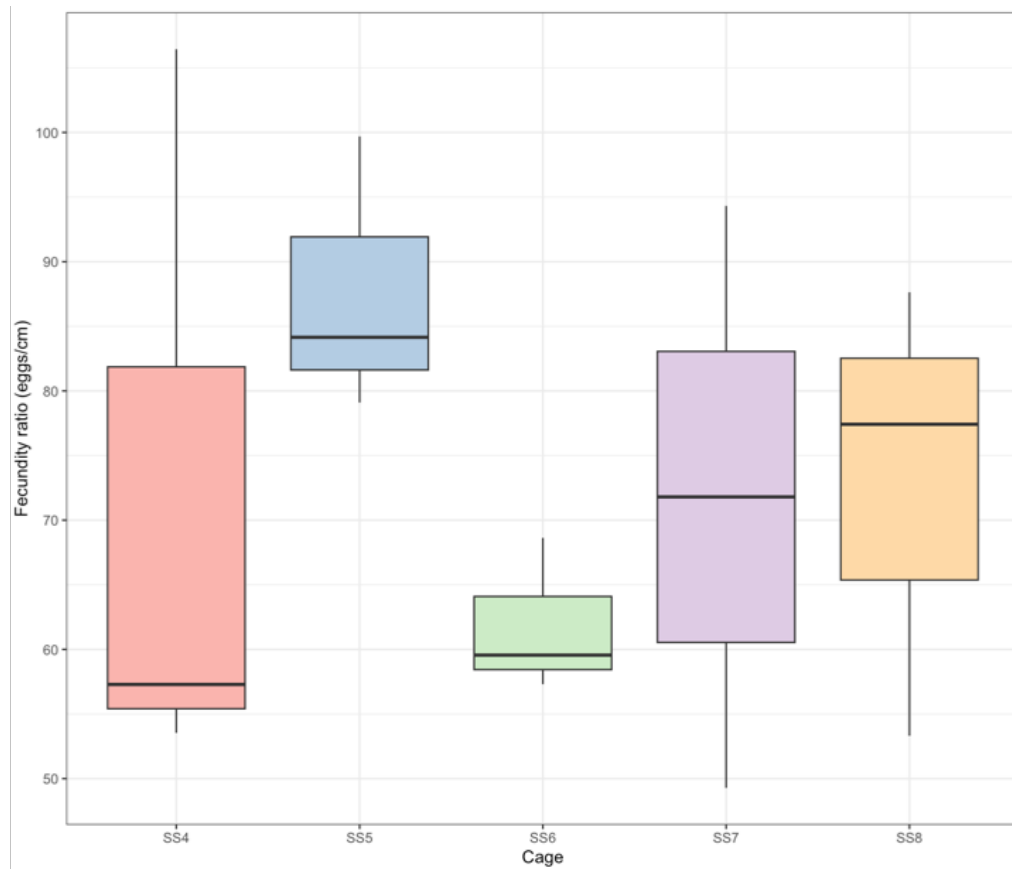


Figure 3. A boxplot of the distribution of fecundity ratios for females across the various feeding treatments (*i.e.*, cages). A single fecundity ratio was calculated for each female within a treatment as the total number of eggs produced by a female divided by her fork length in centimetres.

Length-fecundity relationship

The relationship between female length (cm) and her fecundity was not significant at $\alpha = 0.05$ ($t=2.008$; $p=0.068$). A linear relationship was found to best fit the

data ($R^2 = 0.2515$), and the following equation was developed: $y = 211.05x - 7359.1$, where y provides the fecundity estimate and x represents a female's fork length (Figure 4).

The existing DFO equation (Amiro and MacNeill, 1986) was used to calculate fecundity estimates for this project's females by inputting their fork lengths, and a comparison was made between the DFO estimate and the observed counts. The fecundities of fourteen females were compared, with eleven of those fecundities underestimated by the DFO equation (Figure 5). Differences were significant between the two estimates ($t=3.717$; $p=0.003$), with disparities as few as 18 eggs and as many as over 2000 eggs observed, with both the smallest and largest differences belonging to the control cage SS4 (Table 2).

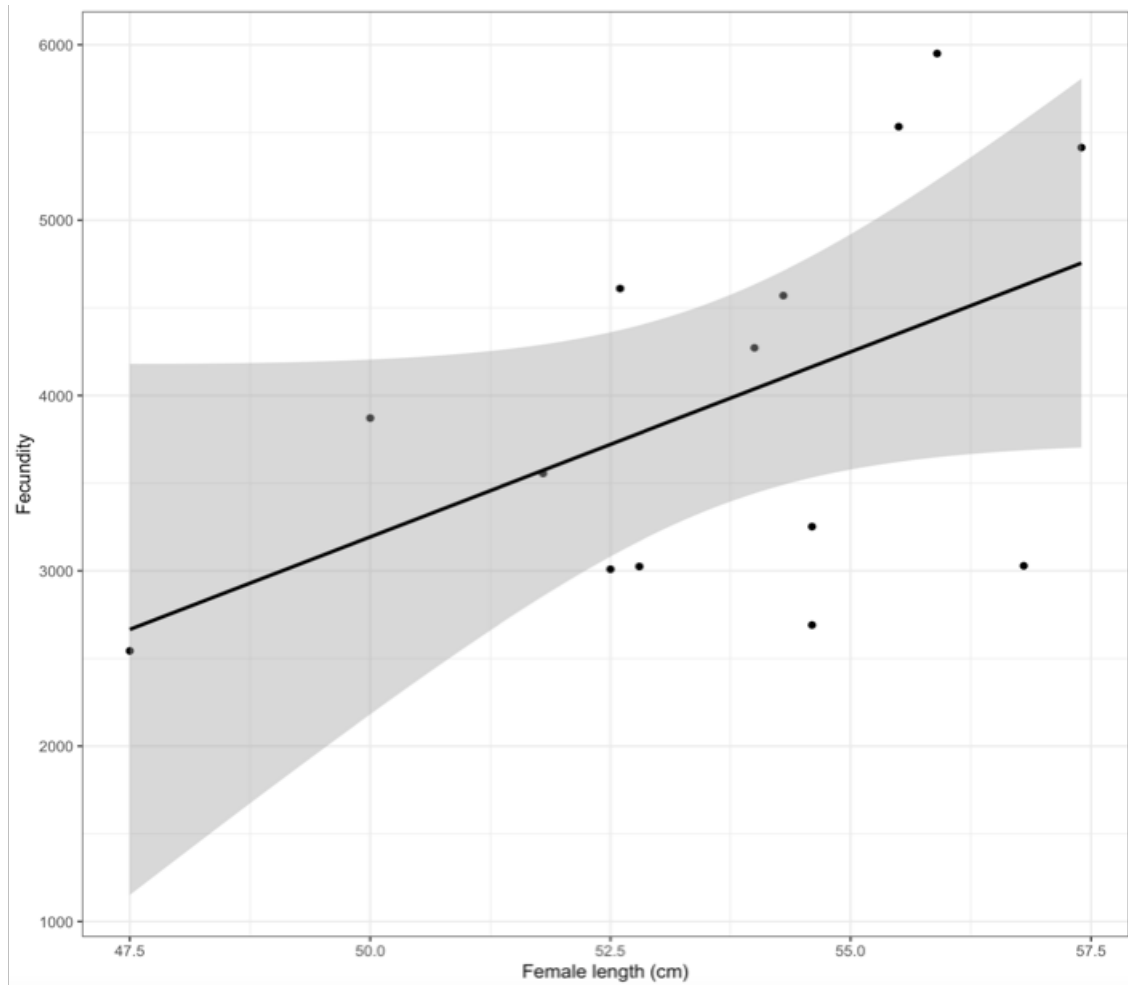


Figure 4. The relationship between fecundity and fork length (cm) in study iBoF females. A linear relationship was found to best fit the data ($R^2 = 0.2515$), with an equation of $y = 211.05x - 7359.1$.

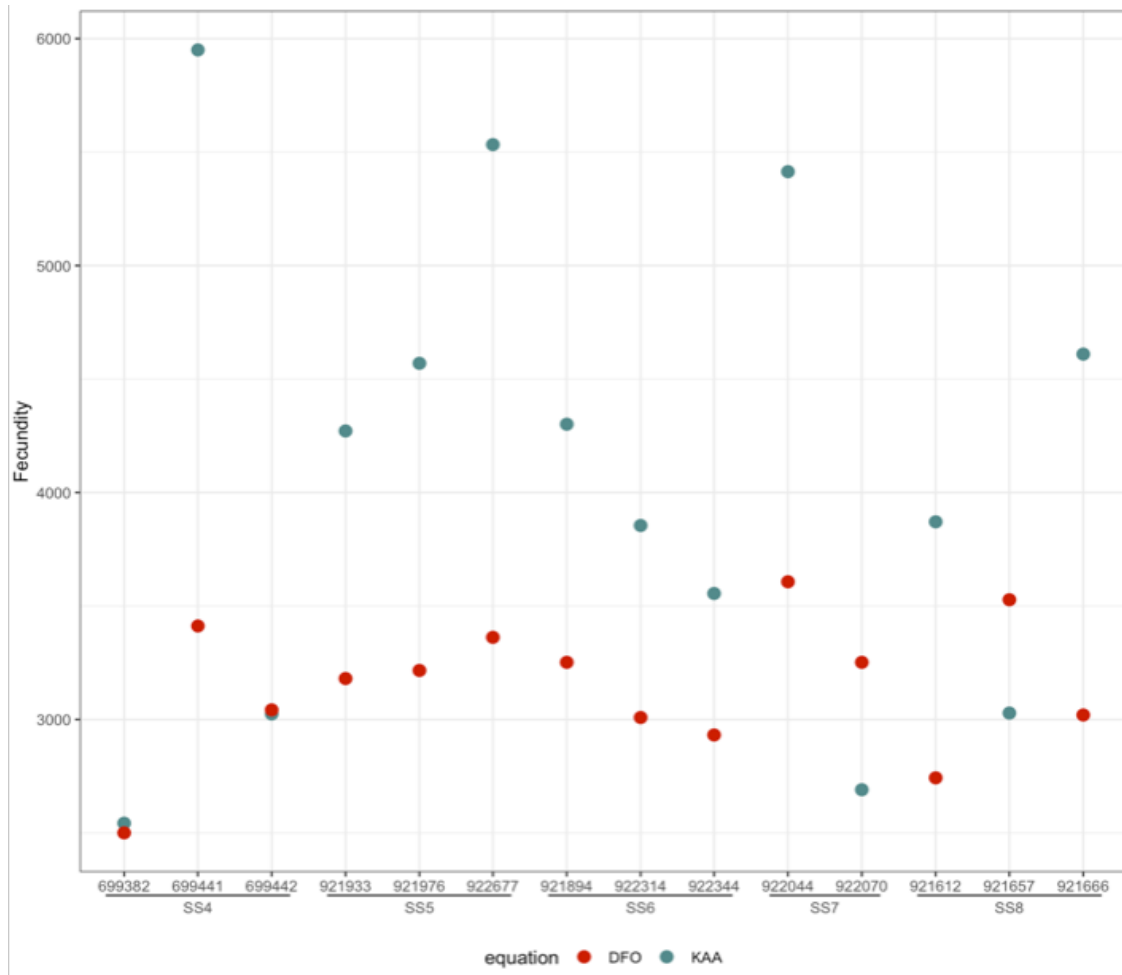


Figure 5. The fecundity estimates observed in the current project (coloured blue) and the fecundity estimates produced by the DFO equation (coloured red) for all females across feeding treatments.

Table 2. Table of female fecundity comparisons between the fecundity estimates based on the existing length-fecundity equation for inner Bay of Fundy Atlantic salmon established by Amiro and MacNeill (1986) and the actual observed fecundities. The Difference is the fecundity estimate from the DFO equation minus observed fecundity.

Regime	PIT	Length (cm)	Equation	Observed	Difference
SS4	699442	52.8	3042.41	3024.55	17.86
SS4	699382	47.5	2500.64	2543.04	-42.40
SS4	699441	55.9	3412.17	5950.12	-2537.95
SS5	922677	55.5	3362.04	5532.92	-2170.88
SS5	921933	54	3180.53	4271.37	-1090.84
SS5	921976	54.3	3216.03	4569.45	-1353.42
SS6	922344	51.8	2931.89	3555.17	-623.27
SS6	921894	54.6	3251.93	4301.11	-1049.18
SS6	922314	52.5	3008.82	3854.90	-846.08
SS7	922070	54.6	3251.93	2690.74	561.19
SS7	922044	57.4	3606.90	5413.93	-1807.03
SS8	921666	52.6	3019.98	4609.93	-1589.95
SS8	921612	50	2742.99	3871.03	-1128.04
SS8	921657	56.8	3527.71	3028.35	499.36

Egg metrics

Egg weight did not vary significantly across the feeding treatments ($F=0.5043$; $p=0.734$), however observable differences existed as with the fecundity data (Figure 6). Cages SS5 and SS8 displayed high variability between females relative to the other cages. Additionally, each of SS4's females each displayed high within-individual variability (Figure 6).

Egg diameter did not vary significantly across the feeding treatments ($F=0.6506$; $p=0.6408$), however differences were observed (Figure 7). Cage SS4 displayed a high degree of variability, as did cage SS6. Some female egg samples were extremely consistent in diameter, with little to no variability across the 30 eggs (Figure 7). However, a limit in precision for diameter measurements (i.e., accurate to only the nearest 0.5mm) impacts the degree of diameter variety.

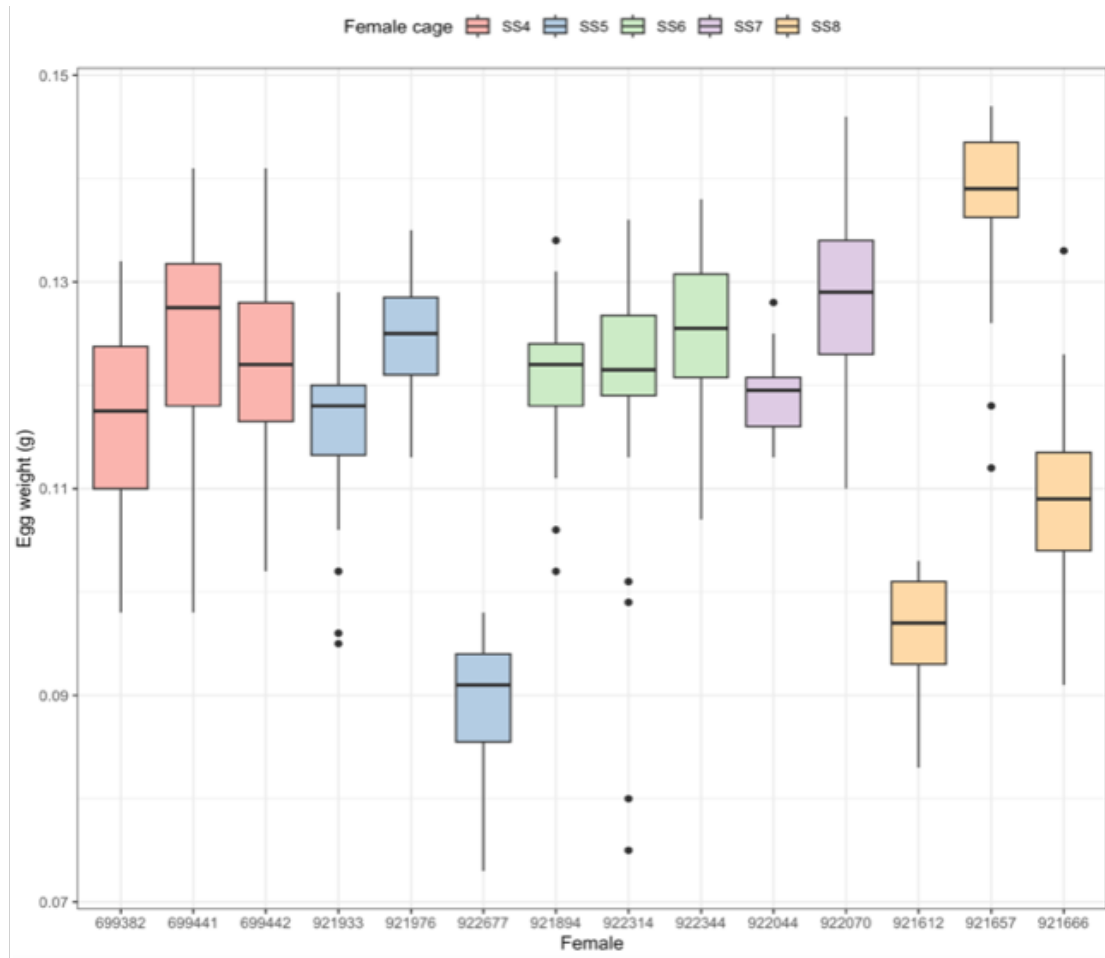


Figure 6. The distribution of egg weight (g) across female (listed as her PIT identification number), with colours representing feeding regimes (i.e., cage); pink (SS4), blue (SS5), green (SS6), purple (SS7), yellow (SS8).

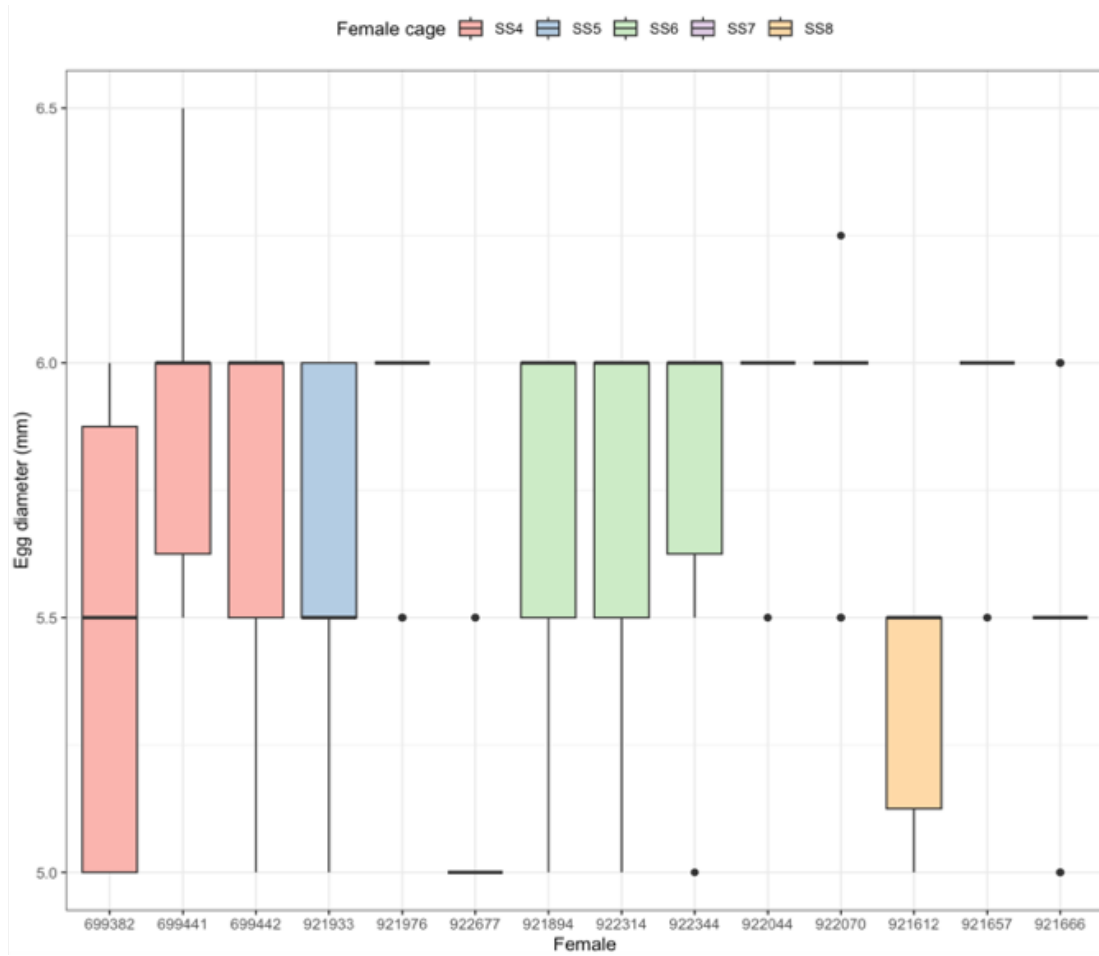


Figure 7. The distribution of egg diameter (mm) across female (listed as her PIT identification number), with the colours representing feeding regimes (i.e., cage); pink (SS4), blue (SS5), green (SS6), purple (SS7), yellow (SS8).

Both egg weight and egg diameter were found to decrease significantly with increasing fecundity ($F=80.09$, $p<0.001$ and $t=3.231$, $p=0.001$, respectively). This indicates that the fewer eggs a female produces, the larger each individual egg will be (Figure 8).

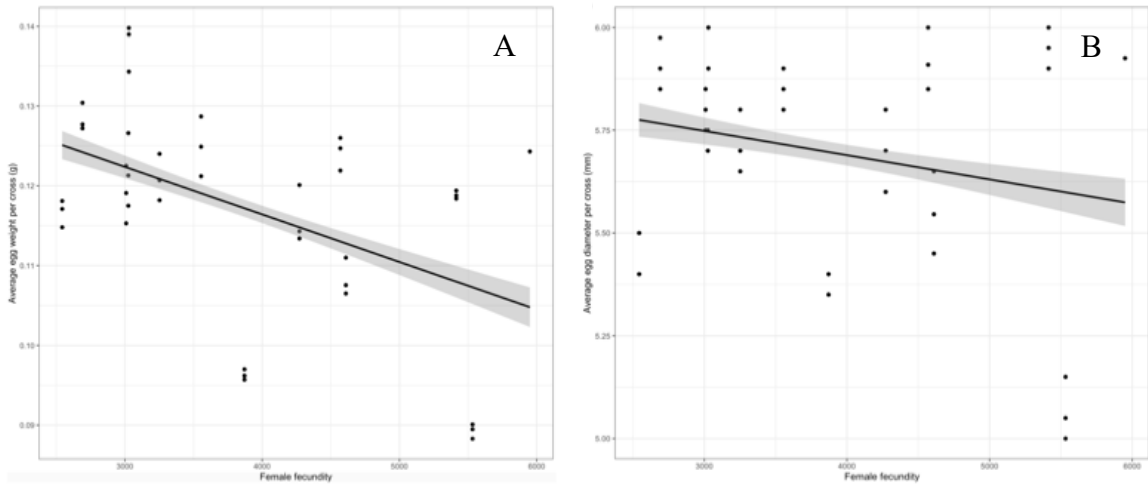


Figure 8. Relationship between A) egg weight (g) or B) egg diameter (mm) and fecundity. Each point represents the average across all eggs in a cross, for all crosses of a female, where each female contributed to three crosses. Therefore, data points in vertical groups of three along same location on the x-axis represent one female.

Egg mortality

Egg mortality to the eyed stage across all treatments was low at <2%, with mortality rates for any individual female ranging from <1% to 8%, and multiple crosses experiencing 100% survivability to the eyed stage.

Egg mortality was found to differ significantly between SS4 and all feeding treatments except cage SS6 (SS4 and SS5 ($t=-2.591$; $p=0.032$), SS4 and SS6 ($t=-1.476$; $p=0.178$), SS4 and SS7 ($t=-2.440$; $p=0.041$), and SS4 and SS8 ($t=-2.521$; $p=0.036$)) (Figure 9). Eggs from rearing group SS6 experienced relatively high mortality, suspected to be due to underlying health issues in one female who, despite being one of the longest females, weighed the least. Variability was again highest in SS4 (Figure 9).

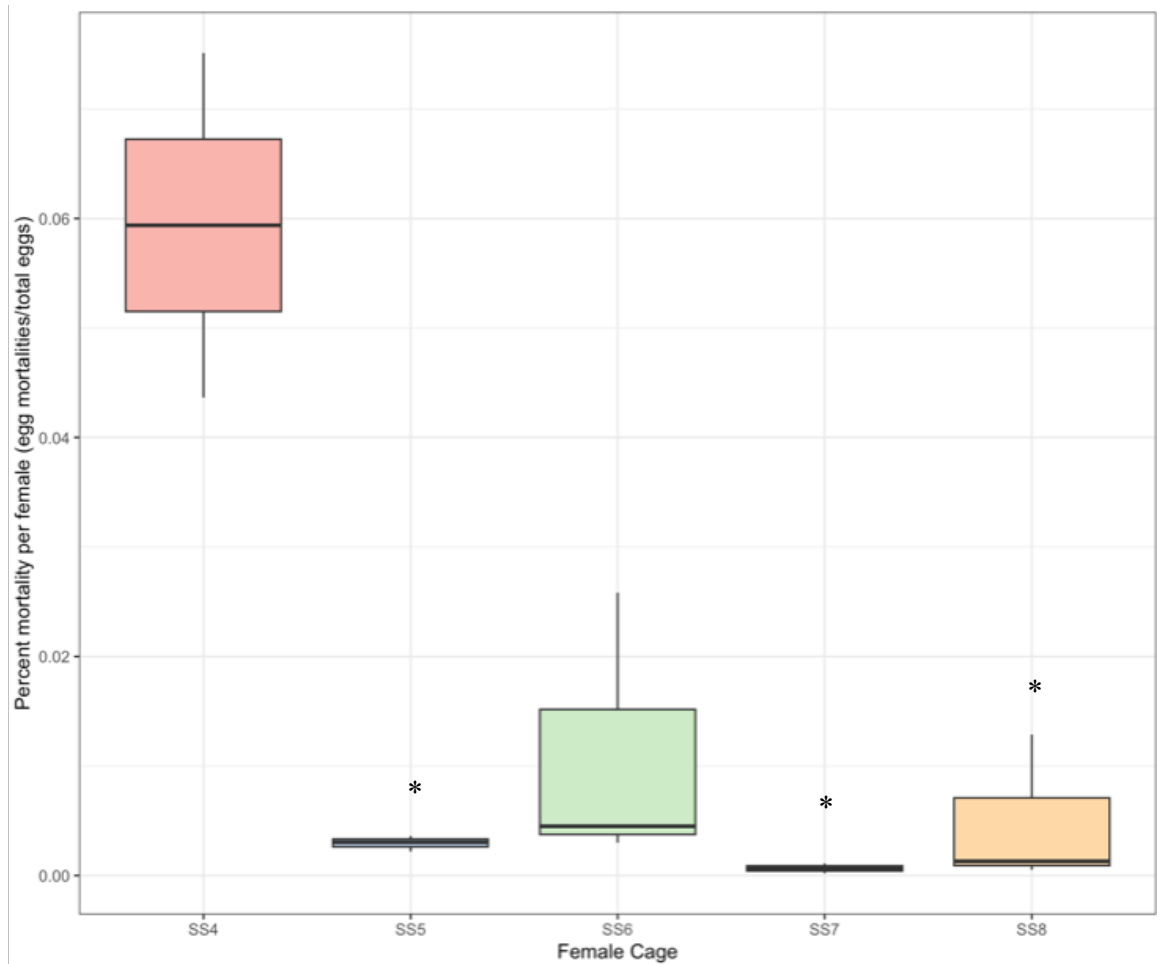


Figure 9. Percent mortality of eggs across feeding. Significant differences from cage SS4 are indicated by an asterisk (*).

Relationship of egg mortality with female fecundity

A significant relationship between egg mortality and egg abundance was observed ($t=-3.112$; $p=0.004$), which suggested that the more eggs present in a cross, the fewer the total number of mortalities that occurred in that cross (Figure 10). However, the fit of the linear model equation was poor ($R^2 = 0.18$).

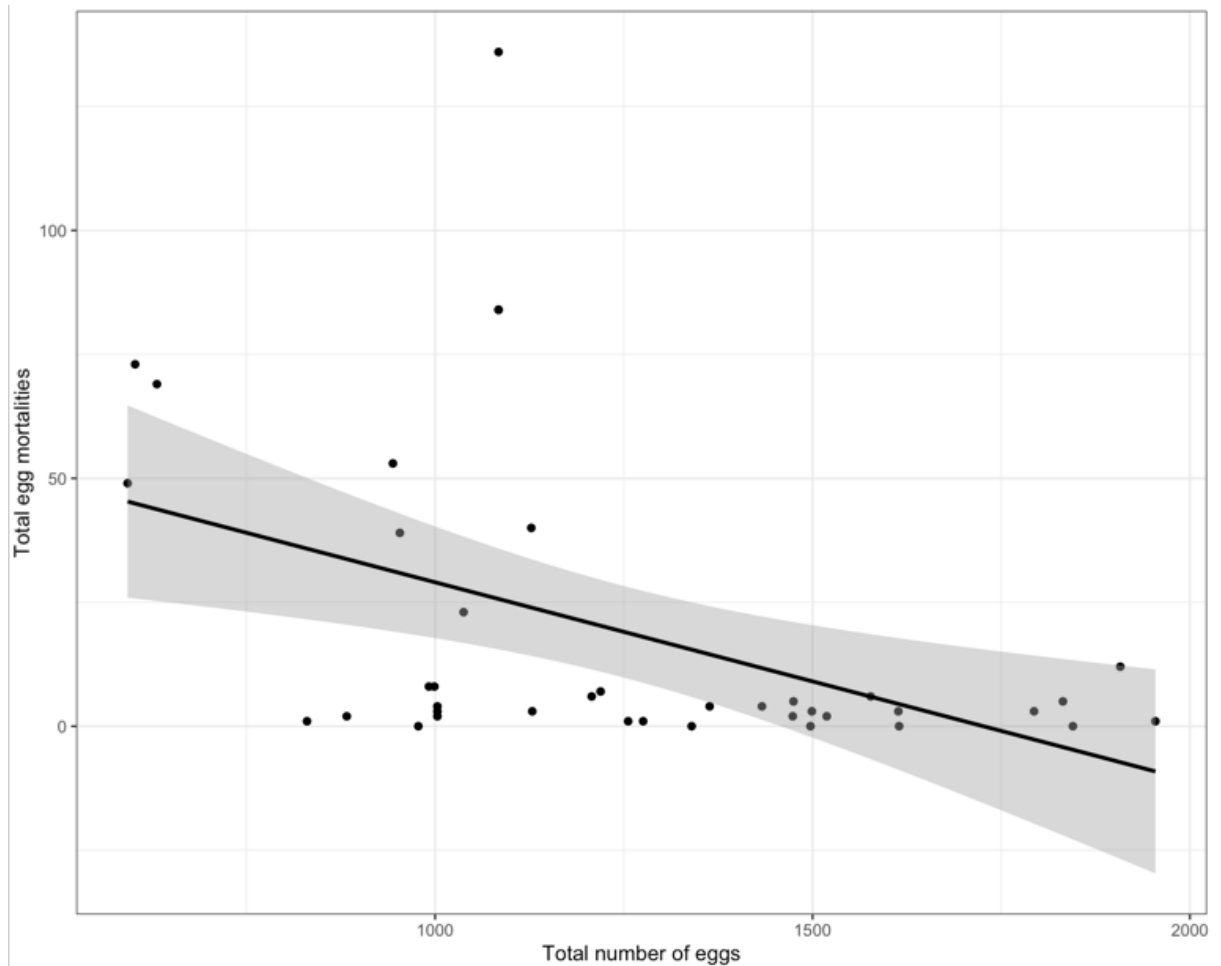


Figure 10. The relationship between the number of egg mortalities to the eyed stage within a cross and the total number of eggs within that cross.

Qualitative results

Spawning and sampling achievements

In total, 39 crosses were completed across four spawning days using 13 females and 12 males. Not all crosses could be completed following a full factorial design (*i.e.*, they could not be completed entirely within a treatment). Two females from SS4 did not contribute to crosses, as one female mortality occurred prior to spawning, and the other female would not give up her eggs at time of spawning. As such, SS4 had only two

females to contribute to spawning for the cage. One adult from cage SS7 originally identified as a female was later confirmed to be a male, reducing the females from this cage to two individuals. This SS7 male however did not produce milt and therefore did not contribute to crosses. The infection by *Saprolegnia spp.* in one tank resulted in pre-spawn mortalities of two SS7 males and one SS8 male, as well as prompted the early release of the remaining two SS7 and two SS8 males, which contributed to the mixed-cage crosses required for later spawning events. All SS4, SS5, and SS6 females were crossed exclusively with their respective cage males, however females from SS7 and SS8 were fertilized with SS5, SS6, SS7, and SS8 males to meet the maximum number of crosses while balancing the depletion of too much milt from any single male. Given that fully developed offspring were not being evaluated (i.e., genetic makeup is not a concern), and there was no significant effect of males on spawning success, using males from other rearing groups was deemed acceptable.

The fecundities of two females from SS6 could not be estimated as the weights of their full egg baskets were not obtained during a spawning/sampling event. As such, these females' fecundities were estimated using the conservative DFO equation.

High fertilization rates of nearly 100% were achieved, with only three individual egg mortalities post-water hardening across all spawning events. Milt quality was determined to be high following microscope investigation of sperm motility, a common measure used to assess milt quality (Aas et. al., 1991; Kommisrud et. al., 2020): every male's milt sample had sperm that were motile for at least a full minute following stimulation with ovarian fluid from stripped females.

Discussion

This study assessed the effectiveness of feeding regime on the fecundity and reproductive success of inner Bay of Fundy Atlantic salmon adults. Fecundity and egg size (*i.e.*, egg weight and egg diameter) did not vary significantly across the feeding treatments (though observable differences across feeding regimes were noted). Egg survival differed significantly between the control group and each experimental treatment, although there were no significant differences between experimental treatments. This information is important for improving the continuing conservation rearing efforts for this critically endangered iBoF population of Atlantic salmon, which are species of ecological importance.

The adult salmon resulting from the initial feeding trials were of good quality. Physical traits, such as the appearance of kypes in the males and protruding vents on the females, the relatively sessile nature of females as ovulation approached, and the brightly-coloured appearance of freshly deposited eggs all indicated good health and quality (Craik, 1985). Though spawning was delayed relative to concurrent Live Gene Bank (LGB) iBoF spawning activity, spawn timing of study adults remained within the natural window for wild iBoF Atlantic salmon. Delays in spawning were likely due to warmer water temperatures and lighting mismatches in the facility relative to the natural environmental state. Spawning was successful, with only one female (SS4) and one male (SS7) not releasing gametes. Fertilization rate was extremely high, at nearly 100% for all crosses, which could in part be due to the good milt quality of the males as assessed by sperm motility. Milt samples were retrieved from every male and observed under microscope, where sperm cells were observed active for over one minute in all cases.

Fecundity and egg size did not vary significantly across the feeding regimes. A lack of significance across the egg production metrics could be a result of a true absence of feeding treatment effect, or due to the small sample size. Cages SS4 (Control) and SS8 (Irregular, once/day) had the greatest variability in egg weight and diameter and received the most and third most total amount of food, respectively over the course of the feeding experiment. The requisite small sample size for the SARA-listed population could also explain the lack of significance. The contrasting egg metrics between feeding regime groups suggests that increasing the sample size could yield significant differences across some of the feeding regimes. Under the SARA permitting, intentional stress or harm to these endangered salmon is strictly prohibited and thus feed limitations do not fully represent that experienced in wild environments, therefore further muting differences otherwise present with more extreme differences between feeding regimes. The prediction that females in feeding regimes with greater food limitation would produce fewer but larger eggs was supported in the case of the most limiting cage (SS6), however this trend was not consistent along the spectrum of food limitation, and there was no trend of greater egg survival with lower fecundity.

Egg survival was overall high, with a total mortality rate of <2% to the eyed stage, however the control cage (SS4), had the highest percent mortality (6%) of all treatments. Egg mortality differed significantly between SS4 (control; most food delivered) and all feeding treatments, except cage SS6 (MWF, once/day; least food delivered). Having a high mortality is an intuitively negative result, and the high variability in mortality between individuals could also be a problematic characteristic of this traditional feeding strategy. Although variability in populations serves as a buffer

against stochastic events, producing many individuals with relatively high egg mortality, outside the natural range can be detrimental to population recovery.

One of the objectives of this study was to establish an updated length-fecundity relationship for the iBoF population from this subset of FSR females, which has not been assessed for iBoF Atlantic salmon since before the population's collapse. When observed fecundity estimates from this study were compared to the estimates of a previously established length-fecundity relationship for iBoF salmon (Amiro & McNeill, 1986), the differences between these two estimates resulted in the DFO equation underestimating fecundity. A key difference between the two equations is that the relationship developed in this study is best fit by a linear model, whereas the relationship established by DFO researchers in the 1980s was an exponential relationship. An important factor varies between the equations, which may explain the discrepancies: DFO established their relationship based on the fecundities and lengths of both one-seawinter (1SW) and multi-seawinter (MSW) females, whereas the fish in the present study were only 1SW. The significant differences between the equations could be that after generations of captive rearing of iBoF salmon for conservation, the equation set by DFO is no longer applicable to present-day females. Human intervention and conservation efforts can and have contributed to considerable changes in fish populations, so it is possible that the populations have shifted to greater egg production as the result of greater feed allocation in captive programs. It is also possible that the past estimates were based on a sample that wasn't representative of the population, especially since the iBoF salmon were already in steep decline by the time the relationship was investigated (Amiro & McNeill, 1986). It is possible that the population has not actually deviated significantly from the past wild-

based length-fecundity model, and instead the differences between estimates can be attributed to our limited sample size and only having 1SW individuals. With so few females, it could be that our data points display only a portion of the full picture, and upon the inclusion of more and/or MSW females, the data points could follow something more aligned with the DFO length-fecundity relationship.

One of the primary goals of the FSR program is the production of high quality, more wild-like fish, and it appears that the traditional, somewhat excessive aquaculture style feeding does not facilitate this. The discernable differences in egg production suggest that there exists a potential for optimal and sub-optimal feeding regimes, which may affect reproductive quality. The results of this study dissuade from the continued practice of the traditional control feeding regime diet that is represented by cage SS4, and instead lean toward the experimental cage of SS5. Data regarding the control treatment (cage SS4) reinforces the idea that the traditional feeding regime may not be the best option for the rearing and conservation of iBoF salmon, as evidenced by the significantly higher mortality experienced in SS4 eggs, as well as the relatively large variability in all factors assessed in this study across females of the SS4 feeding treatment.

Though not the most limited feeding regime (Josh LeBlanc, UNB, personal communication), SS5 was the treatment which provided the most benefits, while still minimizing rearing cost. Females from SS5 had the highest fecundity ratios, meaning they produced more eggs per centimeter length than any other feeding regime's females. The eggs from SS5 also had the lowest mortality rates, with the least variability, implying the prospect for consistently high survivability of SS5 eggs. Females of SS6 produced the largest eggs relative to females of other treatment groups, which may be suggestive of

this cage's capacity to produce more 'wild-like' fish, as wild salmon tend to produce larger eggs than their captive-reared conspecifics (Jonsson et al., 1996). However, confounding factors exist which prevent concrete conclusions from being drawn for this treatment. Due to a measurement error, the fecundities of two SS6 females were not estimated within the study but instead by the DFO, and a suspected underlying health issue in one of the females. An example of how this complicates conclusions for SS6 lies in the fecundity data: SS6 females had the lowest fecundity ratios, however, it is difficult to conclude whether that is because this regime has a negative effect on egg production, or if it is that the fecundities of two females were missed during sampling and likely underestimated by the DFO equation from Amiro & McNeill (1986). Further, SS6 eggs experienced greater mortality than the other experimental groups, however the mortalities are largely accounted for by a single female's crosses – a female who had the lowest weight despite also being one of the largest females by fork length, raising suspicion of an underlying health issue that may be responsible for the unexpected egg mortality. The feeding treatment applied to SS6 was the most limiting regime, and thus is an interesting treatment to investigate further without the influence of these factors. Although the extent of food limitation in SS6 may imply it qualifies to be a conservation-focused rearing regime, it appears SS5 (the second-most limiting regime) exhibited the best egg production and survivability, while minimizing cost of rearing relative to the currently employed commercial-style food input. Fish reared under the SS5 regime have the potential to deposit large numbers of eggs into the rivers, with high rates of survival, resulting in increased juvenile salmon production, contributing to the recovery of the population.

An interesting result was the within-cage fecundity ratio variability being reflected in the range of feeding limitation between treatments, where the variability in fecundity ratios decreased with increasing food-limitation. This is something that has not been observed before in the wild or in captive environments. This variability could be positive if the characteristics (e.g., low mortality, high fecundity) it homogenizes are beneficial to the population, or it could be negative if this diversity/variability in the population is reduced in a meaningful way.

High spawning success of all the rearing groups (e.g., high rates of egg fertilization and egg survival), indicates the high quality of the iBoF salmon in the FSR program. Though the current strategies employed by FSR appear to yield high quality fish (in terms of reproduction), there remains space for improvement to the present strategy. Diet is an important factor in fish maturation, and ultimately fitness (Pérez-Atehortúa, et. al., 2024), so it is intuitive that conservation-based salmon rearing would aim to improve feeding technique. Current conservation efforts involve a necessary captive rearing component, and negative effects of captivity may be reduced by continuing to incorporate more “wild-mimicking” aspects to the rearing process. Population fitness could be impacted if an increased proportion of the population are less fit, simply due to how they are fed.

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Appendix A

Table 3. The parental information for each cross performed in the spawning experiment, identifying the cross ID, as well as the PIT numbers and cage origins of adults which contributed to the cross.

Female PIT	Female cage	Cross ID	Male cage	Male PIT
699442	SS4	1A3	SS4	699384
699442	SS4	2A2	SS4	699383
699442	SS4	3A1	SS4	699390
699382	SS4	4B3	SS4	699390
699382	SS4	5B2	SS4	699383
699382	SS4	6B1	SS4	699384
922070	SS7	7A1	SS7	922022
922070	SS7	8A2	SS8	921603
922070	SS7	9A3	SS8	921663
921666	SS8	10C1	SS8	921663
921666	SS8	11C2	SS8	921603
921666	SS8	12C3	SS7	922022
922677	SS5	13A1	SS5	921921
922677	SS5	14A2	SS5	921969
922677	SS5	15A3	SS5	921975
921933	SS5	16B1	SS5	921975
921933	SS5	17B2	SS5	921921
921933	SS5	18B3	SS5	921969
922344	SS6	19C1	SS6	922324
922344	SS6	20C2	SS6	922373
922344	SS6	21C3	SS6	922351
921894	SS6	22D1	SS6	922324
921894	SS6	23D2	SS6	922351
921894	SS6	24D3	SS6	922373
922314	SS6	25E1	SS6	922373
922314	SS6	26E2	SS6	922351
922314	SS6	27E3	SS6	922324
921612	SS8	28A1	SS5	921921
921612	SS8	29A2	SS5	921975
921612	SS8	30A3	SS5	921969
921976	SS5	31B1	SS5	921969
921976	SS5	32B2	SS5	921921
921976	SS5	33B3	SS5	921975

921657	SS8	34C1	SS6	922351
921657	SS8	35C2	SS6	922324
921657	SS8	36C3	SS6	922373
922044	SS7	37A1	SS6	922373
922044	SS7	38A2	SS6	922351
922044	SS7	39A3	SS6	922324