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Reintroduction of Atlantic salmon in Lake Ontario: the Implications of Genetic Quality on Individual Fitness

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Reintroduction of Atlantic salmon in Lake Ontario: the Implications of Genetic Quality on Individual Fitness

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Reintroduction of Atlantic salmon in Lake Ontario: the Implications of Genetic Quality on Individual Fitness

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17 August 2015

DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is the result of joint research, as follows: my first and second data chapters were co-authored with my supervisor, Dr. Trevor Pitcher, and Dr. Chris Wilson. In each case, my collaborators provided valuable feedback, helped with the project design and statistical analysis, and provided editorial input during the writing of each manuscript; however, in both cases the primary contributions have all been made by the author. Chapter 2 has been prepared as a manuscript that will be submitted to Ecology of Freshwater Fish. Chapter 3 is in the process of being made into a manuscript that will be submitted to Reproductive Biology, respectively.

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ABSTRACT

The genetic health of hatchery broodstock is important for the success of conservation management strategies. This thesis examined the genetic quality of the Atlantic salmon (*Salmo salar*) broodstock used in the Lake Ontario reintroduction effort by examining the potential for outbreeding depression in F_1 juvenile hybrids using a full-factorial breeding design. This study did not find evidence to support the occurrence of outbreeding depression in hybrids. This thesis also examined the relationship between genetic and gamete quality in a strain used in the Lake Ontario restoration effort. Although negative correlations were found between heterozygosity and sperm velocity and longevity, the correlations explained limited variance. Overall, there was little evidence to support the existence of correlations between gamete quality and heterozygosity in Atlantic salmon. This study is the first step into looking at potential implications of stocking multiple stains of Atlantic salmon and assessing the correlation between genetic and reproductive quality in their broodstock.

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CHAPTER 1: GENERAL INTRODUCTION

Darwinian fitness or reproductive fitness is defined as the number of offspring that reach sexual maturity contributed by an individual (see Frankham et al. 2002). An individual's reproductive fitness includes attributes such as their survival, capability of siring offspring, etc. (Frankham et al. 2002). When predicting one's individual fitness, there are many genetic factors that can influence the quantitative trait, such as the level of relatedness or genomic similarity between the parental generation (Edmands 2002; reviewed in Frankham et al. 2002). Many are familiar with the negative impacts of mating individuals of close relatedness (i.e. inbreeding depression) and the negative effects of recessive deleterious alleles being phenotypically expressed as a result of increased homozygosity (Charlesworth and Charlesworth 1999). The opposite end of the spectrum (i.e. outbreeding, or causing increased heterozygosity) does not always result in higher fitness, and in certain cases can be just as negative of an influence on individual fitness as inbreeding (e.g. Edmands 1999; reviewed in Frankham et al. 2011). Known as outbreeding depression, it can arise from the hybridization of two reproductively segregated populations.

Hybridization

The term hybrid is not isolated to the crossing of two different species, it is a term that can also be applied to describe the offspring of intraspecific crosses between individuals of two different populations that are distinguishable by at least one heritable characteristic (e.g. morphology, see Harrison 1990). Unlike interspecific hybridization, intraspecific hybridization often produces fertile individuals; however, the fitness of

offspring can still vary relative to that of their parental populations (Edmands et al. 1999, 2002, 2007; Frankham et al. 2002). When hybrid fitness is inferior relative to the mean of the parental populations it is known as outbreeding depression (or hybrid breakdown, Templeton 1986; Edmands 1999, 2007; Frankham et al. 2002), whereas when the fitness of hybrid offspring is superior relative to the mean fitness of both parental populations it is known as heterosis (or hybrid vigour, Frankham et al. 2002; Edmands and Timmerman 2003; Edmands 2007). Signs of outbreeding depression can include an overall decrease in fertility and survival (Harrison 1990), whereas evidence of heterosis can include an increase in biomass, fertility and growth rate in hybrid offspring compared to that of their parental populations (reviewed in Frankham et al. 2002). The mechanisms responsible for outbreeding depression and heterosis can be divided into extrinsic factors (i.e. factors under environmental influence) and intrinsic factors (factors influenced by the genotype) (Templeton 1986; Edmands and Timmerman 2003). Expressed differences in traits under selection will also vary depending on the underlying genetic architecture (number and effects of contributing genes) (Naish and Hard 2008).

Outbreeding Depression

There are many potential causes that can contribute to outbreeding depression. The potential intrinsic causes behind outbreeding depression can be attributed to both additive and nonadditive genetic effects. One example of an intrinsic effect is underdominance (i.e. heterozygotes have inferior fitness to homozygotes) (Lynch 1991). Underdominance is a nonadditive genetic effect that may encourage outbreeding depression through epistatic interactions that heterozygotes have inherited that are not seen in homozygotes (Waser and Price 1983). Another potential cause of outbreeding

depression is the break-up of co-adapted gene complexes as a result of incompatibilities between the parental populations (Burton 1987; Lynch 1991; Edmands 1999; McGinnity et al. 2003). For example, Gilk et al. (2004) found that F_2 intraspecific Pink salmon hybrids (*Oncorhynchus gorbuscha*) had lower survival rates than the pure counterparts, suggesting it was the result of disrupted co-adapted gene complexes.

Outbreeding depression may also be the result of extrinsic causes such as the loss of local adaptations (Tymchuk et al. 2007). As a result of additive genetic effects, hybrids may have an intermediate phenotype compared to their parental populations, and therefore may have lost critical local adaptations as a result, causing them to be not suited for either parental population's environment (Templeton 1986; Edmands 2007; Tymchuk et al. 2007).

Heterosis

As with outbreeding depression, the intrinsic causes behind heterosis can be attributed to both additive and nonadditive genetic effects. One of the potential nonadditive causes is referred to as overdominance (a.k.a. heterozygote advantage); the explanation that heterozygotes have superior fitness relative to homozygotes as a result of positive allelic interactions (Lynch 1991; Birchler et al. 2003; Edmands and Timmerman 2003). Another possible cause of heterosis is known as dominance; (i.e. when the recessive deleterious alleles of one parent are masked by superior dominant alleles of the other (Lynch et al. 1991; Birchler et al. 2003; Edmands and Timmerman 2003). A third potential cause of heterosis is the added genetic diversity that breeding separate populations provides. When parental populations are small, and highly inbred, the

addition of foreign genetic material may reverse inbreeding depression and result in the expression of hybrid vigour (Frankham et al. 2002; Crespel et al. 2013). An example of a population that experienced heterosis as a result of genetic diversity is the Florida panther (*Puma concolor coryi*) (Johnson et al. 2010). In the 1990's the population of *P. concolor coryi* in Florida was relegated to a few dozen mature individuals that were highly inbred (Johnson et al. 2010). Sexually mature individuals (n=8) from Texas were then transported to Florida in order to reproduce in the hopes of increasing genetic diversity and ultimately promoting hybrid vigour (Johnson et al. 2010). Over a decade after successful reproduction with the individuals from Texas, heterozygosity in the *P. concolor coryi* population increased in Florida-Texas hybrids; their survivorship was higher than that of the pure Florida crosses (Johnson et al. 2010).

In certain cases the added genetic divergence between parental populations leads to a mix of outbreeding depression and heterosis in different generations. For example, Edmands (1999) found that genetic divergence between distinct parental populations of the copepod species, *Trigriopus californicus*, was correlated with hybrid vigour in the F_1 generation and hybrid breakdown in the F_2 and backcross generations. This delayed manifestation of outbreeding depression is the result of the return of deleterious homozygotic combinations between alleles that were present in the parental populations (Templeton 1986; Edmands 2007). In this study, the reduced fitness caused by hybridization was even displayed in the F_3 generation with the variance in displayed fitness ranging from higher than parental fitness to lower than F_2 fitness (Edmands 1999).

Heterozygosity Fitness Correlations

Heterozygosity fitness correlations (HFCs) are correlations between heterozygosity at atleast one locus and measured fitness related traits (e.g. life history traits) (reviewed in Chapman et al. 2009; reviewed in Szulkin et al. 2010). As mentioned in previous paragraphs, although genetic diversity and therefore heterozygosity are generally assumed to have positive effects on fitness, in certain cases negative effects can be seen as well.

When heterozygosity was most commonly measured using allozymes, the prevailing hypothesis was that the correlation with fitness was a direct result of selection on the marker, otherwise known as the direct effect hypothesis (David 1998; Lynch and Walsh 1998; reviewed in Hansson and Westerberg 2002). Now that the majority of HFC studies are done with microsatellites, which are assumed to be neutral markers (Jarne and Lagoda 1996), there are two current prevailing hypotheses to explain observed correlations between fitness related traits and heterozygosity. The first is known as the general effects hypothesis which states; an individual's level of heterozygosity at the microsatellite level is reflective of their heterozygosity at the genome level (David 1998; Lynch and Walsh 1998; reviewed in Hansson and Westerberg 2002; Coltman and Slate 2003). The second is referred to as the local effects hypothesis which states that each marker reflects the heterozygosity of loci under selection in the chromosomal vicinity of the microsatellite due to linkage disequilibrium (David 1998; Lynch and Walsh 1998; reviewed in Hansson and Westerberg 2002). When HFCs are due to general effects, they are assumed to be positive in nature (Szulkin and David 2011). This is due to the fact that it is assumed that general effects are more common in populations with a high degree of

variance in the inbreeding coefficient, and if the heterozygosity at microsatellite markers is reflective of overall heterozygosity, the most heterozygous individuals will be the least inbred and therefore have higher fitness than their more homozygous counterparts (reviewed in Hansson and Westerberg 2002). However, HFC can be quadratic or negative in relation as well, either as a result of local effects (Olano-Marin 2011) or outbreeding depression (Neff 2004). Because life history traits such as gamete quality are particularly sensitive to selection, they make particularly good traits to measure when determining effects of inbreeding and outbreeding.

Gamete Quality Traits

As both sperm and eggs are highly specialized cells, any alterations to their characteristics can affect fertilization rates as well as offspring survival (Gage et al. 2002; Srivastava and Brown 1991). Spermatozoa have many selected characteristics that optimize their chances at fertilizing the ova (Gage et al. 1995; Gage et al. 2002). Characteristics that have been shown to influence fertilization success and therefore the quality of the sperm include velocity (Gage et al. 2004), motility, longevity and density (Gage et al. 1995; reviewed in Snook 2005). Eggs also have characteristics which can influence the survival of the embryo. The volume and mass of the egg are indicative of the energy the embryo will receive until the exogenous feeding stage (Srivastava and Brown 1991), and studies have shown that individuals that emerge from larger eggs have higher survival than those that emerge from smaller eggs (Einum and Fleming 1999, 2000). Heterozygosity has been found to correlate with both sperm (Gage et al. 2006; Fitzpatrick and Evans 2009) and egg quality (Heath et al. 2002; Garcia-Navas et al. 2009;

Wetzel et al. 2012) in various taxa. However, the studies that have examined heterozygosity in relation to sperm and egg quality are limited in number.

Atlantic salmon

Atlantic salmon (*Salmo salar*) are a post-glacial species with distribution across Europe as well as eastern Canada and the North-Eastern United States. Although the vast majority have an anadromous life cycle, migrating to the Atlantic Ocean in order to reach maturity, there are populations that have a completely freshwater life cycle (Ward 1932).

Atlantic salmon were native to Lake Ontario and were an important source of income to the economy of Upper Canada in the 18th century (Dunfield 1985). They formed what is believed to be a landlocked population (Dunfield 1985) until the late-19th century when they were extirpated as a result of anthropogenic activities that range from dam construction, overfishing and forestry (MacCrimmon 1977). Many of the dams that once impeded the upstream migration of Atlantic salmon have either been removed or fish ladders have been incorporated into others through massive restoration efforts in attempts to make conditions more favorable for Atlantic salmon (Stanfield and Jones 2003). In order to determine if Lake Ontario is currently more suitable than previous years for Atlantic salmon, since 1987, fry and fingerlings have been released annually into surrounding tributaries as a pilot study. It was determined that conditions were suitable for Atlantic salmon in Lake Ontario. Therefore, since 2005, the Ontario Ministry of Natural Resources and Forestry (MNRF) and partners have implemented a stocking effort to try and reintroduce the species back into Lake Ontario with the use of 3 separate strains (i.e. LaHave, Sebago and Lac St-Jean). The LaHave strain originates from the

LaHave River in Nova-Scotia (MNRF 2006), and has been reared in captivity since 1989 (Dimond and Smitka 2005). Additional broodstocks were developed from Sebago Lake in Maine and Lac St-Jean in Quebec, and the stocking of these two strains into Lake Ontario tributaries was initiated in 2008 (MNRF 2009), and, as of that year, both populations have been stocked simultaneously. The Sebago Lake strain was chosen as a deliberate ecological contrast to the LaHave strain (Dimond and Smitka 2005) (see Table 1.1), as the wild population is landlocked (Ward 1932) and lake conditions are considered similar to those in Lake Ontario (Toivonen 1971). As the population's freshwater life cycle provides a suitability to a different environment compared to the LaHave strain, and it has been used for previous successful introductions in the state of Maine, it is considered a good candidate for the Lake Ontario reintroduction efforts. The Lac St-Jean strain is another landlocked strain that originates from a lake in central Quebec. The population is used in other stocking efforts and based on geographic proximity, it is speculated that the population is the most genetically similar to the historical population in Lake Ontario. Based on these characteristics, the population is considered another good candidate for Lake Ontario. However, as the survival in captive settings is not as good in the Lac St-Jean strain as it is in the Sebago and LaHave strains, it is not stocked in as high a quantity (MNRF 2009).

Overview of the thesis

The objectives of my thesis were to evaluate fitness related traits in Atlantic salmon to determine the implications of genetic quality (i.e. the potential beneficial or detrimental effects of the genome on characteristics of phenotypic expression) on fitness related traits in the Atlantic salmon broodstock. The objective of Chapter 2 was to

evaluate fitness related traits in juvenile Atlantic salmon crosses between two of the three mentioned allopatric strains (i.e. Sebago and LaHave) in order to determine whether outbreeding depression or heterosis was occurring in the hybrid crosses using a 2 x 2 full factorial breeding design. As fitness of individuals in the hatchery where the stocks are reared is equally as important as those in the wild, Chapter 3 evaluates the occurrence of heterozygosity fitness correlations in the gamete quality of the LaHave strain (the most stocked strain). In order to do this, sperm and egg quality were measured and compared to multilocus heterozygosity using 19 microsatellite markers.

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Table 1.1: Approved populations of Atlantic salmon for Lake Ontario. The table represents a comparison between the known conditions of the current 3 approved candidate populations and the speculated characteristics of the Lake Ontario population

	Ontario (extirpated)	LaHave (Nova-Scotia)	Sebago (Maine)
Freshwater life cycle	Yes	No	Yes
Anadromous	Yes	Yes	No
Oligotrophic	Yes	No	Yes
Genetic Similarity	Yes	No	?

CHAPTER 2: EFFECTS OF INTRASPECIFIC HYBRIDIZATION BETWEEN TWO STRAINS OF ATLANTIC SALMON (*SALMO SALAR*) ON JUVENILE SURVIVAL AND FITNESS-RELATED TRAITS: IMPLICATIONS FOR RESTORATION ECOLOGY

SYNOPSIS

Hybridization between species or divergent conspecific populations may result in hybrid offspring exhibiting either superior (heterosis) or inferior (outbreeding depression) fitness relative to their parental populations. As both heterosis and outbreeding depression have previously been demonstrated in salmonids, consequences of interbreeding between divergent populations may therefore be relevant to salmonid conservation programs and restoration efforts. Atlantic salmon (*Salmo salar*) were once native to Lake Ontario but were extirpated by the late-19th century as a result of anthropogenic causes. Multiple allopatric populations of Atlantic salmon are currently being stocked in an effort to re-establish a self-sustaining population in Lake Ontario. This study evaluated whether interbreeding between individuals from Sebago Lake (Maine) and the LaHave River (Nova-Scotia) will result in the expression of heterosis or outbreeding depression in juveniles. This was accomplished by generating full-factorial 2x2 mating crosses between the two strains and comparing multiple traits (growth and survival) associated with individual fitness between the four cross types (pure within-population breeding and reciprocal hybrids). Hybrid juveniles did not display any signs of outbreeding depression or heterosis. Despite these results, further studies on reproductive fitness and comparative fitness of backcross and second-generation hybrids are recommended to assess potential consequences for this and similar restoration efforts.

Introduction

Although hybridization is often overlooked in the conservation and restoration management of endangered and extirpated populations, it is relevant, as it can be either a potent tool or significant challenge (Edmands 2007, Frankham et al. 2011). Unlike interspecific hybridization, intraspecific hybridization (hybridization within species) often produces viable and fertile individuals; however, the fitness of offspring can be higher (heterosis) or lower (outbreeding depression) than that of their parental populations. Evidence of heterosis can include an increase in biomass, fertility and growth rate in hybrid offspring compared to that of their parental populations (reviewed in Frankham et al. 2002), whereas signs of outbreeding depression can include an overall decrease in fertility and survival (Harrison 1990). As heterosis or neutral outcomes are not considered detrimental to conservation efforts, the focus of selecting candidate populations should be on the prevention of outbreeding depression. Outbreeding depression is considered a likely outcome when breeding populations with fixed chromosomal differences, if they have lived in different environments for over 20 generations and if they have not had any gene flow for over 500 years (Frankham et al. 2011). Therefore, in order to avoid the onset of outbreeding depression when more than one population is being used in reintroduction efforts, it is best to choose parental populations that have low genetic divergence from one another as well as similar local adaptations (Edmands 1999, 2002).

The mechanisms responsible for outbreeding depression can be divided into extrinsic factors (i.e. factors under environmental influence) and intrinsic factors (factors influenced by the genotype) (Templeton 1986; Edmands and Timmerman 2003). Expressed differences in traits under selection will also vary depending on their

underlying genetic architecture (number and effects of contributing genes) as well as the potential correlation with other traits (Naish and Hard 2008). An example of allopatric crosses which have resulted in outbreeding is, Gilk et al. (2004) which crossed allopatric populations of Pink salmon (*Oncorhynchus gorbuscha*) which resulted in lower return rates for the F_1 generation and lower survival for the F_2 generation. Other studies have similarly cautioned against interbreeding between wild and hatchery fish populations with the same population of origin as they may have divergent genetic traits. For example, Araki et al. (2007) saw a 37.5% decrease per generation in the reproductive success of captively reared steelhead trout (*O. mykiss*) due to underlying genetics. As the consequences of lower survival and overall fitness are a possible outcome of outbreeding depression, it is therefore a concern for any conservation or reintroduction effort that utilizes multiple populations.

Atlantic salmon (*Salmo salar*) were once native to Lake Ontario, but were extirpated in the late 19th century as a result of anthropogenic activities such as dam construction, land clearing, and overfishing (MacCrimmon 1977). Land restoration efforts as well as restoration efforts in many tributaries have made conditions more favorable for Atlantic salmon (Ontario Ministry of Natural Resources and Forestry (MNRF) 2003; Stanfield and Jones 2003). Restoration began in earnest in 2005, using an anadromous strain that originates from the LaHave River in Nova-Scotia (MNRF 2005). Additional broodstock was developed from Sebago Lake in Maine, and stocking into Lake Ontario tributaries was initiated in 2008 (MNRF 2008), and, as of this point, both populations have been stocked simultaneously. The Sebago Lake strain was chosen as a deliberate ecological contrast to the LaHave strain (Dimond and Smitka 2005), as the wild

population is landlocked (Ward 1932) and lake conditions are considered similar to those in Lake Ontario (Toivonen 1971). As the population's freshwater life cycle provides a suitability to a different environment compared to the LaHave strain, and it has been used for previous successful introductions in the state of Maine, it is considered a good candidate for the Lake Ontario reintroduction efforts. However, as the strains have been separated for over 500 years and living in different environmental conditions for over 20 generations, the manifestation of outbreeding depression is a possible outcome of breeding these two populations (see Frankham et al. 2011).

This study evaluated the implications of interbreeding (intraspecific hybridization) between these two Atlantic salmon strains for the Lake Ontario reintroduction effort, testing specifically for juvenile heterosis or outbreeding depression in a controlled hatchery environment that is consistent with current hatchery practices. As the manifestation of heterosis and outbreeding depression can also vary with life stages (Fraser et al. 2010; Granier et al. 2011; Crespel et al. 2013), the importance of examining the fitness of the juvenile stage is critical as there is high mortality in this stage; with up to 90% of juveniles dying by the smolt migration phase (Thorstad et al. 2011). The potential for outbreeding depression or heterosis was assessed by measuring survival and multiple fitness-related traits (length, mass, Fulton's condition, and growth rate) over time at ecologically relevant stages by crossing individuals from the Sebago and LaHave populations in order to create family blocks composed of both pure strains and their reciprocal hybrid siblings.

Materials and Methods

Full-factorial breeding design

To assess potential fitness effects resulting from intraspecific hybridization, reproductive adults from both the Sebago and LaHave strains maintained at the MNRF Codrington Fisheries Research Facility (44.18.05°N, 78.29.40°W) were selected haphazardly in order to create 20 distinct half-sibling family blocks . These 20 family blocks were created using a 2x2 breeding design (blocked factorial breeding design); using one male and female from each strain to produce half-sibling family blocks consisting of a pure Sebago cross (S/S), a pure LaHave cross (L/L) and their reciprocal hybrids (LaHave dam/Sebago sire (L/S) and Sebago dam/LaHave sire (S/L)). Each adult was used in only one 2x2 cross, resulting in 20 independent family blocks. The full factorial breeding design allows for the separate evaluation of intrinsic genetic factors and maternal effects (both additive and environmental) and paternal effects (Pitcher and Neff 2006, 2007; Neff et al. 2011). The eggs for the blocks were fertilized on two separate dates (November 22, 2012 (n=8 blocks) and December 4, 2012 (n=12 blocks)). After fertilization, the eggs from each separate cross were randomly allocated into the cells of two separate incubation stacks, each containing five trays with 16 cells per tray.

Fertilized Egg Survival

Fertilization success assays for all family blocks took place between December 21, 2012 and January 10, 2013, and survival of the fertilized eggs was monitored three times a week from January 14, 2013 until the latest date of hatching (March 4, 2013). If the eggs changed from transparent or translucent to opaque, they were deemed dead. To

examine if the dead eggs had been fertilized pre-mortality, they were submersed in acetic acid (5%) (see Hoysak and Liley 2001); if the eggs turned white after exposure to the acetic acid, they were considered fertilized; eggs that remained clear were considered unfertilized. Only eggs that had been fertilized were considered in the survival comparisons.

Rearing

Once the alevin had absorbed their yolk sacs and manual feeding began, up to 100 individuals (mean +/- s.e.: 97 ± 1.2) from each full sibling cross were transported from the incubation trays and randomly allocated into separate 40 L family rearing tanks at the University of Windsor Great Lakes Fish and Research Centre in LaSalle, Ontario. The facility is equipped with a scaled down recirculation system to ensure that the water quality in all the tanks is similar, not unlike those found at the provincial Atlantic salmon hatchery Normandale. Water quality (dissolved oxygen, pH, and temperature) was examined daily to ensure families were being held at optimal water conditions. On April 29, 2013, each tank was manually thinned down haphazardly to 50 individuals by removing individuals present throughout the water tank, with the exception of two tanks which began with 16 and 13 fish due to low egg survival. During the first half of the rearing stage (March 2013 to August 2013), the tanks were thinned on three occasions in order to accommodate growth (April 29, June 21 and August 2013), and to keep densities at relatively consistent levels in order to limit density effects on the early growth of the fish during this critical growth period.

Length, Mass & Fulton's Condition Factor

Length, mass and Fulton's condition factor (K) were measured a total of five times during juvenile development between April 2013 to February 2014; the measurements covered the significant life stages from button-up to smolting (fry, parr and smolt). The first measurement took place during the "button-up" stage (shortly after the yolk sac was absorbed), the second measurement took place during the fry stage, 20 fish per tank were haphazardly selected in order to be weighed and measured. In order to prevent bias, individuals were netted from all areas of the water column. The third, fourth and fifth measurements took place when they were parr, during later period of parr stage and during their smolting period (after they had lost their parr marks). Ten fish per cross type (i.e. pure LaHave cross, pure Sebago cross, Sebago dam x LaHave sire cross or LaHave dam x Sebago sire cross) within each family block were haphazardly selected to be weighed and measured. The first two measurements took place at two week intervals in order to account for the difference in fertilization dates. The fish were anaesthetized using MS-222, each individual's mass (+/- 0.001 g) was then taken using an electronic scale (Denver Instrument TP 323) and digital images (with a size standard) taken of each fish were analysed using Image J (http://imagej.nih.gov/ij/) in order to assess fork length. Fulton's condition factor was calculated as $K= (W/L^3)*10\ 000$ (Ricker 1975).

Survival

The survival of the juveniles was analyzed during four time periods: egg stage (January 2013 - February 2013), fry stage (April 2013 - June 2013), early parr stage (October 2013 - December 2013), and late parr stage (December 2013 - February 2014)

by comparing the change in the number of offspring in each tank that had occurred over that period of time.

Statistical Analyses

Survival was examined at four separate time points using a binomial generalized linear mixed effects model with LaPlace approximation using version 1.1-7 of the lme4 package (Bates et al. 2014) in R 3.1.1 (R Core Team 2014). Cross type was evaluated as a fixed effect in the model, whereas dam ID, sire ID and dam ID x sire ID interaction were evaluated as random factors. Due to biological significance, density, dam ID and sire ID were included in all final models, whereas position effects (i.e. stack, tray, cell) and dam ID x sire ID were included in the final model if deemed statistically significant. A likelihood ratio test fitted with maximum likelihood (ML) was used in order to generate p-values between a full model and a reduced model without the variable in question.

Analyses of length, mass and Fulton's condition factor data at the five time points were also completed using version 1.1-7 of the lme4 package in R to generate linear mixed effects models. Cross type and density were entered in the model as fixed effects, while dam ID, sire ID and dam ID x sire ID interaction were entered in the model as random factors. Due to biological significance, despite the results from the AIC, density, dam and sire effects were always included in the full model. The significance of cross type was assessed using an F-test with a Kenward-Roger degree of freedom estimation in the package pbkrtest (Halekoh and Højsgaard 2014) whereas, the p-values for the random factors were generated using a likelihood ratio test fitted with ML between a full model and a reduced model without the variable in question.

Of the 20 2x2 mating crosses that were established, 6 factorial crosses were discarded due to accidental mixing, missing measurements of certain tanks at one or more time points and equipment failure which lead to the mortality of one tank in a family block during the later portion of the rearing period. In order to better control for factors such as parental ID and uneven sampling of treatment groups that could affect the results, any such experimental error with one (or more) of the tanks in a full factorial cross, led to the discard of all tanks in that family, thereby excluding that family from any further statistical analysis.

Results

Summary statistics of survival and the fitness-related traits for the remaining 14 family blocks are presented in Table 2.1. Cross type did not affect the survival of eggs ($p=0.96$), fry ($p=0.47$), early parr ($p=0.25$) or late parr ($p=0.90$) (Table 2.1). Dam ID effects had a large influence over the survival of eyed eggs and early parr, but were not significant for survival of other life stages (Table 2.1). Dam ID x sire ID interaction only had a significant influence over the survival of the fry stage (Table 2.1). Sire ID effects had no significant influence over the survival of the individuals during any of the life stages (Table 2.1).

Over the five intervals, cross type had no significant effect on any of the characteristics associated with fitness (Table 2.2). Dam ID effects were significant for length, mass and condition at early life stages, but decreased in importance at the parr and smolt life stages (Table 2.2). Sire ID effects also explained part of the variance of mass and length during the early measurements and condition during the later measurements

(Table 2.2). Interaction effects (dam ID x sire ID) contributed to the variance of the early condition measurements but little to the rest of the fitness related traits. Tank density had significant effects on length and mass at the majority of the measurement time points (Table 2.2).

Discussion

The results of this study show no significant influence of hybridization on survival or any of the fitness related traits measured in several stages of ontogeny of juvenile Atlantic salmon. Understanding the potential effect of intraspecific hybridization of Atlantic salmon, particularly in the juvenile life stages, is critical to the successful reintroduction of the species back to Lake Ontario if the program intends to continue using more than one population simultaneously that have been separated for over a thousand generations, as it has the potential to cause complications for hybrid offspring.

Outbreeding depression has been identified as a significant concern for interbreeding between divergent populations (Edmands 2007, Frankham et al. 2011), and has previously been documented in hatchery-reared salmonids (e.g. Araki et al. 2007, Granier et al. 2011), the severity of which has varied across studies. However, the detection of outbreeding depression or heterosis can also be the result of the environmental surroundings. For example, using inbred crosses, Houde et al. (2011) detected heterosis or outbreeding depression in a limited number of their families of Atlantic salmon depending on rearing environment (due to the loss of local adaptations) (Houde et al. 2011). A subsequent study found that the strength of both inbreeding and outbreeding depression varied annually along with environmental quality (Rollinson et al.

2014). In years with poor environmental quality, maternal effects accounted for greater variance in juvenile fitness than years with closer to optimum environmental quality (Rollinson et al. 2014). Therefore, there are various reasons for why the crossing of allopatric populations did not result in the expression of outbreeding depression in the setting of this study.

The most likely reason for our lack of observation of outbreeding depression had to do with the fact that we were looking at the F_1 generation. When outbreeding depression is observed in the F_1 generation it is typically the result of the loss of local adaptations as the result of an intermediate phenotype (Lynch 1991; Edmands 2007). Intrinsic incompatibilities between parental populations (e.g. the break-up of co-adapted gene complexes) (Burton 1987; McGinnity et al. 2003; McClelland and Naish 2007; Tymchuk et al. 2007) typically result in outbreeding depression in the F_2 generation or later when the parental genomes are subject to recombination (Dobzhansky 1948; Lynch 1991; Edmands 1999; Birchler et al. 2003; Edmands and Timmerman 2003; McGinnity et al. 2003; McClelland et al. 2005; Tymchuk et al. 2007). Outbreeding depression as a result of the breakup of co-adapted gene complexes has been demonstrated in intraspecific salmonid hybrids, for example, Gilk et al. (2004) found reduced survival in F_2 intraspecific *Oncorhynchus gorbuscha* hybrids relative to their pure counterparts, suggesting it was the result of disrupted co-adapted gene complexes. Another example is with Atlantic salmon in McGinnity et al. (2003), F_2 hybrid crosses saw significantly higher egg mortality than backcrosses as well as other crosses by the same sire, it was therefore most likely outbreeding depression. If co-adapted gene complexes are disrupted

in the Sebago x LaHave hybrids, the effects of outbreeding depression would more likely be observed in the F_2 generation if it existed.

It is also possible that the environmental setting of the experiment made for a lack of outbreeding depression. Benign hatchery environments mostly test intrinsic factors (Tymchuk et al. 2007), that is the effect caused by the genotype. However, the phenotype is the product of an interaction between genotype and the environment, it is believed that extrinsic factors such as the loss of local adaptations have a stronger influence over outbreeding depression than do intrinsic factors such as the disruption of co-adapted gene complexes for species with many unique and highly local populations (Allendorf et al. 2001; Edmands and Timmerman 2003; reviewed in Garcia de Leaniz et al. 2007; Tymchuk et al. 2007; Vandersteen et al. 2012). In the F_1 generation, outbreeding depression is also more likely to be due to extrinsic factors than intrinsic ones (Lynch 1991, Edmands 2007). As the environmental surroundings will affect the capability for detecting both outbreeding depression and heterosis (Burton 1987; Edmands 2007; Tymchuk et al. 2007; Vandersteen et al. 2012; Crespel et al. 2013), it is therefore possible that any outbreeding depression resulting from the loss of local adaptations was negated by a relaxed environmental setting (Tymchuk et al. 2007). For example, in Tymchuk et al. (2007), F_3 hybrid rainbow trout displayed outbreeding depression related to growth and survival in certain environmental surroundings; as a result, the cause of the outbreeding depression is thought to be primarily the result of the loss of local adaptations that was simply expedited by intrinsic factors. In Vandersteen et al. (2012), it was found that survival of rainbow trout fry differed not only in the geographic area but also seasonally, as the survival of different genotypes varied between summer and winter.

Another study on Brook trout (*Salvelinus fontinalis*) by Crespel et al. (2013) found environmental interactions affected the presence of heterosis in certain hybrid crosses but not others, also suggesting that environmental influence has a varying effect on different genotypes. Therefore, in the case of this study, it is possible that if the juveniles had lost a local adaptation of importance, the outbreeding depression might have only been capable of being detected in more challenging natural settings. If one of the populations being used in the restoration effort is indigenous to the environment, the addition of a second phenotype may cause the offspring to be less suited for the environment compared to the local population, and may hinder the reintroduction effort rather than assist it. In such cases, it would be worth choosing populations with similar local adaptations in order to avoid an intermediate phenotype.

As this study examined the early life stage of Atlantic salmon, another potential reason for the lack of detection of outbreeding depression or heterosis are maternal effects. These environmental or genetic effects can influence the offspring's phenotype on a per subject basis in early life stages by overshadowing the influence of the offspring's genotype (reviewed by Wolf and Wade 2009). Therefore, the phenotypic expression of outbreeding depression can be outweighed by maternal effects during early life stages (Edmands 2007). Previous studies, have demonstrated hybrid fitness has been shown to vary as a result of maternal effects (McGinnity et al. 2003; Debes et al. 2013). McGinnity et al. (2003) found maternal effects affected the survival of egg stage as well as eyed egg stage in the early juvenile stage of farmed x wild Atlantic salmon hybrids, and Debes et al. (2013) found that the actual genotype of the hybrid wild-farm offspring had little effect on survival, whereas maternal effects accounted for almost all of the variance in

survival between hybrid types. As our study found significant dam ID effects (i.e. the effect of the inherited maternal genotype as well as maternal effects) on the egg survival and early size measurements, it is possible that maternal effects are masking interaction effects that may have otherwise lead to outbreeding depression later in life. For example, Heath et al. (1999) found that juvenile growth in Chinook salmon was predominantly the result of maternal effects, however, as the individuals matured the effect of any maternal effect on the offspring's phenotype decreased. Another study by Houde et al. (2015) on the Sebago and LaHave populations of Atlantic salmon supported such findings, with juvenile survival and fitness being primarily influenced by maternal environmental effects during the egg stage and that phenotypic influence was then primary controlled for by nonadditive genetic effects in the fry stage. It is important to mention the egg survival in my study (~50%) was much lower than those typically seen in other studies involving Atlantic salmon (84%) (e.g. Taranger and Hansen 1993) or other salmonids (~90%) (e.g. Cho et al. 2002). It is possible that the low egg survival seen in this study was the result of environmental settings such as the water temperature. For example, Taranger et al. (1993) found that Atlantic salmon eggs that were in warm water conditions (13-14°C) had a 15.5% lower survival than those placed in cold water (5-7°C) and a 7.9% lower survival than the control (8-10°C). As our water temperature typically ranged from 9-12°C, it is possible that the warmer water temperatures were not favorable for the Atlantic salmon eggs. However, as the egg survival in our study were consistent with the egg survival in other studies using these broodstocks of Atlantic salmon (Houde et al. 2015) and that they were all exposed to the same water conditions throughout the experiement, it is assumed that the low egg survival did not influence the outcome of the results. Therefore, it would

demonstrate the importance of testing lifelong fitness of the hybrids to insure that heterosis or outbreeding depression is not being masked by maternal effects in the study.

Another possibility is that the neutral genetic distance between the parental populations (i.e. LaHave and Sebago) was not great enough to invoke any phenotypic expression of outbreeding depression or heterosis, as the occurrence and severity of both has previously been shown to be positively correlation with parental divergence (Edmands and Timmerman 2003; Edmands 2007; McClelland and Naish 2007; Fraser et al. 2010). Although the Sebago and LaHave populations have been isolated from one and other for approximately a thousand generations, the neutral genetic distance between the two populations is relatively low (as measured by F_{ST}=0.038, He et al. 2015). It is therefore possible that the rate at which these populations of Atlantic salmon are diverging is not rapid enough to observe any form of heterosis or outbreeding depression. Makinen et al. (2014) detected low genetic divergence between domesticated populations of Atlantic salmon (F_{ST}<0.03). Although factors such as the fact that Atlantic salmon have been under domestication for over 10 generations (Hutchings and Fraser 2008), as well as the polygenic nature of most selected traits may have lead to the lack in findings of divergent artificial selection (Makinen et al. 2014) , other studies have also suggested that selection in a hatchery environment may cause convergent selection on the same genes between populations of salmonids with 5 to 7 generations of selection and 4 generations respectively (Roberge et al. 2006; Sauvage et al. 2010). As both of the populations in this study have been domesticated for several generations, it is possible that since being in hatchery settings, their broodstocks have been under convergent natural selection for domesticated environments.

In summary, the simultaneous use of both the Sebago and LaHave populations does not appear to result in the expression of outbreeding depression or heterosis during the juvenile life stages of the F_1 generation. Future research should investigate adult fecundity and reproductive fitness (gamete quality and survival) of F_1 hybrids, as well as potential evidence of outbreeding depression in $F_{2,}$ backcrosses and subsequent generations. As with any reintroduction which uses multiple populations simultaneously, hybridization when it results in outbreeding depression remains a potential hindrance for conservation efforts. Therefore, the use of multiple allopatric populations should be done with caution after research has gone into studying the full effects hybridization may have.

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Table 2.1: Mean percentage of survival of F_1 cross types of Atlantic salmon (*Salmo salar*) during four life stages with the significance (p) of cross type ("Cross") as fixed effect as well as dam, sire and their interaction as random effects to the variance of the fitness characteristic (see methods for details).

	Pure Crosses		Hybrids		Significance			
Survival (%)	LL	SS	LS	SL	Dam	Sire	Interaction	Cross
Egg Stage	42.26	48.55	45.32	49.28	**<0.001**	>0.99	>0.99	0.96
Fry Stage	71.23	75.14	79.36	76.43	0.620	0.20	**<0.001**	0.47
Early Parr Stage	94.98	93.44	95.89	98.57	**0.02**	>0.99	>0.99	0.25
Late Parr Stage	92.44	94.38	90.62	90.42	>0.99	0.31	>0.99	0.90

Significant fixed and random effects ($p < 0.05$) are indicated in bold. Original egg number ($p=0.44$) was used in the place of density during the egg stage. Stack, tray and cell effects were also kept in the model as they were significant ($p<0.001$). The cells were kept as separate entities when calculating the mean survival %**.**

Table 2.2 Means (± standard error) of F_1 cross types of Atlantic salmon (*Salmo salar*) with the significance (p) of cross type ("Cross") as fixed effect as well as dam, sire and their interaction as random effects to the phenotypic variance of the fitness characteristic (see methods for details).

Characteristic (units)	Pure Crosses		Hybrids		Significance (p)				
	LL	SS		LS	SL		Dam	Sire	Interaction	Cross
Fork length (cm)										
April 2013(button-up)	35.5 ± 0.230	35.9 ± 0.22	35.5 ± 0.23	35.8 ± 0.22		**<0.001**	**<0.001**	>0.99	0.99	
August 2013(fry)	61.5 ± 0.473	62.9 ± 0.42	60.1 ± 0.41	60.6 ± 0.40		**<0.001**	**<0.001**	>0.99	0.41	
October 2013(parr)	82.7 ± 1.13	84.1 ± 1.22	79.7 ± 0.98	79.5 ± 0.97		>0.99	>0.99	>0.99	0.57	
December 2013(parr)	90.6 ± 1.39	92.8 ± 1.57	88.3 ± 1.29	88.2 ± 1.14		>0.99	>0.99	>0.99	0.84	
February 2014(smolt)	104.7 ± 1.47	107.3 ± 1.80	104.0 ± 1.42	102.4 ± 1.34		>0.99	>0.99	>0.99	0.67	
Mass (g)										
April 2013(button-up)	0.46 ± 0.010	0.48 ± 0.010	0.47 ± 0.010	0.48 ± 0.00978		**<0.001**	**0.01**	>0.99	0.91	
August 2013(fry)	2.81 ± 0.065	2.96 ± 0.058	2.55 ± 0.048	2.63 ± 0.0502		**0.002**	**0.004**	>0.99	0.09	
October 2013(parr)	6.02 ± 0.283	6.55 ± 0.298	5.39 ± 0.22	5.45 ± 0.218		>0.99	>0.99	>0.99	0.59	
December 2013(parr)	9.14 ± 0.484	9.81 ± 0.523	8.40 ± 0.40	8.10 ± 0.362		>0.99	>0.99	>0.99	0.93	
February 2014(smolt)	13.50 ± 0.596	14.64 ± 0.745	13.06 ± 0.53	12.19 ± 0.507		>0.99	>0.99	>0.99	0.66	

Condition (10 000 × g/mm^3)								
April 2013(button-up)	0.099 ± 0.000627	0.101 ± 0.00053	0.100 ± 0.00065	0.100 ± 0.00064	**0.02**	>0.99	**<0.001**	0.95
August 2013(fry)	0.117 ± 0.000942	0.117 ± 0.00074	0.115 ± 0.00081	0.116 ± 0.00101	0.21	0.49	**<0.001**	0.86
October 2013(parr)	0.099 ± 0.00102	0.104 ± 0.00095	0.101 ± 0.00083	0.103 ± 0.00088	**<0.001**	**<0.001**	>0.99	0.74
December 2013(parr)	0.112 ± 0.000628	0.113 ± 0.00060	0.113 ± 0.00050	0.111 ± 0.00062	0.31	**0.006**	0.19	0.30
February 2014(smolt)	0.111 ± 0.000702	0.110 ± 0.00073	0.110 ± 0.00070	0.107 ± 0.00055	**0.02**	0.10	0.22	0.23

Significant fixed and random effects ($p < 0.05$) are indicated in bold. Density was $p<0.05$ for all measurements with the exception of fork length April 2013 ($p=0.19$) and condition (August $p=0.55$, December 0.38 and February 0.06)

CHAPTER 3: HETEROZYGOSITY AND GAMETE QUALITY TRAITS IN HATCHERY-REARED ATLANTIC SALMON (*SALMO SALAR*)

SYNOPSIS

This chapter examined the relationship between gamete quality traits and multi-locus heterozygosity in male and female hatchery-reared Atlantic salmon (*Salmo salar*). Multi-locus heterozygosity (MLH; based on 19 polymorphic microsatellite markers) was correlated with sperm (including velocity, linearity, density, longevity and motility) and egg (including egg diameter, wet egg mass, dry egg mass, fecundity) quality metrics as well as Fulton's condition factor. For females, there was no significant relationship between MLH and any of the gamete quality metrics. For males, although significant negative correlations were found between MLH and sperm velocity traits and longevity traits, the correlation explained limited variance. Also, there was no significant correlation between sperm linearity, motility nor density and MLH. Neither sex displayed any significant correlation between Fulton's condition factor and MLH. Although negative correlations between certain sperm quality traits were found, overall there was little evidence to support the existence of heterozygosity fitness correlations between gamete quality traits potentially due to a lack of variability in the inbreeding coefficient or indicative of a lack of heterozygosity fitness correlations with gamete quality traits in Atlantic salmon.

Introduction

Heterozygosity fitness correlations (HFC) are statistical correlations between an individual measure of heterozygosity (e.g. multi-locus heterozygosity (MLH)) and traits that are related to fitness (reviewed in Chapman et al. 2009; Szulkin et al. 2010), and they have become a widely utilized tool in evolutionary ecology (Mitton 1993; Coltman and Slate 2003; David 1998; reviwed in Chapman et al. 2009; reviewed in Szulkin et al. 2010). Most HFC studies are currently carried out with the use of polymorphic microsatellites, which are predominantly considered to be neutral markers (Jarne and Lagoda 1996). The occurrence of HFCs in neutral markers is explained by what are known as local and general effects. Local effects occur when a neutral marker reflects overdominance of loci under selection within their chromosomal vicinity as a result of linkage disequilibrium (LD) (David 1998; Lynch and Walsh 1998, reviewed in Hansson and Westerberg 2002). General effects occur as the result of the heterozygosity at the markers being reflective of the individual's overall heterozygosity, and as a result of being less inbred, heterozygotes will have a higher fitness than homozygotes as a result of being less inbred) (David 1998; Lynch and Walsh 1998; reviewed in Hansson and Westerberg 2002; Coltman and Slate 2003).

Based on the general effects hypothesis, theory suggests that if HFCs are the result of heterozygotes being less inbred than homozygotes, HFCs would be more prevalent in populations that have high variance in the values of inbreeding coefficients (David 1998; reviewed in Coltman and Slate 2003) due to a higher amount of additive genetic variance within such populations. However, an empirical review by Chapman et al. (2009) found no evidence of HFCs being more common in populations with high variance in

inbreeding coefficients, suggesting that certain studies were finding local effects rather than general ones or the presence of publication bias towards significant results in all populations. Traits that are more closely associated with fitness (e.g. life history traits), or those that are affected by multiple loci also usually show stronger associations in HFC studies compared to traits that are not strongly associated with fitness (Coltman and Slate 2003; reviewed Chapman et al. 2009; reviewed in Szulkin et al. 2010). As spermatogenesis and oogenesis are extremely sensitive to selection pressures, it is expected that gamete quality will be one of the first traits to reveal evidence of correlations with heterozygosity (Gage et al. 2006; Fitzpatrick et al. 2009). Although many studies have examined the relationship between heterozygosity and life history traits (reviewed in Chapman et al. 2009; reviewed in Szulkin et al. 2010), few studies have examined the relationship between heterozygosity and gamete quality (see Table 3.1 for a summary of studies).

There are several characteristics adapted by spermatozoa to increase chances of fertilization and are therefore representative of sperm quality such as motility, longevity (Gage et al. 1995), velocity (Gage et al. 2004) and density (reviewed in Snook 2005). Heterozygosity has been found to correlate positively with sperm quality within (Gage et al. 2006) and among (Fitzpatrick and Evans 2009) species. For example, Gage et al. (2006) found a negative correlation between mean heterozygosity and the production of abnormal sperm within and across wild rabbit populations (*Oryctolagus cuniculus*) using 29 microsatellite loci. The strongest correlations were found within the most fragmented populations, suggesting a possible relationship to inbreeding (Gage et al. 2006). Other studies have also found heterozygosity to be negatively correlated with abnormal sperm

percentage and positively associated with motile sperm in endangered mammals (Fitzpatrick and Evans 2009).

Just as with sperm, egg quality is also important for offspring fitness. Egg quality is important for survival as it is the only source of energy the embryo receives until it reaches the exogenous feeding stage (Pickova et al. 1997). Therefore, the quality such as the volume, mass and fecundity are important to an individual's overall fitness as they reflect the energy content the individual is allocating to offspring (Srivastava and Brown 1991). Heterozygosity has been found to correlate with egg quality and quantity in multiple studies across a variety of taxa (see Table 3.1 for summary of studies) (e.g. Heath et al. 2002; Garcia-Navas et al. 2009; Wetzel et al. 2012). For example, heterozygosity was positively correlated with clutch size in house sparrows (*Passer domesticus*) (Wetzel et al. 2012). Another study found correlation between heterozygosity and clutch size as well as egg pigmentation pattern in blue-tits (*Cyanistes caeruleus*) (Garcia-Navas et al. 2009).

Although gamete quality characteristics are well documented in Atlantic salmon (Gage et al. 1995; 1998; 2004; Lush et al. 2014), there is a lack of studies that have evaluated their correlation to heterozygosity. Atlantic salmon (*Salmo salar*) are an externally fertilizing fish species with a distribution across eastern North America (Holm et al. 2009). There are many characteristics adapted by gametes in Atlantic salmon in order to optimize their chances of fertilization success as well as hatching success. For example, sperm velocity has been shown to be an important characteristic for fertilization success (Gage et al. 1995, 2004). It is also well documented that individuals emerging from larger eggs have higher survival than individuals emerging from smaller eggs

(Einum and Fleming 2000), suggesting egg diameter and egg mass as important characteristics to embryo health and survival. As many salmonid populations, including Atlantic salmon, are currently being supported by hatcheries for restoration and reintroduction, the gamete quality of these hatchery reared fish and their offspring is crucial for future health of these populations. However, it has been demonstrated that the lack of selection that hatcheries pose cause divergence in the evolution of gamete quality selection (e.g. Heath et al. 2002). In salmonids, Coho salmon (*Oncorhynchus kisutch*) reared in hatchery settings displayed lower than average reproductive success in the wild than their wild counterparts (Thériault et al. 2011). Kekäläinen et al. (2013) found that sperm motility in Arctic charr was lower in hatchery populations than their wild counterparts As there is currently a reintroduction effort taking place for Atlantic salmon in Lake Ontario, the gamete quality of the broodstock is an important characteristic for the success of the initiative.

The objective of this chapter is to examine and describe the existence of correlations between gamete quality and MLH through either local or general effects using a population of captively reared Atlantic salmon as a case study. The LaHave population is the most common strain currently used in Atlantic salmon stocking efforts in Lake Ontario and originates from the LaHave River in Nova-Scotia (Stanfield and Jones 2003), and has been breed in captivity for several generations using rotational line crosses (RLC) a breeding technique that exclusively breeds different year classes in order to avoid inbreeding. As this population is the main broodstock for reintroduction efforts around the province (MNRF 2009), it would be of use to know if genetic variance can explain chances at reproductive success. If HFCs do not exist in the LaHave broodstock

of Altantic salmon, I would expect no correlation between the gamete quality traits and heterozygosity. However, if HFCs do exist in the gamete quality of the LaHave broodstock, than I would expect a positive correlation between the gamete quality traits and heterozygosity.

Methods

Broodstock

All Atlantic salmon used in this study were from the LaHave broodstock maintained at the Ontario Ministry of Natural Resources and Forestry (MNRF) Harwood Fish Culture Station (44° 8'13.31"N 78°10'5.59"W). The broodstock was created using a breeding technique known as rotational line crosses (RLC) in which unrelated broodstock are crossed in order to create 3 new lines to be used in rotation (see Figure 3.1; for further information on RLC see Kincaid 1977). The female broodstock were composed of 3 year classes generated in 2007, 2008 and 2009 from a year class at MNRF Normandale Fish Culture Station whose parents had been created from wild gametes. The male broodstock had two year classes created in 2007 and 2008 also generated from the same Normandale Fish Culture Station year class.

Milt Collection and Sperm Quality Assessment

Males were anesthetized using MS-222 (~0.110 g/L), a weight (± 1.0 g) and a fork length (± 1.0 mm) were recorded and milt samples were collected from 38 individuals (aged 6 years (n=29) and 7 years (n=9)). In order to avoid contamination from water or mucus, the area extending from the individual's pelvic fins to the anal fin was thoroughly dried before the individual was stripped. Milt was then collected by gently applying

pressure to the abdomen of the individual and collected in 532 mL Whirl-Pak bags (Nasco, Newmarket, Ontario). The bags were then placed in a cooler until analysis 1-2 hours after collection.

In order to estimate sperm motility traits, sperm activity was recorded using a video camera (XC-ST50, Sony, Japan) mounted on a microscope (CX41 Olympus, Melville, New York) through a 10x objective (as per Pitcher et al. 2009). An aliquot of milt was placed on the edge of a chamber on a 2X-CEL glass slide (Hamilton Thorne, Massachusetts) and activated using 10 µL of hatchery water maintained at 7°C using a Bionomic controller (model BC-110, 20/20 Technology Inc., Wilmington, North Carolina). The HTM-CEROS sperm analysis system was used to analyze the selected sperm traits (i.e. path velocity (VAP), curvilinear velocity (VCL), progressive velocity (VSL) Linearity (i.e. the degree of straightness per unit of distance traveled by a sperm cell), % motility (i.e. the percentage of motile spermatozoa in the recording over the number of total sperm cells in the recording) and longevity (i.e. the point in time during the recording in which 95 % of the cells were immobile)). The settings of the system were placed at the following: Image capture was set at 60 frames/sec, cell detection was set at a contrast of 13 and a minimum cell size of 3 pixels, the cell intensity defaults were set at 3-50, the progressive cell settings were set at a VAP of 26.0 and STR of 80.0 and the slow cell cut-off was set at 20.0-20.0. Percent motility was analyzed 5s post activation, whereas, velocity and linearity were analyzed at 5 seconds as well as 10 seconds post activation. Five and 10 seconds post-activation were used as the standard times because according to the findings of Liley et al. (2002) fertilization success declines after the 10 second time point. The reason percent motility was not analyzed at 10 seconds or 15

seconds post activation and velocity and linearity were not analyzed at 15 seconds post activation was due to the poor correlation coefficients (r^2<0.215) for the different tracks taken by the same male, which would have increased error in the measuring of the metric. The mean value of all recordings per male was used as the value in statistical analysis. Two recordings of sperm activity were performed for every male (with the exception of 3 individuals of which only 1 recording was collected) and the recordings were analyzed (see below) and averaged to insure consistency between measured traits.

To estimate sperm density, an aliquot of 1.5 µL of milt was pipetted into a mixture of 125 mL of gluteraldehyde and 500 µL of Cortland's saline solution (7.25 g/L NaCl; 0.38 g/L KCl; 0.47 g/LMgSO4: 7H2O; 0.4 g/L Na2HPO4: H2O; 1.0 g/L NaHCO3; 0.22 g/L $MgCl_2$; 1.0 g/L $C_6H_{12}O_6$). The mixture was kept in a cooler until it was transported to a refrigerator (4°C) until analysis could be performed (within 24h). 10 µL of the solution was pipetted onto a haemoctometer and left to sit for ~10 minutes to insure that all the sperm cells had settled. The haemocytometer was placed onto a microscope (CX41 Olympus, Melville, New York) and recorded using a negative contrast 20x objective. The cells were counted using a standardized procedure, where the cells in each of the 4 corner squares as well as the centre square was counted. The mean of the 5 counted squares was then multiplied by 25 to represent to total number of squares and then by 10. Then the value is multiplied by the volume of the diluted milt solution (625 µL) to obtain a value to the number of sperm cells estimated to be in 1mL of milt (as per Pitcher et al. 2009a).

Egg Collection and Quality Assessment

Females were anaesthetized using MS-222 (~0.110 g/L), a weight (±1.0 g) and a fork length (±1.0mm) were recorded and eggs were collected from 41 individuals (aged 5 years (n=9), 6 years (n=20), and 7 years (n=12)) Manual pressure was then placed on the individual's abdomen and eggs were then collected in 532 mL whirl-pak bags and placed in a cooler until wet weight was measured between 2-3 hours after collection.

To estimate the quantity of eggs that each female produced, the volume of eggs that each female produced was measured in litres, following which a count was done on a subsample and multiplied by the total volume to calculate the fecundity of each female. To estimate wet egg mass, eggs were placed into a strainer to remove ovarian fluid and between 20-71 eggs (MEAN ± S.D. = 38.64 ± 10.64) were placed in weigh boats and weighed with an electronic scale (Denver Instrument TP 323) in order to calculate the mass of each individual egg (±0.001 g) and a digital image taken. Three replicates were done per female. The egg diameter of 10 eggs was measured in each of the 3 replicates using Image J (http://imagej.nih.gov/ij/) to get an average diameter of each egg (± 0.1 mm). 15 mL of eggs were then stored in falcon tubes and placed on dry ice until they were transported to a -80°C freezer where they were stored until the dry weight was measured. In order to estimate dry egg mass, eggs were removed from the -80°C freezer and left to thaw for ~2-3 hours before 10 eggs were counted and placed in aluminum foil weighing dishes. Three replicates were done per female. The eggs were then placed in a dry oven for 24h at 55°C (Einum and Fleming 2000), followed by a desiccator for 12 hours to insure that all moisture had been removed before weighing (±0.0001 g) with an electronic scale (Denver Instrument SI 234).

Heterozygosity

The MLH of individual males and females was calculated using 19 different microsatellite loci, with a polymorphic range of 7-48 alleles (see Table 3.2 for further information on each locus and Figures 3.2 and 3.3 for the distribution of MLH in both males and females). Amplification was done for the following 6UM of forward and reverse primer 6 UM dNTP, 30 BSA ug/ml, 60 µL of Promega buffer, 1.5 U/µl of Taq DNA polymerase, 60 ng DNA and 94.5uL of autoclaved ddH2O. Primers were tested implementing the following protocol: 96°C for 3 min, 35 cycles of 95°C for 45sec, varying temperature for each locus for 1 min and 72°C for 1min, followed by 72°C for 10min and finally 4°C until use. Heterozygosity was measured using multi-locus heterozygosity (MLH; the total number of loci at which each individual was heterozygous and dividing it by the total number of loci analyzed).

In order to make sure that the alleles were in Hardy-Weinberg equilibrium (HWE), the expected and observed heterozygosity was calculated for all of the loci using GenAlEx (see Table 3.2). In males, Ssa197 and SsaA119 were not in HWE, and in females Ssa202 are not in HWE. As the number of loci not in HWE were minimal, they are not expected to affect the outcome of the results.

Statistical analysis

Females

All statistics were performed in R version 3.2.1 (R Core Team 2015). A PCA was run on age, mass and length of the individuals as the three factors were highly correlated.

The first two principal components generated out of the PCA referred to from here on in the text as “SizeA” and “SizeB” were used in subsequent analysis.

In order to test for the presence of general effects (i.e. the effect influenced by an individual’s overall heterozygosity as reflected by the microsatellites), the dependent variables (i.e. fecundity, mean egg diameter, mean wet weight, mean dry weight and Fulton’s condition factor) were analyzed individually using a general linear regression (LM) with MLH, SizeA and SizeB as fixed factors in the model. Interaction factors were kept in the model if they provided a better fit to the model using the Akaike Information Criterion (AIC). As both SizeA and SizeB were biologically relevant, they were kept as fixed factors in al final models despite the AIC values. In order to assess whether any observed correlations were due to general or local effects, a univariate linear regression was run (i.e. using MLH) and a multivariate linear regression was run (i.e. using every individual locus as separate binary factors in the model). The results of the two models were then compared using an ANOVA to determine if either model explained significantly more variance.

Males

A PCA was performed on mass and length in order to generate uncorrelated principal components to use in a subsequent analysis. The dependent variables (VAP, VCL, VSL, % motility, linearity (squared, in order to generate a normal distribution), density (log transformed), longevity, percent motility and Fulton’s condition factor) were analyzed using an LM with MLH, age and the first principal component generated from mass and length. Interaction terms were also included in the model if selected by AIC. In

order to assess whether any observed correlations were due to general or local effects, a univariate linear regression was run (i.e. using MLH) and a multivariate linear regression was run (i.e. using every individual locus as separate binary factors in the model). The results of the two models were then compared using an ANOVA to determine if either model explained significantly more variance. If the univariate model explains the most variance, than that is suggestive of general effects. However, if the multivariate method explains significantly more variance, than this is indicative of local effects (see Szulkin et al. 2010 for further information on the univariate vs. multivariate comparison).

Results

Females

MLH did not correlate with fecundity (b=1802.20, t(37)=1.24, p=0.22), wet egg mass (b=5.28, t(37)=0.16, p=0.87) , dry mass (b= -8.00, t(36)= -0.56, p=0.58) or egg diameter (b=0.03, t(37)= 0.082, p=0.94) (see Table 3.5). MLH also did not correlated with Fulton's condition factor (b=-0.004, t(37)=-1.60, p=0.12).

Multivariate models did not significantly predict any more variance in the egg quality traits than the univariate models (wet weight F=1.56, p=0.19), (dry mass F=1.34, p=0.28), (diameter F=1.21, p=0.36) with the exception of fecundity (F=2.035, p=0.08). The loci SSsp2201, SSsp2215, SsaA124, SsaA119 were significantly different between homozygotes and heterozygotes, with homozygotes at alleles SSsp2201, SsaA124 and SsaA119 having higher fecundity than heterozygotes, while heterozygotes at locus SSsp2215 had higher fecundity than homozygotes. Fulton's condition factor also did not

show any increased correlation under the multivariate analysis (F=0.84, p=0.65) (see Table 3.6).

Males

Curvelinear velocity at 5 seconds post activation was correlated with MLH explaining 7.9% of the variance (*b*=-169.04, t(35)=-2.075, p=0.04) and the age of the individual (*b*=32.79, t(35)=2.147, p=0.04) (see Table 3.7 & Fig. 3.4). VAP at 5 seconds post activation was also correlated with the individual's age (*b*=30.92, t(35)=2.121, p=0.04) as well as the individual's MLH which explained 7.9% of the variance (*b*=-161.06, t(35)=-2.071, p=0.05) (see Table 3.5, Fig. 3.5). VSL at 5 seconds post activation was also correlated with MLH explaining 7.4% of the variance (*b*=-124.55, t(35)=-1.972, p=0.057)(see Fig. 3.6). At 10 seconds post activation, heterozygosity was still significantly correlated with VSL explaining 7.9% of the variance (*b*=-56.037, t(34)=-2.053, p=0.048) but was no longer significantly correlated with VCL (*b*=-54.434, t(34)=-1.674, p=0.103) nor VAP (*b*=-60.107, t(34)=-1.80, p=0.08). However, age did still have a significant effect on both VCL and VAP (*b*=22.566, t(34)=3.637, p=<0.001) and (*b*=20.60, t(34)=3.23, p=0.003) as well as a correlation with VSL (*b*=10.674, t(35)=2.050, p=0.048) at 10 seconds post activation. Longevity also correlated with MLH which explained 6.4% of the variance (*b*=-22.37, t(33)=-1.97,p=0.057) as well as age (*b*=5.486, t(33)=2.540, p=0.0160) (see Figure 3.7).

Multi-locus heterozygosity did not significantly correlate with linearity at 5 seconds post activation (*b*=-7.079, t(35)=-0.003, p=0.997) nor at 10 seconds post activation (*b*=-3544.30, t(34)=-1.47, p=0.149). However age did have a significant

influence on both factors (*b*=-1117.609, t(34)=-2159, p=0.0378) (*b*=-1028.60, t(34)=-2.28, p=0.0287). MLH was not correlated with density (*b*=3.63, t(35)=1.82, p=0.0767), but was correlated with the principal component generated by the length and weight (*b*=0.3323, t(35)=2.809, p=0.008). Motility at 5 seconds post activation was not significantly correlated with MLH (*b*=-0.329, t(34)=-1.01, p=0.32).

The condition of individuals was not significantly correlated with MLH (*b*=0.007, t(33)=1.57 , p=0.126). However, the size principal component (*b*=-0.007, t(33)=-1.798,p=0.08) as well as the size:age interaction (*b*=0.00126, t(33)=1.986,p=0.055) had influence on the variance in condition between males.

None of the multivariate methods that were examined explained any more variance in any of the fitness related traits that were measured VCL 5s (F=0.83, p=0.66), VCL 10s (F=0.80, p=0.68), VAP 5s (F=0.92, p=0.57), VAP 10s (F=0.90, p=.59), VSL 5s (F=1.27, p=0.32), VSL 10s (F=1.26, p=0.33), squared linearity 5s (F=1.00, p=0.50), squared linearity 10s (F=1.19, p=0.37), Motility 5s (F=0.80,p=0.68), longevity (F=1.79,p=0.14), density(F=0.48, p=0.94) and Fulton's condition factor (F=1.85, p=0.12), suggesting that any observed correlation was the result of general effects and not local effects.

Discussion

In this study, MLH was found to correlate differently with distinct gamete quality traits between the sexes. For males, although negative correlations between MLH and velocity at 5 seconds post activation (VCL, VAP and VSL), 10 seconds post activation for VSL and longevity were found, the variance explained by the MLH ranged between

6.4% and 7.9% at maximum. Therefore, although the results were statistically significant, the correlation might not be highly biologically relevant. Since the multivariate model did not explain any additional variance to the univariate model, it is assumed that these negative correlations, if biologically relevant, were the result of general effects. In females, MLH was not found to correlate with any of the egg quality traits or Fulton's condition factor. Although this study showed no significant different between the variance explained by the multivariate and univariate models on average, the multivariate model for fecundity did explain a variance of 11.4% compared to 0.39% of the variance in the univariate model, suggesting possible local effects with certain loci. As studies have found varying levels of HFCs with different reproductive traits as well as varying results between the sexes, there are several potential reasons for the variance in observations in the correlation between gamete quality of both sexes and MLH.

A potential reason for the finding of negative HFCs for velocity and longevity of spermatozoa is that they are not measures of fitness in the LaHave broodstock. Although studies have found that velocity (Gage et al. 2004) and longevity (Gage et al. 1995) increase fertilization success, it is possible that the increased fertilization success of these traits does not necessarily correlate with an increase in reproductive success. Although in the wild it is speculated that asynchronise release of gametes and the presence of multiple males will lead to factors such as longevity of sperm having a stronger effect on paternity, in the hatchery setting where the broodstock is reared, the RLC breeding tactic only uses one male's sperm per female. Therefore, sperm competition between males does not exist, resulting in factors factors such as longevity having a potentially negligible effect on paternity in this type of setting. Although studies have found velocity to be a key

determinant in fertilization success (Gage et al. 2004; Pitcher et al. 2009b), other studies have found little influence of sperm velocity on fertilization success or hatching success. For example, Linhart et al. (2005) found no correlation between the sperm velocity of Common carp (*Cyprinus carpio*) and fertilization success or hatching success. It is therefore possible that velocity does not correlate with reproductive success in this population.

Another potential reason for finding negative HFCs in the velocity and longevity of spermatozoa is outbreeding depression. Outbreeding depression has been found in the offspring of several separated populations (e.g. Gilk et al. 2004; Goldberg et al. 2005), and manifests as the fitness related traits (e.g. reproductive traits) being worse than either parental population (Frankham et al. 2002). Although outbreeding depression is better documented in cases of the crossing of separated populations, it can also manifest within populations (reviewed in Frankham et al. 2010; reviewed in Szulkin and David 2011). Optimum levels of outbreeding within a population do exist just the same as optimum levels of inbreeding, and the two levels are often not very divergent (Willi and Van Buskirk 2005). Under scenarios of outbreeding depression, HFCs are expected to be negative or quadratic (Marshall & Spalton 2000; Neff 2004a). For example, Neff (2004a) found that individual bluegill sunfish with intermediate levels of genetic divergence had better reproductive success than individuals that were highly outbred or highly inbred. As the Atlantic salmon in our study are bred haphazardly with rotational line crosses to insure minimal inbreeding and maximum genetic diversity is maintained, it is possible that with the low variation in MLH levels we are seeing individuals with intermediate heterozygosity (e.g. 70%) with higher fitness than those at the extreme end (e.g. 100%).

Therefore, if outbreeding depression has manifested in the LaHave population of Atlantic salmon, the negative relation between velocity and heterozygosity as well as longevity and heterozygosity would be expected. If our sample had individuals with lower amounts of heterozygosity it is possible that we would have also seen a quadratic relationship rather than a negative one. It is also, perhaps, this lack of variance in the data which lead to no observable correlation between MLH and the measured egg quality traits in the females.

The presence of HFCs in non-inbred populations may also be reflective of heterozygotes at one particular locus displaying higher fitness than homozygotes at that particular locus rather than general effects (Pogson and Zouros 1994; Thelen and Allendorf 2001). For example, Wetzel et al. (2012) found a relation between clutch size and individual MLH in sparrows, however, the relation between egg volume and MLH displayed a more complicated relationship that was indicative that there were multiple loci influencing the outcome variable (Wetzel et al. 2012). Although Heath et al. (2002) found significant general effects with fecundity in Chinook salmon, locus *Omy207* was a significant local influence in all measured female reproductive traits measured in the study (i.e. fecundity, egg size and egg survival). Although for the most part, my study showed no overall significant evidence of local effects, locus SSsp2201 explained over 8% of the variance in the fecundity model , possibly indicating local effects for this reproductive trait. Therefore, it is possible that local effects are more important for reproductive traits in the LaHave broodstock, which is why no general correlations were found with females.

Life history traits are also known to be plastic (reviewed in Nylin and Gotthard 1998)). Hatchery environments have relaxed selection, which may lead to adaptations that are not suited for natural environments (Lynch and O'Hely 2001; Wedekind 2002; Heath et al. 2003; Thériault et al. 2011; Kekäläinen et al. 2013). Because food sources in hatcheries are not limited once fish reach the exogenous feeding stage, there is no directional selection to favour individuals to expend the energy to produce larger eggs as would be required in the wild (e.g., Srivastava and Brown 1991; Lush et al. 2014). Instead, evolution in hatcheries often will drive egg selection towards smaller yet more numerous eggs instead of fewer and larger eggs (Einum and Fleming 1999; Heath et al. 2003). As the individuals used in this study are the third generation of captively reared individuals, there is no directional selection to lead individuals possessing higher degrees of genetic diversity (i.e. heterozygosity) to have adapted eggs of higher quality (i.e. larger eggs) than individuals that are more homozygous. Certain studies have suggested that heterozygotes have increased homeostasis in varying environments for maintaining a positive phenotype in traits with high plasticity (Marshall and Jain 1968; Gillespie & Turelli 1989), and, although the LaHave broodstock used in this study was the third generation in a line produced from wild gametes in 2007 (personal communication), three generations of hatchery environment rearing may have been enough to influence the selection of traits. Makinen et al. (2014) found that after 5 generations of hatchery environment, convergent evolution between strains of Atlantic salmon took place. If three generations was enough for convergent evolution to start taking place, it would possibly lend explanation to our lack of observing HFCs in female gametes.

Another potential reason for the lack of correlations between heterozygosity and the female gamete quality traits is low additive variance. Traits closely related to fitness (e.g. reproductive traits and survival) have been under strong natural selection for long periods of time and will therefore have seen most of the additive variance fixed as a result (Lynch and Walsh 1998; reviewed in Van Buskirk and Willi 2006). Therefore, variance for these traits will be primarily non-additive variance (Lynch and Walsh 1998; reviewed in Van Buskirk and Willi 2006). Under these conditions, additive genetic variance increases with inbreeding due to the addition of 'low quality' additive variance that is reintroduced in the population once inbreeding occurs (reviewed in Van Buskirk and Willi 2006). In Gage et al. (2006), there was variance in the inbreeding coefficients between the populations of rabbits that were used in the study, whereas in this study the LaHave population has no inbreeding as a result of RLC employed by the hatchery. Therefore, the needed additive genetic variance in order to detect HFCs may not have been present. The fact that the variance was so low due to a lack of inbreeding meant that HFCs (i.e. fecundity, egg diameter, egg mass) could not have been easily detected in many of the gamete quality traits we measured unless the relationship was strong.

There are also several methods for which to measure genetic diversity (e.g. mean d^2 Coulson et al. 1998) Standardized heterozygosity (SH) (Coltman et al. 1999), Interrelatedness (IR)). A meta-analysis, by Chapman et al. (2009), suggested that the results obtained from SH, IR and MLH are all strongly correlated and will lead to similar results in most studies. However, many studies that have observed HFCs in reproductive traits have used mean d^2 (Heath et al. 2002; Neff 2004a; Manias et al. 2014). For example, although Manias et al. (2014) found a positive correlation between clutch size

and heterozygosity as well as egg volume and heterozygosity, the two traits only correlated when heterozygosity was measured as per d^2 and none of the other metrics that were used (i.e. IR, HL, MLH). Although mean d^2 can be a more powerful measure of genetic variation, the measure is more correlated with genetic deviance over long periods of time (e.g. speciation) (Coulson et al. 1998; reviewed in Coltman and Slate 2003; Neff 2004b). As a result, mean d^2 would not have been an appropriate alternative measure of genetic variance for our study.

Finally, as the strength and consistency of HFCs has been questioned in several meta-analysis reviews (e.g. Chapman et al. 2009; Szulkin et al. 2010) it is possible that this study simply did not have the power necessary in order to detect HFCs. For example, in Table 3.1 it is shown that many studies that have found statistically significant HFCs have sample sizes of $n \geq 100$. Since our sample size consisted of n=42 females and n=38 males, if HFCs of small or even moderate effect do exist, than we may have needed to double our sample size in order to detect it.

In conclusion, the majority of the correlations were non-significant, with the exception of the negative correlations I found between sperm velocity and longevity with heterozygosity as well as potential local effects associated with fecundity in females. However, it is worth mentioning that these statistically significant correlations explained little variance in the gamete quality traits. Therefore, it would appear that the heterozygosity present in the LaHave broodstock is currently at sufficient levels to not affect the quality of gametes they produce, and therefore should not affect their reproductive success.

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Table 3.1: Summary of existing literature evaluating the occurrence of HFCs of gamete quality related traits. MLH indicates multilocus heterozygosity, SH indicates standardized heterozygosity, HL indicates homozygosity by weighed locus, mean d^2 is a measure of allelic divergence and IR stands for interrelatedness.

Taxa	Trait	Sample Size (n)	Number of loci	Measure of heterozygosity	Local or general effects	Direction	Source study
Chinook salmon (*O. tshawytscha*)	-GSI	100	7	Mean d^2	General	(+)	Heath et al. 2002
	-fecundity	100		Mean d^2	General & Local	(+)	
	-egg volume			Mean d^2	Local	(+)	
	-hatching success			Mean d^2	Local	(+)	
Rabbits	-abnormal sperm	91	29	MLH	General	(-)	Gage et al. 2006
Blue-tits (*Cyanistes caeruleus*)	-number of eggs sire by a male	1496	79	MLH	General	(+)	Olano-Marin et al. 2011
	-number of recruits			MLH	General	(+)	
	-Clutch size			MLH	General	(+)	
Blue-tits (*Cyanistes caeruleus*)	-Female hatching success	1496	79	SH	Local	(-)	Olano-Marin et al. 2011
	-Female local recruit			SH	Local	(-)	
Blue-tits (*Cyanistes caeruleus*)	-clutch size	22	6	MLH	General	(+)	Foerster et al. 2003
	-Number of recruits per male			MLH	General	(+)	
Blue-tits (*Cyanistes caeruleus*)	-clutch size	269	14	MLH	General	(+)	Garcia-Navas et al. 2009
	-Eggshell pattern			MLH	General	(+)	

Bluegill sunfish (*Lepomis macrochirus*)	Reproductive success	142?	11	Mean d^2	General	Quadratic	Neff 2004a
European shag (*Phalacrocorax aristotelis*)	Reproductive success for females	85	7	MLH HL	General	(+) (-)	Velando et al. 2015
	-Survival			HL		(-)	
Whiskered tern (*Chlidonias hybrid*)	-clutch size -egg size -hatching success	40	8	-MLH -d^2 -IR -inverse hl	General	(+)	Minias et al. 2014
House sparrow (*Passer domesticus*)	-clutch size -egg size -nestling survival -hatching success	791	21	MLH MLH MLH MLH	General Local	(+) (+) No Cor No Cor	Wetzel et al. 2012

*All results reported in the table are statistically significant. (+) represents a positive linear correlation. (-) represent a negative linear correlation. No Cor stands for no correlation.

Table 3.2: Hardy Weinberg equilibrium of the 19 loci used to calculate heterozygosity. The loci that are out of Hardy-Weinberg equilibrium are in bold. Na is the number of alleles, Ho is the observed heterozygosity, He is the expected heterozygosity.

			Males n= 38			**Females n=43**		
Locus	**Size range**	**Na**	**Ho**	**He**	**Na**	**Ho**	**He**	**Source Paper**
SSsp1605	227-267	8	0.833	0.808	10	0.881	0.813	Paterson et al. 2004
SSsp2201	270-370	23	0.929	0.940	24	0.929	0.934	Paterson et al. 2004
SSsp2215	145-193	13	0.857	0.880	13	0.929	0.901	Paterson et al. 2004
Ssa197	163-203	9	**0.929**	**0.844**	11	0.905	0.842	O'Reilly et al. 1996
Ssa202	272-312	11	0.854	0.869	11	**0.829**	**0.867**	O'Reilly et al. 1996
SSspG7	123-219	14	0.881	0.896	15	0.786	0.869	Paterson et al. 2004
SSsp2213	152-204	11	0.881	0.851	9	0.857	0.801	Paterson et al. 2004
SSsp2216	195-259	14	0.929	0.908	17	0.976	0.916	Paterson et al. 2004
SsaA124	178-204	6	0.714	0.726	6	0.800	0.734	King et al. 2005
SsaD190	234-378	23	0.927	0.924	19	0.973	0.911	King et al. 2005
SsaF43	106-144	7	0.881	0.791	8	0.675	0.810	Olafsson et al. 2010
SSsp2210	110-152	3	0.167	0.154	4	0.381	0.344	Paterson et al. 2004
SsaA119	180-188	4	**0.357**	**0.514**	3	0.488	0.488	King et al. 2005
SsaD157	308-452	22	0.878	0.922	25	0.927	0.920	King et al. 2005
SsaD486	174-208	8	0.548	0.607	6	0.750	0.630	King et al. 2005
SsaD58	300-412	21	0.738	0.871	22	0.780	0.911	King et al. 2005
Ssa171	222-276	14	0.951	0.896	14	0.949	0.896	O'Reilly et al. 1996
SsaA86	174-220	9	0.707	0.673	10	0.825	0.744	King et al. 2005
SsaD144	172-284	20	0.976	0.933	20	0.974	0.920	King et al. 2005

Table 3.3: Summary of the heterozygosity and gamete quality metrics for the hatchery-reared female LaHave Atlantic salmon (*Salmo salar*) (n=42) from the broodstock at the Harwood Fish Culture Station. Mean, maximum value, minimum value and standard deviation as well as standard error of the population mean.

Trait	Mean	Maximum	Minimum	S.E.
Heterozygosity index (%)	0.821	1.00	0.5625	0.0175
Fecundity (# of eggs)	5679.039	8654	1653.3	284.47
Mean egg diameter (mm)	6.27	7.025	5.52	0.0482
Mean wet weight (mg)	167.85	220.82	111.32	3.674
Mean dry weight (mg)*	59.70	76.87	38.56	1.565
Fulton's condition factor (g/cm^3)	0.01632	0.02022	0.00944	0.0003
Mass (g)	4568.3	7927	1335	224.89
Fork length (inches)	25.49	31	18	0.435

*N=41 for the dry egg weight

Table 3.4: Summary of the heterozygosity and gamete quality metrics for the hatchery-reared male LaHave Atlantic salmon (*Salmo salar*) (n=38) from the broodstock at the Harwood Fish Culture Station. Mean, maximum value, minimum value and standard deviation as well as standard error of the population mean. VCL stands for curvelinear velocity, VAP stands for average path velocity, VSL stands for progressive velocity.

Trait	**Mean**	**Maximum**	**Minimum**	**S.E.**
Heterozygosity (%)	0.787	0.895	0.632	0.0131
VCL (µm/s) at 5sec post activation	114.00	190.7	44.5	7.00
VCL (µm/s) at 10 sec post activation	69.79	110.05	35.5	3.00
VAP (µm/s) at 5 sec	105.03	181.35	40.5	6.67
VAP (µm/s) at 10 sec	63.82	104.85	30.1	3.02
VSL (µm/s) at 5 sec	81.38	160.4	37.1	5.21
VSL (µm/s) at 10 sec	52.73	97.35	27.7	2.34
Linearity (degree/µm) at 5 sec	72.84	87	45	1.66
Linearity (degree/µm) at 10 sec	76.99	91.5	50.5	1.41
Motility (%)	60.67	91.02	32.59	2.61
Density (cells/ml)	2961349	16250000	187500	628164.1
Longevity (s)	26.47	40.75	14.25	1.02
Mass (g)	4087.3	10409	1310	368.49
Fork Length (inches)	25.87	48	18	0.999
Fulton's condition factor (m/cm^3)	0.0138	0.018029	0.00274	0.00044

Table 3.5: General linear regression between egg quality traits and multilocus heterozygosity for females. All values represent those in the full model (df=37) while controlling for size. Unstandardized b value, t value and p vaue for the multilocus heterozygosity final model as well as the difference in the adjusted r^2 of the final model and the final model without heterozygosity.

Traits	**Unstandardized b values**	**t values**	**P values**	**Difference in Adjusted R^2**
Fecundity	1802.20	1.235	0.22	0.0039
Wet Egg Mass	5.28	0.160	0.87	-0.0225
Dry Egg Mass	-8.00	0.560	0.58	-0.0169
Egg Diameter	0.03	0.082	0.94	-0.0184
Fulton's condition factor	-0.005	1.602	0.12	0.0320

Table 3.6: Summary of the multivariate analysis of weight of each locus on fecundity. The adjusted R2 value given to each locus was calculated by subtracting the adjusted R^2 value of the model without the locus in question from that of the full multivariate model.

Locus	Adjusted R^2
SSsp1605	0.017
SSsp2201	0.080
SSsp2215	0.024
Ssa197	-0.011
Ssa202	-0.012
SSspG7	-0.0086
SSsp2213	-0.0002
SSsp2216	-0.011
SsaA124	0.006
SsaD190	0.030
SsaF43	-0.011
SSsp2210	0.0008
SsaA119	0.027
SsaD157	0.00
SsaD486	0.005
SsaD58	0.005
Ssa171	0.011
SsaA86	0.022
SsaD144	-0.0029

Table 3.7: General linear regression between sperm quality traits and heterogosity for males (n = 38). Unstandardized b value, t value and p vaue for the multilocus heterozygosity final model selected through AIC as well as the difference in the adjusted R^2 of the final model and the final model without heterozygosity. Age was a covariate in all models with the exception of density. The size principal component was a covariate in all three velocity measures at 10 sec post activation, longevity and condition. Finally, age:size interaction was a covariate in the models for condition and longevity.

Traits	**Unstandardized b values**	**T values**	**P values**	**Difference in Adjusted R^2**
VCL 5s	-169.04	-2.075	0.04	0.079
VCL 10s	-54.434	-1.674	0.103	0.038
VAP 5s	-161.06	-2.071	0.046	0.078
VAP 10s	-60.107	-1.796	0.081	0.049
VSL 5s	-124.55	-1.972	0.057	0.074
VSL 10s	-56.037	-2.053	0.048	0.079
Linearity 5s	-7.079	-0.003	0.99	-0.026
Linearity 10s	-3544.3	-1.474	0.15	0.028
Motility 5s	-0.3294	-1.014	0.32	0.0008
Longevity	-22.366	-1.974	0.057	0.064
Density	3.631	1.824	0.077	0.052
Fulton's Condition factor	0.007	1.566	0.13	0.034

Figure Captions

Figure 3.1 Rotational Line Crossing depiction of the parental generation and the following crosses. P represents the parental generation, F_1 represents the first generation of crosses and F2 the second generation that are offspring of crosses. The solid lines represent the females used to produce the line. The dashed lines represent the males used to produce the line. (adapted from Kincaid 1977)

Figure 3.2 Distribution of multilocus heterozygosity (MLH) for the male Atlantic salmon in this study (n=38). MLH was measured as the number of heterozygous loci divided by the total number of loci.

Figure 3.3 Distribution of multilocus heterozygosity (MLH) for the female Atlantic salmon in this study (n=42). MLH was measured as the number of heterozygous loci divided by the total number of loci.

Figure 3.4 Negative relationship between curvelinear velocity (VCL) at 5 seconds post activation and multilocus heterozygosity (*b*=-169.04, t(35)=-2.075, p=0.04, dashed line) and at 10 seconds post activation (*b*=-54.434, t(34)=-1.674, p=0.103, solid line).

Figure 3.5 Negative relationship between average path velocity (VAP) at 5 seconds post activation and multilocus heterozygosity (*b*=-161.06, t(35)=-2.071, p=0.05, dashed line) and at 10 seconds post activation (*b*=-60.107, t(34)=-1.80, p=0.08, solid line).

Figure 3.6 Negative relationship between progressive velocity (VSL) at 5 seconds post activation and multilocus heterozygosity (*b*=-124.55, t(35)=-1.972, p=0.057, dashed line) and at 10 seconds post activation (*b*=-56.037, t(34)=-2.053, p=0.048, solid line).

Figure 3.1

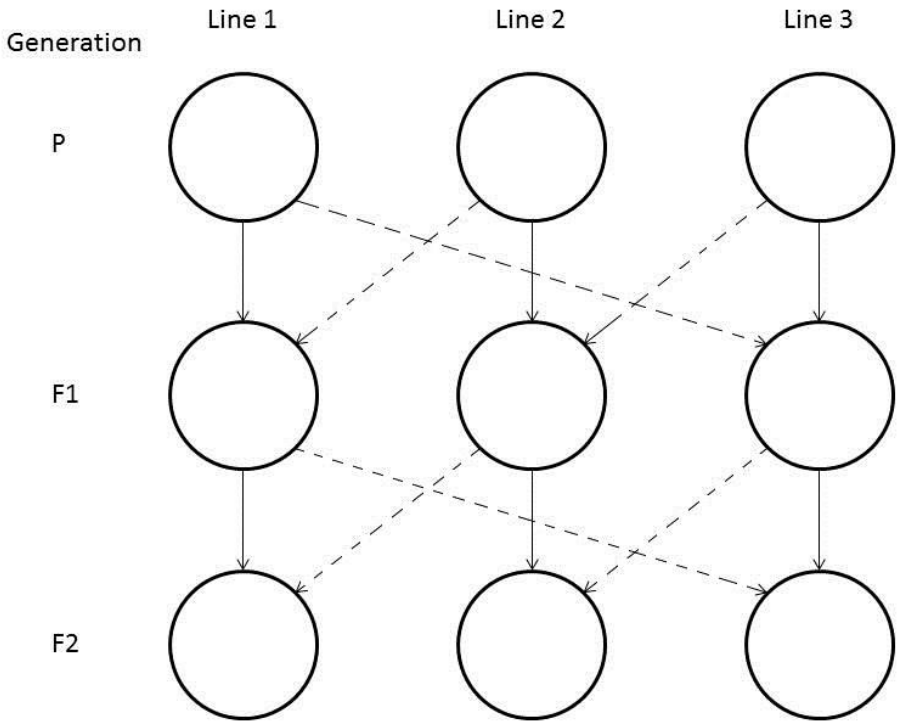


Figure 3.2

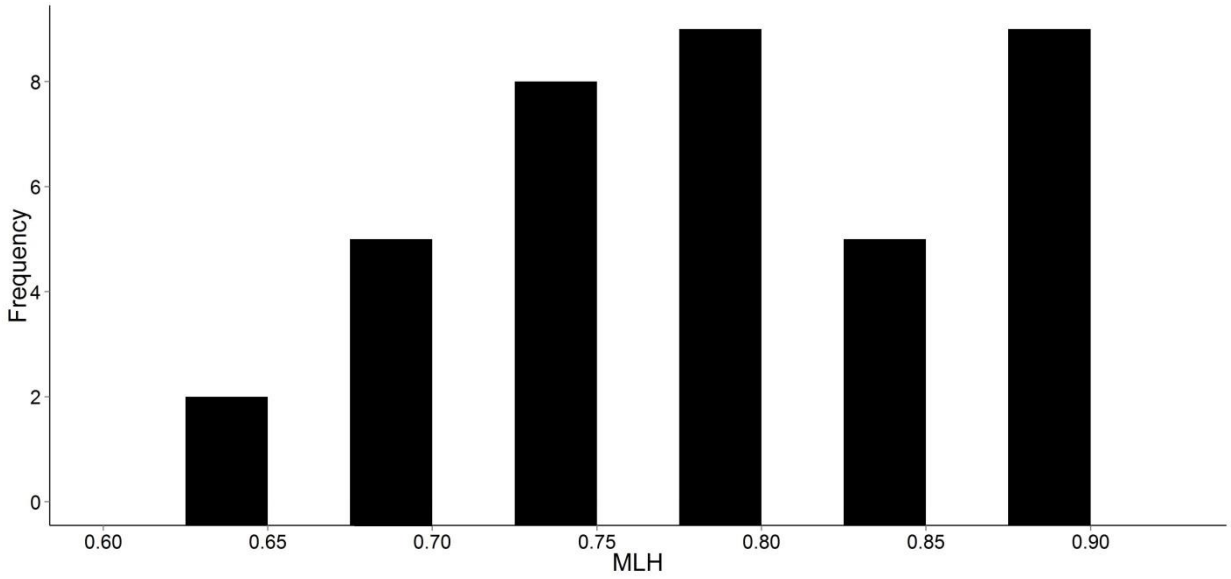


Figure 3.3

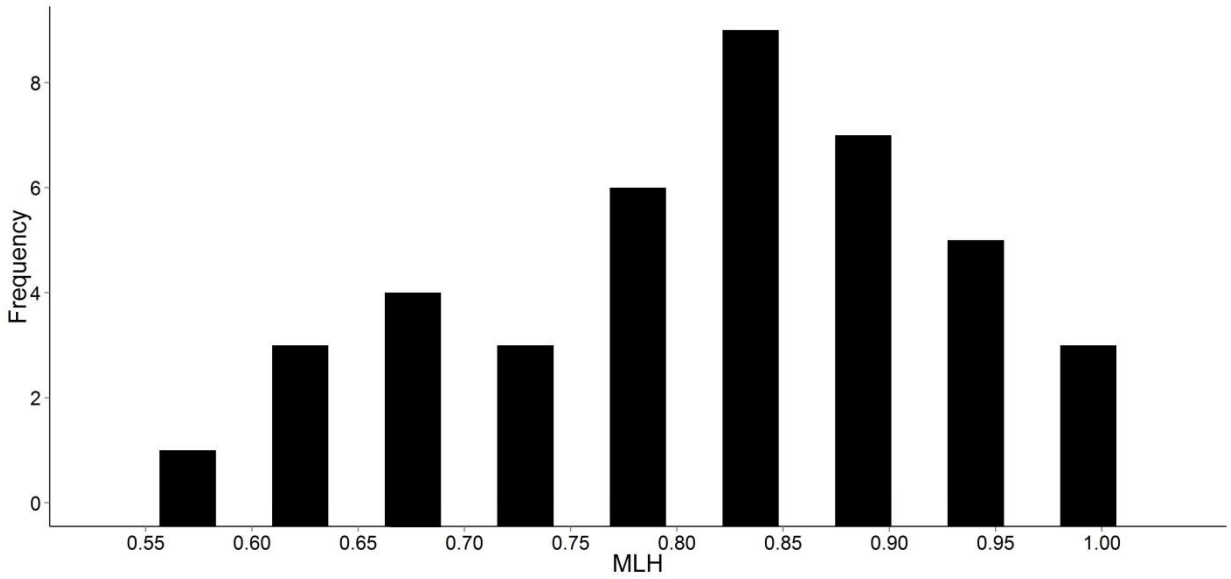


Figure 3.4

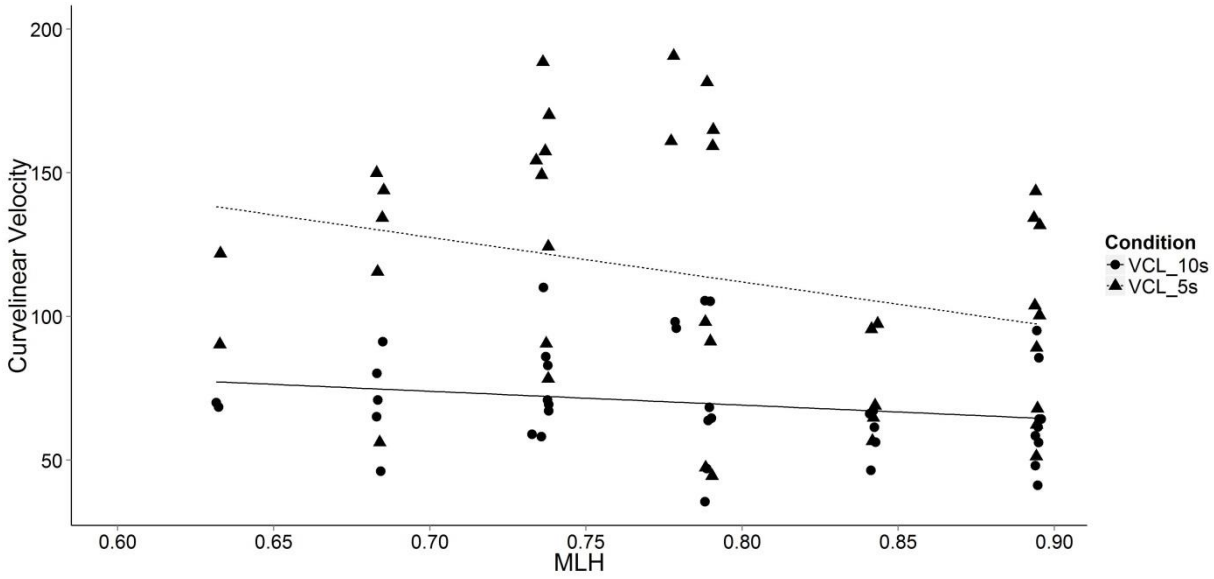

Curvelinear Velocity
200
150
100
50
0.60
0.65
0.70
0.75
0.80
0.85
0.90
MLH
Condition
VCL_10s
VCL_5s

Figure 3.5

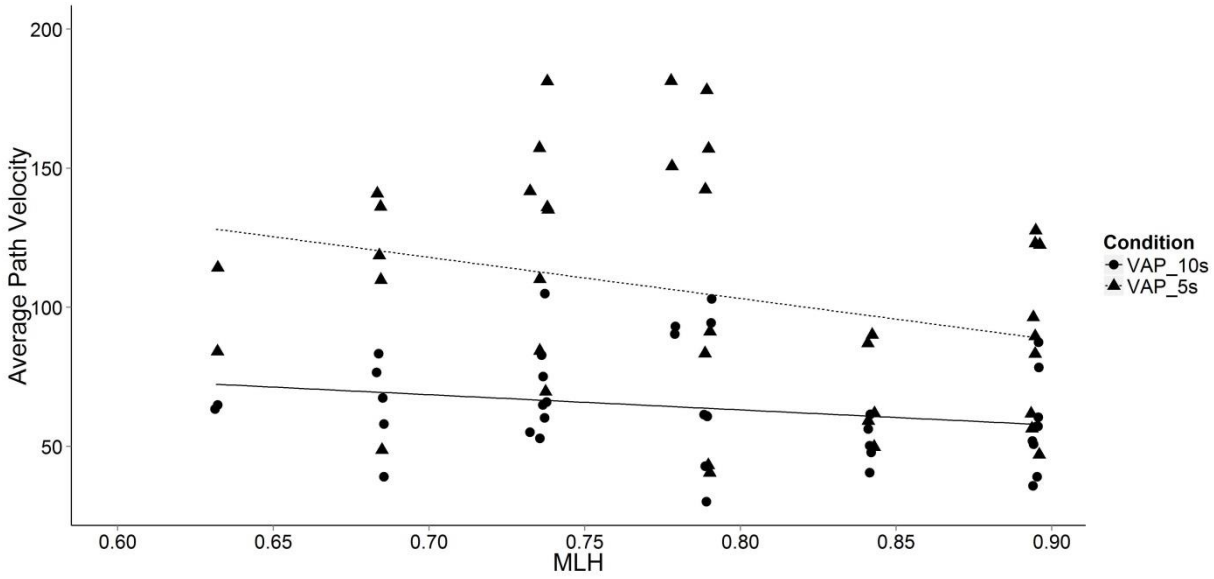

Average Path Velocity
200
150
100
50
0.60
0.65
0.70
0.75
0.80
0.85
0.90
MLH
Condition
VAP_10s
VAP_5s

Figure 3.6

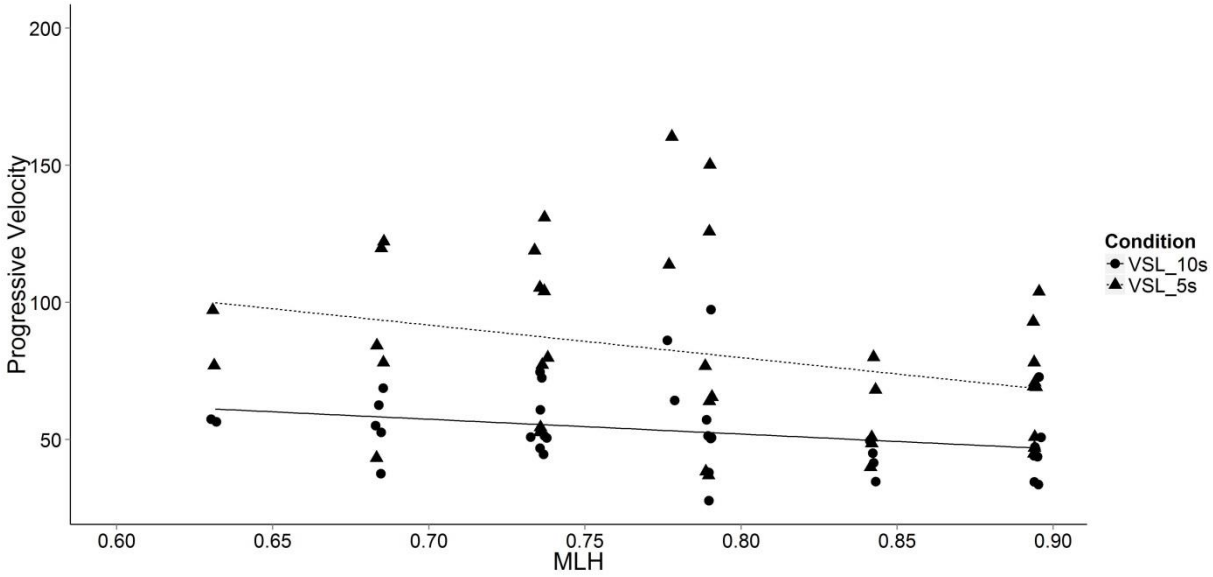
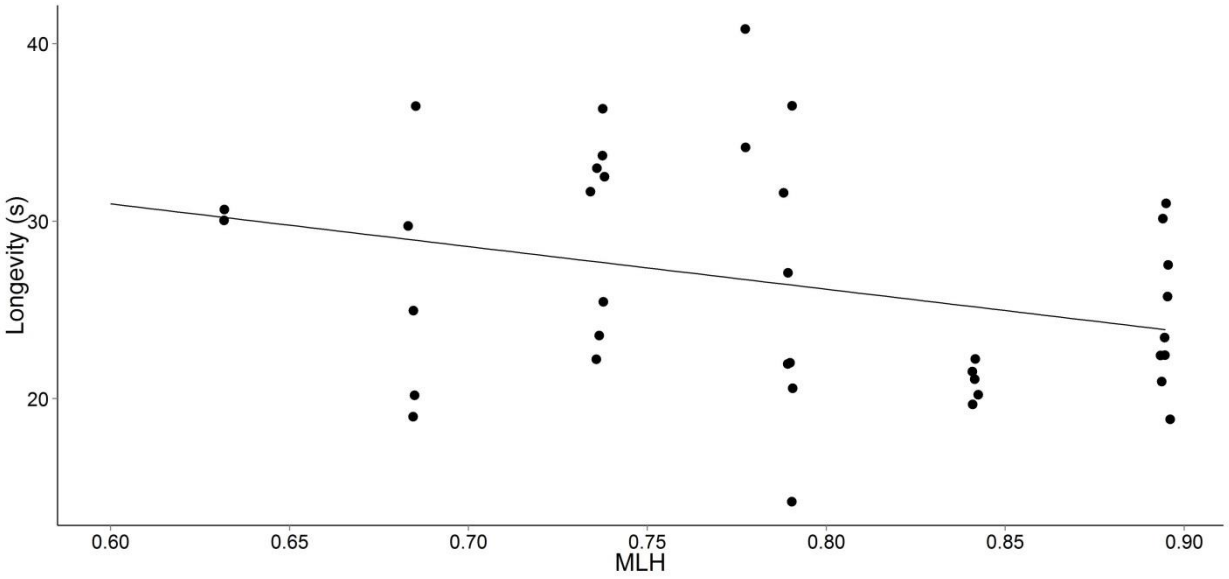

Progressive Velocity
200
150
100
50
0.60
0.65
0.70
0.75
0.80
0.85
0.90
MLH
Condition
VSL_10s
VSL_5s

Figure 3.7



CHAPTER 4: GENERAL DISCUSSION

Summary

In this thesis, I examined the relationship between genetic quality and phenotypic expression of fitness related traits (e.g. survival, morphology and gamete quality) in Atlantic salmon and how it may impact the reintroduction efforts to Lake Ontario. The purpose of this chapter is to summarize my findings of both Chapter 2 and Chapter 3, address the caveats that have come up in previous chapters and make suggestions regarding the future direction studies should take in order to further the research that was done in this thesis.

Chapter 2

The manifestation of outbreeding depression becomes increasingly likely when crossing populations that have been separated for >500 years or have lived in different environments for 20 generations or more (Frankham et al. 2011). As the reintroduction effort to Lake Ontario is simultaneously stocking populations that have been segregated since the last glacial period (Ward 1932; MNRF 2009) outbreeding depression is a potential outcome. This chapter examined the likelihood of outbreeding depression manifesting in the juvenile life phase of F_1 hybrids between the Sebago and LaHave strains of Atlantic salmon by using a 2 x 2 full factorial design in order to compare fitness related traits between the pure strains and their reciprocal hybrids while controlling for maternal and paternal effects. The results of this study showed no significant influence of hybridization on survival or any of the fitness related traits measured in juvenile Atlantic salmon.

Changes to Environmental Settings

As my study only tested for the occurrence of outbreeding depression under intrinsic factors (Tymchuk et al. 2007), it is possible that the environmental setting of the experiment made for a lack of outbreeding depression. As phenotype is the result of an interaction between genotype and the environment, a change in extrinsic factors such as environment can alter the individual fitness of *S. salar* as much as intrinsic factors depending on whether or not they are suited for their surroundings (Garcia de Leaniz et al. 2007). Isolated populations of species that display specific local adaptations for small geographic areas, such as salmonids, are severely affected by the loss of these local adaptations (Templeton 1986; Tymchuk et al. 2007; Vandersteen et al. 2012). In fact, it is believed that the loss of local adaptations has a stronger influence over outbreeding depression than do intrinsic factors such as the disruption of co-adapted gene complexes for these local populations (Allendorf et al. 2001; Edmands and Timmerman 2003; Tymchuk et al. 2007). Therefore, if outbreeding depression were to result from the loss of local adaptations, it may be masked by a relaxed environmental settings such as the one used in this study (Tymchuk et al. 2007; Crespel et al. 2013). A genotype that performs perfectly well in a hatchery setting may perform differently in the parental environment (Tymchuk et al. 2007; Crespel et al. 2013). Several examples of this are documented in salmonids. In Tymchuk et al. (2007), F_3 hybrids *O. mykiss* displayed signs of outbreeding depression in relation to growth and survival only under certain environmental conditions, suggesting that the outbreeding depression was the result of extrinsic factors simply expedited by intrinsic factors. The strength of environmental influences may also vary depending on genotype. Different genotypes of *O. mykiss* had varying survival depending

on geographic area as well as seasonality (Vandersteen et al. 2012). Crespel et al. (2013) found a similar result with Brook trout (*Salvelinus fontinalis*) in which environmental interactions affected certain hybrid crosses but not others. Therefore, in the case of this study, it is possible that if the juveniles had lost a local adaptation of importance, any resulting outbreeding depression might not have been possible to detect in a hatchery setting. Therefore, I recommend that future studies examining the effect of outbreeding, on F_1 generations specifically, should conduct the study in both parental environments if possible in order to detect outbreeding depression caused by extrinsic factors. In the event where the individuals are being transplanted to a novel environment which is not similar to either parental environments, it would perhaps be of equal value to also test the individuals in multiple settings of the new environment during various life stages in order to assess if they would suffer from signs of outbreeding depression in the intended environment. In the case of future studies examining Atlantic salmon in Lake Ontario, testing the individuals in multiple tributaries around the lake to insure that they are not going to suffer outbreeding depression in relation to the environment would be recommended.

F_2, F_3 and backcrosses

As outbreeding depression as a result of intrinsic factors is most likely to manifest in the F_2 generation or later when the original parental genomes on the same chromosome are subject to recombination (Dobzhansky 1948; Lynch 1991; Edmands 1999 McClelland et al. 2005; Tymchuk et al. 2007), it is possible that outbreeding depression could still manifest in the F_2 generation of the Sebago and LaHave crosses of Atlantic salmon. If the F_1 generation displays no evidence of outbreeding depression, they will be equally as

likely to reproduce as their pure strain counterparts resulting in F_2 generation hybrids and backcrosses. Outbreeding depression resulting from the breakup of co-adapted gene complexes has been suggested in studies on intraspecific salmonid hybrids. For example, McGinnity et al. (2003) found that F_2 hybrid *S. salar* had higher egg mortality than backcrosses as well as crosses by the same sire, suggesting that the outbreeding depression was the result of intrinsic incompatibilities. Another study, Edmands (1999), demonstrated that outbreeding depression can last until the F_3 generation, future studies looking at outbreeding depression as a result of hybridization should conduct the study to the F_3 generation (with the inclusion of backcrosses) in order to determine that there is no breakdown of co-adapted gene complexes that may lead to outbreeding depression.

Life History Traits and fitness beyond the juvenile life stage

Certain traits (e.g. life history traits) are reported to be more closely linked to inbreeding depression than morphological traits (DeRose and Roff 1999; originally noted by Falconer 1989) they may be more correlated with outbreeding depression than morphological traits as well. For example the loss of local adaptations has been shown to influence behaviour and homing ability (Gilk et al. 2004). For example, *O. mykiss* x *O. clarki* hybrids migrated earlier than non-hybridized *O. clarki* populations due to altering reactions to photoperiod (Corsi et al. 2012). As migration affects spawning time and success, life history traits may be a better indicator of outbreeding depression over an individual's lifespan rather than early growth traits (McClelland et al. 2005). Therefore, any future studies should evaluate the lifelong fitness of individuals if possible rather than just the juvenile stage.

Chapter 3

Heterozygosity fitness correlations (HFC) are correlations between fitness related traits and a measure of heterozygosity that can occur in life history traits as well as morphological traits (reviewed in Chapman et al. 2009; reviewed in Szulkin et al. 2010) and they can manifest through general or local effects (David 1998, Lynch and Walsh 1998). As heterozygosity is thought to be correlated with genetic diversity and a lack of inbreeding, HFCs are generally expected to be positive in nature and can be indicative of inbreeding depression. It is important to know the correlation of heterozygosity and gamete quality in populations. The LaHave population is the most common for stocking efforts in Lake Ontario, Chapter 3 examined the correlation between multilocus heterozygosity and sperm and egg quality within the broodstock which was accomplished using 19 microsatellite markers. As heterozygosity is indicative of genetic diversity, it was predicted that heterozygosity and the gamete quality traits would be positively correlated. However, I found a negative correlation between sperm velocity as well as sperm longevity and multilocus heterozygosity.

The results found in the study are possibly indicative of outbreeding depression in the LaHave strain of Atlantic salmon. As all of the individuals in this study were > 50% heterozygous, it is possible that the individuals closer to the central position of heterozygosity (i.e. 50%) have the highest degree of fitness while those at either extreme (i.e. 0% or 100%) have the lowest degree of fitness. For example, Neff (2004) found a quadratic relationship between reproductive performance and heterozygosity with intermediate levels displaying the highest level of reproductive success while individuals at either extreme (i.e. inbred and outbred individuals) displayed the lowest. As, our

population had little variance in the inbreeding coefficient, it is possible that if there was more variability in the multilocus heterozygosity in our population that the relationship found between velocity/longevity and multilocus heterozygosity would have been a quadratic one (Neff 2004). Therefore, for future studies researching HFCs in hatchery populations, the experimental design should include individuals inbred and outbred in order to obtain a full spectrum of inbreeding coefficients in order to better assess the direction of any observed correlation.

Although no general effects were found between multilocus heterozygosity and the egg quality traits, local effects may be present between locus SSsp2201 and fecundity. To further the studies around the local effects, I recommend using a linkage map (i.e. a chromosomal map displaying the relative positions of loci) in order to analyze any selected loci in the chromosomal vicinity using existing genome maps out of Europe. Although questions surrounding the usage of European Atlantic salmon maps for North-American Atlantic salmon have arisen (Lubieniecki et al. 2010), a recent study has found that although chromosomal changes do exist, as a whole on a finer scale, the genome remains largely conservative and can therefore be used in reference to North-American populations of Atlantic salmon (Brenna-Hansen et al. 2012).

Conclusion

Conservation management rarely takes into account outbreeding depression when selecting candidate populations for reintroduction or when breeding individuals in the hatchery. This is partially the result of most effort going into avoiding inbreeding and the consequences associated with it. This thesis examined outbreeding depression and how it

may play a role in the reintroduction of Atlantic salmon on two fronts; first through looking at hybridization and second through looking at the gamete quality of the hatchery broodstock. This study found no evidence to support the occurrence of outbreeding depression as a result of hybridization, however negative correlations between sperm quality and heterozygosity was observed in the LaHave broodstock. It is imperative that conservation management responsible for the broodstock of fish that are going to be stocked consider outbreeding depression as just as severe a risk as inbreeding depression and construct methods to avoid its consequences.

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APPENDICES

Appendix 1: Mean length, mass and Fulton's condition factor of the cross types (pure LaHave LL, pure Sebago SS, dam Sebago and LaHave sire SL and LaHave dam and Sebago sire LS) of Atlantic salmon within each family block as measured in April 2013 with one unit of standard deviation

Family Block	Dam	Sire	Hybrid Type	Mean Length (mm)	Mean Mass (g)	Mean Condition ($g/mm^3*10000$)
1	L6	L10	LL	33.07±3.30	0.37±0.11	0.1010±0.0171
1	S15	S27	SS	35.08±2.39	0.43±0.10	0.0966±0.0074
1	L6	S27	LS	34.09±2.37	0.37±0.09	0.0911±0.0048
1	S15	L10	SL	35.56±2.69	0.45±0.12	0.0982±0.0061
2	L4	L14	LL	35.97±2.15	0.47±0.10	0.0988±0.0077
2	S13	S35	SS	33.30±1.82	0.36±0.06	0.0966±0.0046
2	L4	S35	LS	33.09±3.36	0.36±0.12	0.0944±0.0094
2	S13	L14	SL	34.05±2.65	0.40±0.10	0.0976±0.0060
4	L1	L19	LL	34.27±2.94	0.41±0.12	0.0988±0.0051
4	S10	S36	SS	35.78±3.14	0.48±0.12	0.1017±0.0067
4	L1	S36	LS	34.57±2.83	0.41±0.12	0.0960±0.0072
4	S10	L19	SL	35.12±3.03	0.44±0.14	0.0977±0.0101
7	L8	L16	LL	32.61±2.99	0.34±0.10	0.0932±0.0077
7	S11	S29	SS	33.66±2.38	0.39±0.09	0.0993±0.0073
7	L8	S29	LS	32.62±2.93	0.33±0.09	0.0929±0.0056
7	S11	L16	SL	33.90±2.93	0.39±0.12	0.0969±0.0104
8	L7	L20	LL	34.67±3.56	0.39±0.12	0.0889±0.0086
8	S5	S33	SS	33.79±2.06	0.39±0.08	0.0985±0.0985
8	L7	S33	LS	33.94±2.75	0.37±0.10	0.0928±0.0928
8	S5	L20	SL	35.84±1.79	0.46±0.08	0.0986±0.0986
9	L118	L100	LL	38.05±4.53	0.52±0.21	0.0905±0.0905
9	S116	S100	SS	36.90±3.64	0.52±0.16	0.0995±0.0995
9	L118	S100	LS	36.33±3.69	0.52±0.17	0.1044±0.0117
9	S116	L100	SL	36.53±3.88	0.47±0.15	0.0931±0.0078
10	L119	L101	LL	35.43±3.48	0.50±0.17	0.1074±0.0063
10	S117	S101	SS	37.47±4.94	0.54±0.21	0.0971±0.0052
10	L119	S101	LS	36.64±3.13	0.58±0.16	0.1143±0.0079
10	S117	L101	SL	35.49±3.20	0.49±0.13	0.1078±0.0081
11	L120	L102	LL	40.03±1.25	0.73±0.10	0.1132±0.0096
11	S118	S102	SS	35.73±2.66	0.51±0.13	0.1105±0.0056
11	L120	S102	LS	38.78±3.73	0.56±0.19	0.0919±0.0079
11	S118	L102	SL	38.37±3.28	0.58±0.15	0.1018±0.0152
12	L121	L103	LL	36.01±3.87	0.52±0.17	0.1072±0.0078

12	S119	S103	SS	36.71±3.43	0.48±0.13	0.0950±0.0068
12	L121	S103	LS	37.40±2.98	0.58±0.14	0.1085±0.0060
12	S119	L103	SL	36.12±3.84	0.45±0.15	0.0915±0.0055
13	L122	L104	LL	34.82±4.10	0.45±0.17	0.1010±0.0093
13	S120	S104	SS	35.08±3.54	0.43±0.14	0.0950±0.0072
13	L122	S104	LS	37.09±3.63	0.57±0.18	0.1080±0.0078
13	S120	L104	SL	32.92±3.67	0.38±0.16	0.0997±0.0100
14	L123	L106	LL	34.81±2.98	0.43±0.11	0.0991±0.0081
14	S121	S105	SS	36.17±3.64	0.51±0.15	0.1057±0.0076
14	L123	S105	LS	31.98±3.01	0.36±0.12	0.1043±0.0109
14	S121	L106	SL	36.84±2.67	0.54±0.12	0.1051±0.0083
16	L125	L108	LL	33.82±3.19	0.43±0.13	0.1065±0.0083
16	S124	S107	SS	34.66±3.41	0.42±0.14	0.0963±0.0066
16	L125	S107	LS	35.82±3.01	0.48±0.13	0.1009±0.0059
16	S124	L108	SL	33.92±2.94	0.40±0.10	0.0997±0.0148
17	L126	L109	LL	37.54±3.49	0.51±0.14	0.0946±0.0053
17	S125	S108	SS	39.50±3.28	0.68±0.18	0.1069±0.0072
17	L126	S108	LS	37.50±2.38	0.57±0.12	0.1062±0.0059
17	S125	L109	SL	39.56±2.87	0.71±0.17	0.1115±0.0073
20	L131	L113	LL	38.63±1.76	0.58±0.07	0.0999±0.0052
20	S128	S113	SS	38.61±2.88	0.66±0.02	0.1121±0.0074
20	L131	S113	LS	41.39±2.33	0.72±0.12	0.1007±0.0055
20	S128	L113	SL	36.67±4.44	0.56±0.22	0.1061±0.0094

Appendix 2: Mean length, mass and Fulton's condition factor of the cross types (pure LaHave LL, pure Sebago SS, dam Sebago and LaHave sire SL and LaHave dam and Sebago sire LS) of Atlantic salmon within each family block as measured in August 2013 with one unit of standard deviation

Family Block	Dam	Sire	Hybrid Type	Mean Length (mm)	Mean Mass (g)	Mean Condition ($g/mm^3*10000$)
1	L6	L10	LL	60.96±5.93	3.05±0.74	0.1356±0.0226
1	S15	S27	SS	58.43±6.18	2.77±0.82	0.1355±0.0186
1	L6	S27	LS	60.22±7.13	2.50±0.73	0.1123±0.0089
1	S15	L10	SL	56.71±4.93	2.49±0.81	0.1338±0.0246
2	L4	L14	LL	65.12±4.90	3.27±0.73	0.1178±0.0155
2	S13	S35	SS	61.44±4.39	2.91±0.74	0.1231±0.0119
2	L4	S35	LS	56.96±6.92	2.58±0.85	0.1362±0.0167
2	S13	L14	SL	57.28±5.58	2.57±0.89	0.1324±0.0173
4	L1	L19	LL	59.11±6.97	2.62±0.94	0.1227±0.0148
4	S10	S36	SS	58.90±6.24	2.76±0.90	0.1315±0.0144
4	L1	S36	LS	55.17±5.92	2.28±0.65	0.1349±0.0237
4	S10	L19	SL	61.93±5.44	3.18±0.80	0.1321±0.0141
7	L8	L16	LL	54.53±7.17	2.19±0.78	0.1336±0.0223
7	S11	S29	SS	58.96±5.02	2.54±0.65	0.1221±0.0100
7	L8	S29	LS	54.15±3.96	1.98±0.43	0.1231±0.0104
7	S11	L16	SL	53.28±6.85	2.08±0.87	0.1323±0.0135
8	L7	L20	LL	56.70±6.04	2.21±0.56	0.1204±0.0170
8	S5	S33	SS	59.03±3.87	2.53±0.60	0.1209±0.0074
8	L7	S33	LS	53.87±5.74	2.00±0.64	0.1250±0.0152
8	S5	L20	SL	57.57±4.85	2.46±0.66	0.1272±0.0153
9	L118	L100	LL	67.28±6.70	3.89±1.52	0.1223±0.0105
9	S116	S100	SS	66.48±7.45	3.25±1.09	0.1069±0.0116
9	L118	S100	LS	62.86±4.81	3.00±0.67	0.1191±0.0084
9	S116	L100	SL	63.94±6.00	2.85±0.83	0.1065±0.0073
10	L119	L101	LL	63.08±4.61	2.89±0.74	0.1128±0.0070
10	S117	S101	SS	67.37±5.28	3.54±0.82	0.1141±0.0111
10	L119	S101	LS	63.14±5.43	2.90±0.77	0.1127±0.0053
10	S117	L101	SL	62.76±5.17	2.93±0.82	0.1158±0.0082
11	L120	L102	LL	71.85±10.45	3.91±1.15	0.1049±0.0141
11	S118	S102	SS	62.86±4.61	2.81±0.65	0.1112±0.0053
11	L120	S102	LS	63.77±5.22	2.78±0.68	0.1060±0.0118
11	S118	L102	SL	65.26±3.20	2.71±0.53	0.0964±0.0075
12	L121	L103	LL	65.12±5.78	3.11±0.79	0.1100±0.0060
12	S119	S103	SS	62.68±6.03	3.00±0.91	0.1187±0.0061
12	L121	S103	LS	63.71±4.48	2.77±0.58	0.1058±0.0085

12	S119	L103	SL	59.53±5.52	2.53±0.80	0.1165±0.0071
13	L122	L104	LL	60.07±9.93	2.47±1.38	0.1063±0.0075
13	S120	S104	SS	62.00±7.15	3.01±1.20	0.1208±0.0096
13	L122	S104	LS	61.18±5.75	2.57±0.76	0.1100±0.0175
13	S120	L104	SL	54.00±5.30	2.07±0.55	0.1303±0.0194
14	L123	L106	LL	61.03±5.39	2.71±0.69	0.1168±0.0082
14	S121	S105	SS	64.78±6.02	3.03±0.95	0.1085±0.0080
14	L123	S105	LS	57.59±5.88	2.29±0.70	0.1172±0.0110
14	S121	L106	SL	64.22±5.39	2.92±0.71	0.1087±0.0082
16	L125	L108	LL	52.20±7.18	2.02±0.86	0.1382±0.0187
16	S124	S107	SS	62.59±7.01	2.85±0.97	0.1132±0.0079
16	L125	S107	LS	62.44±5.63	2.57±0.78	0.1034±0.0091
16	S124	L108	SL	57.26±6.77	2.15±0.80	0.1113±0.0116
17	L126	L109	LL	61.28±8.01	2.40±0.89	0.1011±0.0102
17	S125	S108	SS	65.87±5.52	3.42±0.73	0.1185±0.0065
17	L126	S108	LS	59.52±5.93	2.50±0.67	0.1163±0.0100
17	S125	L109	SL	64.66±6.22	2.63±0.69	0.0995±0.0322
20	L131	L113	LL	66.21±4.33	3.15±0.62	0.1072±0.0038
20	S128	S113	SS	68.87±4.25	3.42±0.68	0.1035±0.0060
20	L131	S113	LS	69.28±5.82	3.77±1.04	0.1115±0.0019
20	S128	L113	SL	62.06±5.50	2.79±0.81	0.1139±0.0091

Appendix 3: Mean length, mass and Fulton's condition factor of the cross types (pure LaHave LL, pure Sebago SS, dam Sebago and LaHave sire SL and LaHave dam and Sebago sire LS) of Atlantic salmon within each family block as measured in October 2013 with one unit of standard deviation

Family Block	Dam	Sire	Hybrid Type	Mean Length (mm)	Mean Mass (g)	Mean Condition ($g/mm^3*10000$)
1	L6	L10	LL	90.98±11.64	7.83±3.46	0.1005±0.0167
1	S15	S27	SS	81.75±11.11	6.38±2.41	0.1113±0.0045
1	L6	S27	LS	84.31±9.41	5.79±1.90	0.0934±0.0023
1	S15	L10	SL	79.22±8.69	5.71±1.84	0.1107±0.0072
2	L4	L14	LL	83.40±8.51	6.36±1.96	0.1070±0.0035
2	S13	S35	SS	96.89±11.64	8.87±2.88	0.0951±0.0036
2	L4	S35	LS	74.06±8.01	4.50±1.42	0.1073±0.0027
2	S13	L14	SL	80.77±7.59	5.27±1.60	0.0970±0.0045
4	L1	L19	LL	83.99±12.94	6.38±2.94	0.1009±0.0044
4	S10	S36	SS	97.39±14.99	10.80±4.81	0.1104±0.0055
4	L1	S36	LS	88.81±14.35	7.57±3.63	0.1012±0.0035
4	S10	L19	SL	90.04±11.72	7.38±2.99	0.0955±0.0050
7	L8	L16	LL	72.35±7.94	4.42±1.59	0.1120±0.0064
7	S11	S29	SS	83.65±14.42	7.13±3.94	0.1123±0.0042
7	L8	S29	LS	74.57±15.86	4.79±3.81	0.1002±0.0047
7	S11	L16	SL	80.08±13.81	5.48±3.15	0.0977±0.0049
8	L7	L20	LL	79.42±12.53	5.00±2.76	0.0933±0.0089
8	S5	S33	SS	87.94±12.53	6.83±2.97	0.0946±0.0035
8	L7	S33	LS	86.277±10.01	6.37±2.51	0.0953±0.0030
8	S5	L20	SL	83.34±6.60	5.08±1.43	0.0857±0.0055
9	L118	L100	LL	91.29±13.10	7.88±4.23	0.0967±0.0065
9	S116	S100	SS	75.01±9.87	4.95±2.49	0.1101±0.0068
9	L118	S100	LS	77.61±7.13	4.31±0.99	0.0909±0.0054
9	S116	L100	SL	79.01±11.57	5.71±2.56	0.1092±0.0032
10	L119	L101	LL	85.03±8.04	5.83±1.88	0.0918±0.0046
10	S117	S101	SS	87.39±7.77	7.53±1.95	0.1110±0.0058
10	L119	S101	LS	79.77±10.18	5.40±2.29	0.1001±0.0064
10	S117	L101	SL	97.48±16.79	11.03±4.78	0.1150±0.0078
11	L120	L102	LL	91.72±16.57	7.47±4.60	0.0896±0.0102
11	S118	S102	SS	79.12±8.11	5.83±1.76	0.1146±0.0050
11	L120	S102	LS	79.21±5.20	4.71±1.11	0.0930±0.0063
11	S118	L102	SL	72.12±6.90	4.28±1.21	0.1117±0.0058
12	L121	L103	LL	83.84±9.62	6.95±2.48	0.1135±0.0071
12	S119	S103	SS	87.46±16.50	6.90±4.51	0.0915±0.0073
12	L121	S103	LS	82.66±10.45	5.73±2.08	0.0985±0.0161

12	S119	L103	SL	78.31±12.16	5.94±2.84	0.1153±0.0029
13	L122	L104	LL	77.23±16.00	5.92±4.61	0.1130±0.0061
13	S120	S104	SS	84.18±11.46	6.18±2.82	0.0976±0.0043
13	L122	S104	LS	75.57±13.31	5.32±3.30	0.1114±0.0054
13	S120	L104	SL	79.80±5.45	4.96±1.05	0.0963±0.0034
14	L123	L106	LL	82.67±11.29	5.65±2.90	0.0940±0.0051
14	S121	S105	SS	78.88±10.82	5.58±2.62	0.1074±0.0038
14	L123	S105	LS	76.93±11.93	5.35±2.60	0.1100±0.0048
14	S121	L106	SL	76.99±5.39	5.09±1.03	0.1102±0.0031
16	L125	L108	LL	83.80±19.15	6.27±4.97	0.0914±0.0056
16	S124	S107	SS	101.11±26.26	10.86±7.76	0.0955±0.0002
16	L125	S107	LS	80.54±11.57	5.28±2.63	0.0953±0.0063
16	S124	L108	SL	75.89±13.79	4.76±3.15	0.0982±0.0042
17	L126	L109	LL	82.99±9.93	5.36±2.29	0.0890±0.0057
17	S125	S108	SS	87.12±10.82	6.20±2.54	0.0895±0.0026
17	L126	S108	LS	79.14±8.65	5.70±1.96	0.1105±0.0054
17	S125	L109	SL	79.13±12.07	5.16±2.51	0.0978±0.0089
20	L131	L113	LL	79.76±8.86	4.60±1.54	0.0873±0.0053
20	S128	S113	SS	80.76±7.74	5.78±1.66	0.1075±0.0054
20	L131	S113	LS	92.93±22.71	9.88±7.42	0.1096±0.0009
20	S128	L113	SL	74.75±8.92	4.57±1.72	0.1057±0.0049

Appendix 4: Mean length, mass and Fulton's condition factor of the cross types (pure LaHave LL, pure Sebago SS, dam Sebago and LaHave sire SL and LaHave dam and Sebago sire LS) of Atlantic salmon within each family block as measured in December 2013 with one unit of standard deviation

Family Block	Dam	Sire	Hybrid Type	Mean Length (mm)	Mean Mass (g)	Mean Condition ($g/mm^3*10000$)
1	L6	L10	LL	96.91±12.22	11.63±5.46	0.1219±0.0170
1	S15	S27	SS	89.53±14.77	8.77±3.93	0.1147±0.0074
1	L6	S27	LS	88.06±11.82	8.12±3.49	0.1135±0.0043
1	S15	L10	SL	89.94±7.48	8.45±2.29	0.1138±0.0043
2	L4	L14	LL	87.46±10.78	7.85±2.83	0.1142±0.0059
2	S13	S35	SS	108.92±7.89	14.97±3.52	0.1143±0.0036
2	L4	S35	LS	86.24±14.27	7.78±3.90	0.1137±0.0039
2	S13	L14	SL	88.40±8.70	7.89±2.21	0.1118±0.0053
4	L1	L19	LL	92.68±15.78	9.49±4.84	0.1104±0.0051
4	S10	S36	SS	110.87±18.79	16.42±7.84	0.1127±0.0054
4	L1	S36	LS	97.52±16.38	11.26±5.65	0.1139±0.0049
4	S10	L19	SL	96.35±15.40	10.60±5.32	0.1099±0.0052
7	L8	L16	LL	85.58±18.75	8.03±6.30	0.1120±0.0057
7	S11	S29	SS	94.06±15.03	10.65±5.17	0.1198±0.0037
7	L8	S29	LS	82.49±19.36	7.60±6.05	0.1181±0.0054
7	S11	L16	SL	88.25±16.18	8.60±5.31	0.1141±0.0032
8	L7	L20	LL	85.82±15.71	8.02±4.83	0.1157±0.0063
8	S5	S33	SS	90.13±16.34	9.36±4.85	0.1177±0.0051
8	L7	S33	LS	93.80±11.53	9.43±3.49	0.1101±0.0058
8	S5	L20	SL	86.75±8.21	7.28±2.10	0.1090±0.0053
9	L118	L100	LL	96.12±15.77	11.01±6.43	0.1143±0.0053
9	S116	S100	SS	87.740±17.39	8.47±5.54	0.1123±0.0044
9	L118	S100	LS	81.220±9.18	6.58±2.04	0.1188±0.0052
9	S116	L100	SL	89.49±15.04	8.51±4.10	0.1118±0.0049
10	L119	L101	LL	92.82±10.68	9.76±3.63	0.1172±0.0042
10	S117	S101	SS	97.99±16.40	10.41±5.34	0.1044±0.0023
10	L119	S101	LS	86.71±14.69	8.01±4.39	0.1126±0.0034
10	S117	L101	SL	111.84±23.39	18.01±10.98	0.1163±0.0058
11	L120	L102	LL	100.50±23.19	12.89±9.00	0.1151±0.0076
11	S118	S102	SS	92.06±10.57	9.52±3.33	0.1177±0.0038
11	L120	S102	LS	89.66±14.30	8.97±4.22	0.1176±0.0067
11	S118	L102	SL	84.20±7.54	6.92±1.98	0.1129±0.0052
12	L121	L103	LL	93.95±13.91	9.70±4.54	0.1100±0.0049
12	S119	S103	SS	93.89±24.64	11.31±9.39	0.1138±0.0077
12	L121	S103	LS	91.24±11.54	8.78±3.39	0.1112±0.0053
12	S119	L103	SL	94.96±17.21	10.41±5.44	0.1122±0.0051

13	L122	L104	LL	85.84±21.77	8.57±8.13	0.1125±0.0050
13	S120	S104	SS	91.08±15.56	9.14±5.13	0.1113±0.0031
13	L122	S104	LS	84.56±13.45	7.11±3.52	0.1094±0.0034
13	S120	L104	SL	89.13±7.78	8.12±2.06	0.1124±0.0040
14	L123	L106	LL	88.63±14.41	8.50±5.23	0.1124±0.0033
14	S121	S105	SS	90.16±17.12	8.84±5.00	0.1107±0.0054
14	L123	S105	LS	86.59±17.42	7.96±5.03	0.1110±0.0046
14	S121	L106	SL	88.63±10.61	8.00±3.21	0.1107±0.0043
16	L125	L108	LL	92.96±21.78	10.12±8.11	0.1090±0.0045
16	S124	S107	SS	110.57±31.83	16.89±13.27	0.1107±0.0011
16	L125	S107	LS	86.85±15.13	7.87±4.86	0.1110±0.0051
16	S124	L108	SL	76.21±8.41	5.13±1.77	0.1118±0.0028
17	L126	L109	LL	91.24±12.83	8.66±3.91	0.1075±0.0059
17	S125	S108	SS	91.93±12.65	8.78±3.60	0.1075±0.0045
17	L126	S108	LS	91.50±12.03	8.73±3.28	0.1090±0.0045
17	S125	L109	SL	82.11±11.29	6.15±2.43	0.1103±0.0164
20	L131	L113	LL	89.31±9.75	7.91±2.65	0.1072±0.0048
20	S128	S113	SS	90.52±9.62	8.38±2.64	0.1100±0.0042
20	L131	S113	LS	103.70±30.01	14.65±12.92	0.1112±0.0022
20	S128	L113	SL	86.52±11.40	7.16±2.75	0.1059±0.0037

Appendix 5: Mean length, mass and Fulton's condition factor of the cross types (pure LaHave LL, pure Sebago SS, dam Sebago and LaHave sire SL and LaHave dam and Sebago sire LS) of Atlantic salmon within each family block as measured in February 2014 with one unit of standard deviation

Family Block	Dam	Sire	Hybrid Type	Mean Length (mm)	Mean Mass (g)	Mean Condition ($g/mm^3*10000$)
1	L6	L10	LL	111.15±2.91	15.82±2.90	0.1153±0.0215
1	S15	S27	SS	103.44±13.77	12.75±4.50	0.1108±0.0062
1	L6	S27	LS	107.48±16.27	13.84±6.55	0.1057±0.0041
1	S15	L10	SL	100.97±12.24	11.36±4.18	0.1062±0.0043
2	L4	L14	LL	110.48±14.24	15.08±5.25	0.1088±0.0063
2	S13	S35	SS	125.35±7.92	20.83±3.78	0.1050±0.0026
2	L4	S35	LS	102.16±16.64	11.99±5.88	0.1057±0.0081
2	S13	L14	SL	103.98±8.85	11.79±2.24	0.1043±0.0078
4	L1	L19	LL	110.17±15.74	14.83±5.83	0.1065±0.0046
4	S10	S36	SS	128.44±19.03	25.12±10.48	0.1132±0.0031
4	L1	S36	LS	113.74±17.16	17.23±7.50	0.1119±0.0059
4	S10	L19	SL	110.56±16.04	15.17±6.81	0.1059±0.0053
7	L8	L16	LL	97.00±14.65	11.02±4.50	0.1140±0.0075
7	S11	S29	SS	114.26±11.49	17.52±5.28	0.1148±0.0094
7	L8	S29	LS	96.84±13.76	11.63±4.73	0.1216±0.0076
7	S11	L16	SL	103.97±19.35	13.69±7.35	0.1135±0.0066
8	L7	L20	LL	104.79±17.58	14.01±7.07	0.1141±0.0069
8	S5	S33	SS	103.81±18.73	13.90±6.55	0.1163±0.0068
8	L7	S33	LS	111.38±11.89	15.13±4.62	0.1068±0.0052
8	S5	L20	SL	98.51±4.50	10.36±1.29	0.1081±0.0045
9	L118	L100	LL	105.74±14.28	14.15±5.85	0.1147±0.0062
9	S116	S100	SS	100.51±21.46	12.84±7.95	0.1155±0.0096
9	L118	S100	LS	97.70±9.725	10.77±2.65	0.1144±0.0071
9	S116	L100	SL	101.89±16.08	12.07±5.33	0.1089±0.0075
10	L119	L101	LL	107.45±12.60	14.63±5.15	0.1143±0.0042
10	S117	S101	SS	120.16±19.90	18.96±9.14	0.1040±0.0015
10	L119	S101	LS	104.01±16.00	13.12±5.62	0.1102±0.0043
10	S117	L101	SL	125.60±19.33	23.86±10.72	0.1162±0.0002
11	L120	L102	LL	119.18±35.79	22.58±18.13	0.1177±0.0004
11	S118	S102	SS	107.43±10.69	14.60±4.51	0.1144±0.0039
11	L120	S102	LS	105.65±13.36	13.73±4.68	0.1130±0.0072
11	S118	L102	SL	96.62±12.65	10.02±3.58	0.1069±0.0040
12	L121	L103	LL	106.62±16.39	13.68±6.72	0.1057±0.0047
12	S119	S103	SS	104.48±24.98	14.73±11.68	0.1145±0.0067
12	L121	S103	LS	105.99±11.82	13.00±4.43	0.1061±0.0089
12	S119	L103	SL	111.78±19.06	16.32±7.49	0.1103±0.0065

13	L122	L104	LL	95.51±15.65	10.70±5.65	0.1136±0.0058
13	S120	S104	SS	105.37±17.86	13.64±7.23	0.1079±0.0043
13	L122	S104	LS	104.40±21.12	13.38±8.05	0.1059±0.0026
13	S120	L104	SL	105.35±13.76	13.10±4.70	0.1084±0.0047
14	L123	L106	LL	104.02±17.41	13.09±7.73	0.1078±0.0031
14	S121	S105	SS	104.26±18.26	12.82±6.85	0.1045±0.0039
14	L123	S105	LS	99.96±19.47	11.75±6.97	0.1078±0.0059
14	S121	L106	SL	104.62±10.26	12.48±4.20	0.1062±0.0043
16	L125	L108	LL	106.02±22.51	14.50±10.31	0.1084±0.0049
16	S124	S107	SS	128.39±33.13	26.27±18.64	0.1130±0.0003
16	L125	S107	LS	102.38±17.79	12.31±6.63	0.1069±0.0070
16	S124	L108	SL	96.79±20.99	11.36±8.43	0.1111±0.0049
17	L126	L109	LL	103.50±14.14	12.69±5.61	0.1086±0.0069
17	S125	S108	SS	107.99±9.61	13.63±3.10	0.1067±0.0041
17	L126	S108	LS	104.55±14.24	13.41±5.61	0.1117±0.0067
17	S125	L109	SL	101.78±15.86	11.65±5.72	0.1044±0.0047
20	L131	L113	LL	101.10±12.18	11.32±4.04	0.1056±0.0048
20	S128	S113	SS	101.68±10.39	11.43±3.46	0.1059±0.0034
20	L131	S113	LS	119.55±3.18	18.34±0.65	0.1077±0.012
20	S128	L113	SL	95.35±11.32	9.13±3.34	0.1021±0.004

APPENDIX 6: MORPHOMETRY AND SWIMMING ABILITY OF INTRASPECIFIC HYBRID ATLANTIC SALMON (SALMON SALAR) IN THE JUVENILE LIFE STAGE

Introduction

Hybridization is the crossing of individuals from segregated populations and can lead to a phenomena called outbreeding depression (Frankham et al. 2002). When outbreeding depression occurs in the first generation, it is usually the result of an intermediate phenotype, where the hybrid offspring has lost local adaptations of importance and is no longer suitable for either parental environment (Lynch 1991; Edmands 2007). Salmonids are a highly philopatric species that often have local adaptations. Local adaptations between allopatric populations of salmonids often result in distinct morphological differences (Taylor 1991). These morphological differences can be explained by abiotic factors in the environment such as depth, temperature, DO, etc. (Turan 2000), and can influence swimming ability. Atlantic salmon have previously been shown to exhibit environmentally induced morphology with more fusiform shape in environments with faster flow (Taylor 1986; Drinan et al. 2012). Faster flowing water usually results in more streamline bodies in order to decrease swimming costs (Boily and Magnan 2002). As the reintroduction effort of Atlantic salmon to Lake Ontario is introducing multiple allopatric populations simultaneously (i.e. LaHave and Sebago) (Dimond and Smitka 2005, MNRF 2009), it is possible that morphological differences between the populations will result in an intermediate phenotype in juveniles and therefore affect their swimming ability. The objective of this appendix is to summarize and compare the swimming performance as well as the morphometric characteristics of the reciprocal LaHave x Sebago hybrids compared to the pure strains in order to determine if outbreeding depression resulting from the loss of local adaptations is a potential occurrence in the juvenile life phase.

Methods

Swimming ability & Morphometrics

Swim performance was measured between December 18, 2013 and January 20 2014. Five fish per cross type in each family block (20 fish per family block) had their swimming ability tested using a swim flume of 32 L (Swim-30, Loligo Systems, Tjele, Denmark). The individual was acclimated at 0.28 m/s current for 3 minutes, and, once the acclimation period was over, the swim flume was increased to 0.53 m/s and the trial began. Every 2 minutes the current was increased by 0.31 m/s until the fish was no longer able to swim or the maximum current of 1.63 m/s was reached. These recordings will be used to calculate the critical swimming speed (Ucrit); an important measurement that is used to represent a fish's maximum swimming ability in short durations (Fisher et al. 2005). Critical swimming speed (U_{crit}) will be calculated as: $U_{crit} = U_f + U_s*(T_f/T_s)$, where U_f is the speed of the last completed interval, U_s is the interval speed increment, T_f is the time spent during the last interval and T_s in the length of the interval (Fisher et al. 2005).

An area with lower current was detected in the swim flume (see Figure A1, Table A1), if a fish remained in that area they were disqualified from the data analysis. The sire identity in family block 5 was unknown and therefore individuals from family block 5 were disqualified as a result. There were technical difficulties with the camera resulting in data missing for family blocks 8 and 19 from the morphometric analysis, however the swimming data was still used.

Immediately after the swimming trials, the fish were anaesthetized using MS-222, their weight was taken and pictures of the right side of their body were taken using a Canon (Power Shot A570 IS) digital camera. Morphometrics of each individual will be assessed using tpsDig2

software which uses 18 landmarks on the fish's body in order to assess and compare overall body shapes as well as caudal peduncal width and depth between hybrid groups (see Colborne et al. 2011) and partial warp scores generated using CoordGen8. This data will be used in combination with the swim flume data in order to determine if hybrid and pure strain fish differ in terms of body shape and ultimately swimming ability.

Statistical analysis

Swimming Ability

The Ucrit was run in an ANCOVA against cross type with mass as a covariate.

Morphometry

The partial warp scores that were generated through CoordGen8 were then run through a Discriminant Function Analysis in R 3.2.1 using the MASS package (version 7.3-43). The scores generated were then analyzed using an ANOVA with cross type as the independent variable and the morphology scores as the dependent variable. This was then visually examined using thine plate splines created in tpsRegr. Any significant results were then analysed using a Tukey's post hoc test.

Results

Swimming Ability

The ANCOVA demonstrated that there was no significant difference between Ucrit of the four cross types ($F=1.84$, $p=0.37$) out of the 324 individuals used in the study.

Morphometry

Out of the 32 partial warp scores of n=323 individuals that were generated and put through the DFA, three significant discriminant factors were produced. A one way ANOVA displayed significant results in body morphology explained by cross type with the first discriminant factor ($F=21.93$, $p<0.001$). A Tukey post hoc comparison revealed significant difference between SL and LL ($p<0.001$), SS and LL ($p<0.001$), SL and LS ($p=0.007$) as well as SS and LS ($p<0.001$). The second discriminant factor also displayed a significant difference between cross types ($F=13.09$, $p<0.001$). A Tukey's post hoc analysis revealed differences between the LS and LL ($p<0.001$), SL and LS ($p<0.001$) and SS and SL ($p=0.003$). There was also a significant difference between cross types in the third discriminant factor ($F=12.84$, $p<0.001$) with a Tukey post hoc revealing differences between LS and LL ($p<0.001$), SL and LL ($p<0.001$) and SS and LS ($p<0.001$) and SS and SL ($p<0.001$).

Conclusion

In conclusion, all groups differed over various morphological traits. The first discriminant factor differed most strongly between the two pure strains with the reciprocal hybrids being intermediate. The difference with the second discriminant factor seemed to be between groups with different sires. This further supports Chapter 2 in demonstrating the effects of hybridization in the F_1 generation as primarily being the loss of local adaptations through intermediate phenotypes, and may also provide some evidence of dam strain and sire strain effects affecting juvenile morphology.

Acknowledgments

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Table A1: Mean Velocity in each portion of the swim flume. Section 6 (in bold) had a significantly lower velocity than the other sections.

Section	Hz	Mean Velocity
1	10	0.275842
1	15	0.415576
1	20	0.562296
2	10	0.271184
2	15	0.408589
2	20	0.556473
3	10	0.257211
3	15	0.386464
3	20	0.508731
4	10	0.273513
4	15	0.413247
4	20	0.555309
5	10	0.271184
5	15	0.421398
5	20	0.557638
6	10	**0.242073**
6	15	**0.36434**
6	20	**0.47962**
7	10	0.267691
7	15	0.410918
7	20	0.549487
8	10	0.264198
8	15	0.410918
8	20	0.550651
9	10	0.230429
9	15	0.355024
9	20	0.473798

Figure Captions

Figure A1: Graphical representation of the nine zones in which water velocity was measured in the Loligo Swim-30 swim flume used in the study.

Figure A2: Graphical display of the morphological scores of the four different cross types (pure LaHave, pure Sebago, Sebago dam x LaHave sire and LaHave dam x Sebago sire) created using a Discriminant Function Analysis

Figure A1

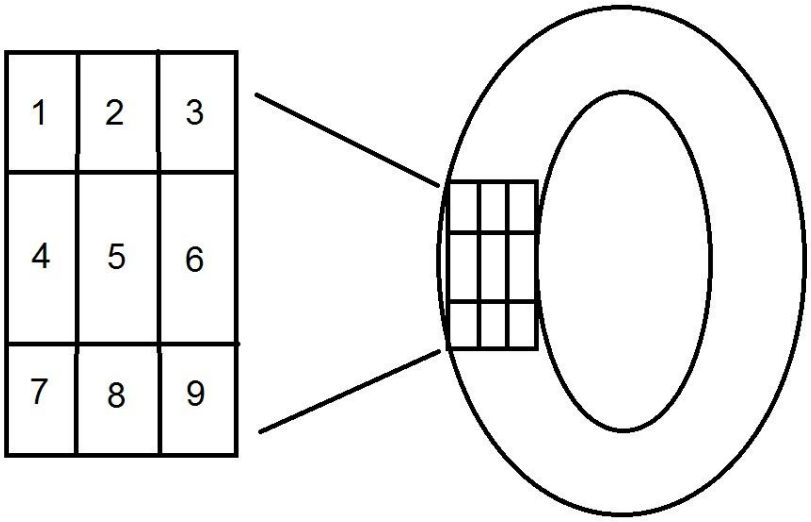
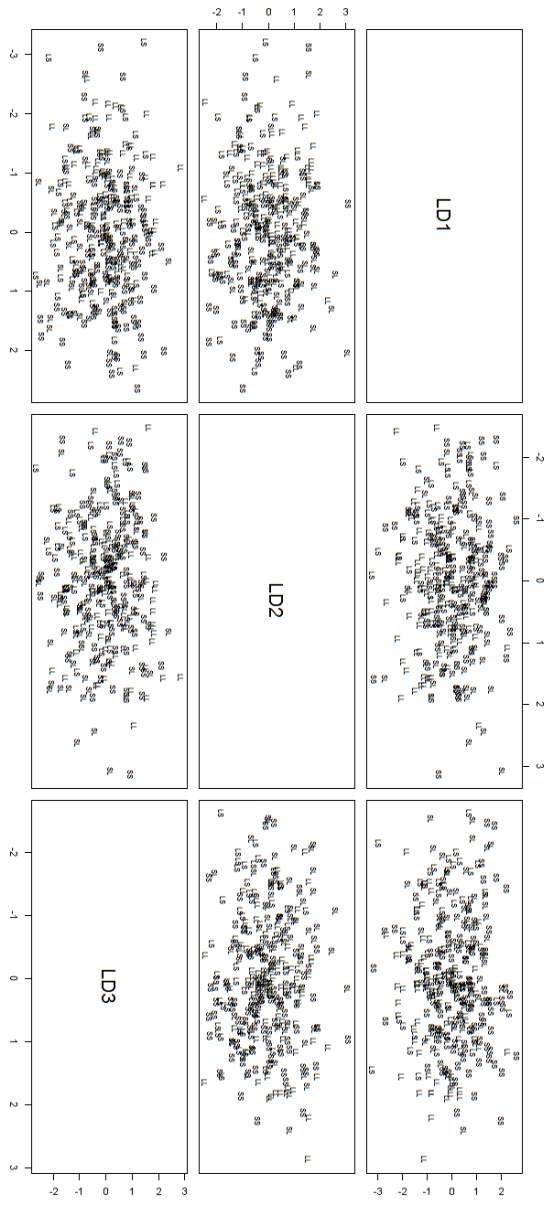


Figure A2



Appendix 7 Female Raw Data as presented per individual. Wet and dry weight is measured on a per egg basis. Fecundity was calculated by multiplying the number of eggs per litre produced by the female with the number of litres she produced. Fulton's condition factor was measured as mass over length^3. Multilocus heterozygosity was measured as a percentage of heterozygous loci. Egg diameter (mm) with one standard error, the coefficient of variation was calculated as the standard deviation/mean then converted to a percentage.

Female ID	Age (years)	Fork Length (inches)	Weight (g)	Fulton's Condition factor in g/cm^3	MLH %	Mean Egg diameter (mm) ± 1 S.E.	Mean Wet Egg weight (mg)±1 C.V.	Mean dry egg weight (mg) ± 1 C.V.	Fecundity
LHF1	5	26	4300	0.0149	0.842	6.04±0.052	162.57±2.529	64.123±9.942	6978
LHF2	5	26.5	5315	0.0174	1.000	6.40±0.050	178.22±1.245	76.81±9.077	6328.4
LHF3	5	22	3022	0.0173	0.611	5.98±0.062	152.14±1.779	53.283±25.221	3857.7
LHF4	5	25	3975	0.0155	0.895	5.88±0.035	139.35±1.750	45.093±4.584	5511
LHF5	5	24	2140	0.0095	1.000	5.52±0.059	111.32±3.134	38.563±4.988	6062.1
LHF6	5	25	3014	0.0118	0.684	6.26±0.051	163.38±1.794	75.325±13.095	3894.4
LHF7	5	24	2869	0.0127	0.944	6.01±0.054	138.16±1.775	51.233±7.490	4070.5
LHF9	5	26	4909	0.0170	0.947	6.11±0.093	160.44±4.665	54.004±5.663	8275.2
LHF10	5	18	1335	0.0140	0.944	5.80±0.036	139.17±2.657	51.77±6.631	1653.3
LHF11	7	29.5	6185	0.0147	0.895	6.09±0.060	149.15±2.606	53.803±10.679	8266.5
LHF13	7	27	6210	0.0193	0.563	6.63±0.094	205.59±3.993	74.56±10.547	4426.65
LHF14	7	22	3201	0.0184	0.842	6.07±0.065	158.01±2.918	67.043±2.576	2413.8
LHF15	7	25	4490	0.0175	0.737	6.50±0.111	160.26±5.628	43.855±11.432	4327
LHF16	7	26	5220	0.0181	0.842	7.03±0.093	220.82±5.201	69.273±2.669	6034.5
LHF17	7	25	4290	0.0168	0.895	6.40±0.037	166.65±1.663	55.883±4.759	5020.4
LHF18	7	28.5	6065	0.0160	0.842	6.56±0.046	177.93±1.876	76.873±11.603	6274.15
LHF19	7	26	5010	0.0174	1.000	6.65±0.041	196.65±5.521	71.67±3.659	4502.4
LHF20	7	27	6211	0.0193	0.737	6.33±0.038	166.46±7.289	54.05±5.867	6723.6
LHF21	7	29	6540	0.0164	0.769	6.64±0.043	190.12±2.843	72.203±1.036	8254.4
LHF23	7	29	6100	0.0153	0.842	6.38±0.043	177.65±3.585	60.693±4.823	6815.2
LHF24	7	22	3495	0.0200	0.684	6.26±0.054	168.05±1.397	56.403±3.546	4868
LHF25	7	31	7927	0.0162	0.632	6.38±0.052	161.98±1.720	61.13±1.0642	8654
LHF26	7	24	4010	0.0177	0.889	6.68±0.054	216.70±13.324	NA	4219.2

LHF27	7	27	5114	0.0159	0.842	6.34±0.053	166.46±3.900	66.767±12.3830	5192.4
LHF28	7	27	5960	0.0185	0.684	6.08±0.060	137.94±2.010	50.417±5.380	7302.4
LHF29	7	23	3359	0.0169	0.632	6.26±0.040	174.59±3.034	64.688±2.714	3620.7
LHF30	7	27	5065	0.0157	0.895	5.99±0.058	151.59±4.529	49.927±3.255	7164.3
LHF31	7	26	4760	0.0165	0.790	6.37±0.047	155.80±1.503	54.893±3.312	4502.4
LHF32	7	30	6290	0.0142	0.790	6.13±0.059	154.65±3.615	57.73±6.643	6206.4
LHF33	6	27	4727	0.0147	0.842	6.48±0.054	186.10±2.538	72.837±10.759	6389.6
LHF34	6	24	4580	0.0202	0.790	5.98±0.038	154.79±1.244	55.553±3.740	7240.8
LHF35	6	26	4593	0.0160	0.842	6.39±0.043	196.91±2.220	66.181±4.576	6490.5
LHF36	6	26	5130	0.0178	0.790	6.46±0.063	185.46±4.293	63.705±2.298	7240.8
LHF37	6	26	3828	0.0133	0.947	5.91±0.057	150.03±3.867	55.24±0.205	7164.3
LHF38	6	24	3690	0.0163	0.737	6.32±0.057	166.34±3.694	54.231±3.180	4759.7
LHF39	6	28	5609	0.0156	0.947	6.39±0.039	182.26±1.883	58.463±8.014	7302
LHF40	6	28	6020	0.0167	0.790	6.66±0.039	211.26±2.416	76.207±6.727	8046
LHF41	6	20	2125	0.0162	0.895	6.09±0.060	163.09±0.326	55.527±9.803	2326
LHF42	6	20	2030	0.0155	0.684	5.72±0.044	130.60±4.664	47.32±14.319	1957.5
LHF43	6	22.5	3387	0.0182	0.842	6.09±0.059	168.61±1.321	51.673±3.710	5689.2
LHF44	6	26	5200	0.0181	0.889	6.75±0.102	184.55±NA	58.965±4.665	6815.2

Appendix 8 Male Raw Data All sperm quality values are the mean taken between all measured tracks. Fulton's condition factor (g/cm^3), multilocus heterozygosity was the percentage of heterozygous loci per individual, average path, curvelinear and progressive velocity were measured as units of distance per second traveled, linearity is the straightness with which the cell travelled per unit of distance, motility is the percentage of cells in a recording that are motile, longevity is the point when which 95% of the cells died and density is the number of cells per 1ml of milt.

Male ID	Age (years)	Fork Length (inches)	Weight (g)	Fulton's condition factor (g/cm^3)	MLH %	VAP 5s (µm/s)	VAP 10s (µm/s)	VCL 5s (µm/s)	VCL 10s (µm/s)	VSL 5s (µm/s)	VSL 10s (µm/s)	Lin 5s (°/µm)	Lin 10s (°/µm)	%Motility	Longevity (s)	Density
LH1	7	23	3128	0.0157	0.895	122.95	78.4	134.3	85.65	78.15	50.85	57.5	60.5	0.56423	31	6656250
LH2	7	22	2517	0.0144	0.789	142.3	94.35	159.3	105.25	76.8	51.3	49	50.5	0.78190	36.5	1437500
LH4	7	28	5594	0.0156	0.737	110.1	75.1	124.3	83	54.5	44.6	45	57	0.5749	25.5	593750
LH5	7	30	7430	0.0168	0.778	150.65	90.3	161.05	95.95	113.75	64.25	69	66.5	0.6302	34.25	812500
LH6	7	32	7858	0.0146	0.737	135.05	64.85	149.25	69.45	79.85	51.3	53.5	75	0.71421	32.5	343750
LH7	7	19	1722	0.0153	0.789	178	103	181.55	105.45	150.2	97.35	83	91.5	0.74125	31.5	3906250
LH8	7	48	4979	0.0027	0.778	181.35	93.1	190.7	98.15	160.4	86.2	83	89	0.86124	40.75	1625000
LH9	7	18	1638	0.0171	0.842	59.2	50.25	64.75	56.2	48.7	45	76.5	78.5	0.47099	21.5	1500000
LH10	7	34	7467	0.0116	0.842	61.85	47.8	69	66.1	50.8	41.5	77.5	68	0.65492	21	187500
LH11	6	22	2406	0.0138	0.684	136.1	58.05	143.9	65.15	119.75	52.6	83.5	85	0.91022	25	1375000
LH12	6	18	1515	0.0159	0.842	49.8	40.5	56.65	46.45	39.95	34.7	73.5	76	0.72655	22.25	2656250
LH13	6	19	1388	0.0123	0.684	140.85	76.55	149.95	80.25	122.2	68.75	79.5	85.5	0.52394	36.5	2750000
LH14	6	24	2417	0.0107	0.737	157.3	104.85	170.15	110.05	104.1	74.6	60	68	0.79600	33.75	2250000
LH16	6	22	2171	0.0124	0.789	83.4	60.75	91.35	68.35	64.05	50.75	71.5	76.5	0.58461	22	1125000
LH18	6	30	5747	0.0130	0.684	109.85	67.4	115.5	70.9	78.05	55.05	70	78.5	0.39814	20.25	343750
LH19	6	20	1707	0.0130	0.737	69.7	60.2	78.3	67.2	52.7	50.6	69	77	0.32768	23.5	1343750
LH20	6	22	3002	0.0172	0.895	61.75	50.8	68	56.15	50.95	44.05	75.5	79	0.50049	25.75	687500
LH21	6	30	6509	0.0147	0.895	83.3	60.4	89.15	64.35	70.85	44.1	78.5	70	0.38421	21	4406250
LH22	6	24	3665	0.0162	0.842	90.15	56.15	95.6	61.4	80.05	50.05	83.5	80	0.32591	20.25	843750
LH23	6	22	2215	0.0127	0.895	122.5	87.4	131.75	95.15	103.9	72.8	77	77	0.36927	22.5	2687500
LH24	6	23	2504	0.0126	0.684	118.7	83.3	134.3	91.25	84.25	62.5	63.5	69.5	0.39023	29.75	2718750
LH27	6	23	3272	0.0164	0.789	156.95	61.35	164.95	63.8	125.8	57.15	75	89	0.75598	22	3250000
LH28	6	26	3856	0.0134	0.789	43.25	30.1	47.4	35.5	38.35	27.7	82	79	0.65891	14.25	1531250

LH29	6	26	4489	0.0156	0.737	181.2	82.8	188.6	85.95	131	72.45	68.5	84.5	0.81442	36.25	7937500
LH30	6	28	2845	0.0079	0.842	87.05	61.55	97.45	67.15	68.15	49.55	72.5	76	0.67428	19.75	16250000
LH31	6	26	3825	0.0133	0.789	91.3	60.95	98.1	64.55	65.55	50.4	67.5	79	0.73670	27	3750000
LH32	6	30	5768	0.0130	0.789	40.5	42.85	44.5	47	37.05	37.95	83.5	82.5	0.51968	20.5	1125000
LH33	6	30	5388	0.0122	0.632	84.05	63.4	90.3	68.5	77.05	56.45	86.5	81	0.52173	30.75	343750
LH34	6	30	5685	0.0128	0.737	84.4	52.9	90.55	58.2	77.25	46.8	85	81	0.5643	22.25	1906250
LH35	6	21	2109	0.0139	0.632	114.2	64.85	121.95	69.95	97.25	57.45	79.5	83	0.53106	30	437500
LH36	6	31	6546	0.0134	0.684	48.7	39.1	56.2	46.1	43.3	37.5	79	82	0.63636	19	500000
LH37	6	32	7567	0.0141	0.895	56.45	39	62.3	48.1	47	34.6	77	71.5	0.46666	18.75	1937500
LH38	6	18	1310	0.0137	0.737	135.9	65.9	157.5	70.9	105.4	60.8	66	84	0.73437	33	15906250
LH39	6	36	10409	0.0136	0.895	96.45	57.1	103.85	61.6	69.2	43.75	69	73.5	0.63146	23.5	2031250
LH40	6	18	1723	0.0180	0.895	127.6	51.9	143.6	58.5	93	46.45	63.5	80	0.67729	30.25	11500000
LH41	6	22	2717	0.0156	0.895	89.6	57.25	100.4	64.3	69.3	47.3	71	73.5	0.79129	27.5	812500
LH42	6	27	4036	0.0125	0.733	141.75	55	154.3	59	118.95	50.9	76.5	85.5	0.77419	31.75	2625000
LH43	6	29	6192	0.0155	0.895	47	35.8	51.3	41.2	44.9	33.6	87	82	0.33333	22.5	437500

Appendix 9 : Summary of the 19 tetra and dinucleotide loci used in the study originally derived from the studies O'Reilly et al. 1996, Paterson et al. 2004, King et al. 2005 and Olafsson et al. 2010. Listed in the primer sequence as well as the number of base pairs. (For further information see referenced sources).

Locus	Primer sequence 5'-3'	Base Pairs	GenBank accession number	Authors
AY081812	F: CGCAATGGAAGTCAGTGGACTGG R: CTGATTTAGCTTTTTAGTGCCCAATGC	438bp	AY081812.1	Paterson et al. 2004
AY081807	F: TTTAGATGGTGGGATACTGGGAGGC R: CGGGAGCCCCATAACCCTACTAATAAC	520bp	AY081807.1	Paterson et al. 2004
AY081810	F: ACTAGCCAGGTGTCCTGCCGGTC R: AGGGTCAGTCAGTCACACCATGCAC	461bp	AY081810.1	Paterson et al. 2004
SSU43694	(*a*) GGG TTG AGT AGG GAG GCT TG (*b*) TGG CAG GGA TTT GAC ATA AC*	473bp	U43694.1	O'Reilly et al. 1996
SSU43695	(*a*) CTT GGA ATA TCT AGA ATA TGG C (*b*) TTC ATG TGT TAA TGT TGC GTG*	344bp	U43695.1	O'Reilly et al. 1996
AY081813	F: CTTGGTCCCGTTCTTACGACAACC R: TGCACGCTGCTTGGTCCTTG	613bp	AY081813.2	Paterson et al. 2004
AY081809	F: ATGTGGAGGTCAACTAACCAGCGTG R: CATCAATCACAGAGTGAGGCACTCG	520bp	AY081809.1	Paterson et al. 2004
AY081811	F: GGCCCAGACAGATAAACAAACACGC R: GCCAACAGCAGCATCTACACCCAG	520bp	AY081811.1	Paterson et al. 2004
AF525202	F: CTCCTGCACCTGACTTCTATTC R: ACAGGCTATCACAGAACAGTTG	410bp	AF525202.1	King et al. 2005
AF525206	F: GGCATTGGAGGTAAGGACAC R: CCAGACCACTGAACTTCTCATC	406bp	AF525206.1	King et al. 2005
SsaF43	Missing	136bp	NA	Olafsson et al. 2010
AY081808	F: AAGTATTCATGCACACACATTCACTGC R: CAAGACCCTTTTTCCAATGGGATTC	349bp	AY081808.1	Paterson et al. 2004
AF525201	F: TCTGGAAGTTTCCCTACTTCTG R: TCTTTAACTGTTGCCTTAACGAC	571bp	AF525201.1	King et al. 2005
AF525204	F: ATCGAAATGGAACTTTTGAATG	451bp	AF525204.1	King et al. 2005

	R: GCTTAGGGCTGAGAGAGGAATAC			
AF525208	F: TCGCTGTGTATCAGTATTTTGG R: ACTCGGATAACACTCACAGGTC	544bp	AF525208.1	King et al. 2005
AF525210	F: TAGAGTTTGTTCTCTGGCTTTG R: AGACCCTAGGACTGGCTACTG	550bp	AF525210.1	King et al. 2005
SSU43693	(*a*) TTA TTA TCC AAA GGG GTC AAA A (*b*) GAG GTC GCT GGG GTT TAC TAT*	436bp	U43693.1	O'Reilly et al. 1996
AF525200	F: TCTCCCAGTGGTTCTAGATGAG R: GGAGCTAAACTTCAAAGCACAG	522bp	AF525200.1	King et al. 2005
AF525203	F: TTGTGAAGGGGCTGACTAAC R: TCAATTGTTGGGTGCACATAG	439bp	AF525203.1	King et al. 2005

APPENDIX 10: WRITTEN PERMISSION FROM CO-AUTHORS

Wilson, Chris (MNRF) <chris.wilson@ontario.ca> Aug 18 (11 days ago)

to Trevor, me

Agreed – please use this as my permission to use both the hybrid and heterozygosity data for your thesis.

Cheers,

Chris.

_____

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From: Trevor Pitcher [mailto:tpitcher@uwindsor.ca]
Sent: August-18-15 3:30 PM
To: Chantal Audet
Cc: Wilson, Chris (MNRF)
Subject: Re: Written Approval to Deposit Thesis

Chantal,

Consider this email my permission to use the data collected for chapter 2 and 3 in your thesis.

Best,

Trevor

_____

Trevor E. Pitcher, PhD

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