



# Effects of intraspecific hybridisation between two hatchery-reared strains of Atlantic salmon, *Salmo salar*, on juvenile survival and fitness-related traits

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**Abstract** Intraspecific hybridisation may result in hybrid offspring exhibiting superior (heterosis) or inferior (outbreeding depression) fitness relative to their parental populations. As both have been demonstrated in salmonids, consequences of interbreeding between divergent populations are relevant to their conservation. Atlantic salmon *Salmo salar* L. were extirpated from Lake Ontario by the late 19th Century due to anthropogenic causes. Multiple allopatric populations of hatchery-reared Atlantic salmon are being stocked in an effort to re-establish a self-sustaining population. This study evaluated whether interbreeding between Sebago Lake (Maine) and LaHave River (Nova Scotia) individuals will result in heterosis or outbreeding depression in juveniles. This was accomplished through full-factorial  $2 \times 2$  mating crosses between the strains and comparing multiple fitness-related traits between the cross types. Hybrid juveniles displayed no signs of outbreeding depression nor heterosis. Further studies on comparative fitness of backcross and  $F_2$  hybrids are recommended to assess potential consequences for this and similar restoration efforts.

**KEY WORDS:** Atlantic salmon, fitness, hybridization, juveniles, reintroduction, stocking.

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## Introduction

Although hybridisation has often been 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 hybridisation, intraspecific hybridisation (hybridisation within species) often produces viable and fertile individuals, but 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 with those 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 the most likely outcome when breeding populations with fixed chromosomal differences, if they have lived in different environments for over 20 generations

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and if they have not had any gene flow for over 500 years (Frankham *et al.* 2011; for examples, see Marshall & Spalton 2000; Neff 2004). Therefore, 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 (Edmunds 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; Edmunds & 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 & Hard 2008). An example of allopatric crosses resulting in outbreeding depression was reported by Gilk *et al.* (2004). Crossing allopatric populations of pink salmon *Oncorhynchus gorbuscha* (Walbaum) resulted in lower return rates for the F<sub>1</sub> generation and lower survival for the F<sub>2</sub> generation. Other studies have similarly cautioned against interbreeding between wild and hatchery fish populations (even in cases where the hatchery stock was derived from the same population) as they may have divergent genetic traits. For example, Araki *et al.* (2007) found a 37.5% decrease per generation in the reproductive success of steelhead trout *Oncorhynchus mykiss* (Walbaum) reared in captivity due to the effects domestication had on genetic integrity. 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 uses multiple populations.

Atlantic salmon *Salmo salar* L. was native to Lake Ontario, but was extirpated in the late 19th Century as a result of anthropogenic activities such as dam construction, land clearing and overfishing (MacCrimmon 1977). Many of the dams that once impeded the upstream migration of Atlantic salmon have either been removed or have incorporated fish ladders, and restoration efforts in many tributaries have made conditions more favourable for Atlantic salmon (Ontario Ministry of Natural Resources (2004); Stanfield & Jones 2003). Reintroduction began in earnest in 2005, using an anadromous strain in provincial hatcheries that originates from the LaHave River in Nova Scotia (Ontario Ministry of Natural Resources 2006). Additional hatchery-based broodstock was developed from Sebago Lake in Maine, and stocking into Lake Ontario tributaries was initiated in 2008 (Ontario Ministry of Natural Resources 2009), and, as of that point, both populations have been stocked simultaneously. The Sebago Lake strain was chosen as

an ecological contrast to the LaHave strain (Dimond & Smitka 2005), as the wild population is landlocked (Ward 1932) and lake conditions are considered similar to those in Lake Ontario (Toivonen 1971). The population's freshwater life cycle is suitable to a different environment than the LaHave strain, and it has been used for previous successful introductions in the state of Maine, so it was 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 (Ward 1932), outbreeding depression is a possible outcome of breeding these two populations (see Frankham *et al.* 2011).

This study evaluated the implications of hybridisation 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: where wild gametes are collected and then fertilised and reared in a hatchery setting before being released as fry. Therefore, it is important to know whether the crossing of the allopatric populations being used in the reintroduction effort (i.e. LaHave and Sebago) will result in heterosis or, more disconcerting, outbreeding depression, as it would be a potential hindrance to population stability for such programmes in the future. 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 before the smolt migration phase (Thorstad *et al.* 2010). 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 to create family blocks composed of both pure strains and their reciprocal hybrid siblings.

## Materials and methods

### Full-factorial breeding design

Adults from the Sebago and LaHave strains maintained at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Codrington Fisheries Research Facility (44.18.05° N, 78.29.40° W) were selected haphazardly to create 20 distinct half-sibling family blocks using a 2 × 2 breeding design (blocked factorial breeding design). One male and female from each strain was used

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  $2 \times 2$  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 & Neff 2006, 2007; Neff *et al.* 2011). The eggs for the blocks were fertilised on two separate dates [22 November 2012 ( $n = 8$  blocks) and 4 December 2012 ( $n = 12$  blocks)]. After fertilisation, the eggs from each cross were randomly allocated into the cells of two separate incubation stacks, each containing five trays with 16 cells per tray.

#### *Fertilised egg survival*

Fertilisation success assays for all family blocks took place between 21 December 2012 and 10 January 2013, and survival of the fertilised eggs was monitored three times a week from 14 January 2013 until the last date of hatching (4 March 2013). If the eggs changed from translucent to opaque, they were deemed dead. To examine whether the dead eggs had been fertilised pre-mortality, they were submersed in acetic acid (5%) (see Hoysak & Liley 2001); if the eggs turned white after exposure to the acetic acid, they were considered to have been fertilised, whereas eggs that remained clear were considered unfertilised. Only eggs that had been fertilised were considered in the survival comparisons.

#### *Rearing*

Once the alevins had absorbed their yolk sacs and manual feeding began, up to 100 individuals (mean  $\pm$  SE:  $97 \pm 1.2$ ) 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 recirculation system to ensure that the water quality in all the tanks is similar. Water quality (dissolved oxygen, pH and temperature) was examined daily to ensure families were being held at optimal water conditions (e.g. water temperature was kept at  $11.94 \text{ }^\circ\text{C} \pm 1.29$ , DO was  $10.62 \text{ mg L}^{-1} \pm 0.66$  and pH was  $7.50 \pm 0.29$ ). On 29 April 2013, each tank was reduced to 50 individuals, 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 to accommodate growth (29 April, 21 June and August 2013), and keep

densities relatively consistent to limit density effects on the early growth of the fish during this critical growth period.

#### *Length, mass and Fulton's condition factor*

Length, mass and Fulton's condition factor (K) were measured five times during juvenile development between April 2013 to February 2014, covering the key life stages from button-up to smolting (fry, parr and smolt). The first measurement took place during the 'button-up' stage (shortly after yolk absorption), the second was during the fry stage; 20 fish per tank were randomly selected to be weighed and measured. The third, fourth and fifth measurements were during later period of parr stage and during the smolting period (after they had lost their parr marks). Ten fish per cross type (i.e. pure LaHave cross, pure Sebago cross, Sebago dam  $\times$  LaHave sire cross or LaHave dam  $\times$  Sebago sire cross) within each family block were randomly selected to be weighed and measured. The first two measurements took place at 2-week intervals to account for the difference in fertilisation dates. The fish were anaesthetised using MS-222, each individual's mass ( $\pm 0.001 \text{ g}$ ) was then taken using an electronic scale (Denver Instrument TP 323, Denver Instruments Company, Arvada, CO, USA) and digital images (with a size standard) taken of each fish were analysed using Image J (<http://imagej.nih.gov/ij/>) to assess fork length. Fulton's condition factor was calculated as  $K = (W/L^3) \times 10\,000$  (Ricker 1975).

#### *Survival*

The survival of the juveniles was analysed during the four time intervals: egg stage (January 2013 to February 2013), fry stage (April 2013 to June 2013), early parr stage (October 2013 to December 2013) and late parr stage (December 2013 to 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 during four time intervals using a binomial generalised linear mixed effects model with Laplace approximation using version 1.1-7 of the lme4 package (Bates *et al.* 2015) in R 3.1.1 (R Core Team 2014). Cross type was evaluated as a fixed effect in the model with four potential categories (LL, SS, LS and SL), whereas dam ID, sire ID and dam ID  $\times$  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  $\times$  sire ID were included in the final model if deemed statistically significant. A likelihood ratio test fitted with maximum likelihood (ML) was used 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  $\times$  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 & 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,  $2 \times 2$  mating crosses that were established, six factorial crosses were discarded due to accidental mixing and equipment failure during the later portion of the rearing experimental error with one (or more) of the tanks in the cross; as a result, the family could not be used in any further statistical analysis.

## Results

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 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 1). Dam ID  $\times$  sire ID interaction only had a significant influence over the survival of the fry stage (Table 1). Sire ID effects had no significant influence

over the survival of the individuals during any of the life stages (Table 1).

Over the five measurement points, cross type had no significant effect on any of the fitness characteristics (Table 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). 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). Interaction effects (dam ID  $\times$  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).

## Discussion

Hybridisation had no significant influence 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 hybridisation of Atlantic salmon, particularly in the juvenile life stages, is critical to the successful reintroduction of the species back to Lake Ontario if the programme 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 been documented in hatchery-reared salmonids (Araki *et al.* 2007; Granier *et al.* 2011; Crespel *et al.* 2013) but 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)

**Table 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 Materials and methods section for details)

Survival (%)	Pure Crosses		Hybrids		Significance			
	LL	SS	LS	SL	Dam	Sire	Interaction	Cross
Egg stage	42.26	48.55	45.32	49.28	<b>&lt;0.001</b>	>0.99	>0.99	0.96
Fry stage	71.23	75.14	79.36	76.43	0.620	0.20	<b>&lt;0.001</b>	0.47
Early parr stage	94.98	93.44	95.89	98.57	<b>0.02</b>	>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.** Means ( $\pm$ standard error) of F<sub>1</sub> 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 Materials and methods for details)

Characteristic (units)	Pure Crosses		Hybrids		Significance ( <i>P</i> )			
	LL	SS	LS	SL	Dam	Sire	Interaction	Cross
<b>Fork length (mm)</b>								
May 2013 (button-up)	35.5 $\pm$ 0.230	35.9 $\pm$ 0.22	35.5 $\pm$ 0.23	35.8 $\pm$ 0.22	<b>&lt;0.001</b>	<b>&lt;0.001</b>	>0.99	0.99
August 2013 (fry)	61.5 $\pm$ 0.473	62.9 $\pm$ 0.42	60.1 $\pm$ 0.41	60.6 $\pm$ 0.40	<b>&lt;0.001</b>	<b>&lt;0.001</b>	>0.99	0.41
October 2013 (parr)	82.7 $\pm$ 1.13	84.1 $\pm$ 1.22	79.7 $\pm$ 0.98	79.5 $\pm$ 0.97	>0.99	>0.99	>0.99	0.57
December 2013 (parr)	90.6 $\pm$ 1.39	92.8 $\pm$ 1.57	88.3 $\pm$ 1.29	88.2 $\pm$ 1.14	>0.99	>0.99	>0.99	0.84
February 2014 (smolt)	104.7 $\pm$ 1.47	107.3 $\pm$ 1.80	104.0 $\pm$ 1.42	102.4 $\pm$ 1.34	>0.99	>0.99	>0.99	0.67
<b>Mass (g)</b>								
May 2013 (button-up)	0.46 $\pm$ 0.010	0.48 $\pm$ 0.010	0.47 $\pm$ 0.010	0.48 $\pm$ 0.00978	<b>&lt;0.001</b>	<b>0.01</b>	>0.99	0.91
August 2013 (fry)	2.81 $\pm$ 0.065	2.96 $\pm$ 0.058	2.55 $\pm$ 0.048	2.63 $\pm$ 0.0502	<b>0.002</b>	<b>0.004</b>	>0.99	0.09
October 2013 (parr)	6.02 $\pm$ 0.283	6.55 $\pm$ 0.298	5.39 $\pm$ 0.22	5.45 $\pm$ 0.218	>0.99	>0.99	>0.99	0.59
December 2013 (parr)	9.14 $\pm$ 0.484	9.81 $\pm$ 0.523	8.40 $\pm$ 0.40	8.10 $\pm$ 0.362	>0.99	>0.99	>0.99	0.93
February 2014 (smolt)	13.50 $\pm$ 0.596	14.64 $\pm$ 0.745	13.06 $\pm$ 0.53	12.19 $\pm$ 0.507	>0.99	>0.99	>0.99	0.66
<b>Condition (10 000 <math>\times</math> g mm<sup>-3</sup>)</b>								
April 2013 (button-up)	0.099 $\pm$ 0.000627	0.101 $\pm$ 0.00053	0.100 $\pm$ 0.00065	0.100 $\pm$ 0.00064	<b>0.02</b>	>0.99	<b>&lt;0.001</b>	0.95
August 2013 (fry)	0.117 $\pm$ 0.000942	0.117 $\pm$ 0.00074	0.115 $\pm$ 0.00081	0.116 $\pm$ 0.00101	0.21	0.49	<b>&lt;0.001</b>	0.86
October 2013 (parr)	0.099 $\pm$ 0.00102	0.104 $\pm$ 0.00095	0.101 $\pm$ 0.00083	0.103 $\pm$ 0.00088	<b>&lt;0.001</b>	<b>&lt;0.001</b>	>0.99	0.74
December 2013 (parr)	0.112 $\pm$ 0.000628	0.113 $\pm$ 0.00060	0.113 $\pm$ 0.00050	0.111 $\pm$ 0.00062	0.31	<b>0.006</b>	0.19	0.30
February 2014 (smolt)	0.111 $\pm$ 0.000702	0.110 $\pm$ 0.00073	0.110 $\pm$ 0.00070	0.107 $\pm$ 0.00055	<b>0.02</b>	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 May 2013 (*P* = 0.19) and condition (August *P* = 0.55, December 0.38 and February 0.06).

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 lack of observation of outbreeding depression was that the study focussed on examining the F<sub>1</sub> generation. When outbreeding depression is observed in the F<sub>1</sub> generation, it typically reflects the loss of local adaptations resulting from 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 & Naish 2007; Tymchuk *et al.* 2007) typically result in outbreeding depression in the F<sub>2</sub> generation or later when the parental genomes are subject to recombination (Dobzhansky 1948; Lynch 1991; Edmands 1999; Birchler *et al.* 2003; Edmands & Timmerman 2003; McGinnity *et al.* 2003;

McClelland *et al.* 2005; Tymchuk *et al.* 2007). Outbreeding depression as a result of the break-up of co-adapted gene complexes has been demonstrated in intraspecific salmonid hybrids; for example, Gilk *et al.* (2004) found reduced survival in F<sub>2</sub> intraspecific *O. gorbuscha* hybrids relative to their pure counterparts, suggesting it was the result of disrupted co-adapted gene complexes. Similarly in Atlantic salmon, McGinnity *et al.* (2003) reported that F<sub>2</sub> hybrid crosses saw significantly higher egg mortality than any other cross types by the same sire; it was therefore most likely outbreeding depression. If co-adapted gene complexes are disrupted in the Sebago  $\times$  LaHave hybrids, the effects of outbreeding depression would more likely be observed in the F<sub>2</sub> 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, and it is believed that extrinsic factors, such as the loss of local adaptations, have a stronger influence over outbreeding depression than do intrinsic factors (e.g. the disruption of co-adapted gene complexes) for species with many unique and highly local populations (Allendorf & Thorgaard 1984; Edmands & Timmerman 2003;

reviewed in Garcia de Leaniz *et al.* 2007; Tymchuk *et al.* 2007; Vandersteen *et al.* 2012). In the F<sub>1</sub> 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 outbreeding depression (Burton 1987; Edmands 2007; Tymchuk *et al.* 2007; Vandersteen *et al.* 2012), 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; Crespel *et al.* 2013). For example, Tymchuk *et al.* (2007) found F<sub>3</sub> hybrid rainbow trout (*Oncorhynchus mykiss*) displayed outbreeding depression related to growth and survival in certain environmental surroundings; 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. Vandersteen *et al.* (2012) found that survival of rainbow trout fry differed not only in the geographical 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 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 with 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 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 is 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 & Wade 2009). Therefore, the phenotypic expression of outbreeding depression can be outweighed by maternal effects during early life stages (Edmands 2007). Previous studies have shown hybrid fitness 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 × wild Atlantic salmon hybrids. 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 this study found significant dam ID effects on the egg survival and early size measurements, it is possible that maternal effects are masking interaction effects that may have otherwise led to outbreeding depression. Therefore, it suggests the importance of testing lifelong fitness of hybrids to ensure that fitness is not being influenced by maternal effects.

Another possibility is that the neutral genetic distance between the parental LaHave and Sebago populations was insufficient to invoke any phenotypic expression of heterosis or outbreeding depression, as the occurrence and severity of heterosis or outbreeding depression have previously been shown to be positively correlated with parental divergence (Edmands & Timmerman 2003; Edmands 2007; McClelland & Naish 2007; Fraser *et al.* 2010). Although the Sebago and LaHave populations have been isolated from one and other for a thousand generations before captivity (Ward 1932), 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. Although factors such as Atlantic salmon being under domestication for over 10 generations (Hutchings & Fraser 2008), as well as the factor that most selected traits are polygenic in nature, may have led 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 5–7 generations of selection and four generations, respectively (Roberge *et al.* 2006; Sauvage *et al.* 2010). As both of the populations in this study are domesticated, it is possible that as being in hatchery settings, their broodstock have been under convergent natural selection for domesticated environments. Even in the case of strong genetic distance, it is possible that during early life stages, other factors have greater influence on fitness-related traits. When evaluating outbreeding in brown trout (*Salmo trutta*) using a full-factorial cross, Stelkens *et al.* (2014) did not find any significant effect of genetic distance over embryo survival, but maternal environmental effects were significant and possibly masked the effects of genetic distance. As noted by Stelkens *et al.* (2014), no significant sire effects were found in this study, and with a lack of additive genetic effects, it is still possible that environmental factors

(maternal or otherwise) masked part of the genetic effects and therefore hybridisation effects.

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<sub>1</sub> generation in a realistic hatchery setting. Future research should investigate adult fecundity and reproductive fitness (gamete quality and survival) of F<sub>1</sub> hybrids in hatcheries as well as in the wild and should include F<sub>2</sub>, backcrosses and subsequent generations. As with any reintroduction that uses multiple populations simultaneously, hybridisation 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 hybridisation may have on the fitness of individuals in hatcheries and in the wild.

### Acknowledgments

This study was funded by the NSERC Strategic Project Grant (to TEP and CCW), NSERC Discovery Grant (to TEP), CFI (to TEP), the Ontario Ministry of Economic Development and Innovation (to TEP) as well as the Ontario Ministry of Natural Resources and Forestry (OMNRF) and was approved by an Animal Care Committee. We thank Bill Sloan and Scott Ferguson from the OMNRF Codrington Fisheries Research Facility for providing technical expertise, Craig Black for helping to initiate this research and Katelynn Johnson for helping to produce the crosses. We also thank the Great Lakes Fish Research Centre in LaSalle for providing the logistical support to conduct the research as well as Aleksa Zubic, Sumeet Bhardwaj and Jade Laycock for helping with the maintenance or care of the fish during the rearing stage. Lastly, we thank my colleagues and laboratory members Jason Lewis, Adriana Forest and Sarah Lehnert for their assistance and support during this project.

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