

Maternal and paternal effects on fitness correlates in outbred and inbred Atlantic salmon (*Salmo salar*)

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Abstract: Small populations are at risk of fitness reductions due to inbreeding depression and the loss of within-population genetic diversity. Although this risk can be mitigated by interpopulation outbreeding, any increases in genetic variability may be offset by reductions in fitness attributable to outbreeding depression. Here, we evaluate the risks of inbreeding and outbreeding by quantifying changes in survival and seven other fitness-related traits expressed in early life (e.g., specific growth rate, development time), using three small and neighbouring populations of Atlantic salmon (*Salmo salar*) reared under a common-garden experimental protocol. After accounting for parental (maternal and paternal) effects on several traits (which differed between pure and F₁ outbred parents), we detected no significant cross type-level differences between inbred and pure (non-inbred, within-population) cross types, outbred and pure cross types, or inbred and outbred cross types. The extent to which parental effects on fitness-related traits might be considered beneficial or detrimental cannot be reliably determined in the absence of information on the adaptive significance of the trait values in the local environment.

Résumé : Les petites populations risquent une réduction de leur fitness à cause de la dépression consanguine et de la perte de diversité génétique à l'intérieur de la population. Bien que ce risque puisse être mitigé par des croisements exogames entre les populations, toute augmentation de la variabilité génétique peut être neutralisée par des réductions de la fitness attribuables à la dépression exogame. Nous évaluons ici les risques de l'endogamie et de l'exogamie en mesurant les changements dans la survie et dans sept autres traits reliés à la fitness qui se manifestent dans les premiers stades de vie (par ex., le taux de croissance spécifique, la durée du développement) chez trois petites populations avoisinantes de saumons atlantiques (*Salmo salar*) élevées selon un protocole expérimental de jardin commun. Après avoir tenu compte des effets parentaux (maternels et paternels) sur plusieurs traits (qui diffèrent entre les parents purs et exogames de F₁), nous ne décelons aucune différence en fonction des modes de croisement, entre les types de croisement endogames et purs (sans endogamie, au sein même de la population), entre les types de croisement exogames et purs, ni entre les types de croisement endogames et exogames. Il n'est pas possible de déterminer avec assurance dans quelle mesure les effets parentaux sur les traits reliés à la fitness peuvent être avantageux ou nuisibles sans avoir de renseignements sur l'importance adaptative de la valeur de ces traits dans l'environnement local.

[Traduit par la Rédaction]

Introduction

Many species are increasingly composed of small, isolated populations because of widespread, human-induced habitat change and loss (Frankham 2005). Such species may have an elevated risk of extinction because of a variety of threats, including those of a genetic nature. For example, small population size often results in disproportionately faster rates by which genetic diversity is lost via genetic

drift (Frankel 1974; Frankham 2005). Inbreeding at low abundance can also reduce individual fitness because of the expression of recessive deleterious alleles (i.e., inbreeding depression: Charlesworth and Charlesworth 1987; Saccheri et al. 1998; Crnokrak and Roff 1999). In turn, either factor may reduce the capacity of populations to adapt to environmental change or further hinder population recovery after other demographic threats, such as habitat alteration, have been removed (Frankham et al. 2002; Spielman et al. 2004).

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To some extent, these genetic threats can be mitigated with well-planned supportive or captive breeding programs (cf., Frankham 2008; Fraser 2008). Alternatively, there may be some merit to interbreeding small but genetically related populations, especially once captive breeding has become essential to avoid extirpation in the wild. Interbreeding may produce outbred offspring with higher fitness because the offspring contain new beneficial alleles and have increased locus heterozygosity (i.e., heterosis, Emlen 1991; Whitlock et al. 2000; Vergeer et al. 2004). Similarly, interbreeding has the potential to generate greater genetic variability in outbred individuals, which may increase the ability of a population to persist in stochastically variable environments and may ultimately contribute positively to population growth (i.e., “genetic rescue”; Tallmon et al. 2004).

Nevertheless, intentional interbreeding of even apparently closely-related populations is not without risks. Outbred individuals generated may, alternatively, have reduced fitness relative to pure parents, owing to outbreeding depression, either through the loss of local adaptation (extrinsic outbreeding depression) or the disruption of co-adapted gene complexes (intrinsic outbreeding depression) (Dobzhansky 1950; Templeton 1986). Furthermore, although a few empirical studies have shown that the severity of outbreeding depression can be positively related to genetic differentiation at neutral genetic markers (Edmands 1999; 2002; but see McClelland and Naish 2007), theoretical work suggests that interbreeding between populations of small genetic differentiation may generate variable effects (Edmands and Timmerman 2003). In fact, Lynch (2000) suggests that the interaction of genomes from populations may not be predictable because the processes of fixation and mutation are random among populations.

The question of whether or not to interbreed populations is thus an important, understudied, and contentious issue in conservation biology (Edmands 2007). This may be especially true for a growing number of species rehabilitation projects involving declining populations of salmonid fishes (Fraser 2008). Indeed, salmonids exhibit a high degree of population diversity in phenotypic traits, putative local adaptations, and population genetic differentiation even at fine geographic scales, with the latter having been routinely screened at neutral genetic markers and applied in management contexts for 40 years (Utter 2004; Fraser 2008, references therein). Nonetheless, it is largely unknown whether population genetic data are useful as indicators of quantitative trait diversity, inbreeding depression, or outbred fitness in salmonids (Mavarez et al. 2009). In part, this is because few studies have examined the fitness outcomes of crossing multiple populations (Gilk et al. 2004; McClelland and Naish 2007; Fraser et al. 2010). We are unaware of any empirical study on a salmonid that has examined potential trade-offs between inbreeding and outbreeding depression concurrently.

Wild Atlantic salmon (*Salmo salar*) populations worldwide have suffered extirpations and large declines in population size (WWF 2001). Live Gene Banking (captive breeding) efforts of endangered Inner Bay of Fundy (IBoF) populations in eastern Canada have resulted in an increase in the number of juveniles in rivers that receive support, but this increase is not projected to result in the recovery of

IBoF populations while salmon mortality at sea remains unusually high (DFO 2008). There are concerns that inbreeding and reduced within-population genetic diversity may also be impeding the recovery of IBoF Atlantic salmon (DFO 2008). There are also concerns that the use of outbreeding to mitigate genetic threats associated with inbreeding and genetic drift in small, fragmented populations — even between neighbouring populations — may result in outbreeding depression, given the literature on inter-population outbreeding in salmonids (e.g., Gharrett et al. 1999; McGinnity et al. 2003; Fraser et al. 2008).

Our objective was, thus, to evaluate the relative fitness of inbred and interpopulation outbred cross types, using three small and declining neighbouring Atlantic salmon populations of the IBoF. Fitness-related traits were measured during the early-life history stages in a common-garden laboratory environment. Observations were taken on survival and several salmonid fitness-related traits (e.g., egg diameter and development time to 50% hatch). In light of the foregoing theoretical and empirically-based concerns, we suggest there may be considerable merit in comparing the relative fitness of inbred and outbred cross types, using neighbouring populations, as a basis for providing advice to those managing the recovery of endangered Atlantic salmon in eastern Canada and of populations of species at heightened extinction risk elsewhere.

Materials and methods

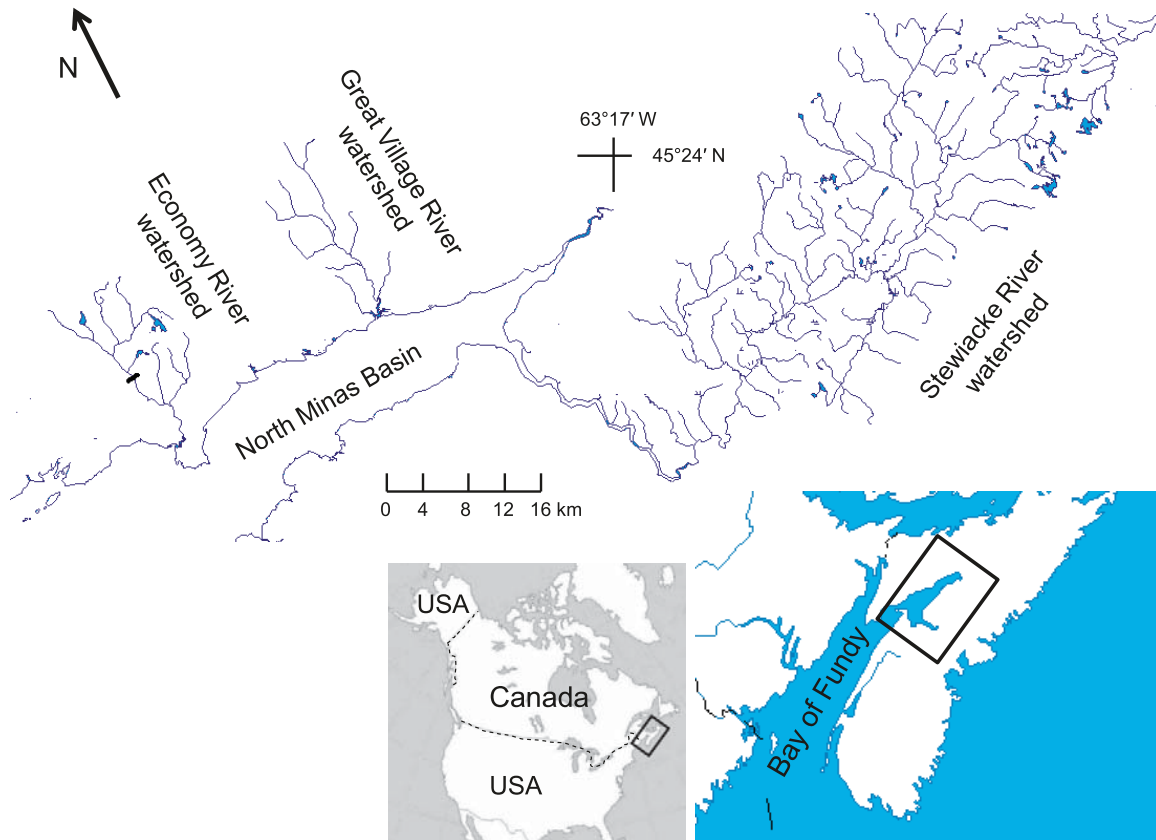
Study populations

Three Nova Scotian populations of Atlantic salmon from the Inner Bay of Fundy, North Minas Basin, i.e., Stewiacke (STW and S), Great Village (GRV and G), and Economy (ECO and E) rivers, were used to generate the cross types analyzed here (Fig. 1). Genetic data suggest that genetic differentiation between IBoF populations may be small (F_{ST} range = 0.01–0.04; Fraser et al. 2007), that gene flow between populations may be high (Fraser et al. 2007), and that populations may have similar numbers of differentially-expressed functional genes (Tymchuk et al. 2010). On the other hand, pair-wise DNA mitochondrial genetic differentiation (Φ_{ST} , ECO vs. STW = 0.0198, ECO vs. GRV = 0.4545, GRV vs. STW = 0.0587; Verspoor et al. 2002) and pair-wise DNA microsatellite genetic differentiation (F_{ST} , ECO vs. STW = 0.0938–0.0968, ECO vs. GRV = 0.0673, GRV vs. STW = 0.0345–0.0361; Tymchuk et al. 2010) suggest that there may be large genetic differences between some rivers. In addition, DNA microsatellite data suggest that the ECO and GRV populations, but not the STW population, have experienced genetic bottlenecks in the recent past (A.L.S. Houde, D.J. Fraser, P. O'Reilly, and J.A. Hutchings, unpublished data). Genetic bottlenecks suggest the loss of locus heterozygosity and, thus, a greater likelihood of heterosis in the first outbred generation (Tallmon et al. 2004).

Generation and recovery of 2003 parental cross types

Wild, age 1+ year salmon parr were collected by electrofishing in 2001 from STW, GRV, and ECO, and reared at the Coldbrook Biodiversity Facility, Coldbrook, Nova Scotia. Genotype information at 9 or more of 11 loci (*Ssa197*, *Ssa202*, O'Reilly et al. 1996; *SSsp1605*, *SSsp2201*,

Fig. 1. Location of the experimental river populations in the North Minas Basin region of the Inner Bay of Fundy. The bold line on the Economy River watershed represents a waterfall barrier to upstream passage of Atlantic salmon.



SSsp2210, *SSsp2213*, *SSsp2215*, *SSsp2216*, *SSspG7*, Paterson et al. 2004; *SsaD486*, and *SsaD144*, King et al. 2005) had been acquired previously, as described in O'Reilly and Harvie (2009).

Using the wild fish as parents, families were generated in fall 2003 for pure (noninbred within-population cross types: ECO, GRV, and STW) and first-generation outbred (F_1 E.S and F_1 G.S: pure 1 \times pure 2) cross types at the Mersey Biodiversity Facility, Milton, N.S. The offspring were pooled, transferred to Coldbrook, N.S., in July 2004, and raised to sexual maturity under common-environmental conditions (temperature, dissolved oxygen, pH) at different densities. An exclusion-based family assignment simulation in FAP 3.6 (Taggart 2007), using the information of known families (i.e., known female–male mated pairs) at five loci (i.e., *Ssa197*, *SSsp1605*, *SSsp2215*, *SSsp2216*, and *SSspG7*), determined that 99.8% of offspring could be unambiguously assigned to each family. The 2003 offspring were genotyped at these five microsatellite loci and assigned back to known families, using an Excel® Exclusion-based macro (Carolyn Harvie, Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, N.S., unpublished data). Only the offspring that matched the original wild parents at all five loci were accepted as candidate parents in the production of the next generation.

Generation of 2007 experimental cross types

Cross types were carried out within and among the three populations, using a combination of the adults recovered

from the 2003 parental cross types and the original wild fish as parents (Fig. 2). The 18 cross types generated contained 3 pure (noninbred, within-population) cross types, 6 inbred cross types (inbreeding coefficients, $F = 0.125$ and 0.25 per within-population mating, assuming a base population inbreeding coefficient of $F = 0$, Wang et al. 2002), and 9 interpopulation outbred (hybrid) cross types (first-generation, second-generation, and back-cross outbred cross types). The original wild fish were used because the number of families from some of the 2003 parental cross types was low (e.g., ECO = four available families). To mitigate the potential confounding individual maternal and individual paternal representation of the families in comparing the cross types, the same 10 females and the same 10 males from a parental cross type, i.e., ECO, GRV, STW, F_1 E.S, and F_1 G.S, were used in a balanced design to generate 20 families per cross type.

Cross types were generated on 5 and 8 November 2007 at Coldbrook. Females and males were anaesthetized, using tricaine methanesulphonate (MS-222) for handling ease, and were later revived. All the eggs contained within each female were divided equally among the number of families that were to be generated using that female. The eggs were fertilized immediately, using a volume of milt proportional to the amount of eggs (~ 1 mL to 500 eggs), and left undisturbed for 90 s to permit fertilization. Water was then added and the eggs were allowed to fertilize for another 90 s. Each family was haphazardly placed into one of the six sections of different egg trays contained within an incubation trough.

Table 1. Number of families and number of individual parents per cross type for the Aquatron data.

Families		Total number of individuals		Number of individuals for the family compositions of F ₁ and BC ₁ outbred cross types			
Cross type	<i>N</i>	Females	Males	Females-group 1	Males-group 2	Females-group 2	Males-group 1
Pure cross types (females × males)							
ECO	17	9 (5)	10 (6)				
GRV	11	7 (2)	8 (5)				
STW	12	7 (0)	9 (2)				
Inbred cross types (females × males)							
ECO.1/4	6	5 (2)	5 (2)				
ECO.1/8	8	5 (1)	7 (5)				
GRV.1/4	5	5 (2)	3 (1)				
GRV.1/8	2	2 (1)	2 (1)				
STW.1/4	7	7 (0)	7 (0)				
STW.1/8	5	4 (0)	4 (0)				
F₁ outbred cross types (females group 1 × males group 2 and females group 2 × males group 1)							
E.G	15	15	14	8 (5)	7 (5)	7 (3)	7 (4)
E.S	14	14	14	8 (5)	8 (2)	6 (0)	6 (4)
G.S	16	16	16	8 (3)	8 (2)	8 (1)	8 (5)
BC₁ outbred cross types (females group 1 × males group 2 and females group 2 × males group 1)							
E.ES	16	16	15	8 (4)	7	8	8 (5)
G.GS	16	15	13	7 (2)	7	8	6 (4)
S.ES	16	16	16	7 (1)	7	9	9 (3)
S.GS	17	16	16	8 (0)	7	8	9 (3)
F₂ outbred cross types (females × males)							
E.S	16	8	8				
G.S	19	10	8				

Note: Total *N* for families = 218. Females were mated once per cross type except in the generation of pure and F₂ outbred cross types, in which the majority were mated twice. Numbers in parentheses are the number of wild parents. F₁ and BC₁ outbred cross types are expressed as group 1–group 2 in cross type column. For example, E.ES is composed of females–ECO (group 1) × males–F₁ E.S (group 2) and females–F₁ E.S (group 2) × males–ECO (group 1).

March; alevin total length (total length from the tip of the snout to the last trace of tail visible); yolk sac length, and yolk sac width at hatch on 13 April; and alevin total length at yolk sac absorption on 19 May, for 15 randomly chosen individuals. ImageJ was calibrated to the 6 mm beads in the Kritter Keeper sections for size measurements (nearest 0.1 mm). Yolk sac volume was calculated as yolk sac length × (yolk sac width)² × $\pi/6$ (Koskinen et al. 2002). Specific growth rate (*G*) was calculated as 100 [ln(length of unfed fry) – ln(length just after hatch)]/Δ*D*, and yolk sac conversion efficiency was calculated as (fork length at first feeding – 203 fork length just after hatch)/yolk sac volume (Fraser et al. 2010).

Analysis

Early-life history traits of families were analyzed for differences among pure and inbred cross types as a test of genetic effects due to inbreeding, and among pure and outbred cross types as a test of genetic effects due to outbreeding. Data were analyzed in R 2.9.0 (available from the R Project for Statistical Computing, www.r-project.org) and statistical significance was set at the $\alpha = 0.05$ level. Early-life history traits were first examined for nuisance effects, i.e., tray position/ tank effects and density effects (number of eggs per Kritter Keeper section), and for simple maternal effects, i.e.,

relationships with maternal length, using linear or generalized linear models and Pearson correlations (Table 2).

Model selection for significant effects

Forward step-wise model selection, using Akaike Information Criteria, was used to generate an ordered list of significant effects. Variables used for model selection were maternal ID, paternal ID, maternal environment, paternal environment, maternal cross type, paternal cross type, cross type, as well as variables for any significant nuisance effects or relationships with maternal length from previous tests. Parental environment referred to the captive (F₁ captive) and wild environmental origin of the parents. Linear models were used for normal data and weighted binomial generalized linear models were used for proportional data. If a binomial model was overdispersed, the model was reconstructed as a quasi-binomial model. Nonsignificant effects, starting with nonsignificant interactions, were removed one at a time after an analysis of variance of a linear model, or an analysis of deviance of a binomial or quasi-binomial model.

Individual maternal effects (i.e., maternal ID) and individual paternal effects (i.e., paternal ID), if retained by the selection process, were examined for significant differences among parental cross types (i.e., ECO, F₁ E.S, GRV, F₁

Table 2. Final model structures for analysing nuisance effects and simple maternal effects for early-life history traits.

Trait	Error*	Link	Position/tank ID	Density	Maternal length [†]
Egg survival					
At Coldbrook	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
At day 33	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
Alevin survival					
At day 59	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
Development time					
At 50% hatch	Normal	Identity	ANOVA	Pearson	LM, Pearson
Size					
Egg diameter	Normal	Identity	ANOVA	Pearson	LM, Pearson
Length at hatch	Normal	Identity	ANOVA	Pearson	LM, Pearson
Yolk sac volume	Normal	Identity	ANOVA	Pearson	LM, Pearson
Length at yolk absorption	Normal	Identity	ANOVA	Pearson	LM, Pearson
Energy conversion					
Specific growth rate	Normal	Identity	ANOVA	Pearson	LM, Pearson
Yolk sac conversion efficiency	Normal	Identity	ANOVA	Pearson	LM, Pearson

Note: GLM, generalized linear model; ANODEV, analysis of deviance of GLM. LM, linear model; ANOVA, analysis of variance; Pearson, Pearson correlation.

*Quasi-binomial, error correction for overdispersion, i.e., originally binomial models that contained residual scaled deviance degrees of freedom that were roughly not equal to the residual degrees of freedom.

[†]Pearson correlation was examining the correlation of individual maternal effect sizes (from the GLM or LM) and maternal length.

G.S, and STW) and parental environments (i.e., captive and wild). Significant differences among the parental cross types and parental environments were determined by plotting the 95% confidence intervals (CIs) of the effect sizes.

Cross type effects in simple models

Early-life history traits were examined for differences among cross types, using simple models. Any parental effect variable (e.g., maternal ID, paternal ID, maternal environment, and paternal environment), except maternal length, in the final model was removed and replaced with a single cross type effect variable (cross type) to generate the simple model. In addition, any variables of tray position or tank ID in the final model were modeled as random effects in the simple model. Linear, binomial, or quasi-binomial models were used for models that did not include random effects. For models that did include random effects, linear mixed-effects models, using the function `lmer` in the `lme4` package of R, were used for normal data. Proportional data were logit-transformed to increase normality (Crawley 2005), using `logit` in the `car` package of R, before being used in linear mixed-effects models. All mixed-effects models used restricted maximum likelihood estimation and Laplace approximations to estimate parameter values.

Significant differences among the effect sizes for cross type were determined by plotting the CIs of the effect sizes. For mixed-effects models, CIs were generated by Markov Chain Monte Carlo sampling of the posterior distribution of effect size estimates, using `mcmcsm` with 5000 samples and `HPDinterval` functions in the `lme4` package.

Cross type effects in mixed-effects models

If individual maternal effects or individual paternal effects were generated by model selection along with a cross type effect, there were missing effect sizes for some of the cross

types. This was most likely due to a loss of statistical power because of the loss of families at Coldbrook that resulted in the loss of information on females and males within cross types. To obtain cross type effect estimates, individual maternal and paternal effects were treated as two random intercepts in mixed-effects models. Tray position and individual tank effects, if present in model selection, were also treated as random intercepts. Any significant variables that remained from model selection were treated as fixed effects in the mixed model. Significant differences among the effect sizes for cross type were determined by plotting the CIs of the effect sizes.

Results

Although nuisance factors, such as tank effects and density, had a significant influence on some early-life history traits (Table 3), few were selected in the final models when they were included in the list of variables for model selection (Table 4). In addition, the model selection procedure revealed that most early-life history traits were significantly influenced by maternal effects. Furthermore, in the simple models, there were some significant differences between pure cross types and those resulting inbred and outbred cross types (Fig. 3). However, there were no significant cross type-level differences between inbred and pure cross types, or between outbred and pure cross types, once individual parental effects had been incorporated into mixed-effects models. Asterisks denote significant differences (i.e., non-overlapping confidence intervals) and “ns” denotes non-significant differences (i.e., overlapping confidence intervals). Individual parental effects extracted from the final model were grouped to examine confidence intervals for the parental cross type and parental environment result columns. Cross type effect column displays the confidence interval

Table 3. Nuisance effects examined and relationship with maternal length for early-life history traits.

Trait	Position/tank ID, <i>p</i>	Density, <i>p</i>	Maternal length, <i>p</i>
Egg survival			
At Coldbrook	<<0.001*	0.159	0.002*, +
At the Aquatron (day 33)	<<0.001*	0.004*, –	0.267
Alevin survival			
At day 59	0.141	0.003*, +	0.272
Development time			
At 50% hatch	0.042*	<<0.001*, 0.27	0.161
Size			
Egg diameter	<<0.001*	<<0.001*, 0.14	<<0.001*, 0.82
Length at hatch	<<0.001*	<<0.001*, 0.09	0.050*, 0.28
Yolk sac volume	<<0.001*	<<0.001*, 0.20	<<0.001*, 0.80
Length at yolk absorption	<<0.001*	<<0.001*, –0.24	0.117
Energy conversion			
Specific growth rate	0.042*	0.032*, –0.15	–0.776
Yolk sac conversion efficiency	0.010*	<<0.001*, –0.32	<<0.001*, –0.67

Note: Displayed are *p*-values and Pearson correlations or the direction of the relationship. Asterisks denote significant *p*-values.

results for the cross type effect in a mixed-effects model (different than the final model) that treated individual parental effects as random effects.

Egg survival

We found no evidence of significant inbreeding or outbreeding effects on egg survival at Coldbrook (fertilization to eyed stage), although a cross type effect was detected in model selection. However, upon examination of the cross type CIs, there were no significant egg survival differences between inbred and pure cross types, outbred and pure cross types, or inbred and outbred cross types in mixed-effect models (Fig. 4). While there was a positive correlation between maternal length and egg survival, when maternal length was included in the list of variables for model selection, it was not selected. In addition, there was a significant effect of tray position in the incubation trough on egg survival at the Coldbrook. The egg mortality may have been caused by vibrations generated at the base of the incubation trough by the formalin dispenser, which could have disrupted egg development (Purser and Forteach 2003).

We found no evidence for differential egg survival at the Aquatron at day 33 (eyed stage to hatch) among cross types. While there was a significant negative correlation between initial egg density and egg survival, when initial egg density was included in the list of variables for model selection, it was not selected.

Alevin survival

There was no evidence for differential alevin survival at day 59 (hatched until yolk sac absorption) among cross types. However, small but significant maternal cross type effects were detected. Alevins with ECO mothers had higher survival (95% CI difference; $1.67 \pm 1.00\%$) than alevins with F₁ G.S mothers (Fig. 5). While there was a significant positive correlation between initial egg density and alevin

survival, when initial egg density was included in the list of variables for model selection, it was not selected.

Development time at 50% hatch

We found no evidence for differential development time at 50% hatch among cross types. However, significant maternal environment effects were detected, with offspring of captive mothers requiring more time (95% CI difference; 9.71 ± 6.21 degree-days) to hatch than offspring of wild mothers (Fig. 5). While there was a significant positive correlation between initial egg density and the development time at 50% hatch, individual maternal effects (i.e., maternal ID) were more important than density effects in model selection.

Size of eggs, alevins at hatch, yolk sac, and alevins at yolk sac absorption

We found no evidence of significant inbreeding or outbreeding effects on size traits. A cross type effect was detected for all size traits in model selection, however, upon examination of the cross type CIs, there were no significant size trait differences between inbred and pure cross types, outbred and pure cross types, or inbred and outbred cross types in mixed-effect models (Fig. 4). However, significant maternal cross type effects were detected (Fig. 5). Offspring with F₁ G.S mothers had larger egg diameter (95% CI difference; F₁ E.S = 0.30 ± 0.17 , GRV = 0.31 ± 0.17 mm), yolk sac volume (ECO = 15.75 ± 7.54 , F₁ E.S = 10.83 ± 7.11 , GRV = 11.99 ± 7.11 mm³), and length at yolk absorption (F₁ E.S = 2.00 ± 1.11 , GRV = 2.38 ± 1.11 mm), than offspring with ECO (yolk sac volume only), F₁ E.S, and GRV mothers. In addition, significant paternal cross type effects were detected for egg diameter only. Offspring with STW fathers had a smaller egg diameter (F₁ E.S = 0.09 ± 0.05 , GRV = 0.08 ± 0.05 , F₁ G.S = 0.12 ± 0.05 mm) than offspring sired by the remaining paternal cross types, except for offspring with ECO fathers.

Table 4. Model selection results, parental effects, and cross type effects for early-life history traits.

Trait	Final model	Maternal cross type	Maternal environ.	Paternal cross type	Paternal environ.	Cross type effect
Egg survival						
At Coldbrook	Tray position + maternal ID + paternal ID + cross type	ns	ns	ns	ns	*
At the Aquatron (day 33)	Tank ID + maternal ID + paternal ID	ns	ns	ns	ns	
Alevin survival						
At day 59	Maternal ID	*	ns			
Development time						
At 50% hatch	Maternal ID + initial egg density	ns	*			
Size						
Egg diameter	Maternal length + maternal ID + paternal ID + cross type	*	ns	*	ns	ns
Length at hatch	Maternal length + maternal ID + paternal ID + cross type	ns	ns	ns	ns	ns
Yolk sac volume	Maternal length + maternal ID + paternal ID + cross type	*	ns	ns	ns	ns
Length at yolk absorption	Maternal ID + paternal ID + cross type	*	ns	ns	ns	*
Energy conversion						
Specific growth rate	Maternal cross type	*				
Yolk sac conversion efficiency	Maternal length + maternal cross type	ns				

These size trait models defined maternal length as the first variable because of the strong correlations between maternal length and size traits, except length at yolk absorption. In addition, there were significant individual tank effects and a significant positive correlation between initial egg density and size traits. However, tank ID and initial egg density were not included in the list of variables for model selection because the individual tank effects probably reflected the haphazard placement of different sized families (e.g., smaller captive vs. larger wild mother egg diameters) within tanks while the density effect reflected the correlation of maternal length with size traits given that maternal length was also correlated with initial egg density (Pearson correlation, $r = 0.493$, $p = \ll 0.001$).

Specific growth rate and yolk sac conversion efficiency

We found no evidence for differential specific growth rate and yolk sac conversion efficiency among cross types. However, for both specific growth rate and yolk sac conversion efficiency, there were significant maternal cross type effects detected. Offspring with STW mothers experienced a faster specific growth rate (95% CI difference; $ECO = 0.022 \pm 0.013$, $GRV = 0.021 \pm 0.014$) than offspring produced by ECO and GRV mothers (Fig. 5). For yolk sac conversion efficiency, the offspring of STW mothers expressed a higher yolk sac conversion efficiency ($ECO = 0.026 \pm 0.054$, F_1 E.S. $= 0.030 \pm 0.050$, $GRV = 0.023 \pm 0.054$, F_1 G.S. $= 0.038 \pm 0.049$) than the offspring of other maternal cross types, although this difference was not significant.

The model for yolk sac conversion efficiency defined maternal length as the first variable, similar to the analysis of size traits, because of the strong negative correlation between yolk sac conversion efficiency and maternal length. In addition, there were significant individual tank effects and significant negative correlations between initial egg density and energy conversion traits. However, tank ID and initial egg density were not included in the list of variables for model selection for the same reasons as they were not included for size trait model selection.

Discussion

Our objective was to evaluate the relative fitness of inbred and inter-population outbred cross types, using three small neighbouring populations of endangered Atlantic salmon under common-environmental conditions in captivity. A salient feature of our work is that no significant differences between inbred and pure cross types, outbred and pure cross types, or inbred and pure cross types were detected among the fitness-related traits under examination. However, most traits were influenced by parental effects that needed to be incorporated into our analyses before potential differences among cross types could be adequately tested.

Parental effects

Individual parental effects were more important than cross type effects in explaining the variance among families. That is, using model selection, individual parental effects, maternal ID and sometimes paternal ID, would appear before a cross type effect, cross type. In addition, parental effects present in the cross types gave, in some instances, the false impression of significant cross type differences when parental effects were not incorporated into the models. Furthermore, these parental effects were present in cross types that had been generated by considerable numbers of females and males (8–10 individual females or males per cross type). Sometimes parental effects may be ignored in the comparison of cross types generated by several females and males because they are believed not to have a large influence of the mean value of the cross type (e.g., Gharrett et al. 1999; McGinnity et al. 2003; Gilk et al. 2004). The present work can be added to the growing number of studies that have detected individual paternal effects in early-life history traits of salmon (e.g., Pakkasmaa et al. 2001; Petersson and Järvi 2007; Wedekind et al. 2008), a finding that challenges the traditional assumption that there are few individual paternal effects in salmonid early-life history traits (Miller et al. 2004; Smoker et al. 2004; Green 2008).

The individual-specific parental effects that were detected in the present study have also been detected in other studies

Fig. 3. Cross type effect size estimates using simple models. Displayed are 95% confidence intervals, (a) egg survival at Coldbrook, (b) egg survival at the Aquatron (day 33), (c) alevin survival at day 59, (d) development time at 50% hatch, (e) egg diameter, (f) length at hatch, (g) yolk sac volume, (h) length at yolk absorption, (i) specific growth rate, (j) yolk sac conversion efficiency. Numbers at the top are the sample sizes for the cross types. Cross type symbols: ECO and E, Economy; GRV and G, Great Village; and STW and S, Stewiacke; F₁, first-generation outbred; F₂, second-generation outbred (F₁ × F₁); BC₁, backcross outbred (pure × F₁); 1/4, inbreeding coefficient of 0.25; and 1/8, inbreeding coefficient of 0.125.

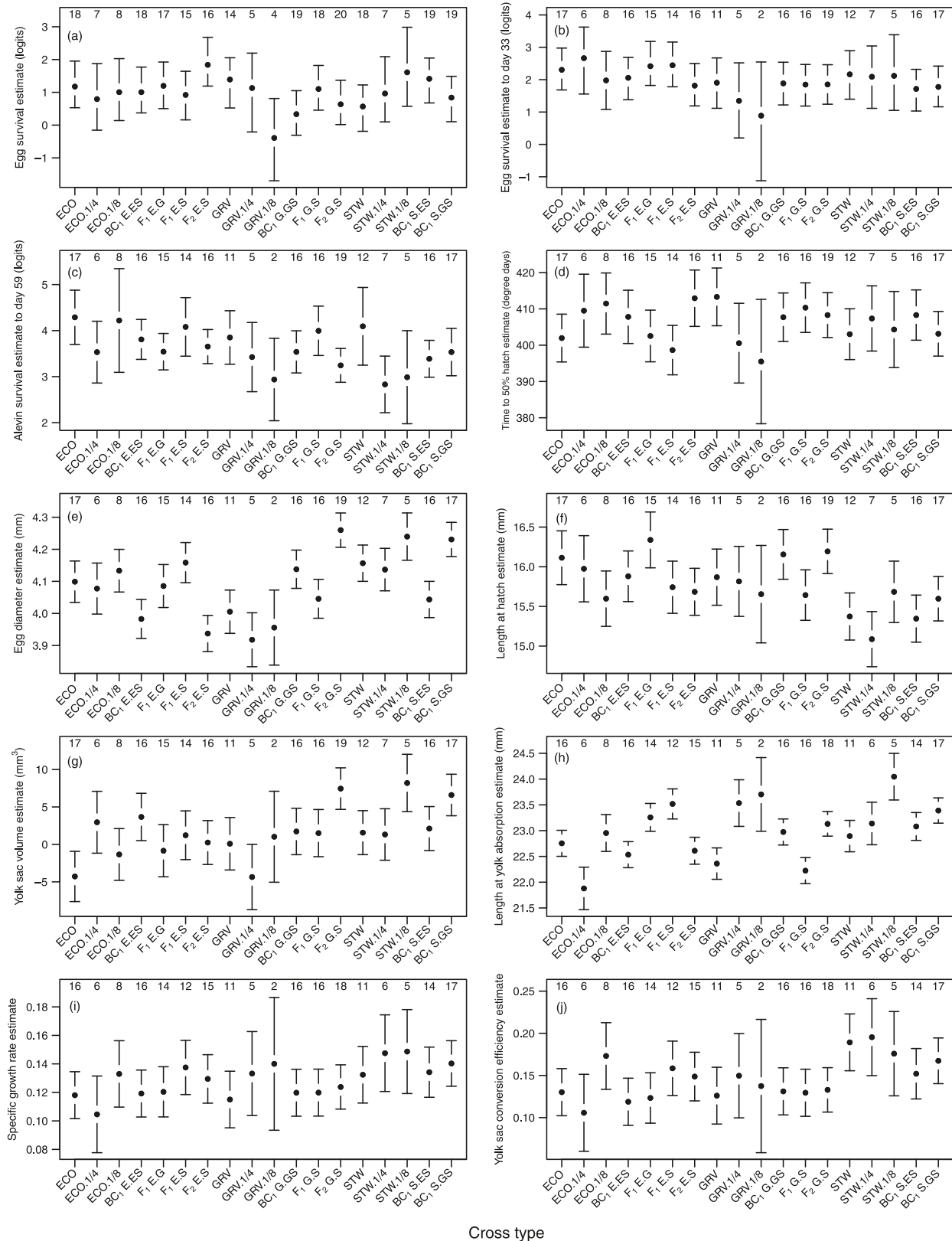
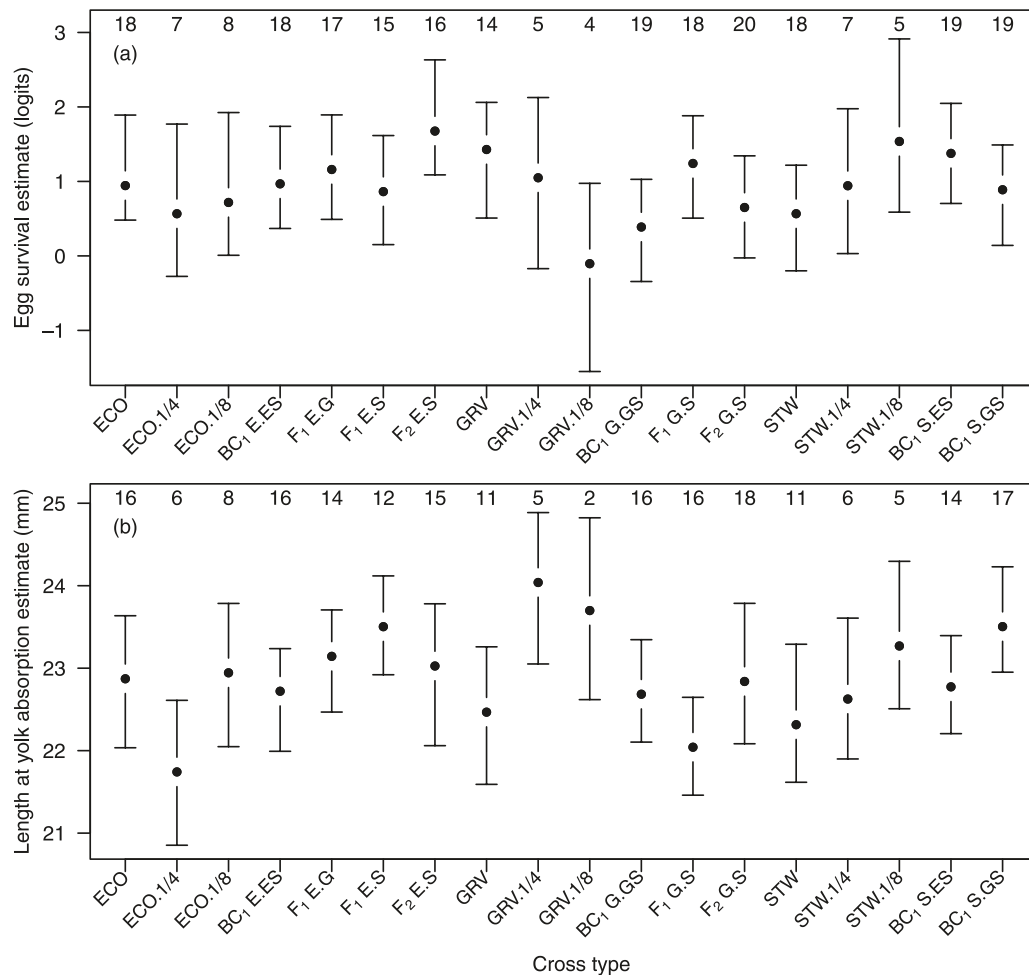


Fig. 4. Cross type effect sizes estimates using mixed-effects models. Displayed are 95% confidence intervals, (a) egg survival at Coldbrook, and (b) length at yolk absorption. Cross type symbols: ECO and E, Economy; GRV and G, Great Village; and STW and S, Stewiacke; F₁, first-generation outbred; F₂, second-generation outbred (F₁ × F₁); BC₁, backcross outbred (pure × F₁); 1/4, inbreeding coefficient of 0.25; and 1/8, inbreeding coefficient of 0.125.



of salmonids. Egg and alevin survival, for example, are known to be affected by individual maternal effects (e.g., Nagler et al. 2000; Vandeputte et al. 2002; Smoker et al. 2004). However, these same studies did not detect individual paternal effects on egg survival. In the case of egg survival at Coldbrook, individual paternal effects may have been detected because individual fathers differ in their ability to fertilize eggs (e.g., sperm velocity, Gage et al. 2004; match to female major histocompatibility alleles, Yeates et al. 2009) and because unfertilized eggs were indistinguishable from dead eggs (representing two different parental effects) in our analyses. Nevertheless, others have detected individual paternal effects on the survival of fertilized eggs in salmonids (e.g., Wedekind et al. 2001; Granath et al. 2004; Wedekind et al. 2008).

Offspring size traits are known to be affected by maternal effects, such as body length (e.g., Beacham and Murray 1985; Bailey and Loudenslager 1986; Berg et al. 2001), and by paternal effects of smaller effect (e.g., Refstie and Steine 1978; Pakkasmaa et al. 2001; Gilbey et al. 2005). In our study, offspring energy conversion traits were not affected by individual maternal effects, contrary to the findings of

Heath et al. (1993), while specific growth rate was not affected by maternal length, a finding similar to that reported by Gilbey et al. (2009). Furthermore, there was a significant negative correlation of yolk sac conversion efficiency with maternal length. It is possible that the energy contained within yolk reserves was directed towards mass growth rather than towards length growth in the offspring of larger females, given that all offspring were approximately the same length at the time of release (mean \pm 1 SD, 22.9 \pm 2.0 mm). This is consistent with research that has found a positive relationship between offspring weight gain and maternal length (e.g., Ojanguren et al. 1996; Vandeputte et al. 2002).

Differences between the offspring of captive and wild parents

There may have been other genetic or epigenetic differences between captive and wild parents that may have affected offspring traits. The “captive” parents were born in captivity, and had spent one generation in captivity (F₁ captive), whereas the “wild” parents were born in the wild, collected as juveniles, and had spent the remainder of their lives in captivity. The difference in birth and rearing environments

Fig. 5. Individual parental effects estimates grouped by parental cross type and parental environment. Displayed are 95% confidence intervals, (a) egg survival at Coldbrook, (b) egg survival at the Aquatron at the Aquatron (day 33), (c) alevin survival at day 59, (d) development time at 50% hatch, (e) egg diameter, (f) length at hatch, (g) yolk sac volume, (h) length at yolk absorption, (i) specific growth rate, (j) yolk sac conversion efficiency. Parental cross type symbols: ECO and E, Economy; GRV and G, Great Village; and STW and S, Stewiackie; and F₁, first-generation outbred. Parental environment symbols are C, captive parent; and W, wild parent.

may have created different maternal and other epigenetic effects between captive and wild parents (Kawecki and Ebert 2004). The one additional generation of domestication selection might have caused genetic differences to have arisen between captive and wild parents because of selection resulting from mate choice in natural spawning environments, compared with the lack thereof in artificial spawning circumstances (Fleming 1994; Wedekind 2002; Pitcher and Neff 2007) or because of the increased expression of rare deleterious alleles in captive populations relative to those found in the wild (Frankham 2008). The above differences may translate into a smaller egg size produced by captive adults relative to those produced by wild adults when examined in the laboratory environment (Heath et al. 2003; Jonsen et al. 1996). However, in the present study there was no significant parental environment effect on egg diameter. There were also no significant parental environment effects on egg or alevin survival.

Regarding the other fitness-related traits examined here, there were no genetic or epigenetic effects that have not been documented previously, with the exception that offspring produced by wild mothers required fewer degree-days to reach the 50% hatch stage. The difference between offspring of both captive and wild mothers was up to 5 degree-days, which would translate to a difference of approximately 1–2 days in the wild, which may have minimal biological significance. One additional consideration is that wild parents may have developed as embryos at colder temperatures in the wild than did captive parents in captivity, which may have been manifested by different maternal or epigenetic effects (see Kawecki and Ebert 2004). In other words, the offspring of wild parents may be “programmed” to develop at faster rate per degree-day than the offspring of captive parents.

Risks of inbreeding vs. outbreeding

After accounting for the influences of maternal length and (or) individual parental effects, inbred and outbred cross types did not differ significantly from their respective pure cross types at a multitude of early-life history traits (165 comparisons per trait).

The lack of significant genetic differentiation between inbred, outbred, and pure cross types in survival may be attributable, in part, to the comparatively benign rearing conditions of the laboratory relative to those that would be experienced by individuals in the wild (Miller 1994; Crnokrak and Roff 1999; Bijlsma et al. 2000). In addition, the outbred cross types, ideally, should have been tested in their pure parental local environments to evaluate the potential for a loss of local adaptation, which is one mechanism by which outbreeding depression can be generated (Kawecki and Ebert 2004; McClelland and Naish 2007). By contrast, one considerable advantage associated with laboratory studies for these early-life stages is the increased accuracy and precision with which survival can be estimated. Further-

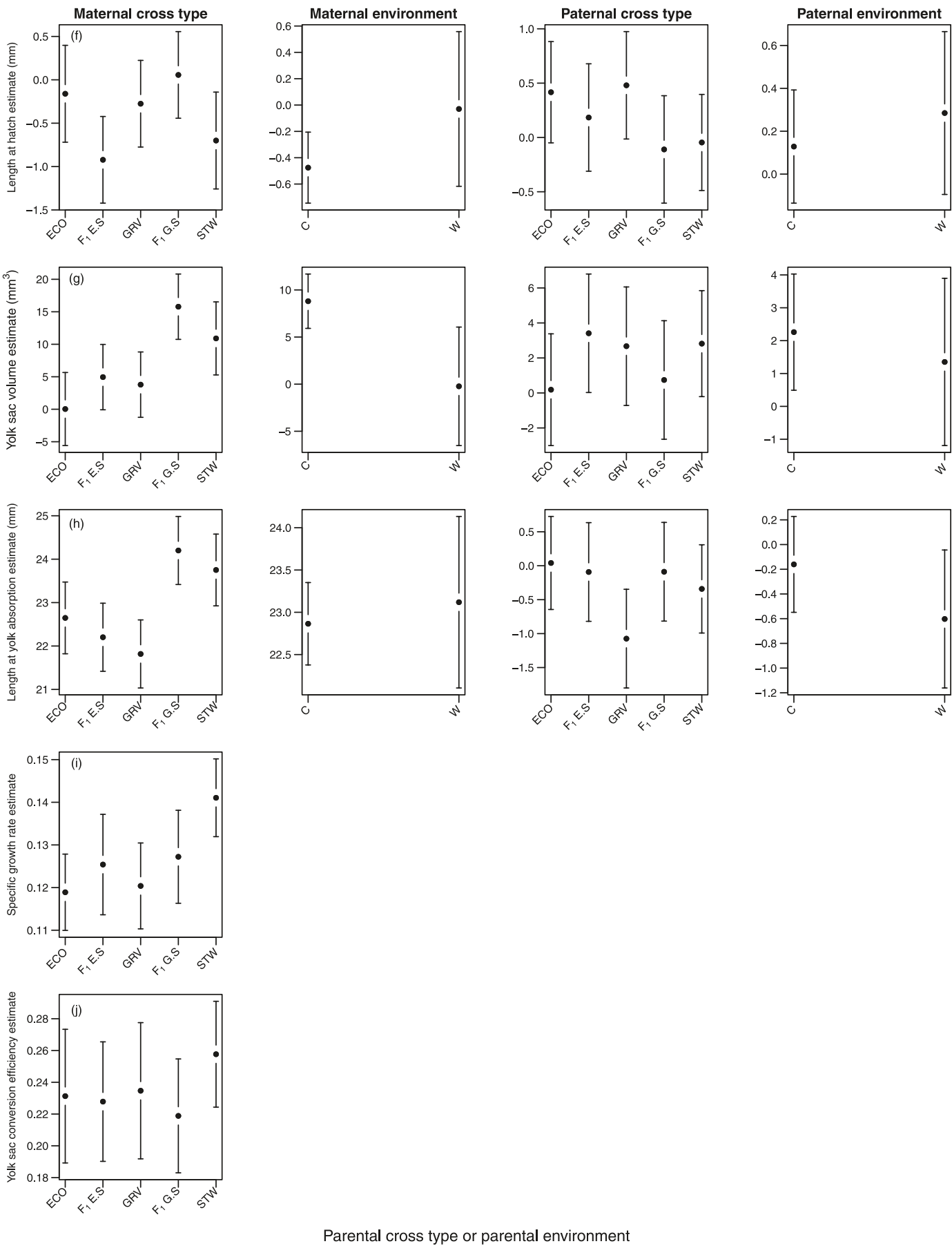
more, there may be additional factors in the wild, such as size-based dispersal (McGinnity et al. 1997), which might result in a nonrandom shift of some fish out of the sampling area; such sampling biases can be eliminated in the laboratory. An additional consideration is that the magnitudes of detrimental outbreeding effects documented in a laboratory setting are likely to be more severe in the wild (Edmands 2007).

Notwithstanding the laboratory environment caveats, we note that inbreeding depression has been documented in rainbow trout (*Oncorhynchus mykiss*) in the laboratory (Kincaid 1976a, 1976b; Gjerde et al. 1983). We are also cognizant of the fact that inbreeding coefficients are a function of current consanguineous matings and inbreeding history (Allendorf and Luikart 2007). Thus, our assumption of a base population inbreeding coefficient of $F = 0$ might not have been valid for ECO and GRV, given their recent population bottlenecks (see Wang et al. 2002). Yet, if ECO and GRV populations were more inbred than the STW population, there was certainly no indication of this in our results: significantly greater inbreeding depression was not detected in the pure or inbred cross types for ECO and GRV populations relative to the pure cross type or inbred cross types of the STW population. On the other hand, assuming greater fixations of deleterious recessive alleles from previous inbreeding for ECO and GRV, inbreeding depression may not have been detected for these two populations because the magnitude of difference in traits between pure and inbred cross types is expected to be much higher in STW relative to ECO and GRV (Allendorf and Luikart 2007).

Significant genetic differences between outbred and pure cross types may also not have resulted from outbreeding in our study because of small genetic differentiation and potentially high gene flow between the Inner Bay of Fundy populations (see Materials and Methods for F_{ST} values). That is, the mixing of populations that are similar genetically and have a history of interbreeding may not express outbreeding depression or heterosis (Edmands and Timmerman 2003; Kawecki and Ebert 2004; Tallmon et al. 2004).

Interestingly, there were differences observed between the offspring of F₁ outbred parents and those of pure parents. For example, F₁ G.S mothers produced offspring that were ~0.3 mm larger in egg diameter than F₁ E.S and GRV mothers. Also, there were paternal cross type effects for egg diameters. That is, F₁ E.S and F₁ G.S fathers produced offspring that were larger in egg diameter than the offspring of STW fathers. These egg size enlargements could be a product of heterosis, given that larger size is associated with higher early-life fitness (Wallace and Aasjord 1984; Beacham and Murray 1985; Einum and Fleming 1999). However, these enlargements, if biologically meaningful, could equally be considered outbreeding depression if smaller egg size in STW salmon represents an adaptive response to the local environment (cf., Hutchings 1991; Taylor 1991; Garcia de Leaniz et al. 2007). However, we are unaware of any ma-





Parental cross type or parental environment

for differences in the three populations' habitats that might account for a smaller size being favoured in the Stewiacke River.

Overall, there appear to be no fitness-related advantages or disadvantages to inbreeding or outbreeding these three populations, in this common-environment laboratory setting and at the early-life stages examined. These results should be interpreted with caution since the relative life-time fitness of these crosses should be measured in more natural settings or, ideally, the local environments. In addition, without prior knowledge on egg size selection in the local environment of these populations, it cannot be determined whether the changes in egg size are a fitness-related advantage or disadvantage. Our work identifies a need to undertake comparative studies of the survival and fitness-related traits between cross types in the natural environment.

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References

- Allendorf, F.W., and Luikart, G. 2007. Conservation and the genetics of populations. Blackwell Publishing, Oxford, UK.
- Bailey, J.K., and Loudenslager, E.J. 1986. Genetic and environmental components of variation for growth of juvenile Atlantic salmon. *Aquaculture*, **57**(1-4): 125–132. doi:10.1016/0044-8486(86)90189-4.
- Beacham, T.D., and Murray, C.B. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. *Can. J. Fish. Aquat. Sci.* **42**(11): 1755–1765. doi:10.1139/f85-220.
- Berg, O.K., Hendry, A.P., Svendsen, B., Bech, C., Arnekleiv, J.V., and Lohrmann, A. 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic salmon families. *Funct. Ecol.* **15**(1): 13–23. doi:10.1046/j.1365-2435.2001.00473.x.
- Bijlsma, R., Bundgaard, J., and Boerema, A.C. 2000. Does inbreeding affect the extinction risk of small populations? Predictions from *Drosophila*. *J. Evol. Biol.* **13**(3): 502–514. doi:10.1046/j.1420-9101.2000.00177.x.
- Charlesworth, D., and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**(1): 237–268. doi:10.1146/annurev.es.18.110187.001321.
- Crawley, M.J. 2005. Statistics: An introduction using R. John Wiley & Sons Ltd., London, UK.
- Crnokrak, P., and Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity*, **83**(3): 260–270. doi:10.1038/sj.hdy.6885530.
- DFO. 2008. Recovery potential assessment for Inner Bay of Fundy Atlantic salmon. Canadian Science Advisory Report 2008/050. Department of Fisheries and Oceans, Dartmouth, NS.
- Dobzhansky, T. 1950. Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila-pseudoobscura*. *Genetics*, **35**: 288–302.
- Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution*, **53**(6): 1757–1768. doi:10.2307/2640438.
- Edmands, S. 2002. Does parental divergence predict reproductive compatibility? *Trends Ecol. Evol.* **17**(11): 520–527. doi:10.1016/S0169-5347(02)02585-5.
- Edmands, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **16**(3): 463–475. doi:10.1111/j.1365-294X.2006.03148.x.
- Edmands, S., and Timmerman, C.C. 2003. Modeling factors affecting the severity of outbreeding depression. *Conserv. Biol.* **17**(3): 883–892. doi:10.1046/j.1523-1739.2003.02026.x.
- Einum, S., and Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc. Biol. Sci.* **266**(1433): 2095–2100. doi:10.1098/rspb.1999.0893.
- Emlen, J.M. 1991. Heterosis and outbreeding depression - a multi-locus model and an application to salmon production. *Fish. Res.* **12**(3): 187–212. doi:10.1016/0165-7836(91)90095-W.
- Fleming, I.A. 1994. Captive breeding and the conservation of wild salmon populations. *Conserv. Biol.* **8**(3): 886–888. doi:10.1046/j.1523-1739.1994.08030863-13.x.
- Frankel, O.H. 1974. Genetic conservation - our evolutionary responsibility. *Genetics*, **78**: 53–65.
- Frankham, R. 2005. Genetics and extinction. *Biol. Conserv.* **126**(2): 131–140. doi:10.1016/j.biocon.2005.05.002.
- Frankham, R. 2008. Genetic adaptation to captivity in species conservation programs. *Mol. Ecol.* **17**(1): 325–333. doi:10.1111/j.1365-294X.2007.03399.x.
- Frankham, R., Ballou, J.D., and Briscoe, D.A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.
- Fraser, D.J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evol. Appl.* **1**: 535–586. doi:10.1111/j.1752-4571.2008.00036.x.
- Fraser, D.J., Jones, M.W., McParland, T.L., and Hutchings, J.A. 2007. Loss of historical immigration and the unsuccessful rehabilitation of extirpated salmon populations. *Conserv. Genet.* **8**(3): 527–546. doi:10.1007/s10592-006-9188-8.
- Fraser, D.J., Cook, A.M., Eddington, J.D., Bentzen, P., and Hutchings, J.A. 2008. Mixed evidence for reduced local adaptation in wild salmon resulting from interbreeding with escaped farmed salmon: complexities in hybrid fitness. *Evol. Appl.* **1**(3): 501–512. doi:10.1111/j.1752-4571.2008.00037.x.
- Fraser, D.J., Houde, A.S., Debes, P.V., O'Reilly, P., Eddington, J.D., and Hutchings, J.A. 2010. Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. *Ecol. Appl.* **20**(4): 935–953. doi:10.1890/09-0694.1.
- Gage, M.J.G., Macfarlane, C.P., Yeates, S., Ward, R.G., Searle, J.B., and Parker, G.A. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* **14**: 44–47.
- Garcia de Leaniz, C.G., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F., Webb, J.H., Vollestad, L.A., Villanueva, B., Ferguson, A., and Quinn, T.P. 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications

- tions for conservation. *Biol. Rev. Camb. Philos. Soc.* **82**(2): 173–211. doi:10.1111/j.1469-185X.2006.00004.x.
- Gharrett, A.J., Smoker, W.W., Reisenbichler, R.R., and Taylor, S.G. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. *Aquaculture*, **173**(1–4): 117–129. doi:10.1016/S0044-8486(98)00480-3.
- Gilbey, J., McLay, A., Houlihan, D., and Verspoor, E. 2005. Individual-level analysis of pre- and post first-feed growth and development in Atlantic salmon. *J. Fish Biol.* **67**(5): 1359–1369. doi:10.1111/j.0022-1112.2005.00831.x.
- Gilbey, J., Cauwelier, E., Jones, C.S., McLay, A., Noble, L.R., and Verspoor, E. 2009. Size-dependent growth of individual Atlantic salmon *Salmo salar* alevins from hatch to first feeding. *J. Fish Biol.* **75**(10): 2820–2831. doi:10.1111/j.1095-8649.2009.02497.x.
- Gilk, S.E., Wang, I.A., Hoover, C.L., Smoker, W.W., Taylor, S.G., Gray, A.K., and Gharrett, A.J. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environ. Biol. Fishes*, **69**(1–4): 287–297. doi:10.1023/B:EBFI.0000022888.28218.c1.
- Gjerde, B., Gunnes, K., and Gjerdem, T. 1983. Effects of inbreeding on survival and growth in rainbow trout. *Aquaculture*, **34**(3–4): 327–332. doi:10.1016/0044-8486(83)90212-0.
- Granath, K.L., Smoker, W.W., Gharrett, A.J., and Hard, J.J. 2004. Effects on embryo development time and survival of intercrossing three geographically separate populations of Southeast Alaska Coho salmon, *Oncorhynchus kisutch*. *Environ. Biol. Fishes*, **69**(1–4): 299–306. doi:10.1023/B:EBFI.0000022882.61940.21.
- Green, B.S. 2008. Maternal effects in fish populations. *Adv. Mar. Biol.* **54**: 1–105. doi:10.1016/S0065-2881(08)00001-1.
- Heath, D.D., Bernier, N.J., Heath, J.W., and Iwama, G.K. 1993. Genetic, environmental, and interaction effects of growth and stress response of Chinook salmon (*Oncorhynchus tshawytscha*) fry. *Can. J. Fish. Aquat. Sci.* **50**(2): 435–442. doi:10.1139/f93-049.
- Heath, D.D., Heath, J.W., Bryden, C.A., Johnson, R.M., and Fox, C.W. 2003. Rapid evolution of egg size in captive salmon. *Science*, **299**(5613): 1738–1740. doi:10.1126/science.1079707.
- Hutchings, J.A. 1991. The threat of extinction to native populations experiencing spawning intrusions by cultured Atlantic salmon. *Aquaculture*, **98**(1–3): 119–132. doi:10.1016/0044-8486(91)90377-J.
- Jonsson, N., Jonsson, B., and Fleming, I.A. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? *Funct. Ecol.* **10**(1): 89–96. doi:10.2307/2390266.
- Kawecki, T.J., and Ebert, D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* **7**(12): 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x.
- Kincaid, H.L. 1976a. Effect of inbreeding on rainbow trout populations. *Trans. Am. Fish. Soc.* **105**(2): 273–280. doi:10.1577/1548-8659(1976)105<273:EOIORT>2.0.CO;2.
- Kincaid, H.L. 1976b. Inbreeding in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* **33**: 2420–2426.
- King, T.L., Eackles, M.S., and Letcher, B.H. 2005. Microsatellite DNA markers for the study of Atlantic salmon (*Salmo salar*) kinship, population structure, and mixed-fishery analyses. *Mol. Ecol. Notes*, **5**(1): 130–132. doi:10.1111/j.1471-8286.2005.00860.x.
- Koskinen, M.T., Haugen, T.O., and Primmer, C.R. 2002. Contemporary fisherian life history evolution in small salmonid populations. *Nature*, **419**(6909): 826–830. doi:10.1038/nature01029.
- Lynch, M. 2000. The limits to knowledge in quantitative genetics. *Evol. Biol.* **32**: 225–237.
- Mavarez, J., Audet, C., and Bernatchez, L. 2009. Major disruption of gene expression in hybrids between young sympatric anadromous and resident populations of brook charr (*Salvelinus fontinalis* Mitchell). *J. Evol. Biol.* **22**(8): 1708–1720. doi:10.1111/j.1420-9101.2009.01785.x.
- McClelland, E.K., and Naish, K.A. 2007. What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. *Conserv. Genet.* **8**(2): 397–416. doi:10.1007/s10592-006-9178-x.
- McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Ferguson, A. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in natural river environment. *ICES J. Mar. Sci.* **54**(6): 998–1008. doi:10.1016/S1054-3139(97)80004-5.
- McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Maoileidigh, N.ó, Baker, N., Cotter, D., O’Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc. Biol. Sci.* **270**(1532): 2443–2450. doi:10.1098/rspb.2003.2520.
- Metcalf, N.B., and Thorpe, J.E. 1992. Early predictors of life history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar*. *J. Fish Biol.* **41**(sb): 93–99. doi:10.1111/j.1095-8649.1992.tb03871.x.
- Miller, P.S. 1994. Is inbreeding depression more severe in a stressful environment? *Zoo Biol.* **13**(3): 195–208. doi:10.1002/zoo.1430130302.
- Miller, L.M., Close, T., and Kapuscinski, A.R. 2004. Lower fitness of hatchery and hybrid rainbow trout compared to naturalized populations in Lake Superior tributaries. *Mol. Ecol.* **13**(11): 3379–3388. doi:10.1111/j.1365-294X.2004.02347.x.
- Nagler, J.J., Parsons, J.E., and Cloud, J.G. 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **184**(1–2): 177–183. doi:10.1016/S0044-8486(99)00309-9.
- National Institutes of Health. 2008. ImageJ: Image processing and analysis in Java [analytical software]. Research Services Branch, National Institutes of Health, Bethesda, Md. Available from rsb.info.nih.gov/ij [accessed 24 June 2008].
- O’Reilly, P.T., and Harvie, C.J. 2009. Conservation of genetic variation in the inner Bay of Fundy Atlantic salmon captive breeding and rearing program. Canadian Science Advisory Secretariat Research Document 2009/095. Department of Fisheries and Oceans, Dartmouth, NS.
- O’Reilly, P.T., Hamilton, L.C., McConnell, S.K., and Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Can. J. Fish. Aquat. Sci.* **53**(10): 2292–2298. doi:10.1139/cjfas-53-10-2292.
- Ojanguren, A.F., Reyes-Gavilán, F.G., and Brāna, F. 1996. Effects of eggs size on offspring development and fitness in brown trout, *Salmo trutta* L. *Aquaculture*, **147**(1–2): 9–20. doi:10.1016/S0044-8486(96)01398-1.
- Pakkasmaa, S., Peuhkuri, N., Laurila, A., Hirvonen, H., and Ranta, E. 2001. Female and male contribution to egg size in salmonids. *Evol. Ecol.* **15**(2): 143–153. doi:10.1023/A:1013873810905.
- Paterson, S., Piernney, S.B., Knox, D., Gilbey, J., and Verspoor, E. 2004. Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (*Salmo salar* L.) microsatellites. *Mol. Ecol. Notes*, **4**(2): 160–162. doi:10.1111/j.1471-8286.2004.00598.x.
- Petersson, E., and Järvi, T. 2007. Characteristics of brown trout

- males influence growth and survival of their offspring. *J. Fish Biol.* **71**(2): 493–509. doi:10.1111/j.1095-8649.2007.01501.x.
- Pitcher, T.E., and Neff, B.D. 2007. Genetic quality and offspring performance in Chinook salmon: implications for supportive breeding. *Conserv. Genet.* **8**(3): 607–616. doi:10.1007/s10592-006-9204-z.
- Purser, J., and Forteach, N. 2003. Salmonids. In *Aquaculture: Farming Aquatic Animals and Plants*. Edited by J.S. Lucas and P.C. Southgate. Blackwell Publishing, Oxford, UK. pp. 295–320.
- Refstie, T., and Steine, T.A. 1978. Selection experiments with salmon III. Genetics and environmental sources of variation in length and weight in Atlantic salmon in the freshwater phase. *Aquaculture*, **14**(3): 221–234. doi:10.1016/0044-8486(78)90096-0.
- R Project for Statistical Computing. 2009. R [statistical software]. R Foundation for Statistical Computing, University of Vienna, Austria. Available from www.r-project.org [accessed 17 April 2009].
- Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., and Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**(6675): 491–494. doi:10.1038/33136.
- Smoker, W.W., Wang, I.A., Gharrett, A.J., and Hard, J.J. 2004. Embryo survival and smolt to adult survival in second-generation outbred Coho salmon. *J. Fish Biol.* **65**(s1): 254–262. doi:10.1111/j.0022-1112.2004.00550.x.
- Spielman, D., Brook, B.W., and Frankham, R. 2004. Most species are not driven to extinction before genetic factors can impact them. *Proc. Natl. Acad. Sci. U.S.A.* **101**(42): 15261–15264. doi:10.1073/pnas.0403809101.
- Taggart, J.B. 2007. FAP: an exclusion-based parental assignment program with enhanced predictive functions. *Mol. Ecol. Notes*, **7**(3): 412–415. doi:10.1111/j.1471-8286.2006.01616.x.
- Tallmon, D.A., Luikart, G., and Waples, R.S. 2004. The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.* **19**(9): 489–496. doi:10.1016/j.tree.2004.07.003.
- Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, **98**(1–3): 185–207. doi:10.1016/0044-8486(91)90383-I.
- Templeton, A.R. 1986. Coadaptation and outbreeding depression. In *Conservation Biology: The Science of Scarcity and Diversity*. Edited by M.E. Soule. Sinauer Associates Inc., Sunderland, Massachusetts. pp. 105–116.
- Tymchuk, W.V., O'Reilly, P., Bittman, J., MacDonald, D., and Schulte, P. 2010. Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. *Mol. Ecol.* **19**(9): 1842–1859. doi:10.1111/j.1365-294X.2010.04596.x.
- Utter, F. 2004. Population genetics, conservation and evolution in salmonids and other widely cultured fishes: some perspectives over six decades. *Rev. Fish Biol. Fish.* **14**(1): 125–144. doi:10.1007/s11160-004-3768-9.
- Vandeputte, M., Quillet, E., and Chevassus, B. 2002. Early development and survival in brown trout (*Salmo trutta fario* L.): indirect effects of selection for growth rate and estimation of genetic parameters. *Aquaculture*, **204**(3–4): 435–445. doi:10.1016/S0044-8486(01)00829-8.
- Vergeer, P., Sonderen, E., and Ouborg, N.J. 2004. Introduction strategies put to the test: local adaptation versus heterosis. *Conserv. Biol.* **18**(3): 812–821. doi:10.1111/j.1523-1739.2004.00562.x.
- Verspoor, E., O'Sullivan, M., Arnold, A.L., Knox, D., and Amiro, P.G. 2002. Restricted matrilineal gene flow and regional differentiation among Atlantic salmon (*Salmo salar* L.) populations within the Bay of Fundy, eastern Canada. *Heredity*, **89**(6): 465–472. doi:10.1038/sj.hdy.6800166.
- Wallace, J.C., and Aasjord, D. 1984. An investigation of the consequences of egg size for the culture of Arctic charr, *Salvelinus alpinus* (L.). *J. Fish Biol.* **24**(4): 427–435. doi:10.1111/j.1095-8649.1984.tb04813.x.
- Wang, S.Z., Hard, J., and Utter, F. 2002. Salmonid inbreeding: a review. *Rev. Fish Biol. Fish.* **11**(4): 301–319. doi:10.1023/A:1021330500365.
- Wedekind, C. 2002. Sexual selection and life history decisions: implications for supportive breeding and the management of captive populations. *Conserv. Biol.* **16**(5): 1204–1211. doi:10.1046/j.1523-1739.2002.01217.x.
- Wedekind, C., Müller, R., and Spicher, H. 2001. Potential genetic benefits of mate selection in whitefish. *J. Evol. Biol.* **14**(6): 980–986. doi:10.1046/j.1420-9101.2001.00349.x.
- Wedekind, C., Jacob, A., Evanno, G., Nusslé, S., and Müller, R. 2008. Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. *Proc. Biol. Sci.* **275**(1644): 1737–1744. doi:10.1098/rspb.2008.0072.
- Whitlock, M.C., Ingvarsson, P.K., and Hatfield, T. 2000. Local drift load and the heterosis of interconnected populations. *Heredity*, **84**(4): 452–457. doi:10.1046/j.1365-2540.2000.00693.x.
- WWF. 2001. The status of wild Atlantic salmon: a river by river assessment [online]. World Wildlife Fund. Available from assets.panda.org/downloads/salmon2.pdf [accessed 19 May 2010].
- Yeates, S.E., Einum, S., Fleming, I.A., Megens, H.-J., Stet, R.J.M., Hindar, K., Holt, W.V., Van Look, K.J.W., and Gage, M.J.G. 2009. Atlantic salmon eggs favour sperm in competition that have similar major histocompatibility alleles. *Proc. Biol. Sci.* **276**(1656): 559–566. doi:10.1098/rspb.2008.1257.