

# 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 F<sub>1</sub> 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 F<sub>1</sub>), 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

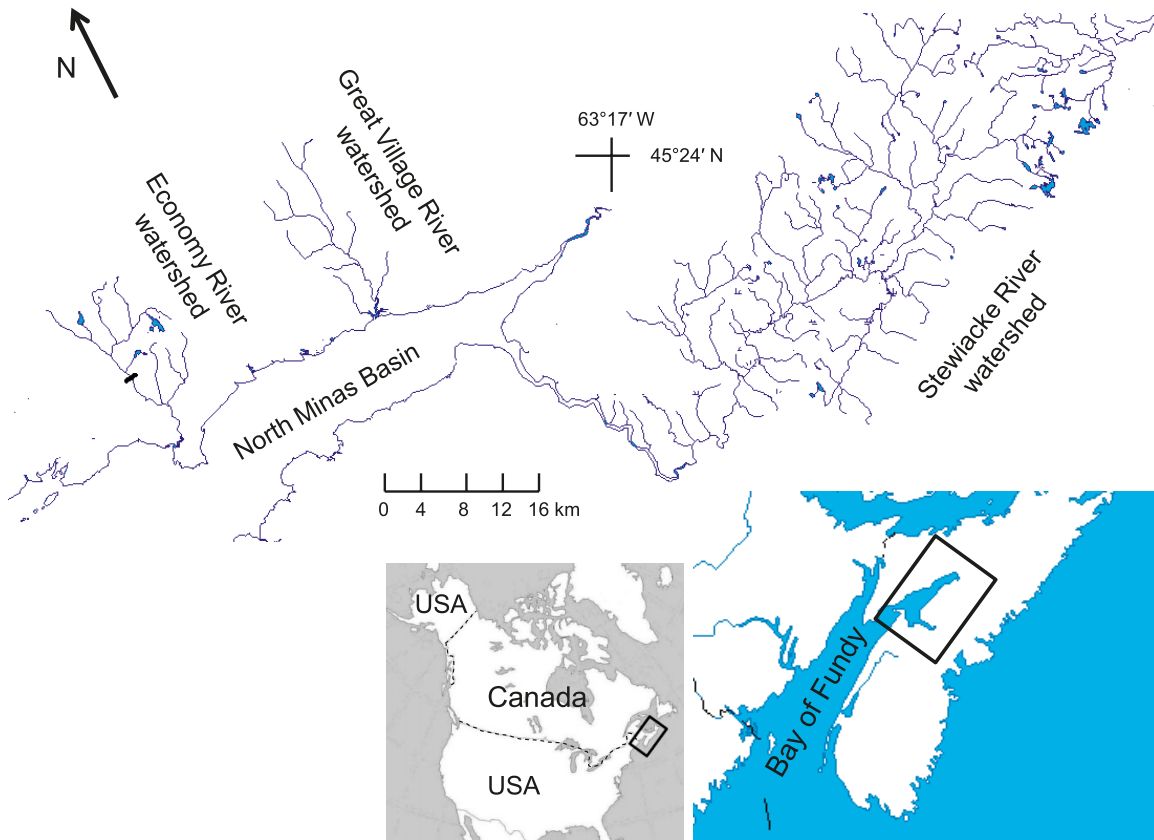
### Study populations

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 ( $\Phi_{ST}$ , 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<sup>®</sup> 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.

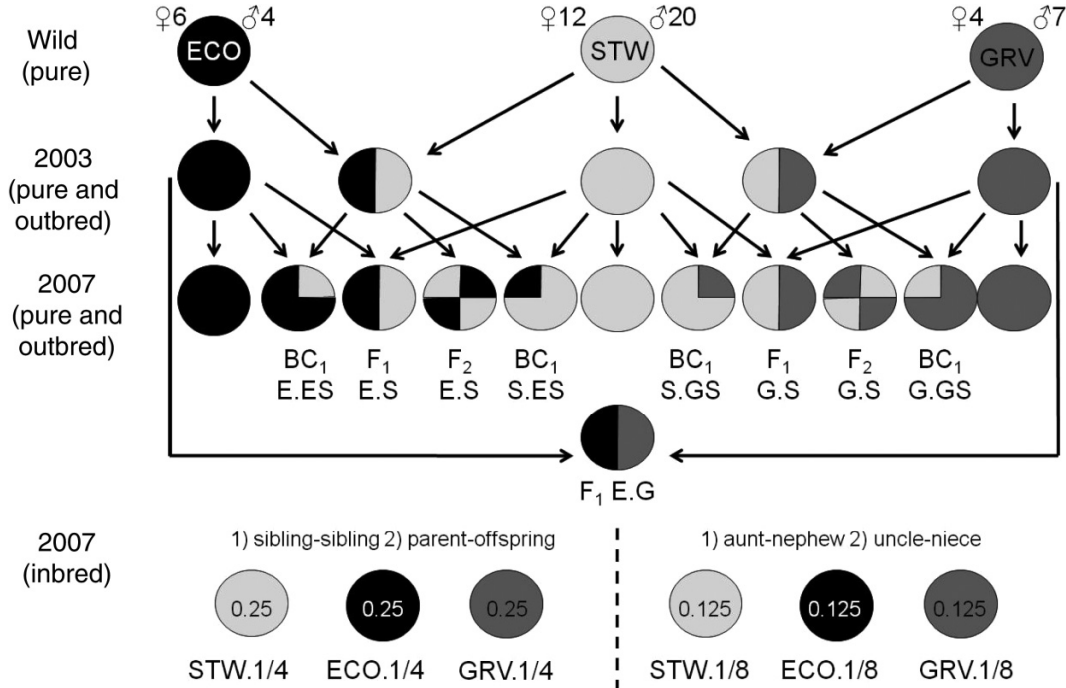
#### Generation of 2007 experimental cross types

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 ( $\sim 1$  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.

**Fig. 2.** Experimental cross types within (pure and inbred) and among (outbred) rivers. Cross type symbols: ECO and E, Economy (black); GRV and G, Great Village (dark grey); and STW and S, Stewiacke (light grey), F<sub>1</sub>, first-generation outbred; F<sub>2</sub>, second-generation outbred (F<sub>1</sub> × F<sub>1</sub>); BC<sub>1</sub>, back-cross outbred (pure × F<sub>1</sub>); 1/4, inbreeding coefficient of 0.25; and 1/8, inbreeding coefficient of 0.125. Arrows represent the parental cross types; ♀, number of wild females; and ♂, number of wild males. The proportion of genes from any one population is reflected by the area of the circle in outbred cross types. The numbers within circles represent inbreeding coefficients (*F*). The phrases above the circles for inbred cross types are examples of the matings used to generate that inbreeding coefficient.



As per the Coldbrook egg-rearing protocol, all eggs were treated with formalin (625 ppm) for 20 min twice a week to prevent the spread of fungus from unfertilized and dead eggs to live eggs. In total, 247 families were produced at Coldbrook.

**Measurement of early-life survival and fitness-related traits**

We first measured egg survival (fertilization to eyed stage and eyed stage to hatch) and alevin survival (post-hatch until yolk sac was completely absorbed) as direct measures of early-life survival. We then measured seven traits that may influence body size and, thus, survival at later life stages (e.g., fry to adult) in salmonids (Metcalf and Thorpe 1992; Pakkasmaa et al. 2001; Koskinen et al. 2002): development time to 50% hatch; egg diameter at eyed stage (307 degree-days); length at hatch; yolk sac volume at hatch; length at yolk sac absorption; specific growth rate and yolk sac conversion efficiency.

Eyed eggs from all cross types were transferred from Coldbrook to the Aquatron Facility, Dalhousie University (Halifax, N.S.) on 19 and 20 March 2008 (294 degree-days) because of rearing space constraints at Coldbrook. The transfer coincided with the development stage during which eggs can be safely transported without causing developmental harm (Purser and Forteach 2003). Because of an unforeseen technical problem at the Coldbrook Biodiversity Facility, 29 families exhibited 100% mortality, thus leaving 218 families at the Aquatron (Table 1). Owing to the mortality, individual maternal and individual paternal representa-

tion of the families was no longer balanced in comparing the cross types. At the Aquatron, the eggs were kept in modified Lee's<sup>®</sup> Kritter Keepers, perforated with 1.5" (3.81 cm) holes patched with pet screen to allow water flow, and divided into two sections (13.8 cm × 17 cm), using perforated plastic board. Coloured plastic beads (13 mm × 6 mm) with numerical identifiers were attached to the centre of each section. A family was haphazardly placed in a Kritter Keeper section and three Kritter Keepers occupied a 67.3 cm diameter × 45.7 cm height circular tank. The water temperature (nearest 0.1 °C) of each tank was recorded daily.

All dead and unfertilized eggs from the period at Coldbrook were removed on 21–23 March. We were unable to discern dead from unfertilized eggs, both of which represent different parental effects. For the period at the Aquatron, dead eggs and alevins were counted and removed daily at the same time of day from 24 March to 25 April for eggs (total of 33 days) and from 22 March to 19 May for alevins (total of 59 days). As the eggs hatched, the number of alevins was recorded daily at the same time of day from 19 March to 26 April. For families that contained large numbers of alevins, digital photographs were collected to precisely count alevins daily, using ImageJ version 1.38 (available from the Research Services Branch of the National Institutes of Health (US)). Development time at 50% hatch was measured in growing degree-days ( $\Delta D = \sum \text{°C per day}$ ) from the spawning date.

Digital photographs of the families were collected for the following: live egg counts and egg diameters on 22 and 23

**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 <sub>1</sub> and BC <sub>1</sub> outbred cross types			
Cross type	<i>N</i>	Females	Males	Females-group 1	Males-group 2	Females-group 2	Males-group 1
<b>Pure cross types (females × males)</b>							
ECO	17	9 (5)	10 (6)				
GRV	11	7 (2)	8 (5)				
STW	12	7 (0)	9 (2)				
<b>Inbred cross types (females × males)</b>							
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)				
<b>F<sub>1</sub> outbred cross types (females group 1 × males group 2 and females group 2 × males group 1)</b>							
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)
<b>BC<sub>1</sub> outbred cross types (females group 1 × males group 2 and females group 2 × males group 1)</b>							
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)
<b>F<sub>2</sub> outbred cross types (females × males)</b>							
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 F<sub>2</sub> outbred cross types, in which the majority were mated twice. Numbers in parentheses are the number of wild parents. F<sub>1</sub> and BC<sub>1</sub> 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–F<sub>1</sub> E.S (group 2) and females–F<sub>1</sub> 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)<sup>2</sup> × π/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 α = 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 (F<sub>1</sub> 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, F<sub>1</sub> E.S, GRV, F<sub>1</sub>

**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 <sup>†</sup>
<b>Egg survival</b>					
At Coldbrook	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
At day 33	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
<b>Alevin survival</b>					
At day 59	Quasi-binomial	Logit	GLM, ANODEV	GLM, ANODEV	GLM, Pearson
<b>Development time</b>					
At 50% hatch	Normal	Identity	ANOVA	Pearson	LM, Pearson
<b>Size</b>					
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
<b>Energy conversion</b>					
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.

<sup>†</sup>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>
<b>Egg survival</b>			
At Coldbrook	<<0.001*	0.159	0.002*, +
At the Aquatron (day 33)	<<0.001*	0.004*, –	0.267
<b>Alevin survival</b>			
At day 59	0.141	0.003*, +	0.272
<b>Development time</b>			
At 50% hatch	0.042*	<<0.001*, 0.27	0.161
<b>Size</b>			
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
<b>Energy conversion</b>			
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 F<sub>1</sub> 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;  $9.71 \pm 6.21$  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 F<sub>1</sub> G.S mothers had larger egg diameter (95% CI difference; F<sub>1</sub> E.S =  $0.30 \pm 0.17$ , GRV =  $0.31 \pm 0.17$  mm), yolk sac volume (ECO =  $15.75 \pm 7.54$ , F<sub>1</sub> E.S =  $10.83 \pm 7.11$ , GRV =  $11.99 \pm 7.11$  mm<sup>3</sup>), and length at yolk absorption (F<sub>1</sub> E.S =  $2.00 \pm 1.11$ , GRV =  $2.38 \pm 1.11$  mm), than offspring with ECO (yolk sac volume only), F<sub>1</sub> 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 (F<sub>1</sub> E.S =  $0.09 \pm 0.05$ , GRV =  $0.08 \pm 0.05$ , F<sub>1</sub> G.S =  $0.12 \pm 0.05$  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
<b>Egg survival</b>						
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	
<b>Alevin survival</b>						
At day 59	Maternal ID	*	ns			
<b>Development time</b>						
At 50% hatch	Maternal ID + initial egg density	ns	*			
<b>Size</b>						
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	*
<b>Energy conversion</b>						
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**

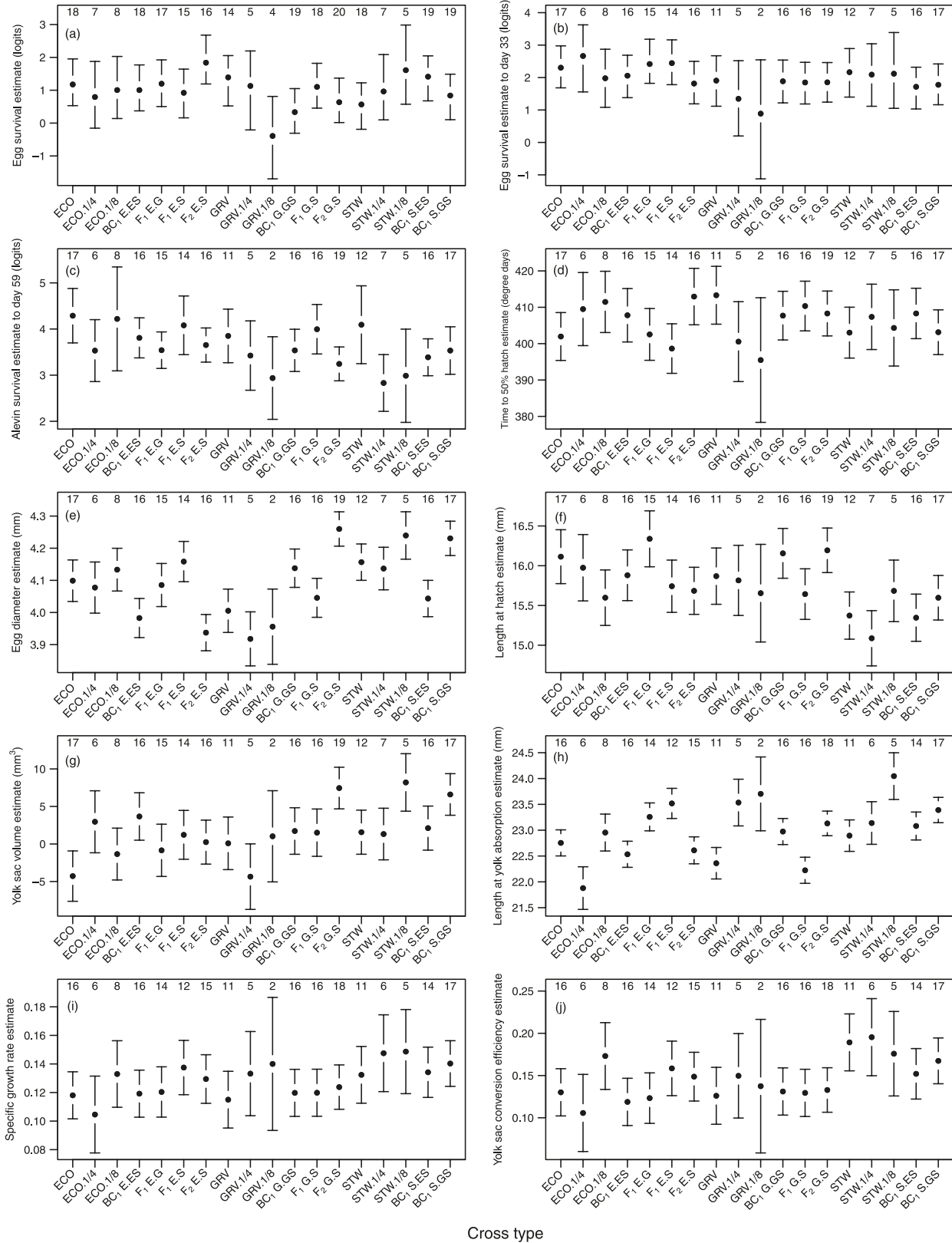
Our objective was to evaluate the relative fitness of inbred and inter-population outbred cross types, using three small neighbouring populations of endangered Atlantic salmon under common-environmental conditions in captivity. A salient feature of our work is that no significant differences between 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**

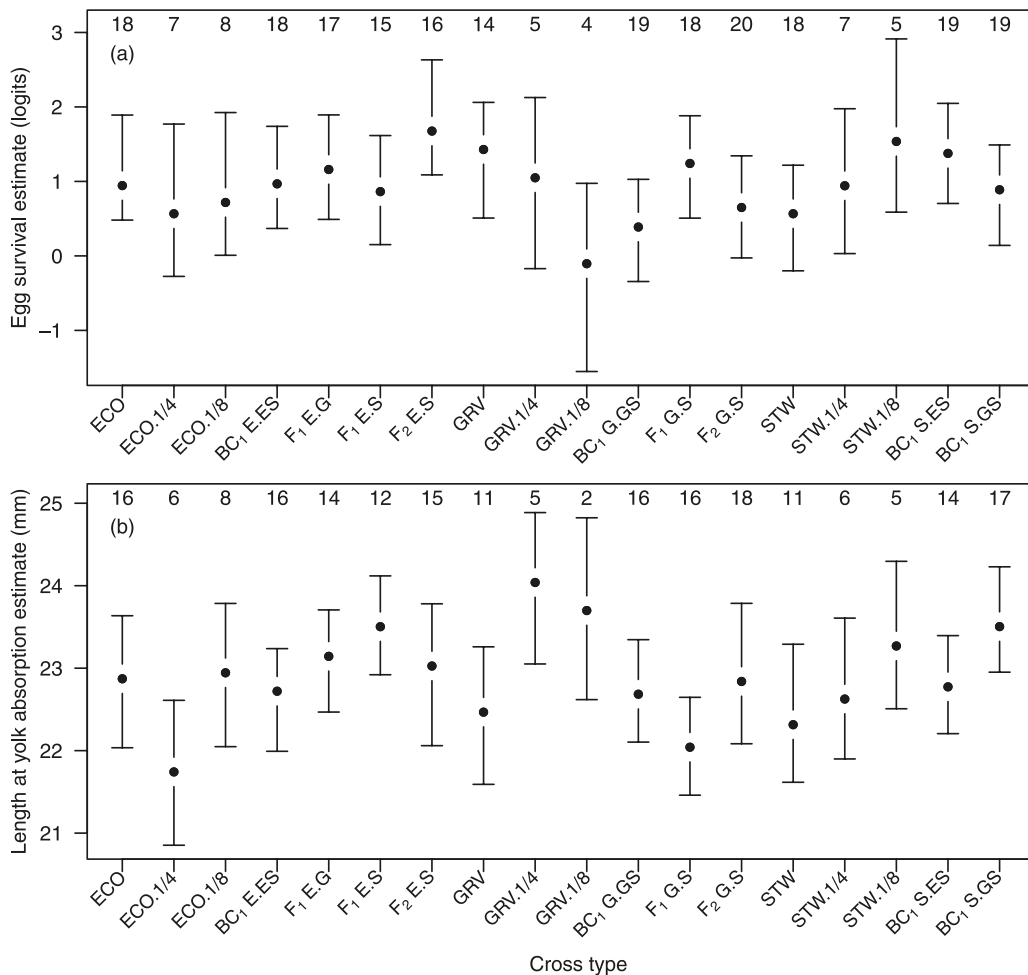
Individual parental effects were more important than cross type effects in explaining the variance among families. That is, using model selection, individual parental effects, maternal ID and sometimes paternal ID, would appear before a cross type effect, cross type. In addition, parental effects present 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; F<sub>1</sub>, first-generation outbred; F<sub>2</sub>, second-generation outbred (F<sub>1</sub> × F<sub>1</sub>); BC<sub>1</sub>, backcross outbred (pure × F<sub>1</sub>); 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; F<sub>1</sub>, first-generation outbred; F<sub>2</sub>, second-generation outbred (F<sub>1</sub> × F<sub>1</sub>); BC<sub>1</sub>, backcross outbred (pure × F<sub>1</sub>); 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 ± 1 SD, 22.9 ± 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 (F<sub>1</sub> 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, Stewiacke; and F<sub>1</sub>, 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-

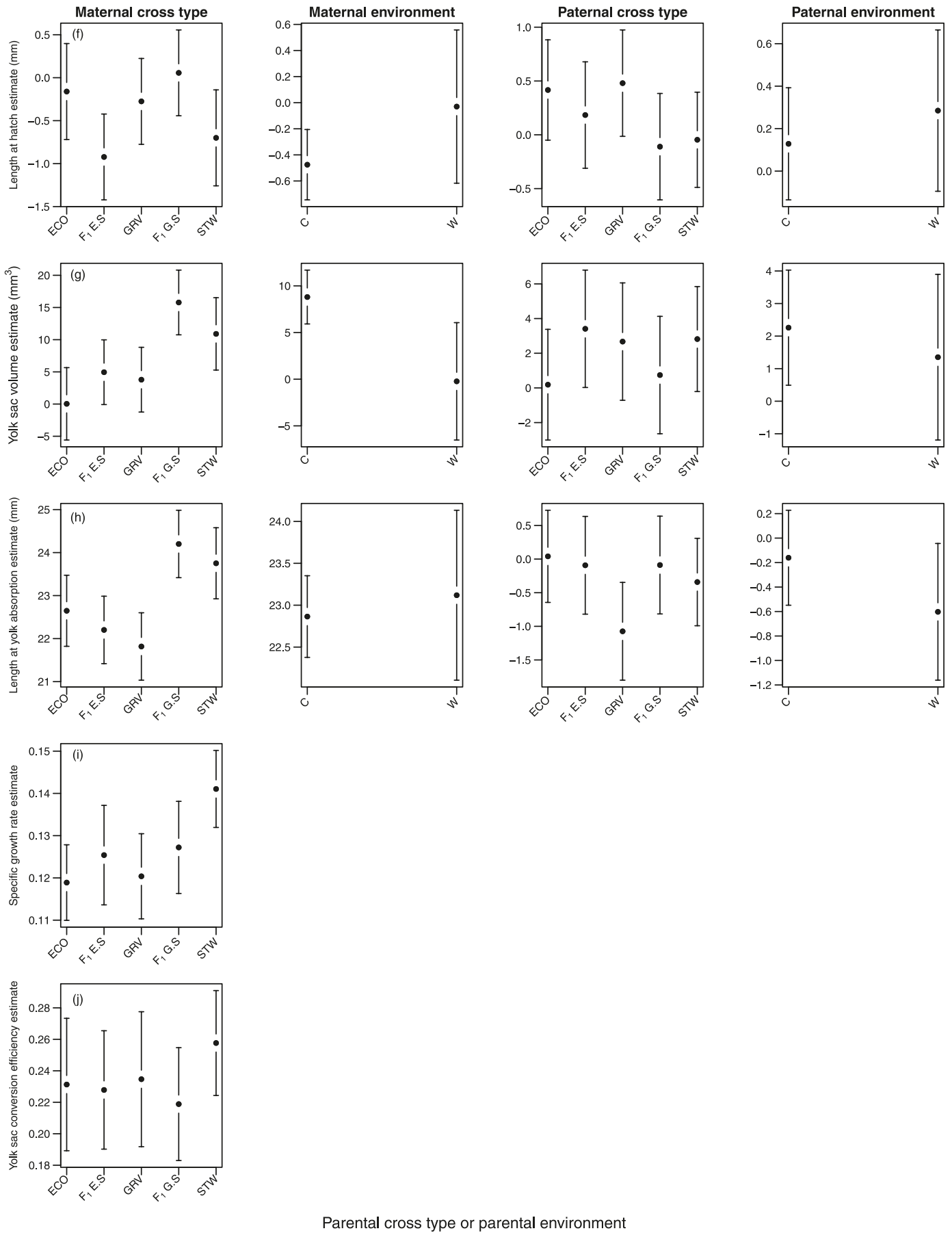
more, there may be additional factors in the wild, such as size-based dispersal (McGinnity et al. 1997), which might result in a nonrandom shift of some fish out of the sampling area; such sampling biases can be eliminated in the laboratory. An additional consideration is that the magnitudes of detrimental 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 F<sub>1</sub> outbred parents and those of pure parents. For example, F<sub>1</sub> G.S mothers produced offspring that were ~0.3 mm larger in egg diameter than F<sub>1</sub> E.S and GRV mothers. Also, there were paternal cross type effects for egg diameters. That is, F<sub>1</sub> E.S and F<sub>1</sub> 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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