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# Mixed evidence for reduced local adaptation in wild salmon resulting from interbreeding with escaped farmed salmon: complexities in hybrid fitness

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## Abstract

Interbreeding between artificially-selected and wild organisms can have negative fitness consequences for the latter. In the Northwest Atlantic, farmed Atlantic salmon recurrently escape into the wild and enter rivers where small, declining populations of wild salmon breed. Most farmed salmon in the region derive from an ancestral source population that occupies a nonacidified river (pH 6.0–6.5). Yet many wild populations with which escaped farmed salmon might interbreed inhabit acidified rivers (pH 4.6–5.2). Using common garden experimentation, and examining two early-life history stages across two generations of interbreeding, we showed that wild salmon populations inhabiting acidified rivers had higher survival at acidified pH than farmed salmon or $F_1$ farmed-wild hybrids. In contrast, however, there was limited evidence for reduced performance in backcrosses, and $F_2$ farmed-wild hybrids performed better or equally well to wild salmon. Wild salmon also survived or grew better at nonacidified than acidified pH, and wild and farmed salmon survived equally well at nonacidified pH. Thus, for acid tolerance and the stages examined, we found some evidence both for and against the theory that repeated farmed-wild interbreeding may reduce adaptive genetic variation in the wild and thereby negatively affect the persistence of depleted wild populations.

## Introduction

Interbreeding between artificially-selected and wild organisms has the potential to lead to several negative consequences for wild populations (Ellstrand 2003; McGinnity et al. 2003; Hails and Morley 2005; Noren et al. 2005; Bekkevold et al. 2006; Bert 2007; Bowman et al. 2007). Here, we address the concern that such interbreeding may result in the loss of adaptive genetic variation. Adaptive genetic variation could be lost if interbreeding generates hybrids that carry maladapted genes from the artificially-selected parent and, thereby, that experience reduced fitness in the wild. This could arise because (i) advertent/inadvertent selection in controlled rearing environments elicits genetic changes in the artificially-selected organism, and/or (ii) the artificially-selected organism is transported to and produced in regions other than where it was derived (Hutchings and Fraser 2008).

Despite containment improvements to aquaculture sea cage technology, large escapes of farmed Atlantic salmon (*Salmo salar*) recur from sea cages (Fiske et al. 2006). Escaped farmed salmon can enter rivers inhabited by wild salmon and interbreed with the latter, potentially leading to fitness reductions in wild salmon populations (McGinnity et al. 2003).

In the Northwest Atlantic, extensive salmon aquaculture occurs at the species' southern limit in close proximity to several regional wild salmon population groups that are rapidly declining. One such group, the Southern Upland in Nova Scotia (Canada), inhabits a series of rivers that are naturally-acidified because of their surface geology, in contrast to rivers from surrounding areas (Ginn et al. 2007) (Fig. 1). Many Southern Upland populations have declined over the past 50 years partly because of an increase in river acidification attributable to acid rain (Lacroix and Knox 2005). Yet, small populations of

 

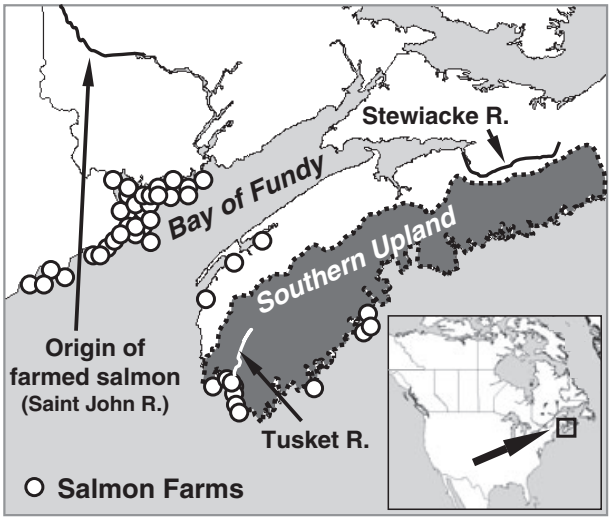


**Figure 1** Map of the location of Atlantic salmon study populations, the Southern Upland of Nova Scotia, and the general location of regional salmon farms.

Atlantic salmon still persist, despite very low mean pH (4.6–5.2) in tributaries and main stems of some rivers. This raises the possibility that some local adaptation to acidified rivers exists, a reasonable hypothesis given that other salmonid populations exhibit differences in acid tolerance (Hurley and Foyle 1989; Donaghy and Verspoor 1997). Concurrently, salmon aquaculture production along the Southern Upland coastline has increased ninefold over the past decade (M.J. Morris, D.J. Fraser and J.A. Hutchings, unpublished data). The farmed strain predominantly utilized here and elsewhere in the Northwest Atlantic originates from a nonacidified source, the St John River, New Brunswick (Canada), for which the mean pH is 6.0–6.5 (Lacroix 1985; Glebe 1998) (Fig. 1). Escaped farmed salmon have also been documented in Southern Upland Rivers but monitoring of these rivers has been limited (M.J. Morris, D.J. Fraser and J.A. Hutchings, unpublished data).

Taken together, Nova Scotia's Southern Upland literally represents the 'acid test' of local adaptation for risk assessment. If wild salmon found here are locally adapted to acidified rivers, a loss of adaptive genetic variation in these populations could result from interbreeding with escaped farmed salmon, given that the farmed salmon originate from a nonacidified source.

Our study's objectives were thus to test two hypotheses: (i) that wild salmon from one acidified (Southern Upland) river were more acid tolerant than both farmed salmon and wild salmon from a nonacidified river (Fig. 1); and (ii) that two generations of interbreeding between farmed salmon and wild salmon from the acidified river would result in outbreeding depression in farmed-wild hybrids (i.e. $F_1$ = farmed × wild; $F_2 = F_1 \times F_1$; backcrosses = $F_1$ × wild), especially in the hybrids that were likely to be more common in nature (i.e. $F_1$, backcrosses). For this latter hypothesis, we were especially interested in whether interbreeding resulted in potentially maladaptive changes to the reaction norms of farmed-wild hybrids, a reaction norm being a linear or nonlinear function that expresses how the phenotypic expression of a trait for a given genotype changes with different environmental conditions (Schlichting and Pigliucci 1998; Hutchings et al. 2007). Indeed, a reaction norm perspective can be used to predict how farmed-wild hybrid genotypes might respond, on average, to changes in pH relative to wild genotypes. In addition, it was important to examine the fitness consequences of two generations of interbreeding between farmed and wild salmon, as outbreeding depression may not be manifested until at least the second generation in which parental gene combinations are broken up by recombination (Edmands 2007).

Owing to the small size and threatened conservation status of Southern Upland wild salmon populations (DFO 2002), it was not feasible to compare the fitness of farmed, wild and hybrid individuals in the wild. Thus, to test our hypotheses, we conducted two common-garden experiments, at several pH levels, to compare (i) survival and growth of newly hatched juveniles, called 'alevins', and (ii) growth of older juveniles (yearlings), called 'parr', between pure and hybrid crosses. These life history stages were chosen because of their high sensitivity to low pH in Atlantic salmon, especially at the alevin stage (Daye and Garside 1977, 1979; Farmer et al. 1980; Lacroix 1985). The different pH levels utilized in each experiment (five for alevins, three for parr) encompassed the range to which salmon from the acidified river would be exposed naturally at these stages.

## Materials and methods

### Cross design and rearing

Unfertilized eggs and sperm used to generate crosses for this study in 2005 were obtained from adult salmon at Dalhousie University's Aquatron Facility. These 2005 adults had been generated from pure and $F_1$ hybrid crosses carried out in 2001 at Dalhousie University and raised their entire lives under common environmental conditions (tank volume, temperatures, food regimes, densities, dissolved oxygen, pH = 7.0) (Fig. 2). The 2005 adults comprised four cross-types (and were based on 10 full-sibling families per cross-type): (i) individuals from a wild population occupying an acidified, Southern Upland river (Tusket = TUSK; mean pH = 4.6–5.2); (ii) farmed salmon (FARM) derived from the St John River, a nonacidified river of the outer Bay of Fundy (mean


 

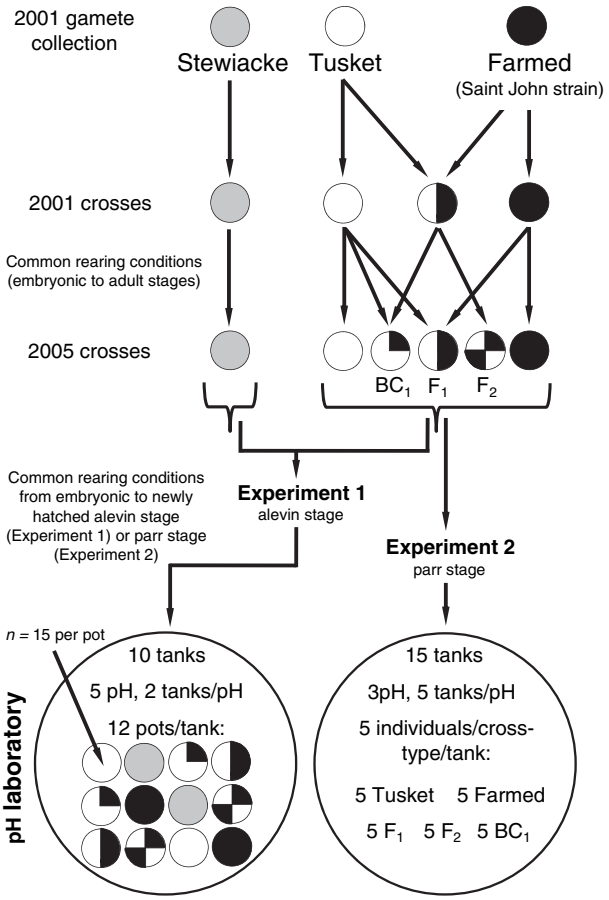


**Figure 2** A general flow diagram of the cross design and experimental set-up.

pH = 6.0–6.5); (iii) individuals from a wild population occupying a nonacidified river of the inner Bay of Fundy (Stewiacke = STEW; mean pH = 6.0–6.5); and (iv) $F_1$ TUSK × FARM hybrids (Figs 1 and 2). The 2001 TUSK and STEW adults originated from the wild. The 2001 FARM adults originated from an aquaculture broodstock that had undergone four generations of artificial selection up to that year, primarily for faster growth (Glebe 1998). The 2001 adults were assumed to represent random samples from each population or broodstock.

Prior to generating our 2005 crosses, all 2005 adults were individually tagged. To assign 2005 adults back to their respective 2001 families within cross-types and thus to avoid inbred (i.e. full- or half-sib) matings, we collected adipose fin clips from each fish (these were also previously collected from the 2001 parents), genotyped all individuals at five polymorphic microsatellite loci, and carried out parentage assignments using PAPA (Duchesne et al. 2002).

All crosses for this study were performed on November 22nd and 25th, 2005, at which time six different cross-types were created: pure TUSK, FARM and STEW, and three hybrids between TUSK and FARM ($F_1$; $F_2 = F_1 \times F_1$; and backcrosses = $BC_1 = F_1 \times$ pure TUSK) (Fig. 2). Two cross dates were necessary because of logistical constraints in having females from different cross-types available on the same day. Cross-types each consisted of six full-sibling families; $F_1$ hybrid families were derived from the same six FARM♀ and TUSK♂ used to generate pure FARM and TUSK families, respectively, with $BC_1$ families comprising a mixture of the same males and females used to generate pure TUSK and $F_2$ families (the ratios of TUSK: $F_1$♀ and ♂ were 1:1). For each cross-type, the 2005 adult males and females used to generate the crosses originated from different full-sibling families created in 2001.

Initially 500 eggs from each family were randomly allocated to one of 36 100 L circular tanks (diameter = 0.66 m, height = 0.43 m). Under common environmental conditions (temperature, dissolved oxygen, pH = 7.0), eggs were first kept in the dark at temperatures between 3 and 4°C until hatching occurred in March 2006, and dead eggs were removed every 4 days during incubation. A subset of the newly hatched alevins from each family was then transferred to another lab housing apparatuses for manipulating pH where experiment 1 was carried out (see below; Fig. 2). Owing to space limitations with concurrent work, remaining alevins were pooled within cross-type and with equalized family sizes into four lots once exogenous feeding began, and randomly assigned to one of four of the 100 L tanks (May 10, 2006). Alevins in all tanks were then maintained at the same density and fed the same regime of commercial dry feed for an additional 235 days (May 10, 2006–February 1, 2007). At this point, a subset of parr (from each tank of each cross-type) was transferred to the pH lab where experiment 2 was also carried out (see below; Fig. 2).

### Experiment 1: acid tolerance in relation to alevin survival and growth

At five different pH levels, we compared survival and growth of newly hatched alevins from different cross-types during the yolk absorption period up to 23 days following the initiation of exogenous feeding. This began when alevin yolk sacs had been completely re-absorbed or 'buttoned-up' into the body cavity (Beacham and Murray 1990). On the day that a sufficient representation (more than one-third) of alevins had hatched from each family (March 25, 2006), equal numbers of randomly collected alevins from each family ($n = 50$) were pooled within cross-type into separate buckets ($n = 300$) and transported to another lab housing apparatuses for manipulat-

 

ing pH. Here, for each cross-type, two sets of 15 alevins were each randomly assigned to one of 12 total pots nested within 10 circular tanks (diameter = 0.66 m, height = 0.43 m) (Fig. 2). Pots in each tank were of equal size, separated by equal distances, and attached to a plexiglass grid. Pot bottoms were drilled out and filled with a thin-mesh screen to ensure sufficient oxygenation for alevins.

We then randomly assigned two of the 10 tanks to one of five pH levels: 4.6, 4.9, 5.2, 5.7 and 7.0. The three lowest pH levels (4.6–5.2) corresponded to the range experienced upon hatching by wild alevins inhabiting the acidified river (TUSK) (Lacroix and Knox 2005). The pH was gradually reduced to the target pH in each tank to acclimate alevins from pH 7.0 and was recorded daily. The same air, water flow (replacement every 5 h) and temperatures (1 SD: ±0.1°C) were maintained for all tanks throughout the experiment (69 days), with the overall temperature increasing from 5 to 9°C over the experiment. Once yolk absorption was reached after day 46 (May 10, 2006), and after accounting for mortalities, we fed alevins in individual pots the same proportion of commercial dry feed daily. Dead alevins were counted and removed daily throughout the experiment (69 days).

For each cross-type and pH, we measured and compared (i) size-at yolk re-absorption (length in mm) and (ii) yolk sac conversion efficiency [estimated as: (size at yolk re-absorption – size at hatch)/yolk sac volume, where size at hatch was the length in mm, and yolk sac volume ($mm^3$) = $LH^2(\pi/6)$, where $L$ and $H$ were the length and height of the yolk sac in mm, respectively; Koskinen et al. 2002]. These traits may be important to fitness because early development, growth and size influence the probability of surviving to maturity in salmonids (Metcalfe and Thorpe 1992; Einum and Fleming 2000). For each trait, alevins in each of the 120 pots were placed into a plastic tray and photographed, using a digital camera mounted overhead. Photos were then imported into IMAGEJ (NIH 2003) where we determined trait measurements relative to a standardized scale measure included in each pot photo. Alevin length was not measured at the end of the experiment (day 69) as variability in cross-type survival after yolk absorption sometimes resulted in very low sample sizes. Mean yolk sac conversion efficiencies were calculated using the two values of each cross-type at each pH.

### Experiment 2: acid tolerance in relation to parr growth

At three pH levels (5.0, 5.5, 7.0), and using the same pH lab as in experiment 1, we compared growth of age 0+ parr between cross-types over a 109-day period. Because of tank space limitations, however, STEW was not included in this experiment, and the remaining five cross-types could not be separated into individual tanks. The experimental set-up consisted of 15 tanks with an even mixture of five fish per cross-type per tank (total $n = 375$) (Fig. 2). Note that there were more tanks used than in experiment 1 because of a lack of constraint in producing only three rather five different pH levels. Three sets of five tanks were exposed to one of three pH levels during the experimental period (Fig. 2). Again, the two lower pH levels encompassed the range that salmon from the acidified river (TUSK) would be exposed to at the age 0+ parr stage in the wild (Lacroix and Knox 2005). Within tanks, each cross-type was tagged with a unique fin clip. Fin clips were assigned evenly to each cross-type across pH levels to avoid any potential impacts on growth from particular fin clips.

Parr of pure TUSK, FARM, and hybrid origin ($F_1$, $F_2$, $BC_1$) were selected from the four 100 L tanks harboring pooled families of each of the different cross-types and transferred to the pH lab (see above; Fig. 2). We selected similarly sized parr from the different cross-types that were 80–86 mm in length, as these were readily available in all cross-types. Over the course of the experiment (February 1 until May 3, 2007), all fish were held under the seasonal photoperiod at ambient water temperature that increased from 5 to 7°C. The same air, water flow (replacement every 5 h) and temperatures (1 SD: ±0.1°C) were also maintained between all tanks at a given time throughout the experiment. All tanks were fed the same regime of commercial dry feed (15 mL, twice daily/tank). Length (in mm) and weight (to the nearest 0.1 g) of all fish was measured four times throughout the experiment (days 1, 40, 75, 109). Attempts were also made to ensure that individuals within cross-types were selected to represent a similar size both within and between pH treatments, such that there were no differences in length and weight between treatments on day 1 (see Results). Minimal mortality (five individuals or 1.3%) occurred during the experiment; in these cases, a replacement fish of similar size and from the same cross-type was uniquely marked and added to the tank to maintain equal tank densities, but the individual was not included as part of our analyses.

### Statistical analyses

For experiment 1, we compared alevin survival among cross-types under varying pH at yolk absorption (day 46) and at the end of the experiment (day 69), using a generalized linear model (GLM) fitted with a binomial error distribution. Two body size traits (length at hatch and yolk sac volume) were included in the model as covariates, along with cross-type and pH as factors, to account for their potential influence on survival. We used factorial



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ANOVA to compare size at yolk absorption among cross-types, and again a GLM with binomial errors to compare yolk sac conversion efficiencies, given that these were calculated as proportions. In all of these models, we were particularly interested in the cross-type × pH interaction term because this would signify how different cross-type reaction norms would respond, on average, to specific changes to pH.

For experiment 2, factorial ANOVAs were used to compare the mean body size of different cross-types on days 1, 40, 75 and 109. Three metrics of body size were analyzed: means of length, weight and condition (k = g/cm$^3$ × 10 000), as well as changes in variability within these (coefficient of variation = CV = SD/mean). As we compared three metrics of mean body size, critical $\alpha$ significance values were taken to be 0.05/3 = 0.0167. GLMs/ANOVAs within pH were performed if significant cross-type × pH interactions were found, and Tukey *post hoc* tests were used to elucidate which cross-types differed significantly at the $P < 0.05$ level. ANOVA assumptions of normality and equal variances were met.

## Results

### Experiment 1: alevin survival, size at yolk absorption, and yolk sac conversion efficiencies

At yolk absorption (day 46), and accounting for initial body size differences (length at hatch, yolk sac volume), only pH had a significant effect on alevin survival (Table 1). However, by 23 days postyolk absorption (day 69), yolk sac volume, cross-type, pH and the cross-type × pH interaction term were all highly significant (Table 1). These results indicated, most notably, that the reaction norms for acid tolerance differed between cross-types, particularly at the low pH (4.6–5.2) experienced by wild alevins inhabiting an acidified river (TUSK) (Fig. 3; Table 1).

Consistent with the hypothesis that wild salmon from acidified rivers are more tolerant of acidity, TUSK alevins exhibited significantly higher survival and survived longer than FARM or STEW alevins at pH 4.6–4.9 (TUSK: 67%, 87%; FARM: 38%, 67%; STEW: 18%, 53%) (Figs 3,4 and S1; Table 1). However, TUSK, FARM and STEW alevins survived equally well at pH = 5.2–5.7, as did TUSK and FARM alevins at pH = 7.0 (Fig. 3; Table 1). In general, all cross-types (pure and hybrids) also had greater survival as the pH increased from 4.6 to 7.0 (Fig. 3, 4 and S1; Table 1).

Consistent with the hypothesis that outbreeding depression is manifested in farmed-wild hybrids, $F_1$ TUSK × FARM hybrids had lower survival than both TUSK and FARM alevins, but only at the lowest pH (4.6) (Fig. 3; Table 1). In addition, both $F_1$ TUSK × FARM hybrids (pH = 4.9) and $BC_1$ hybrids (pH = 4.6) had lower survival than TUSK alevins, albeit this difference was only marginally significant for $BC_1$ hybrids (Fig. 3;

**Table 1.** Results of generalized linear model examining alevin survival at yolk absorption (day 46), and at 23 days postyolk absorption (day 69). Degrees of freedom (df) and *F* values are presented for each factor in the model. Day 69 results of *post hoc* Tukey tests performed on individual pH levels are highlighted below: cross-types having different letters within a given pH differed significantly at the $P < 0.05$ level (see also Fig. 3).

| | | Day 46 | | Day 69 | |
|---|---|---|---|---|---|
| Factor | df | *F* | *P* | *F* | *P* |
| Length at hatch | 1 | 3.57 | 0.07 | 0.42 | 0.52 |
| Yolk sac volume | 1 | 0.11 | 0.74 | 249.01 | <0.0001 |
| Cross-type | 5 | 1.91 | 0.12 | 7.79 | 0.0001 |
| pH | 4 | 12.03 | <0.0001 | 208.6 | <0.0001 |
| Cross-type × pH | 20 | 1.07 | 0.43 | 10.53 | <0.0001 |
| Residuals | 59 | | | | |
| Day 69 Tukey tests | pH = 4.6 | 4.9 | 5.2 | 5.7 | 7.0 |
| T | A | A | A | A | A |
| $BC_1$(T × TF) | B* | A | A | A | A |
| $F_1$(TF) | C | B | B† | A | A |
| $F_2$(TF) | D | A | A | A | B‡ |
| F | E | B | A | A | A |
| S | C | C | B† | A | C |

*T versus $BC_1$(T × TF), $P = 0.052$.

†S and $F_1$(TF) only significantly different from $BC_1$(T × TF).

‡$F_2$(TF) only significantly different from $BC_1$(T × TF) and $F_1$(TF) ($P = 0.053$, $P = 0.053$, respectively), and S.

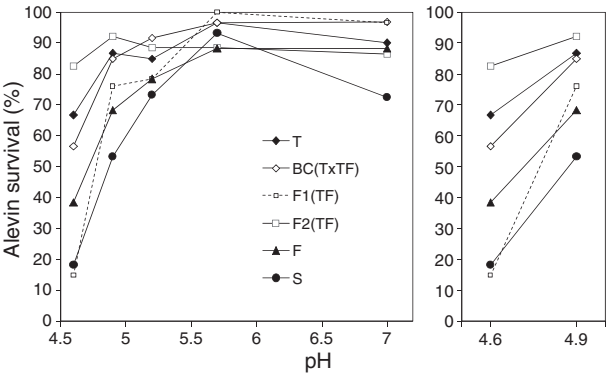


**Figure 3** Cumulative survival of salmon alevins from the six different cross-types, following 69 days of exposure to five different pH levels. An inset graph of the lowest pH treatments is shown at right to highlight the main differences in cross-type reaction norms; see Table 1 for statistical comparisons between cross-types at individual pH levels. Error bars are not given because data points represent the mean percent survival of alevins based on two tank replicates per pH level. Cross-type symbols are the same for both graphs. T = Tusket (wild, acidified river); F = Farmed (from a nonacidified source); S = Stewiacke (wild, nonacidified river); $F_1$(TF) = $F_1$ Tusket × Farmed hybrids; $F_2$(TF) = $F_2$ Tusket × Farmed hybrids; $BC_1$ hybrids = backcross [$F_1$(TF) × T].

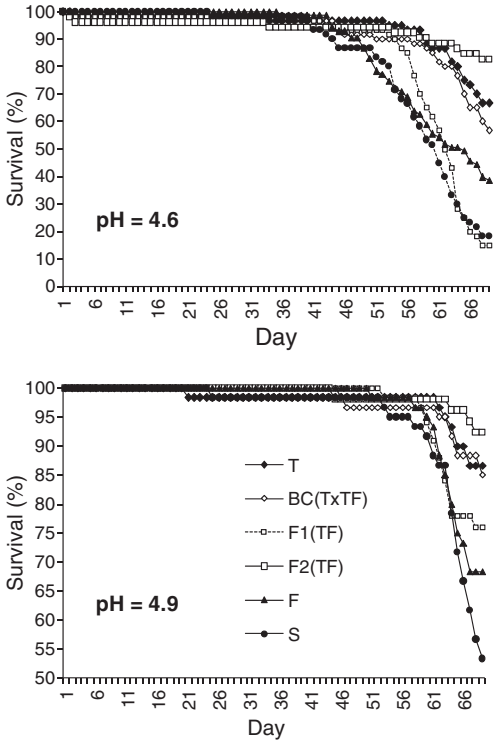


**Figure 4** Cumulative survival of salmon alevins from the six different cross-types, over 69 days of exposure to the lowest pH treatments. Similar graphs for cumulative survival in the remaining three pH treatments are available online as Supplementary material (Fig. S1). Error bars are not given because data points represent the mean percent survival of alevins based on two tank replicates per pH level. Note that values along *y*-axes differ between graphs. See Fig. 3 caption for cross-type code details.

Table 1). The magnitude of survival differences between $F_1$ TUSK × FARM hybrids and TUSK alevins was thus most pronounced as the pH became more acidic (e.g. $F_1$ versus Tusket, pH = 4.6: 15% vs 67%; pH = 4.9: 76% vs 87%; Fig. 3). In contrast, $F_2$ TUSK × FARM hybrids had higher survival than both TUSK and FARM alevins at pH 4.6, and equal survival relative to TUSK alevins at pH = 4.9–5.2 (Fig. 3; Table 1). At the highest pH levels (5.7–7.0), all hybrids generally performed equally well to parental populations (TUSK, FARM) (Fig. 3; Table 1). Overall then, relative to the reaction norm of TUSK alevins, farmed-wild interbreeding led to changes in both the slope and the elevation of hybrid reaction norms. The $F_1$ hybrid reaction norm, and to a much lesser extent, that of $BC_1$ hybrids, had a lower elevation (i.e. reduced survival at low pH) and a steeper slope (i.e. especially reduced survival at the lowest pH); the $F_2$ hybrid reaction norm had a higher elevation and its slope was flattened towards zero (Fig. 3).

At yolk absorption (day 46), cross-type, pH and the cross-type × pH interaction had significant effects on alevin body size (Table 2). While alevins of all cross-types exhibited maximal growth at pH = 5.7 and poorest growth at pH = 4.6, $F_1$ TUSK × FARM hybrids grew especially poorly relative to TUSK and FARM alevins at the lowest pH (4.6) (Fig. 5; Table 2). Conversely, $BC_1$ and $F_2$ TUSK × FARM hybrid alevins generally grew equally well across different pH levels relative to TUSK alevins (Fig. 5; Table 2). The relationship between yolk sac conversion efficiencies and pH did not differ between cross-types (Table 2).

**Table 2.** Results of ANOVA examining size at yolk absorption (day 46), and results of generalized linear model examining yolk sac conversion efficiencies (days 1–46). Degrees of freedom (*df*) and *F* values are presented for each factor in the model. For size at yolk absorption, day 46 results of *post hoc* Tukey tests performed on individual pH levels are highlighted below: cross-types having different letters within a given pH differed significantly at the $P < 0.05$ level (see also Fig. 5).

| | Size at yolk absorption | | | Yolk sac conversion efficiencies | | |
|---|---|---|---|---|---|---|
| Factor | df | *F* | *P* | df | *F* | *P* |
| Cross-type | 5 | 66.17 | <0.0001 | 5 | 10.55 | <0.0001 |
| pH | 4 | 240.32 | <0.0001 | 4 | 43.65 | <0.0001 |
| Cross-type × pH | 20 | 2.12 | 0.003 | 20 | 1.21 | 0.31 |
| Residuals | 1560 | | | 59 | | |

| Day 46 Tukey tests | pH 4.6 | 4.9 | 5.2 | 5.7 | 7.0 |
|---|---|---|---|---|---|
| T | A | A | A | A | A |
| $BC_1$(T × TF) | A | A | A | B | A |
| $F_1$(TF) | B | B* | B† | B | A |
| $F_2$(TF) | A | A | A | A | A |
| F | B | C | C | B | B‡ |
| S | B | C | D | C | B‡ |

*$F_1$(TF) only significantly different from T, F, and S.

†$F_1$(TF) only significantly different from $F_2$(TF) and not significantly different from *F*.

‡F and S significantly different from T, $BC_1$(TF) and $F_2$ (TF) but not $F_1$(TF).

### Experiment 2: parr growth

On days 1 and 40, there were no differences in any body size variables between cross-types (Table 3; only results from day 1 are shown), with one exception on day 1; $F_2$ TUSK × FARM hybrid and TUSK parr were in better condition than $F_1$ TUSK × FARM hybrid or FARM parr (*post hoc* Tukey tests, $P < 0.05$). Even after 75 and 109 days of exposure to pH = 5.0, 5.5, 7.0, the relationship between any body size variable and pH did not differ among cross-types (Table 3; only results from day 109 are shown), indicating no differences in the reaction norms of different cross-types. Parr of all cross-types exhibited


 

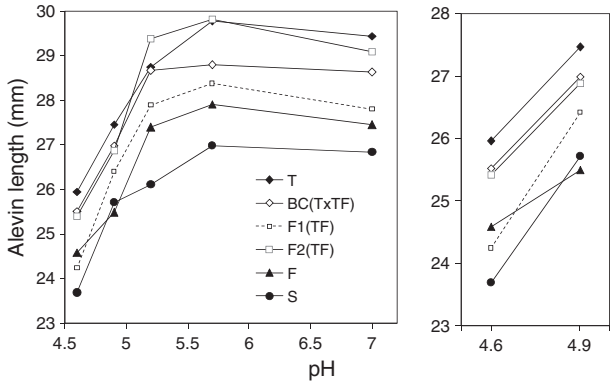


**Figure 5** Size of alevins at yolk absorption (day 46) of the six different cross-types at five different pH (B). See Fig. 3 caption for cross-type code details. An inset graph of the lowest pH treatments is shown at right to highlight differences between $F_1$ Tusket × Farmed hybrid versus pure Tusket or pure Farmed reaction norms; see Table 2 for statistical comparisons between cross-types at individual pH levels. Error bars are not given because data points represent the mean percent survival of alevins based on two tank replicates per pH level.

the poorest growth at the lowest pH (5.0) (Fig. 6). However, across all pH levels, $F_2$ TUSK × FARM hybrids and $BC_1$ hybrids continuously grew faster, being longer and heavier in body size than TUSK or FARM parr, whereas $F_1$ TUSK × FARM hybrids were generally intermediate in body size relative to parental populations (Fig. 6).

## Discussion

### Genetically-based population differences in acid tolerance and potential for local adaptation

Adopting common-garden experimentation, we found that wild alevins from an acidified river (TUSK) had higher survival at acidified pH than either farmed (FARM) or wild (STEW) alevins originating from nonacidified sources. TUSK alevins also survived cumulatively longer than FARM or STEW alevins within the range of pH found in the Tusket River (e.g. pH = 4.6–4.9). The higher survival of TUSK alevins under conditions of their local environment is one prerequisite of local adaptation (Kawecki and Ebert 2004). Indeed, the spring hatching period in Atlantic salmon is potentially the most critical season for survival, as alevins are exposed to abrupt pH reductions from melting snow and ice runoff (Daye and Garside 1979; Lacroix 1985).

On the other hand, more definitive support for local adaptation, which we did not find, might have been provided if: (i) FARM and STEW alevins survived better than TUSK alevins at higher, nonacidified pH levels; and similarly, (ii) TUSK alevins performed better at lower (pH = 4.6–5.2) than higher pH (pH = 5.7–7.0) (Kawecki and Ebert 2004). Additionally, we detected no differences in parr mortality or growth between cross-types that were attributable to the pH normally encountered by wild TUSK parr. Nonetheless, less severe fitness effects were expected at this stage relative to the alevin stage, as alevins are more sensitive to low pH than parr in Atlantic salmon (Daye and Garside 1977, 1979; Lacroix 1989). Given that local adaptation usually entails a physiological cost in environments where it is not needed (Kawecki and Ebert 2004), our overall results cannot conclusively provide evidence that adaptive genetic variation exists in TUSK salmon for tolerating acidity.

It would, however, be premature for several reasons to conclude that adaptive genetic variation relating to pH *does not* exist. First, comparisons of the performance of TUSK salmon at different pH levels (acidified/nonacidified) or relative to FARM/STEW salmon at nonacidified pH might not be definitive tests of local adaptation.

**Table 3.** Results of ANOVA examining body size variable responses of Atlantic salmon parr at: (i) the onset of exposure to varying pH (5.0, 5.5, 7.0) (day 1), and (ii) the end of the exposure period to varying pH (day 109). Degrees of freedom (df) and *F* values are presented for each factor in the model.

| | | Length | | Weight | | Condition | | | Length CV | | Weight CV | | Condition CV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factor | Df | *F* | *P* | *F* | *P* | *F* | *P* | df | *F* | *P* | *F* | *P* | *F* | *P* |
| Day 1 | | | | | | | | | | | | | | |
| Cross-type | 4 | 2.17 | 0.07 | 2.29 | 0.07 | 18.01 | <0.0001 | 4 | 1.65 | 0.17 | 0.94 | 0.45 | 6.04 | 0.0003 |
| pH | 2 | 0.65 | 0.52 | 0.98 | 0.38 | 0.55 | 0.58 | 2 | 2.71 | 0.08 | 1.16 | 0.32 | 2.52 | 0.03 |
| Cross-type × pH | 8 | 0.55 | 0.82 | 0.70 | 0.69 | 2.38 | 0.02 | 8 | 0.42 | 0.89 | 0.50 | 0.85 | 2.32 | 0.04 |
| Residuals | 360 | | | | | | | 60 | | | | | | |
| Day 109 | | | | | | | | | | | | | | |
| Cross-type | 4 | 9.81 | <0.0001 | 8.25 | <0.0001 | 13.67 | <0.0001 | 4 | 3.36 | 0.02 | 1.97 | 0.11 | 0.77 | 0.55 |
| pH | 2 | 8.59 | <0.0001 | 9.82 | <0.0001 | 2.17 | 0.12 | 2 | 1.46 | 0.37 | 1.22 | 0.34 | 0.51 | 0.59 |
| Cross-type × pH | 8 | 0.66 | 0.72 | 0.77 | 0.63 | 1.85 | 0.07 | 8 | 0.62 | 0.70 | 0.36 | 0.92 | 1.08 | 0.39 |
| Residuals | 355 | | | | | | | 60 | | | | | | |

CV, coefficient of variation. Statistical significance is based on $P < 0.0167$ ($\alpha = 0.05/3$: see Materials and methods).

 

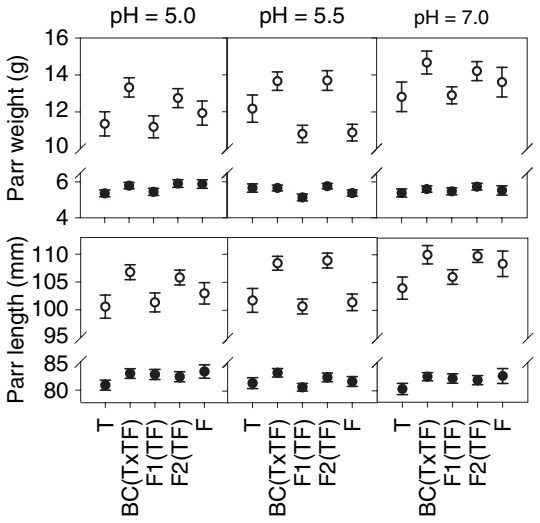


**Figure 6** Body size (length, mass, ±1 SE) of parr from five different cross-types on day 1 (filled circles) of the experiment, and day 109 following exposure to three different pH. See Fig. 3 caption for cross-type code details.

Salmon only require acid tolerance in freshwater stages (e.g. juvenile/spawning) because seawater stages (subadult/adult) of all populations are exposed to a similarly high and relatively homogeneous sea pH of 7.5–8.4. By default then, salmon from acidified rivers (e.g. TUSK) are exposed to, and thus require tolerance to, both acidic and nonacidic pH (although not necessarily both at any one particular stage). This could account for the apparent lack of constraint on TUSK genotypes at higher pH. Secondly, and similarly, the wider range of pH to which salmon from acidified rivers are exposed should favor the evolution of adaptive phenotypic plasticity (Hutchings 2004; Kawecki and Ebert 2004). It could be that pH adaptation in TUSK salmon relates more to tolerating a range of pH than a specific pH *per se*. Third, while our study focused on stages that have previously shown sensitivities to low pH in Atlantic salmon (Daye and Garside 1977; Lacroix 1985), adaptive genetic variation might exist at earlier or later, unexamined life history stages, such as during embryonic development or the parr-smolt transformation (Smith and Haines 1995). Finally, our experimentation may have failed to mimic specific environmental conditions related to pH in which adaptive genetic variation is expressed (Kawecki and Ebert 2004). For instance, our pH exposure trials did not incorporate interactions between acidity and heavy metals, such as aluminum, which can affect the toxicity of pH (Lacroix 1989, 1992). Nonetheless, these latter interactions are unlikely to have affected our results because Southern Upland Rivers have high levels of dissolved organic matter which decrease the toxicity of heavy metals (Farmer et al. 1980; Lacroix 1985).

### $F_1$ versus the $F_2$ generation of farmed-wild interbreeding

Interbreeding between divergent populations often generates $F_1$ heterosis followed by hybrid breakdown in the $F_2$ or later recombinant generations (Edmands 2007). A salient and contrasting result of our study is that $F_1$ TUSK × FARM hybrids showed reduced performance relative to parental populations at acidified pH, whereas we found limited evidence for reduced performance in TUSK × FARM backcrosses, and $F_2$ TUSK × FARM hybrids performed better or equally well to TUSK salmon. Several explanations, relating both to the genetic characteristics of salmon and our experimental design, might account for these discrepancies.

$F_1$ outbreeding depression is normally attributable to a disruption of local adaptation (via extrinsic interactions between genes and the environment), underdominance, or epistatic interactions (Edmands 1999, 2007). These mechanisms may act concurrently, but our study was not designed to disentangle which of them might explain the observed reduction in fitness in $F_1$ TUSK × FARM hybrids. Nevertheless, reduced $F_1$ hybrid performance relative to parental populations was highly environmentally-dependent and only detectable as the pH became more acidic. This suggests that extrinsically-based disruption of local adaptation was involved. However, we emphasize that only TUSK males and FARM females were used to generate our $F_1$ hybrids. Reciprocal $F_1$ hybrids (TUSK female × FARM male) may not experience as great a reduction in fitness at acidified pH as the $F_1$ hybrids in our study. On the other hand, available data suggest that mating between wild males and farmed females may be more representative of what takes place in the wild (Fleming et al. 2000).

In contrast to $F_1$ hybrids, we found limited evidence for reduced performance in TUSK × FARM backcrosses, and $F_2$ TUSK × FARM hybrids occasionally performed better than, but most often equally well to, TUSK salmon. The general lack of $F_2$ outbreeding depression might suggest that co-adapted gene complexes related to acid tolerance do not exist in salmon, at least at the life history stages examined. Or, perhaps there has been insufficient time to evolve tightly-linked co-adapted gene complexes given Atlantic salmon only colonized the Southern Upland region 12 000 years ago after the last glaciation (Pielou 1991). Yet alternative explanations might explain the lack of $F_2$ outbreeding depression and complicate interpretations of the mechanisms underlying hybrid fitness.

For example, salmonids are well-known for exhibiting pronounced maternal effects in many of the traits evaluated here (alevin size, yolk sac size, size at hatch, parr growth). These maternal effects can be due to either

environmental or genetic causes, or both (e.g. Einum and Fleming 2000; McClelland et al. 2005; Perry et al. 2005). All females used to generate the 2005 crosses in our study were raised under common environmental conditions except that for logistical reasons, cross-types had to be kept in individual, separate holding tanks from 6 months postexogenous feeding onwards. Thus, we cannot entirely discount the possibility that tank effects might have led to environmentally-driven maternal effects. These in turn could have affected comparisons of the performance of certain cross-types relative to one another that were based on different generations of interbreeding (e.g. $F_1$ versus $F_2$ hybrids).

We believe it is more likely, however, that maternal effects with a genetic basis could have influenced hybrid fitness. For instance, if first-generation interbreeding led to $F_1$ hybrid females with heterosis, maternal heterosis might have masked negative fitness effects in their $F_2$ hybrid offspring (Tave et al. 1990; Falconer and MacKay 1996). Interestingly, the mean diameter (±1 SE) of $F_1$ TUSK × FARM hybrid female eggs was slightly larger than TUSK females (5.99 ± 0.29 vs 5.85 ± 0.34 mm) or FARM females (5.46 ± 0.27 mm), despite an intermediate body length of $F_1$ TUSK × FARM hybrid females (TUSK = 53.4 ± 1.2 cm; $F_1$ TUSK × FARM = 59.4 ± 0.5 cm; FARM = 64.5 ± 0.6 cm). Accordingly, $F_2$ alevins derived from $F_1$ TUSK × FARM females had larger yolk sacs than any cross-type, including TUSK alevins (data not shown), and importantly, yolk sac volume had a significant influence on alevin survival in our analyses. Similarly, $F_1$ TUSK × FARM hybrid fitness may have been affected because only FARM females were used to generate them, but under common environmental conditions, FARM females produced smaller eggs with smaller yolk sacs than TUSK females.

Many studies of interbreeding between divergent populations that find $F_1$ heterosis and $F_2$ hybrid breakdown are also based on diploid organisms, yet salmonids are residual tetraploids and some gene loci are still duplicated (Allendorf and Thorgaard 1984). The larger number of loci involved in genetic interactions than in a diploid organism might diminish fitness effects in $F_2$ hybrids (Etterson et al. 2007; McClelland and Naish 2007), particularly under greater environmental stress (Edmands 2007), as was observed at acidified pH. Similarly, whereas diploids are expected to exhibit the greatest amount of heterosis after one generation of interbreeding (Falconer and MacKay 1996), heterosis in other polyploids is not fully attained until later generations (Bingham et al. 1994). Later generation heterosis in polyploids might also appear elevated if considerable inbreeding existed within parental populations (Etterson et al. 2007). This is a further possibility in our study given that the TUSK salmon population is small (<100–250 annual spawning adults; Amiro et al. 2000; DFO 2002) and that farmed salmon strains can exhibit reduced genetic diversity (Hutchings and Fraser 2008).

Fitness comparisons made in this study were also initiated after salmon embryos had hatched. At earlier embryonic stages, concurrent work suggests that a partial inviability might exist in $F_2$ TUSK × FARM hybrids as they have reduced survival relative to parental populations (D. J. Fraser and J. A. Hutchings, unpublished data). Our study, therefore, used only the remaining $F_2$ gene combinations that survived the hatching period, and on average, these genotypes might have had superior fitness to either parental population at the stages examined. Finally, on a related note, we point out that spawning salmon may preferentially spawn in upwelling areas that have higher pH (Lacroix 1992). If such areas exist within acidified rivers, and if salmon selectively use them, then the adverse effects from farmed-wild interbreeding documented here at the alevin stage might be buffered somewhat in the wild.

### Conservation and management implications

Marked differences in pH between Southern Upland Rivers and the ancestral source river of regional farmed salmon provided a benchmark for evaluating the risk posed to small and declining fish populations from interbreeding with their escaped farmed counterparts. We showed that wild salmon inhabiting acidified rivers had higher survival at acidified pH than farmed salmon or $F_1$ farmed-wild hybrids, the hybrids that will be most commonly generated in the wild. Interbreeding also resulted in maladaptive (i.e. survival-reducing) changes to the reaction norms for acid tolerance in $F_1$ hybrids. It is unlikely that these fitness reductions were due to advertent/ inadvertent selection during the farming process *per se*, but rather to the ancestral characteristics of the farmed individuals. The transfer and production of these farmed individuals into different geographical regions than where they originated then sets the stage for interbreeding of potentially maladapted farmed individuals with wild individuals when the former escape (Hutchings and Fraser 2008). For mitigating the effects of farmed-wild interbreeding, our results are thus directly relevant to ongoing debates regarding the use of farmed strains derived from local or nonlocal wild populations relative to where the farming is taking place (Hutchings and Fraser 2008). They are also relevant for considering the scale at which a farmed strain can be considered 'local'.

We also found, however, that later generation ($F_2$, $BC_1$) farmed-wild hybrids generally performed equally well, if

 

not better than, wild salmon at acidified pH. Furthermore, we did not find definitive evidence for the existence of adaptive genetic variation relating to pH in wild salmon. These results have two implications. First, divergent mechanisms likely affect the performance of farmed-wild hybrids between $F_1$ and later generations. Secondly, our results provided some evidence both for and against the hypothesis that repeated farmed-wild interbreeding may lead to a dilution of adaptive genetic variation and potentially affect the persistence of wild populations.

We caution, nevertheless, that although our results do not point to one clear answer, this should not be used as justification for societal or governmental inaction with respect to mitigating the potentially negative impacts of aquaculture on wild species. First, our study focused on the response of only a few traits related to pH. Adaptive genetic variation in wild salmon, or outbreeding depression in multi-generational farmed-wild hybrids, could exist at other, unexamined traits. Secondly, we were logistically unable to examine the lifetime performance of wild, farmed and multi-generational hybrid salmon. Interestingly, in the only study that has done so to date, later generation ($F_2$, $BC_1$) farmed-wild hybrids exhibited similar, equal or greater fitness at embryo to smolt stages relative to wild Atlantic salmon, but lower overall lifetime success (see McGinnity et al. 2003). Thirdly, while later generation ($F_2$, $BC_1$) farmed-wild hybrids in our study exhibited equal if not superior fitness relative to wild salmon, their generation ultimately depends on the survival of $F_1$ hybrids. Our results suggest that $F_1$ hybrid survival may be much poorer relative to wild salmon. Fourthly, even if true $F_2$ farmed-wild hybrids were produced in the wild, at present it cannot be ruled out that outbreeding depression in fishes may be generated in $F_3$ or later generations after further recombination. For instance, this has been observed in some plant and invertebrate studies (Edmands 1999; Fenster and Galloway 2000). Clearly then, the generality of our findings as they pertain to other animals, fishes or salmonid populations awaits further studies of (i) the genetic architecture underlying fitness in multi-generational hybrids at a variety of traits; (ii) the lifetime fate of hybrids in the wild; and (iii) the degree to which multi-generational, farmed-wild interbreeding influences overall wild population growth rates or productivity.

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## Supplementary material

The following supplementary material is available for this article online:

**Figure S1.** Cumulative survival of salmon alevins from the six different cross-types, over 69 days of exposure to the three highest pH treatments (to accompany Fig. 4).

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1752-4571.2008.00037.x (This link will take you to the article abstract).

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