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Relative risks of inbreeding and outbreeding depression in the wild in endangered salmon

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Abstract

Conservation biologists routinely face the dilemma of keeping small, fragmented populations isolated, wherein inbreeding depression may ensue, or mixing such populations, which may exacerbate population declines via outbreeding depression. The joint evaluation of inbreeding and outbreeding risks in the wild cannot be readily conducted in endangered species, so a suggested 'safe' strategy is to mix ecologically and genetically similar populations. To evaluate this strategy, we carried out a reciprocal transplant experiment involving three neighboring populations of endangered Atlantic salmon (*Salmo salar*) now bred in captivity and maintained in captive and wild environments. Pure, inbred, and outbred (first and second generation) cross types were released and recaptured in the wild to simultaneously test for local adaptation, inbreeding depression, and outbreeding depression. We found little evidence of inbreeding depression after one generation of inbreeding and little evidence of either heterosis or outbreeding depression via genetic incompatibilities after one or two generations of outbreeding. A trend for outbreeding depression via the loss of local adaptation was documented in one of three populations. The effects of inbreeding were not significantly different from the effects of outbreeding. Hence, at the geographic scale evaluated (34–50 km), inbreeding for one generation and outbreeding over two generations may have similar effects on the persistence of small populations. The results further suggested that outbreeding outcomes may be highly variable or unpredictable at small genetic distances. Our work highlights the necessity of evaluating the relative costs of inbreeding and outbreeding in the conservation and management of endangered species on a case-by-case basis.

Introduction

Human-induced fragmentation and depletion of many natural populations have resulted in a growing vulnerability to inbreeding depression and a loss of genetic diversity (Frankham 2005). Theoretical studies predict that active mixing of inbred populations can potentially rectify these problems (Vergeer et al. 2004; Pertoldi et al. 2007). Nevertheless, while even low levels of gene flow may restore inbred populations to more demographically and genetically healthy states because of increased heterozygosity (Westermeier et al. 1998; Pimm et al. 2006), population mixing can also result in outbreeding depression, wherein outbred cross types have reduced fitness relative to parental populations (Dobzhansky 1950; Templeton 1986). Such outbreeding depression may be extrinsically based, involving the loss of local adaptation, or intrinsically based through the disruption of coadapted gene complexes – the latter usually does not arise until the second or later outbred generations when full recombination of parental genomes occurs (Edmands 2007). Empirical work indicates that multi-generational outbreeding depression can be sufficiently severe in some cases (e.g., Goldberg et al. 2005) as to reduce fitness to a greater

extent than reductions generated by inbreeding depression (Edmands 2007).

A conundrum therefore faces many endangered species conservation programs. Should one maintain small, fragmented populations, isolated from one another, with the risk that inbreeding depression will ensue? Or should one actively or passively allow populations to interbreed, thereby reducing risks posed by inbreeding depression but increasing the probability of outbreeding depression? While a suggested 'safe' strategy may be to mix inbred populations that are as ecologically and genetically similar as possible (Edmands 2007), the joint evaluation of inbreeding and outbreeding in the wild is necessary, but is rarely conducted, to evaluate their relative expected impacts on population viability.

Herein, we assess the relative risks of inbreeding and outbreeding depression in multiple populations of endangered Atlantic salmon (*Salmo salar*), using experimentation carried out in the wild. As with all salmonid fishes, the Atlantic salmon is comprised of ecologically and genetically differentiated populations, many of which have been adversely affected by an array of human activities (Garcia de Leaniz et al. 2007). Captive breeding programs have been increasingly used to rescue severely depleted salmonid populations from extinction because of each population's suspected import to the species' adaptability and long persistence (Fraser 2008). As a result, salmonid captive breeding programs are based on the assumption that populations from different rivers, even at fine geographic scales, represent independently evolving 'units' (Fraser 2008) and attempts are made to minimize outbreeding between them. The Atlantic salmon therefore represents an exemplary vertebrate species with which to evaluate the inbreeding–outbreeding conundrum in small-population conservation.

In the case of inner Bay of Fundy (iBoF) Atlantic salmon, a severely depleted group of phylogenetically related populations in eastern Canada that exhibits characteristics rarely found elsewhere in the species' global distribution (e.g., localized marine migration) (COSEWIC 2006), individual populations are being maintained, in relative isolation, through three generations of captive breeding (O'Reilly and Harvie 2009). With successive generations of captive breeding, the inbreeding–outbreeding conundrum is emerging. On one hand, the numbers of wild fish used to initiate captive broodstocks for most iBoF populations were necessarily small. There were also indications of genetic bottlenecking, reductions in heterozygosity, and possible inbreeding in some of these founder broodstocks (Tymchuk et al. 2010; Appendix S1 in Supporting Materials in the present study). On the other hand, while gene flow may have been extensive among iBoF populations historically (Fraser et al. 2007), mixing of captive populations could still result in a loss of persisting, 'cryptic', fine-scale local adaptation, which might hinder current and future recovery efforts.

There were several reasons why we initiated an experiment to test the 'safe' mixing strategy in iBoF salmon. First, many populations in this group are now thought to be extirpated (COSEWIC 2006), eliminating the risk of genetic introgression of experimental individuals into wild populations. Second, we experimented on juveniles, few of which would have been expected to reproduce in the wild. Third, any fish surviving to maturity in the future will be collected and readily identified as an outbred cross type using molecular data. And fourth, there was a need to evaluate the relative risks of inbreeding and outbreeding in the captive management of these populations.

Our experiment in the wild involved a reciprocal transplant experiment of pure, inbred, and outbred cross types from three iBoF populations to simultaneously test for evidence of local adaptation, inbreeding depression, and outbreeding depression. Specifically, we quantified juvenile survival of different interbred cross types at two time periods following release into the wild (5 months and 1 year after release). Our work represents the first for a fish, and one of only a few across diverse endangered taxa, to use reciprocal transplantation to explicitly test for local adaptation, inbreeding depression, and outbreeding depression simultaneously.

Materials and methods

Study populations

We included salmon from three, captive-bred populations of the iBoF to generate experimental cross types: Economy (denoted ECO and E) (45°22′N 63°54′W), Great Village (GRV and G) (45°22′N 63°36′W), and Stewiacke (STW and S) Rivers (45°8′N 63°22′W). Genetic data either on historical samples or on the last remaining samples collected from wild individuals indicate that these salmon were characterized by relatively small to moderate levels of neutral genetic differentiation (F_{ST} ECO-GRV = 0.0673, ECO-STW = 0.0953, GRV-STW = 0.0353), similar numbers of differentially expressed functional genes (ECO-GRV = 55, ECO-STW = 59, GRV-STW = 54), and that ECO and likely GRV had experienced recent genetic bottlenecks (Tymchuk et al. 2010; Appendix S1); the latter may result in large inbreeding coefficients (Wang et al. 2002).

Production and rearing of cross types

Our study involves three generations of ECO, GRV, and STW salmon families: wild, first generation in captivity, and second generation in captivity (Fig. 1). Wild fish

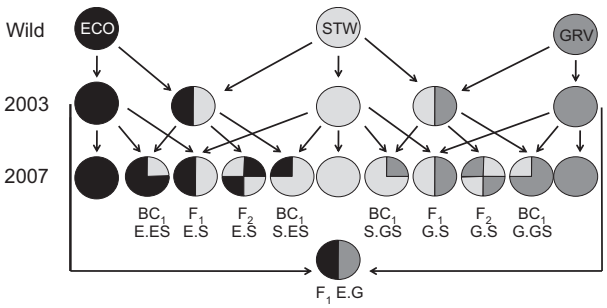

Figure 1 Experimental cross types within (pure) and among (outbred) rivers. Modified from Houde et al. (2011). Cross type symbols: ECO and E = Economy (black), GRV and G = Great Village (dark gray), and STW and S = Stewiacke (light gray), F_1 = first-generation outbred, F_2 = second-generation outbred ($F_1 \times F_1$), and BC_1 = backcross outbred (pure × F_1). Arrows represent the parental cross types. The proportion of genes from any one population is reflected by the area of the circle in outbred cross types.

were captured as juveniles in 2001 from each river and reared until maturity in 2003 at the Coldbrook Biodiversity Facility, Nova Scotia, Canada. The first generation of families was produced in 2003, using the mature wild fish as parents for pure cross types (ECO, GRV, and STW, and intentionally avoiding matings between full and half sibs) and first-generation outbred cross types (F_1 E.S and G.S; no F_1 E.G was produced). Houde et al. (2011) describe the microsatellite genotyping (five loci) of the 2003 surviving offspring (N = 559) and their subsequent assignment to wild parents.

The second generation of families was produced in 2007, using a combination of previously spawned wild and 2003-born individuals as parents. The 2007 cross types comprised three pure cross types, six inbred cross types (inbreeding coefficients, F = 0.125 (1/8) and 0.25 (1/4) for each of ECO, GRV, and STW river populations, assuming a base inbreeding coefficient of F = 0; Wang et al. 2002), and nine outbred cross types, including newly available F_1 E.G (first-generation) outbred cross type, second-generation outbred cross types F_2 ES.ES and GS.GS (F_1 outbred × F_1 outbred), and backcross outbred cross types BC_1 E.ES, G.GS, S.ES, and S.GS (pure × F_1 outbred) (Fig. 1). Twenty families were produced per cross type, using a balanced mating design in which the same ten females and ten males from a parental cross type, i.e., ECO, GRV, STW, F_1 E.S, and F_1 G.S, were represented in each cross type. Within a cross type, the families were mostly full-siblings, except for the pure and F_2 cross types in which each female and male was mated twice to produce twenty different families. Inbred cross types (i.e., F = 1/4 and 1/8) were pooled for analysis to increase statistical power; this was necessary because of the low number of inbred families. Additional details on the production of the 2007 cross types, and a description of the common environmental rearing conditions under which they were raised until release into the wild, are described by Houde et al. (2011). In brief, eggs were fertilized at the Coldbrook Biodiversity Facility and placed in trays within an incubation trough. Eggs were treated with formalin twice a week to prevent the spread of fungus and, because of rearing space limitations, eggs were transferred to the Aquatron Facility, Dalhousie University, at the developmental stage of 294 degree-days. At the Aquatron, the eggs were kept in modified Kritter Keepers and dead individuals were removed daily until the pooling of individuals for wild releases.

Salmon releases in the wild

Between May 19–22, 2008, we released approximately 30 000 unfed salmon fry (salmon ready to commence exogenous feeding, approximately 5 months after egg fertilization) from the different cross types into the wild, according to a reciprocal transplant design and using three sites per river (Fig. 2; Table 1). Fry release involved the removal of small batches of individuals from a site-specific transport container, using a fine-meshed net and allowing the fish to actively swim out of the net into river habitat.

Each site within each of the three rivers contained cross types in which one parent or one grandparent was 'local' to the river and cross types comprising two 'foreign' population controls which were the pure cross types from the other two rivers examined. Within each cross type, attempts were made to equalize the numerical contributions of families from females for which all the planned families involving that female survived. This helped to mitigate potential maternal influences on offspring survival or body size attributes in comparisons among cross types within and between rivers (e.g., Wallace and Aasjord 1984; Einum and Fleming 1999). However, in cases where there were few fry remaining within a cross type, all fry of the complete female families were used; if there were insufficient fry to attain the number per cross type required for release, fry (N = 4290, 14.5%) were obtained from the remaining families per cross type. Attempts were made within each cross type to equalize the contributions of any additional families.

Recapturing released salmon from the wild

We recaptured juvenile salmon from the different release sites, using a backpack electrofisher and a lip-seine net, at 5 months and 1 year after release (September 19–October 15, 2008; April 19–May 13, 2009, respectively). We

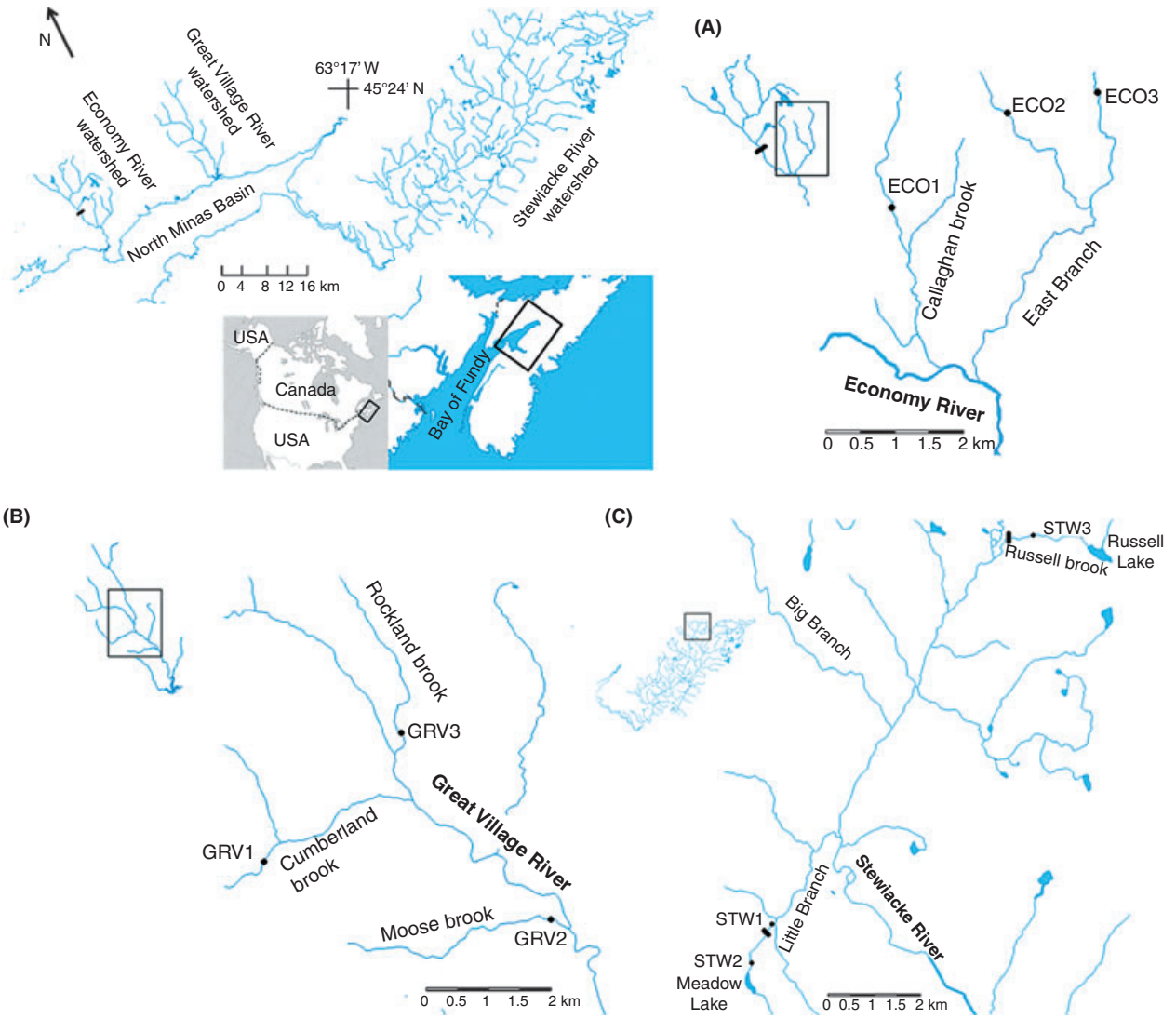

Figure 2 Sites within the experimental rivers. (A) Economy River sites, (B) Great Village River sites, and (c) Stewiacke River sites. Bold lines near the Stewiacke River sites represent bridges.

electrofished 500–600 m downstream of the release points. Approximately 200 juveniles/day (age 0+) and 70 juveniles/day (age 1+), respectively, were captured at most sites in the fall ($N = 1747$) and following spring ($N = 1110$).

Captured juveniles were held within in-river live boxes until the end of electrofishing for the day. Juveniles were then anaesthetized, using clove oil, measured (nearest mm), and weighed (nearest 0.01 g) before a small portion of one of the lobes of the caudal fin was clipped and preserved in 95% ethanol for later genotyping. Following sampling, individuals were allowed to recover and were subsequently released, following the same procedure as the initial fry release. We note that fin clipping 5 months after release (fall) did not significantly affect the recapture rates of different families in the following spring (Appendix S2).

Parentage assignments

Individual tissue samples were genotyped at the same five microsatellite loci used for 2003-born individuals (see Houde et al. (2011) for methodology). Exclusion-based, family assignment simulations in FAP 3.6 (Taggart 2007) predicted a ~97% success rate of unambiguous parentage assignment to a single family given the known 2007 families released into a given river. As expected, when carrying out the actual exclusion-based parentage assignments, we

Table 1. Numbers of fry released and the number of families in brackets at each of the three sites for each experimental river.

	Economy River		Great Village River		Stewiacke River	
Prop.*	Cross type	*N*	Cross type	*N*	Cross type	*N*
1	100% local					
	ECO (10)	167	GRV (6)	165	S (12)	168
	ECO.1/4 + ECO.1/8 (5)	167	GRV.1/4 + GRV.1/8 (6)	167	STW.1/4 + STW.1/8 (5)	167
2						
	75% local (BC_1)					
	E.ES (14)	670	G.GS (17)	670	S.ES (11)	334
					S.GS (14)	324
2						
	50% local (F_1)					
	E.G (10)	334	E.G (10)	334	E.S (11)	336
	E.S (11)	336	G.S (10)	334	G.S (10)	334
2						
	50% local (F_2)					
	ES.ES (9)	668	GS.GS (15)	668	ES.ES (9)	334
					GS.GS (15)	336
2						
	25% local (BC_1)					
	S.ES (11)	668	S.GS (14)†	482	E.ES (14)	335
					G.GS (7)	336
1						
	Foreign controls					
	S (12)	167	S (12)	167	E (10)	167
	G (6)	165	E (10)	168	G (6)	165
Total per site		3342		3155		3336
Total per river		10 026		9465		10 008

*Proportion of fry as a ratio from the percent local (<100%) and foreign control groups relative to the 100% local group.
†S.GS in GRV had an adjusted proportion of 1.5 because the number of fry was limiting.

were able to assign most recaptured juveniles (once released in the wild) to a single experimental family (individual rivers: ECO = 96.7%, GRV = 96.4%, and STW = 96.7%; range across sites: 94-98%; Appendix S3). To resolve ambiguous assignments, juveniles exhibiting at least one mismatch when compared to each of the cross type families ($N = 228$), and those assigning at all loci to more than one parental cross, were genotyped at two additional loci (data not shown), as were all of the parents. Juveniles that exhibited a single mismatch, involving a single allele that differed from a parental allele by a single repeat unit (four base pairs in this study), were assigned to the family for which six of seven loci matched. Juveniles that did not assign to a single cross type family under the above criteria were excluded from all further analyses.

Tests of local adaptation

For each of the two sampling periods (initial release to 5 months and initial release to 1 year after release), we tested for local adaptation in our study populations by using 'local versus foreign' and 'home versus away' criteria (Kawecki and Ebert 2004). We treated each site within a river as a data point that represented a sample of each river, to allow for the application of parametric statistics, i.e., Student's *t*-tests. We used recapture rates (number recaptured/number initially released) of the three pure cross types in our comparisons because the number released at each site was similar, i.e., 165–168 unfed fry (Table 1). Across the three sites in each river, we first compared the recapture rates of 'local' salmon ($N = 3$ within a river) relative to the other two 'foreign' salmon ($N = 6$ within a river), e.g., ECO versus GRV and STW salmon in the ECO River, using one-sided Student's *t*-tests. We pooled the two 'foreign' salmon to improve statistical power because of the low number of sites per river. We then compared the recapture rates of 'home' salmon ($N = 3$) relative to those measured when they were introduced in the two 'away' environments ($N = 6$), e.g., ECO salmon in ECO River versus GRV and STW Rivers, using one-sided Student's *t*-tests and 2×2 Fisher's exact tests. One-sided Student's *t*-tests were deemed appropriate because of the hypothesis that salmonids are

locally adapted (see Fraser et al. 2011), such that 'local' or 'home' salmon are expected to have higher recapture rates than 'foreign' or 'away' salmon.

All statistical testing was conducted in R 2.10.1 (available at http://www.r-project.org/) and all displayed *P*-values for multiple tests have been adjusted, using false discovery rate within sampling periods. Power analyses on statistical tests were conducted using the method of Cohen (1988) with the exception that the pooled standard deviation was used for comparing two samples.

Tests of outbreeding depression and heterosis

For each inter-population outbred comparison within a river, we first evaluated the potential for outbreeding depression via the loss of local adaptation. This was assessed by testing the significance of a linear regression between cross type recapture-rate estimates and the percentage of local genes within each cross type, assuming that additive genetic variation underlies recapture-rate differences (cf. Kawecki and Ebert 2004). Positive relationships between cross type recapture-rate estimates and the percentage of local genes within each cross type would indicate outbreeding depression. Recapture-rate estimates of pooled families by cross type at each site, and pooled families by cross type in each river, were generated using weighted binomial generalized linear models (*glm*). If the binomial model showed overdispersion, we re-analyzed the data using a quasi-binomial model (see Crawley 2005). Cross type recapture estimates, excluding inbred cross types, were tested for dependence on the percentage of local genes using linear regressions (*lm*) using the initial release number of unfed fry for the cross types as weights. There were two linear regressions applied to each site, one for each introgressed population comparison.

Next, we evaluated the potential for heterosis and outbreeding depression via the disruption of coadapted gene complexes in outbred cross types; the former would be reflected in an outbred cross type having higher recapture rates than the parental midpoint value and the latter in BC_1 and F_2 outbred cross types having lower recapture rates than the parental mid-point. Parental midpoints for F_1 and F_2 outbred cross types were calculated as $1/2\ (P_L + P_F)$ and for BC_1 outbred cross types as $3/4P_L + 1/4P_F$, where P_L and P_F were the recapture rates for the local and foreign pure cross types, respectively (Fraser et al. 2010). The magnitudes of change in outbred cross types relative to the parental midpoint were calculated as $[(X_{\text{outbred}}/X_{\text{parental midpoint}}) - 1]$ (Edmands 2007). Each site within a river was treated as a data point that represented a sample ($N = 3$) of each river, to allow for the application of parametric statistics. Two-sided Student's *t*-tests were used to test for differences in outbred cross type values from parental midpoints within rivers.

Genetic basis of outbreeding effects

For inter-population outbred comparison, we adopted two approaches to evaluate the genetic basis of outbreeding effects in outbred cross types (i.e., related to survival): *d/a* ratio tests and joint-scaling tests. For the *d/a* ratio tests, estimates of the additive (a) and dominance (d) parameters were used to test the relative contribution of additive versus dominance effects in outbred recapture rates (Falconer 1989), where $a = (P_L - P_F)/2$ and $d = F_1 - (P_L + P_F)/2$, with P_L and P_F being the recapture rates of the 'local' and 'foreign' pure cross types, respectively. Parameter estimates were generated using linear contrasts in R; 95% confidence intervals (CIs) for parameter estimates were generated using *confint*. The CIs for *d/a* ratios were calculated using Fieller's method (Piepho and Emrich 2005) that is programmed into the *sci.ratio.gen* function in the *mratios* package of R.

Joint-scaling tests (described in Lynch and Walsh 1998) were used to assess genetic inheritance models for the outbred recapture rates. We tested a 'mean-only' model and a simple additive inheritance model. A likelihood ratio test was used to determine which model (i.e., mean-only versus additive) best fits the data. The mean-only model would suggest that there is no genetic divergence, whereas an additive model would suggest genetic divergence among the cross types. If neither the mean-only nor the additive model provided a significant fit, a more complex inheritance model that incorporated dominance effects was generated. A likelihood ratio test was used to determine which mode of inheritance (e.g., additive versus additive–dominance) best fits the data. If neither the mean-only nor the additive model provided a significant fit for comparisons between ECO and GRV, *d/a* ratio tests were used to test for dominance effects because joint-scaling tests incorporating dominance effects require a minimum of four cross types and only three cross types were available (i.e., ECO, GRV, and F_1 E.G).

Tests of inbreeding depression

For each sampling period, we tested for inbreeding depression by comparing the recapture rates of inbred versus pure cross types across the three sites within each river (ECO, GRV, and STW). The magnitude of change in recapture rates of inbred relative to pure cross types was calculated as $[(X_{\text{inbred}}/X_{\text{local pure}}) - 1]$ (Edmands 2007). Each site within a river was treated as a data point that represented a sample ($N = 3$) of each river, to allow for the application of parametric statistics. Significance

was determined by one-sided Student's *t*-tests under the hypothesis that the magnitude of change was less than zero within rivers. One-sided Student's *t*-tests were deemed appropriate because the levels of inbreeding used here (i.e., F = 1/4 and 1/8) should have been sufficient to have generated inbreeding depression (see Ryman 1970; Kincaid 1983; Thrower and Hard 2009), i.e., a negative magnitude of change.

Tests of the risks of inbreeding versus outbreeding

For each inbred versus inter-population outbred comparison within a river, i.e., inbred versus F_1, F_2, BC_1 (25% local genes), or BC_1 (75% local genes), we used two-sided Student's *t*-tests to test for significant differences between the performance of inbred and outbred cross types relative to the pure cross type. Each site within a river was treated as a data point that represented a sample ($N = 3$) of each river, to allow for the application of parametric statistics. Respectively, the magnitude of change in recapture rates of inbred relative to pure cross types and outbred relative to pure cross types were calculated as $[(X_{\text{inbred}}/X_{\text{local pure}}) - 1]$ and $[(X_{\text{outbred}}/X_{\text{local pure}}) - 1]$.

Results

Local adaptation

Five months after release, we found a trend for local adaptation in only one of the three populations: ECO juveniles were recaptured at a higher rate in their 'local' river than 'foreign' GRV and STW juveniles (one-sided Student's *t*-test, $P = 0.030$, power = 0.799; high power to detect large differences). One year after release, a similar pattern was observed, but recapture rates of ECO juveniles were not significantly higher than those of GRV and STW juveniles (one-sided Student's *t*-test, $P = 0.327$, power = 0.461). On the other hand, ECO juveniles did not meet the 'home versus away' criterion of local adaptation, as the one-sided Student's *t*-test was not significant ($P = 0.138$, power = 0.466) and none of the 2×2 Fisher's exact tests was significant (Fig. 3). Among GRV and STW juveniles, there was little evidence of local adaptation for either 'local versus foreign' or 'home versus away' criteria, as neither the one-sided Student's *t*-tests (power mean ± 1SD = 0.047 ± 0.048; low power to detect small differences) nor the 2×2 Fisher's exact tests were significant for these populations ($P > 0.05$).

Outbreeding depression via the loss of local adaptation

Eleven of 12 tests for the ECO sites were characterized by recapture rates for outbred cross types that were positively related to the percent local ECO genes in the cross type. However, excepting the analysis in which the ECO sites were pooled within ECO River (Fig. 4, power = 0.665 ± 0.392), none of these tests was significant (power = 0.465 ± 0.294). Corroborating the finding of little or no evidence for local adaptation in GRV and

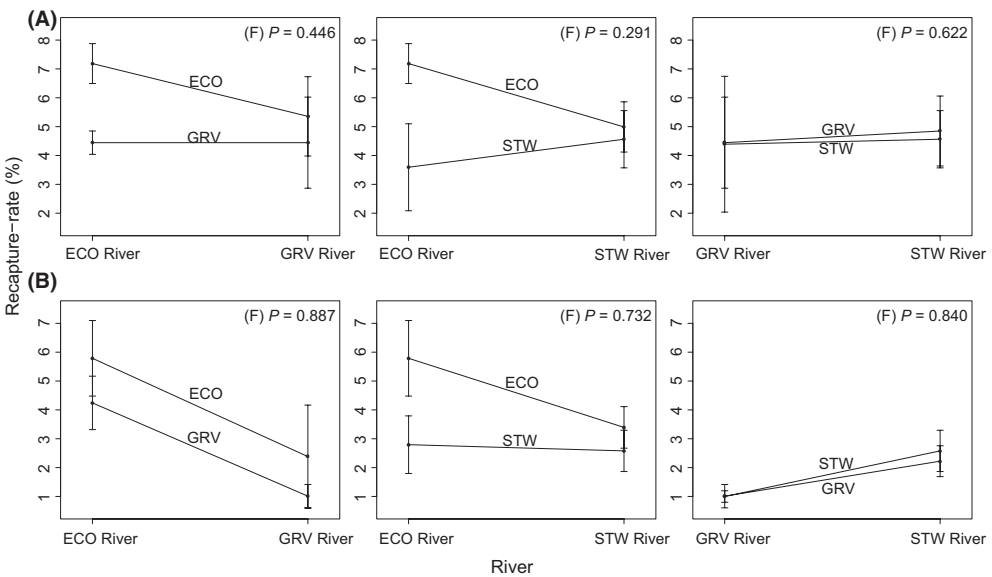

Figure 3. Juvenile recapture-rate means and one standard errors for the three sites per river testing for 'home versus away' interactions. (A) Five months after release and (B) 1 year after release. Displayed is the *P*-value after false discovery rate adjustment for 2×2 Fisher's exact test testing for a positive association between 'home' river and 'home' cross type.

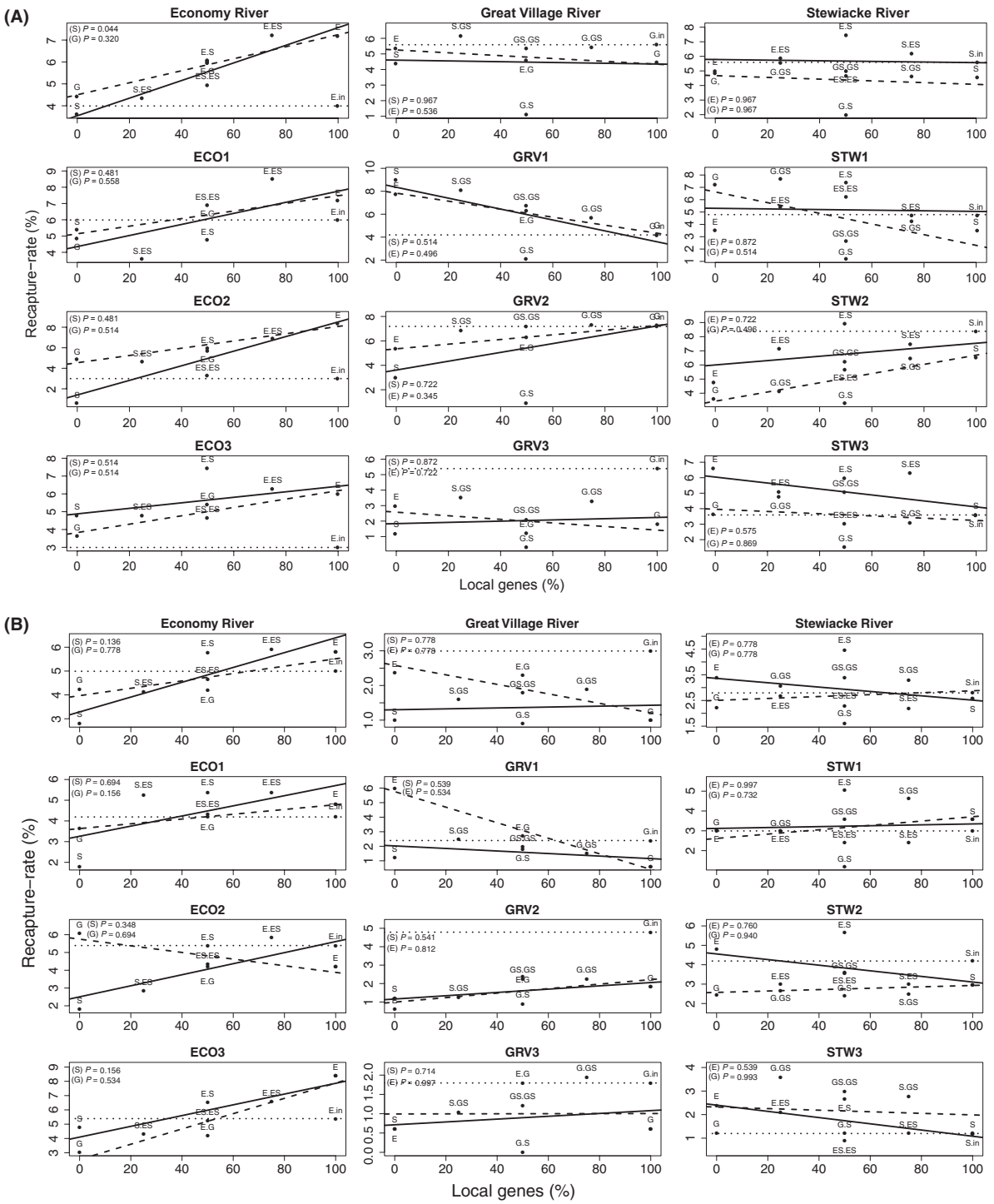

Figure 4. Juvenile recapture rates by percent local genes. (A) Five months after release and (B) 1 year after release. Pooled information for the three sites in the river is displayed in top row of panels. Dotted horizontal lines represent the value of the inbred cross type. Solid and dashed lines represent linear regressions with STW or ECO/GRV genes, respectively. Displayed are the *P*-values after false discovery rate adjustment.

STW juveniles is the observation that few relationships between recapture rates for outbred cross types and percent local GRV or STW genes for GRV or STW sites were positive ($N = 12$ of 24 tests); indeed, the same number of tests was negative ($N = 12$) and none of these tests was significant (power = 0.192 ± 0.243).

Heterosis and outbreeding depression

Overall, there was little indication of heterosis via increased heterozygosity or outbreeding depression via the breakdown of coadapted gene complexes, although the power to detect heterosis and outbreeding depression was low (power = 0.269 ± 0.274). Namely, only two, first-generation outbred cross types (F_1 E.S and G.S) had recapture rates that deviated significantly from parental midpoint values, although significance differed between sampling periods and rivers examined (Table 2). There were no significant deviations from parental midpoint values for the third first-generation outbred cross type, the two second-generation outbred cross types, or the four backcrossed outbred cross types.

Genetic basis of outbreeding effects

All joint-scaling tests revealed no genetic divergence among the cross types as the *P*-values for the likelihood ratio tests were not significant ($P > 0.05$) to reject the mean-only model relative to the simple additive inheritance, except for ECO sites pooled for ECO River (Table 3). The *P*-values of the likelihood ratio tests for ECO sites pooled for ECO River were significant to reject the mean-only model in favor of the simple additive inheritance model.

Inbreeding depression

We found no evidence for inbreeding depression in the three rivers, using pooled inbred cross types with F values of 1/4 (0.25) and 1/8 (0.125) (Table 2), although the power to detect inbreeding depression was low (power = 0.125 ± 0.253). F was calculated conservatively by assuming an initial population F of 0 (Wang et al. 2002).

Risks of inbreeding versus outbreeding

Relative to pure cross types, there was no indication that the risks associated with one generation of inbreeding differed from the risks associated with one or two generations of outbreeding. All inbred versus outbred comparisons of survival were not significantly different (two-sided Student's *t*-tests, $P > 0.05$), although the power to detect differences between inbreeding and outbreeding was low (power = 0.135 ± 0.119).

Discussion

Local adaptation and loss of local adaptation via outbreeding depression

Only one of the three studied populations (ECO) exhibited survival rates supportive of the hypotheses of local adaptation and the loss of local adaptation in outbred cross types. Although ECO did not meet the 'home versus away' criterion of local adaptation (Kawecki and Ebert 2004), it is more important to conservation research that ECO performed better than GRV and STW within ECO River ('local versus foreign' criterion) than how ECO performed in GRV and STW Rivers ('home versus away' criterion). In addition, a recent review of local adaptation in salmonids (Fraser et al. 2011) concluded that lack of detection of a fitness trade-off ('home versus away' criterion) may not be surprising at this small spatial scale (34–50 km). Alternatively, the positive slopes observed between the percentage of 'local' genes and survival ('local versus foreign' criterion) could be explained by parental effects or other genetic differences among populations, such as fixed beneficial mutations (see Kawecki and Ebert 2004). However, upon inclusion of parental effects in mixed-effects models, these relationships remained, albeit with reduced significance (Appendix S4). Also, similar analyses that included parental effects on other fitness-related traits, i.e., juvenile size, condition, and growth, also revealed few relationships (Houde 2009). The inability to detect local adaptation in all three rivers was notable for Atlantic salmon. It suggests that the geographic scale of local adaptation in our study region (the iBoF) may be larger than the scale of 'river' (as concluded by Fraser et al. 2007), the scale at which traditional conservation strategies have most often been applied in this species (Garcia de Leaniz et al. 2007; Fraser 2008).

Heterosis and outbreeding depression

Outbreeding effects depended on the populations that were mixed and did not correspond with neutral genetic or gene expression differences between populations or the inbreeding histories of the three study populations (Fraser et al. 2007; Tymchuk et al. 2010; Appendix S1). Heterosis is predicted to occur in outbred cross types between closely related populations, such as ECO and GRV populations, that have a history of inbreeding (Vergeer et al. 2004; : Pertoldi et al. 2007). Yet, we were unable to detect heterosis in outbred cross types having ECO or GRV population ancestry with the exception of F_1 E.S juveniles in the STW River (but not in the ECO River). Furthermore, outbreeding depression is not predicted to occur in outbred cross types between closely related populations (Edmands and Timmerman 2003; Gilk et al. 2004; but see McClelland and Naish 2007). However, we may not have been able to detect small changes resulting from heterosis or outbreeding depression because of our limited statistical power in having three sites that allowed only three pure-outbred cross type comparisons per river.

Table 2. Magnitudes of change and significance for juvenile recapture rates 5 months and 1 year after release.*

		First-generation outbred‡			Second-generation outbred‡		Backcross outbred‡			
Site	Inbred†	E.G	E.S	G.S	ES.ES	GS.GS	E.ES	G.GS	S.ES	S.GS
5 months after release										
ECO1	−0.166	0.144 (−0.042)	−0.243 (−0.337)	–	0.095 (−0.042)	–	0.263 (0.184)	–	−0.385 (−0.500)	–
ECO2	−0.643	−0.140 (−0.321)	0.325 (−0.290)	–	−0.267 (−0.607)	–	0.067 (−0.181)	–	0.824 (−0.446)	–
ECO3	−0.500	0.120 (−0.100)	0.381 (0.243)	–	−0.139 (−0.225)	–	0.102 (0.047)	–	−0.059 (−0.200)	–
P-value	0.136	0.842 (0.434)	0.719 (0.778)	–	0.707 (0.434)	–	0.494 (0.943)	–	0.843 (0.323)	–
Power	0.638	0.060 (0.190)	0.077 (0.071)		0.093 (0.182)		0.279 (0.051)		0.056 (0.587)	
GRV1	−0.012	0.050 (0.482)	–	−0.683 (−0.506)	–	0.019 (0.588)	–	0.045 (0.337)	–	0.038 (0.907)
GRV2	−0.012	−0.004 (−0.135)	–	−0.825 (−0.876)	–	0.400 (−0.012)	–	0.179 (0.006)	–	0.685 (−0.059)
GRV3	1.964	−0.500 (−0.341)	–	−0.801 (−0.835)	–	0.390 (0.153)	–	0.974 (0.806)	–	1.607 (0.940)
P-value	0.910	0.716 (0.995)	–	0.058 (0.218)	–	0.494 (0.503)	–	0.590 (0.434)	–	0.590 (0.434)
Power	0.008	0.084 (0.050)		0.999 (0.863)		0.242 (0.132)		0.134 (0.168)		0.176 (0.192)
STW1	0.341	–	1.077 (1.083)	−0.779 (−0.665)	0.755 (0.760)	−0.506 (−0.250)	0.498 (0.504)	0.219 (1.167)	0.339 (0.341)	−0.039 (0.210)
STW2	0.280	–	0.575 (0.364)	−0.353 (−0.497)	0.003 (−0.131)	0.227 (−0.045)	0.370 (0.094)	−0.045 (−0.364)	0.225 (0.143)	0.114 (−0.010)
STW3	0.006	–	0.172 (0.667)	−0.585 (−0.581)	−0.411 (−0.162)	0.404 (0.417)	−0.130 (0.421)	0.315 (0.333)	0.454 (0.760)	−0.140 (−0.136)
P-value	0.910	–	0.494 (0.349)	0.260 (0.124)	0.843 (0.845)	0.895 (0.943)	0.590 (0.407)	0.590 (0.723)	0.260 (0.434)	0.843 (0.943)
Power	0.001		0.270 (0.455)	0.669 (0.999)	0.055 (0.062)	0.051 (0.052)	0.123 (0.337)	0.150 (0.083)	0.739 (0.263)	0.054 (0.052)
All sites *P*-value	–	0.674 (0.719)	0.261 (0.382)	0.002* (0.002*)	0.971 (0.818)	0.430 (0.117)	0.261 (0.330)	0.261 (0.330)	0.346 (0.937)	0.346 (0.330)
All sites Power		0.075 (0.081)	0.409 (0.183)	0.999 (0.999)	0.050 (0.060)	0.141 (0.149)	0.399 (0.291)	0.338 (0.284)	0.200 (0.051)	0.204 (0.242)
1 year after release										
ECO1	−0.125	−0.005 (−0.125)	0.627 (0.118)	–	0.318 (−0.094)	–	0.329 (0.122)	–	1.059 (0.094)	–
ECO2	0.286	−0.182 (0.000	0.789 (0.278)	–	0.450 (0.036)	–	0.620 (0.389)	–	0.188 (−0.321)	–
ECO3	−0.357	−0.266 (−0.500)	−0.006 (−0.219)	–	−0.205 (−0.375)	–	−0.123 (−0.217)	–	−0.237 (−0.482)	–
P-value	0.984	0.454 (0.635)	0.454 (0.768)	–	0.498 (0.635)	–	0.454 (0.711)	–	0.498 (0.635)	–
Power	0.082	0.213 (0.135)	0.209 (0.058)		0.090 (0.113)		0.122 (0.064)		0.085 (0.134)	
GRV1	2.952	−0.178 (3.446)	–	0.992 (1.964)	–	1.158 (2.211)	–	0.980 (1.463)	–	1.372 (3.108)
GRV2	1.635	0.985 (0.317)	–	−0.404 (−0.506)	–	0.489 (0.235)	–	0.346 (0.231)	–	−0.080 (−0.315)
GRV3	1.964	1.991 (1.964)	–	−1.000 (−1.000)	–	0.988 (0.976)	–	2.211 (2.201)	–	0.727 (0.712)
P-value	0.984	0.454 (0.635)	–	0.838 (0.883)	–	0.325 (0.635)	–	0.454 (0.635)	–	0.454 (0.635)
Power	0.001	0.147 (0.236)		0.053 (0.051)		0.627 (0.215)		0.242 (0.259)		0.162 (0.109)
STW1	−0.162	–	0.541 (0.417)	−0.637 (−0.665)	−0.270 (−0.329)	0.082 (0.000)	−0.049 (−0.164)	−0.060 (−0.167)	−0.301 (−0.329)	0.347 (0.296)
STW2	0.408	–	0.456 (0.900)	−0.113 (−0.195)	−0.075 (0.207)	0.323 (0.200)	−0.312 (0.003)	0.045 (−0.100)	−0.127 (0.006)	−0.130 (−0.170)
STW3	0.006	–	0.494 (1.250)	−0.003 (0.006)	−0.499 (−0.246)	1.477 (1.500)	−0.002 (0.755)	1.960 (2.000)	−0.197 (0.006)	1.323 (1.333)
P-value	0.984	–	0.044* (0.635)	0.454 (0.635)	0.454 (0.667)	0.454 (0.635)	0.454 (0.667)	0.498 (0.667)	0.324 (0.666)	0.454 (0.635)
Power	0.022		0.999 (0.485)	0.123 (0.140)	0.265 (0.075)	0.143 (0.115)	0.120 (0.072)	0.094 (0.080)	0.585 (0.091)	0.114 (0.104)
All sites *P*-value	–	0.615 (0.299)	0.062 (0.271)	0.771 (0.884)	0.774 (0.299)	0.084 (0.271)	0.771 (0.417)	0.179 (0.271)	0.774 (0.299)	0.179 (0.299)
All sites Power		0.138 (0.199)	0.938 (0.397)	0.088 (0.052)	0.058 (0.219)	0.780 (0.490)	0.075 (0.127)	0.455 (0.409)	0.057 (0.305)	0.427 (0.256)

*Asterisks denote significant *P*-values after false discovery rate adjustment.

†Magnitudes of change of inbred cross types relative to pure cross types calculated as $[(X_{\text{inbred}}/X_{\text{local pure}}) - 1]$. Significance of a negative value prediction (inbreeding depression) for inbred cross types was determined by a one-sided Student's *t*-test.

‡Magnitudes of change of outbred cross types relative to the parental midpoint calculated as $[(X_{\text{outbred}}/X_{\text{parental midpoint}}) - 1]$. In brackets are the magnitudes of change of outbred cross types relative to the local pure cross type calculated as $[(X_{\text{outbred}}/X_{\text{local pure}}) - 1]$. Significance of a zero value prediction (no difference between outbred cross type and mid-parent) was determined by a two-sided Student's *t*-test.

Table 3. Joint-scale analyses of juvenile recapture rates 5 months and 1 year after release.*

		5 months after release			1 year after release		
	Foreign pop.	Mean-only (M)	Additive (A)	Likelihood (M vs. A)	Mean-only (M)	Additive (A)	Likelihood (M vs. A)
Pooled sites by river							
Economy	GRV	4.470 (0.107)	0.253 (0.615)	A (0.040)	0.564 (0.754)	0.230 (0.631)	M (0.563)
	STW	7.629 (0.178)	1.151 (0.886)	A (0.010)	5.755 (0.331)	2.693 (0.610)	M (0.080)
Great Village	ECO	0.144 (0.931)	0.031 (0.860)	M (0.737)	2.274 (0.321)	0.403 (0.526)	M (0.171)
	STW	3.701 (0.593)	3.692 (0.449)	M (0.926)	7.253 (0.203)	6.240 (0.182)	M (0.314)
Stewiacke	ECO	2.615 (0.759)	2.613 (0.624)	M (0.970)	2.081 (0.838)	1.833 (0.766)	M (0.619)
	GRV	3.178 (0.672)	3.105 (0.540)	M (0.786)	4.813 (0.439)	4.714 (0.318)	M (0.754)
Sites							
ECO1	GRV	0.060 (0.971)	0.042 (0.838)	M (0.894)	0.020 (0.990)	0.012 (0.911)	M (0.930)
	STW	1.860 (0.868)	1.083 (0.897)	M (0.378)	1.423 (0.922)	0.452 (0.978)	M (0.324)
ECO2	GRV	0.070 (0.966)	0.001 (0.997)	M (0.792)	0.063 (0.969)	0.005 (0.941)	M (0.810)
	STW	3.085 (0.687)	0.574 (0.966)	M (0.113)	1.763 (0.881)	1.010 (0.908)	M (0.385)
ECO3	GRV	0.070 (0.966)	0.027 (0.869)	M (0.836)	0.126 (0.939)	0.037 (0.847)	M (0.766)
	STW	0.617 (0.987)	0.392 (0.983)	M (0.636)	0.162 (0.999)	0.076 (0.999)	M (0.769)
GRV1	ECO	0.110 (0.946)	0.111 (0.739)	M (1.000)	1.115 (0.573)	0.015 (0.901)	M (0.294)
	STW	1.375 (0.927)	1.225 (0.874)	M (0.698)	1.186 (0.946)	0.692 (0.952)	M (0.482)
GRV2	ECO	0.186 (0.911)	0.014 (0.906)	M (0.678)	0.589 (0.745)	0.332 (0.565)	M (0.612)
	STW	2.400 (0.791)	1.793 (0.774)	M (0.436)	0.345 (0.997)	0.219 (0.994)	M (0.722)
GRV3	ECO	0.124 (0.940)	0.128 (0.721)	M (1.000)	0.141 (0.932)	0.126 (0.723)	M (0.902)
	STW	2.139 (0.830)	2.062 (0.724)	M (0.782)	0.891 (0.971)	0.849 (0.932)	M (0.837)
STW1	ECO	0.568 (0.989)	0.566 (0.967)	M (0.960)	0.265 (0.998)	0.189 (0.996)	M (0.783)
	GRV	1.130 (0.951)	0.965 (0.915)	M (0.684)	0.403 (0.995)	0.376 (0.984)	M (0.870)
STW2	ECO	0.371 (0.996)	0.246 (0.993)	M (0.724)	0.409 (0.995)	0.221 (0.994)	M (0.664)
	GRV	0.281 (0.998)	0.034 (1.000)	M (0.619)	0.182 (0.999)	0.153 (0.997)	M (0.866)
STW3	ECO	0.349 (0.997)	0.321 (0.988)	M (0.868)	0.263 (0.998)	0.209 (0.995)	M (0.816)
	GRV	0.542 (0.991)	0.528 (0.971)	M (0.907)	0.561 (0.990)	0.571 (0.966)	M (1.000)

If $P < 0.05$ then reject the model.

*Displayed are χ^2 values for the mean-only model and additive inheritance model and in the brackets are the *P*-values.

We were unable to detect outbreeding depression by the breakdown of coadapted gene complexes in the second-generation outbred cross types. However, the potential for local adaptation and its loss via outbreeding depression in one of our study's three closely related populations is of concern from a conservation perspective. Furthermore, simple additive inheritance models on outbred recapture rates fit only ECO sites pooled in ECO River with most outbred recapture rates not being different from the mean of the sites or rivers, although there were some deviations from the parental midpoints (i.e., F_1 E.S heterosis and F_1 G.S outbreeding depression). F_1 heterosis and outbreeding depression may be attributed to dominance and epistatic interactions which are less predictable than additive effects (Kawecki and Ebert 2004; Edmands 2007).

Collectively, our results are consistent with the hypothesis that outbreeding outcomes may be highly variable at small genetic distances (Edmands and Timmerman 2003) and that the genetic interaction between population pairs may be difficult to predict because of random mutation and fixation processes (Lynch 2000; see Bougas et al. 2010). Hence, the reality for endangered species conservation is that outbreeding effects may have to be evaluated on a case-by-case basis.

Inbreeding depression

The unpredictability of outbreeding effects was mirrored by a similar lack of consistency in the ability to detect inbreeding depression that had been predicted to exist in ECO and GRV because of presumed large inbreeding coefficients attributable to population bottlenecks (Wang et al. 2002; Tymchuk et al. 2010; Appendix S1). Indeed, similar inbreeding coefficients have been associated with severe inbreeding depression in salmon in both captivity (Kincaid 1983) and in the wild (Ryman 1970; Thrower and Hard 2009). Yet, ECO and GRV did not exhibit inbreeding depression. Possible explanations for these results are that these populations may have naturally mixed with relatives at a sufficiently slow rate such that deleterious alleles may have been purged by selection

(Templeton and Read 1984; Allendorf and Luikart 2007). Alternatively, deleterious alleles may have become fixed by genetic drift, resulting in few differences between pure and inbred cross types (Keller and Waller 2002; Hedrick and Fredrickson 2010). The former explanation seems unlikely because there was an instance of heterosis when ECO was mixed with STW. In addition, the pooling of individuals with different inbreeding coefficients (F = 1/4 and 1/8) may not have generated a high enough level of inbreeding to detect inbreeding depression, at least for a salmonid fish (Gjerde et al. 1983; Pante et al. 2001). Furthermore, similar to outbreeding depression, we may not have been able to detect small changes owing to inbreeding depression because of the limited power in having three sites per river.

Relative risks of outbreeding and inbreeding

According to one analysis using Student's *t*-tests, our results suggest that the risks from one generation of inbreeding do not differ significantly from the risks posed by one or two generations of outbreeding within endangered Atlantic salmon populations. Yet another test, using linear regressions, which had greater mean statistical power (linear regression power = 0.300 ± 0.309 versus Student's *t*-tests power = 0.247 ± 0.249), indicated outbreeding depression via the loss of potential local adaptation in ECO. Furthermore, while limited in statistical power because of having just three comparisons, there was a trend in Student's *t*-tests (P = 0.136, power = 0.638) for inbred ECO to consistently perform more poorly than pure ECO at all three study sites within the ECO river. Our precautionary interpretation of these trends is that both outbreeding and inbreeding might be detrimental to survival for ECO Atlantic salmon.

More generally, over more generations than studied here, either process might affect the persistence of small populations (see Frankham 2005). For example, the decreased recapture rate of the second-generation (F_2) outbred cross type (F_2 ES.ES) relative to first-generation (F_1) cross type (F_1 E.S) juveniles could suggest a negative recapture-rate trend for successive outbred generations (see Dobzhansky 1950). Even reduced F_1 fitness coupled with fitness improvements in successive outbred generations (e.g., F_1 G.S versus F_2 GS.GS juveniles) may also be concerning. It may take several generations of outbreeding for fitness to recover to the same level as pure cross types because of natural selection for beneficial gene combinations (Edmands 2007), and small fitness declines in the earlier outbred generations could lead to population extirpation before there is time to recover in later outbred generations (Hutchings 1991).

One caveat of our work is that we could not assess the entire salmon life cycle because of the current very high mortality at sea experienced by iBoF salmon and other logistic issues (Fraser et al. 2007; DFO 2008). On the other hand, it is likely that these populations share similar adult life histories (COSEWIC 2006). Consequently, phenotypic changes detrimental to survival because of a loss of local adaptation in outbred cross types may be more likely to occur at juvenile than adult life stages (Taylor 1991).

Conservation implications

Our study is insightful given the conundrum of either continuing to accrue inbreeding or to risk outbreeding depression in the management of small fragmented populations and endangered species. In the case of endangered salmon, our study revealed that different management recommendations may be necessary even for closely related populations owing to varying inbreeding and outbreeding risks. For one population (ECO), both inbreeding and outbreeding may be detrimental to survival and it is recommended that pure noninbred ECO broodstock be maintained for conservation purposes. For the other populations, GRV and STW, inbreeding for one generation may not be detrimental to survival, at least during the freshwater phase of their life cycle, and interbreeding GRV and STW may be acceptable in their long-term management because the survival decline in the first outbred generation did not continue into the second generation (both backcrosses and F_2 cross types). Such specific recommendations would not have been possible without experimentation conducted in the wild. The relative costs of inbreeding and outbreeding in the conservation and management of endangered species may, therefore, have to be tested on a case-by-case basis and interpreted very judiciously.

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Data archiving statement

Data for this study are available in Dryad (DOI: 10.5061/dryad.8710).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Genetic estimates of salmon populations acquired using DNA microsatellite information.

Appendix S2. Effects of caudal fin clipping on juveniles traits.

Appendix S3. Percent of juveniles assigned and unassigned to experimental families 5 months and 1 year after release.

Appendix S4. Parental and length-at-release effects in recapture-rate data interpretation.

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