Contributed Paper

Risk Assessment of Inbreeding and Outbreeding Depression in a Captive-Breeding Program

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Abstract: *Captive-breeding programs can be implemented to preserve the genetic diversity of endangered populations such that the controlled release of captive-bred individuals into the wild may promote recovery. A common difficulty, however, is that programs are founded with limited wild broodstock, and inbreeding can become increasingly difficult to avoid with successive generations in captivity. Program managers must choose between maintaining the genetic purity of populations, at the risk of inbreeding depression, or interbreeding populations, at the risk of outbreeding depression. We evaluate these relative risks in a captive-breeding program for 3 endangered populations of Atlantic salmon (Salmo salar). In each of 2 years, we released juvenile F_1 and F_2 interpopulation hybrids, backcrosses, as well as inbred and noninbred within-population crosstypes into 9 wild streams. Juvenile size and survival was quantified in each year. Few crosstype effects were observed, but interestingly, the relative fitness consequences of inbreeding and outbreeding varied from year to year. Temporal variation in environmental quality might have driven some of these annual differences, by exacerbating the importance of maternal effects on juvenile fitness in a year of low environmental quality and by affecting the severity of inbreeding depression differently in different years. Nonetheless, inbreeding was more consistently associated with a negative effect on fitness, whereas the consequences of outbreeding were less predictable. Considering the challenges associated with a sound risk assessment in the wild and given that the effect of inbreeding on fitness is relatively predictable, we suggest that risk can be weighted more strongly in terms of the probable outcome of outbreeding. Factors such as genetic similarities between populations and the number of generations in isolation can sometimes be used to assess outbreeding risk, in lieu of experimentation.*

Keywords: COSEWIC, egg size, environmental quality, hatchery, heterosis, live gene banking, local adaptation, maternal effects, population collapse, population viability

Evaluación del Riesgo de Depresión por Endogamia y Exogamia en un Programa de Reproducción en Cautiverio

Resumen: *Los programas de reproducción en cautiverio pueden ser implementados para preservar la diversidad genética de las poblaciones en peligro, de tal forma que la liberación controlada de los individuos criados en cautiverio a la vida libre puede promover la recuperación. Sin embargo, una dificultad común es que los programas se encuentran dentro del limitado capital de reproducción silvestre, y la endogamia puede volverse cada vez más difícil de evitar con generaciones sucesivas en cautiverio. Los directores del programa deben elegir entre mantener la pureza genética de las poblaciones, con el riesgo de una depresión endogámica, o reproducir entre poblaciones, con el riesgo de una depresión exogámica. Evaluamos estos riesgos relativos en un programa de reproducción en cautiverio para tres poblaciones en peligro de salmón del Atlántico (Salmo salar). Cada 2 años, liberamos juveniles híbridos inter-poblacionales F_1 y F_2, retrocruzamientos, así como cruzas endogámicas y no endogámicas entre poblaciones en nueve arroyos silvestres. El tamaño juvenil*

y la supervivencia se cuantificaron cada año. Se observaron pocos efectos de cruza, pero interesantemente, las consecuencias de la adaptabilidad relativa de la endogamia y exogamia variaron año con año. La variación temporal en la calidad ambiental pudo ser conductora en alguna de estas diferencias anuales, al exacerbar la importancia de los efectos maternales sobre la aptitud juvenil en un año de baja calidad ambiental y al afectar diferentemente la severidad de la depresión endogámica en años diferentes. Sin embargo, la endogamia fue asociada constantemente con un efecto negativo sobre la adaptabilidad, mientras que las consecuencias de la exogamia fueron menos predecibles. Considerando los obstáculos asociados con un estudio concreto de riesgo en vida silvestre y dado que el efecto de la endogamia sobre la aptitud es relativamente predecible, sugerimos que el riesgo puede considerarse más fuerte en términos de un resultado probable de la exogamia. Factores como las similitudes genéticas entre poblaciones y el número de generaciones en aislamiento puede usarse algunas veces para estudiar el riesgo de la exogamia, en lugar de la experimentación.

Palabras Clave: adaptación local, banco de genes vivos, calidad ambiental, colapso de población, COSEWIC, criadero, efectos maternales, heterosis, tamaño del huevo, viabilidad poblacional

Introduction

When populations experience severe declines, captive-breeding programs can be implemented to avoid extirpation or extinction (Fraser 2008). Captive-breeding programs use nurseries or hatcheries to maintain populations that are unable to persist in the wild. These programs are founded with wild broodstock, and they endeavor to preserve genetic diversity ex situ through carefully developed breeding designs and in situ through release of captive-bred animals into remnant populations, thereby increasing the chance of recovery. A common challenge, however, is that the wild broodstock used to found these programs is limited in number (O'Reilly & Doyle 2007) such that inbreeding can become increasingly difficult to avoid with successive generations of captive breeding. Managers may therefore maintain the genetic integrity of their populations, at the risk of inbreeding depression, or they may outbreed their populations with other populations being maintained in captivity, at the risk of outbreeding depression (Edmands 2007). Adopting the better strategy depends on the relative risks of inbreeding and outbreeding depression.

On the one hand, inbreeding decreases heterozygosity, thereby increasing the likelihood that deleterious recessive alleles will be expressed. This can result in reductions in individual fitness or population viability from inbreeding depression (Lynch 1991). Managers working with limited wild broodstock may choose to avoid the risk of inbreeding depression by deliberately outbreeding populations (Edmands 2007). Indeed, outbreeding can introduce new alleles into a population. This can increase heterozygosity, mask deleterious recessive alleles, promote overdominance, or have positive epistatic effects (Lynch 1991).

On the other hand, outbreeding can also result in a loss of fitness by virtue of genetic dissimilarity among populations. When at least 1 population exhibits local adaptation, outbreeding can depress fitness in the local environment as early as the F_1 generation by diluting locally beneficial alleles; this is extrinsic outbreeding depression (Edmands 2007). Incompatibilities in the genetic environment can also lead to outbreeding depression. Detrimental epistatic effects may occur in the first generation of outbreeding if independent allelic assortment disrupts gene–gene coadaptation, or gene–gene coadaptation could be disrupted through crossing over after the first generation of outbreeding (Dobzhansky 1950; Templeton 1986). This is intrinsic outbreeding depression.

Managers may be opposed to outbreeding under the precautionary assumption that each population exhibits local adaptation. As an example, the chief paradigm underlying Atlantic salmon (*Salmo salar*) conservation efforts is that each river group represents 1 population that is adapted to local conditions, such that conservation plans tend to be employed at the scale of single rivers (Garcia de Leaniz et al. 2007). There are reasons to suspect that local adaptation is prevalent in salmonids: salmonids exhibit philopatry to the natal river, populations can exhibit temporally stable genetic structure, and some degree of genetic differentiation usually occurs among populations (Hendry & Stearns 2003). Whether or not local adaptation is common in Atlantic salmon is not well understood (Fraser et al. 2011); nonetheless, deliberate outbreeding as part of any conservation effort has been discouraged historically (Garcia de Leaniz et al. 2007).

Atlantic salmon are declining throughout much of eastern North America (COSEWIC 2010), and captive-breeding programs have been initiated to recover many endangered populations (Fraser 2008). Managers must develop breeding designs that maintain genetic diversity and fitness of populations. Although outbreeding is typically discouraged, captive-breeding programs must face the challenges imposed by limited wild broodstock. This necessarily entails weighing the risks of inbreeding and outbreeding depression. We drew from a captive-breeding program initiated to recover 3 critically endangered populations of Atlantic salmon in the inner Bay of Fundy (iBoF), Canada (O'Reilly & Harvie 2009). We performed a temporally replicated risk assessment
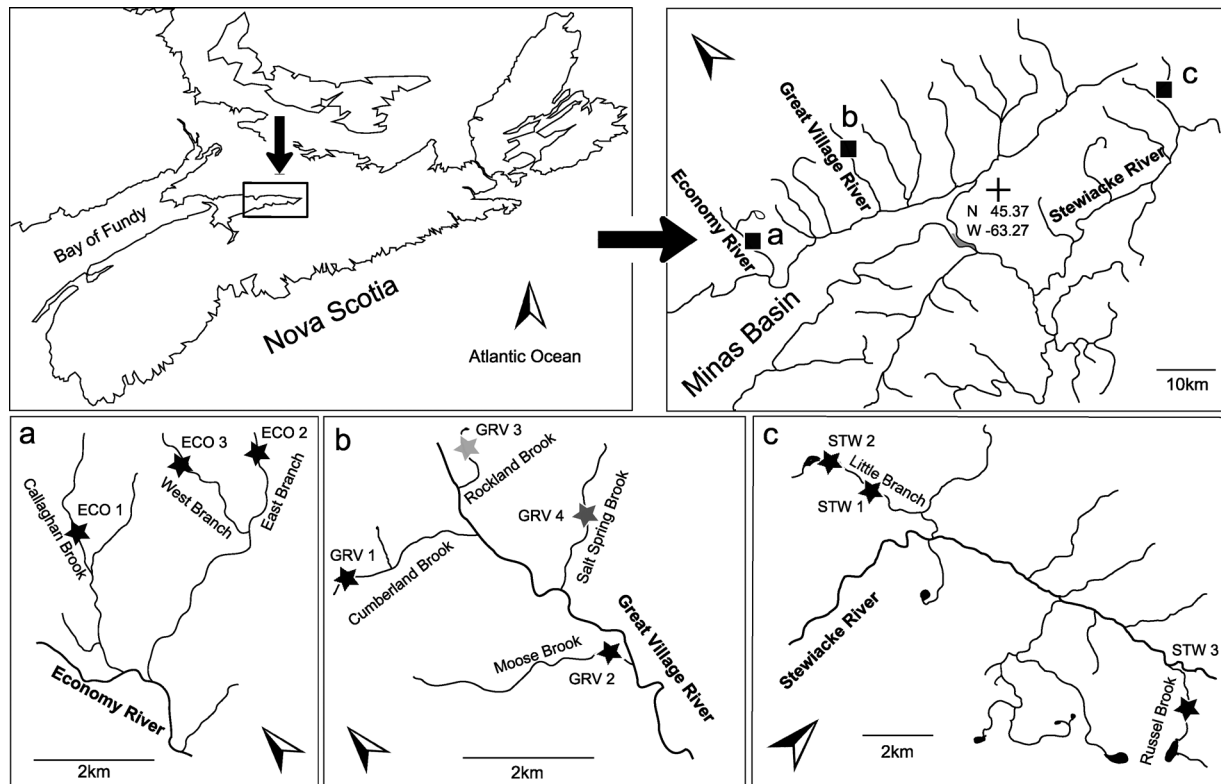
Figure 1. Nova Scotia, Canada, and the location of release streams near the inner Bay of Fundy. Regions depicted by black squares (a, b, and c) in the upper right panel are shown on a smaller scale in subsequent panels (black stars, release points used both in 2008 [Houde et al. 2011a] and 2009; light gray star, release point used only in 2008; dark gray star, release point used only in 2009; Stw1-3, Stewiacke release streams; Eco1-3, Economy; Grv1-3, Great Village).
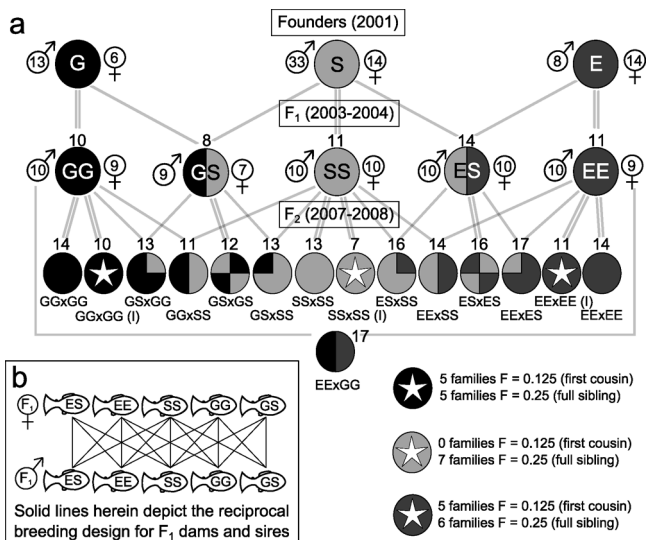
of inbreeding and outbreeding depression, evaluated the genetic basis of outbreeding effects, and tested for local adaptation in these 3 populations. This study is among the most elaborate reciprocal transplants performed in vertebrates. In addition to being directly applicable to the management of Atlantic salmon, the lessons learned from the present work should facilitate the development of inbreeding–outbreeding or translocation risk assessments in many other taxa.

Methods

We studied 3 populations of Atlantic salmon in the iBoF: the Stewiacke River (S), the Great Village River (G), and the Economy River (E). All rivers drain into the Minas Basin, and the maximum distance between river mouths is about 35 km (Fig. 1). Adult salmon in these populations presumably share a common marine environment, but adults migrate to their natal rivers to breed (Amiro 2003). Genetic data suggest that differentiation among rivers (F_{ST}: G−S = 0.0353; E−G = 0.0673; E−S = 0.0953) (Tymchuk et al. 2010) is typical of levels of differentiation in Atlantic Canada (e.g., Dionne et al. 2008).

The iBoF historically supported several productive salmon rivers, but populations collapsed in the 1990s, and it is estimated that <200 adults remain in the wild (COSEWIC 2010). In 2001, the Department of Fisheries and Oceans (DFO) initiated an effort to recover these populations. A total of 56, 52, and 198 wild salmon parr (juveniles at least 1 year old) were collected from the Economy, Stewiacke, and Great Village Rivers, respectively. These wild-born E, G, and S fish were genotyped and reared to maturity in captivity. Levels of relatedness were estimated for wild-born fish, as in Herbinger et al. (2006), to facilitate the implementation of a broodstock management program (O'Reilly & Doyle 2007) intended to minimize loss of genetic variation in subsequent generations.

In 2003 and 2004, E, G, and S fish were spawned (Fig. 2). Three pure within-river crosstypes were generated by crossing wild-born fish that were collected from the same river: pure Stewiacke (S×S = SS), pure Great Village (G×G = GG), and pure Economy (E×E = EE). In addition to these pure SS, GG, and EE crosstypes, wild-born broodstock were used to generate 2 outbred crosstypes (Fig. 2a). Outbred crosstypes were generated by mating E fish with S fish (E×S = ES) and by mating G fish with S fish

Figure 2. (a) Salmo salar from the Great Village (G), Economy (E), and Stewiacke (S) Rivers, Nova Scotia, were bred in captivity over 2 generations to evaluate the effect of inbreeding and outbreeding on juvenile growth and survival in the wild. In autumn 2007 and 2008, we produced 3 pure offspring crosstypes (EE×EE, SS×SS, GG×GG) and 3 inbred offspring crosstypes (mixture of families in which F = 0.125 or 0.25), denoted with an (I). We created 3 first-generation outbred offspring crosstypes (EE×SS, GG×SS, EE×GG), 2 second-generation outbred crosstypes (ES×ES, GS×GS), and 4 backcrosses (EE×ES, SS×ES, GG×GS, SS×GS). Values in male and female symbols are the number of parents contributing to crosstypes of subsequent generations for 2009 releases, and values above a crosstype represent the number of families created for 2009 releases. (b) The 5 crosstypes generated in 2003 and 2004 (EE, SS, GG, ES, and GS) were mated in a reciprocal breeding scheme to create offspring for the present study (solid lines, potential matings among the 5 crosstypes). In this design, any given dam and sire is represented in multiple offspring crosstypes.

(G×S = GS). Fewer wild-born G and E fish were available to generate crosstypes than S fish (e.g., Fig. 2a); thus, the outbred GE crosstype was not created in this captive-breeding program. Pure and outbred crosstypes were reared through 2008 under common environmental conditions, and all captive-bred individuals were genotyped, allowing familial assignment. Details on this program are provided in the Supporting Information and elsewhere (Fraser et al. 2007; O'Reilly & Harvie 2009).

The consequences of inbreeding and outbreeding our focal populations were studied by Houde et al. (2011a, 2011b). We used populations and crosstypes identical to those studied by Houde et al., such that we combined

survival data from Houde et al. (2011a, 2011c) with additional data to produce a temporally replicated experiment. Therefore, offspring in our study were generated over 2 years. Breeding was performed on 5 and 8 November 2007 (Houde et al. 2011a, 2011b) and 31 October and 4 November 2008 at the Coldbrook Biodiversity Facility, in Coldbrook, Nova Scotia, Canada.

In each year, dams of a known crosstype (i.e., EE, GG, SS, ES, or GS) were stripped of their eggs, and eggs of each dam were divided into 4 to 7 groups of approximately equal number. Each group of eggs was fertilized with milt from a different sire (either EE, GG, SS, ES, or GS), thereby creating 4 to 7 maternal half-sib families that differed in crosstype (Fig. 2b). We used a reciprocal breeding design such that dams and sires from a given parental crosstype were represented in multiple offspring crosstypes (Table 1 & Fig. 2b). We generated fifteen offspring crosstypes (Table 1 & Fig. 2a), comprising pure crosstypes (e.g., SS parent × SS parent = SS×SS offspring crosstype), F_1 hybrids (e.g., SS×GG), F_2 hybrids (e.g., ES×ES), and backcrosses (e.g., ES×SS). Inbred crosstypes were generated by mating pure crosstypes of known familial relationship. The inbreeding coefficient for a given inbred family was either $F = 0.125$ (e.g., aunt × nephew) or $F = 0.25$ (e.g., sibling × sibling), assuming a base rate of $F = 0$ (Wang et al. 2002). We pooled inbred families of a given crosstype for analysis (i.e., SS×SS[I], EE×EE[I], and GG×GG[I]) (Table 1). We used between 4 and 25 full-sib parental families (median = 11.5) to generate offspring of a given crosstype (Table 1), and the number of full-sib offspring families generated per crosstype ranged from 5 to 17 (median = 12).

For offspring generated in 2007 and released in 2008, wild-born salmon ($n = 7$ dams, $n = 14$ sires) as well as captive-bred salmon born in 2003 ($n = 33$ dams, $n = 30$ sires) were used as parents (Table 1). For offspring generated in 2008 and released in 2009, 2 wild-born sires were used, but all other parents were born in captivity ($n = 45$ dams, $n = 46$ sires) (Table 1 & Fig. 2). Ideally, the use of wild-born fish as parents should have been avoided, but many captive-born fish had not reached maturity by the first year of this study. Therefore, we used wild-born parents to increase sample size for 2008 releases, but fewer wild-born parents were used for 2009 releases. Differences in the performance of offspring from captive versus wild-born parents are presented in Supporting Information.

We released juveniles into the wild between 19 and 22 May for the 2008 experiment, and on 11 May for the 2009 experiment. In both years, release timing coincided with the point of transition from endogenous to exogenous feeding. We released over 3000 juveniles in each year into each of 3 streams in the Economy, Great Village, and Stewiacke Rivers, following a release scheme designed to test for inbreeding and outbreeding effects (Table 1). One release stream differed between years such that a

Table 1. Number of parental families from which individual parents were drawn and number of individual parents (dams and sires) used to generate full-sib offspring families of *Salmo salar*.

Offspring crosstype	No. of dams[a]		No. of sires[a]		Parental families[b]	Offspring families	Mean no. of offspring released/family stream[c]			Total no. of offspring released/stream[c]		
	wild	captive	wild	captive			Eco	Grv	Stw	Eco	Grv	Stw
2008												
EE×EE	5	2	5	4	9	10	16.7	16.8	16.7	167	168	167
EE×EE(I)[d]	2	1	2	2	4	5	33.4	NA	NA	167	NA	NA
GG×GG	2	1	3	3	6	6	27.5	27.5	27.5	165	165	165
GG×GG(I)[e]	3	3	2	2	7	6	NA	27.8	NA	NA	167	NA
SS×SS	0	7	2	7	9	12	14	14	14	168	168	168
SS×SS(I)[f]	0	3	0	5	7	5	NA	NA	33.4	NA	NA	167
EE×GG	5	5	6	4	13	10	33.4	33.4	NA	334	334	NA
EE×SS	4	7	4	11	17	11	30.5	NA	30.5	336	NA	336
GG×SS	3	7	3	7	15	10	NA	33.4	33.4	NA	334	334
EE×ES	4	10	4	10	16	14	47.9	NA	23.9	671	NA	335
GG×GS	2	12	0	17	12	17	NA	39.4	19.8	NA	670	337
SS×ES	0	12	3	9	15	11	61.8	NA	28.5	680	NA	314
SS×GS	0	13	3	9	14	14	NA	34.4	23.1	NA	482	324
ES×ES	0	6	0	7	6	9	74.2	NA	37.1	668	NA	334
GS×GS	0	8	0	7	4	15	NA	44.5	22.4	NA	668	336
2009												
EE×EE	0	7	0	10	11	14	12.5	12.5	12.5	175	175	175
EE×EE(I)[g]	0	6	1	6	7	11	15.9	NA	NA	175	NA	NA
GG×GG	0	8	0	9	10	14	12.5	12.5	12.5	175	175	175
GG×GG(I)[b]	0	5	0	8	8	10	17.5	NA	NA	175	NA	NA
SS×SS	0	7	0	9	11	13	13.5	13.5	13.5	176	176	176
SS×SS(I)[i]	0	7	0	7	7	7	NA	NA	25	NA	NA	175
EE×GG	0	17	0	17	20	17	20.6	19.3	NA	351	328	NA
EE×SS	0	14	1	13	19	14	25.2	NA	25.2	353	NA	353
GG×SS	0	11	0	11	16	11	NA	34.5	32.2	NA	380	355
EE×ES	0	17	2	15	25	17	41.2	NA	20.6	700	NA	350
GG×GS	0	14	0	14	17	14	NA	50	25.2	NA	700	353
SS×ES	0	16	0	16	24	16	27.6	NA	27.6	442	NA	442
SS×GS	0	13	0	13	16	13	NA	55	37.1	NA	715	482
ES×ES	0	8	0	10	14	16	34.9	NA	17.4	559	NA	279
GS×GS	0	7	0	8	7	12	NA	58.3	29.2	NA	700	350

[a]*Breeding design was reciprocal such that parents could contribute to multiple offspring crosstypes.*
[b]*Full- and half-sib relationships among wild-born dams and sires were estimated following Herbinger et al. (2006).*
[c]*Offspring were released into each of 3 streams in the Stewiacke (Stw), Economy (Eco), and Great Village (Grv) Rivers.*
[d]*Four families, F = 0.25 (e.g., sib×sib; parent×offspring); 1 family, F = 0.125 (e.g., uncle×niece; aunt×nephew).*
[e]*Four families, F = 0.25; 2 families, F = 0.125.*
[f]*Three families, F = 0.25; 2 families, F = 0.125.*
[g]*Six families, F = 0.25; 5 families, F = 0.125.*
[b]*Five families, F = 0.25; 5 families, F = 0.125.*
[i]*Seven families, F = 0.25; 0 families, F = 0.125.*

total of 10 streams were used across both years (Fig. 1). For each of the 8 streams used in both years, release locations within streams in 2009 were <100 m from release locations used in 2008.

We electrofished streams with a backpack electrofisher and a lip-seine net between 19 September and 15 October 2008 and between 25 August and 3 October 2009. Electrofishing began 500 m downstream from the release point, or as near as possible to the 500 m point (Supporting Information). We fished all streams by systematically working upstream until reaching 10 m above the release point. We weighed all juveniles to the nearest 0.01 g, and a sample of fin tissue was obtained from the caudal fin of all individuals and stored individually in 95% ethanol (1.5 mL reaction tubes) until DNA analysis. Juveniles were released.

We used at least 5 tetranucleotide microsatellite loci to genotype each juvenile (details in Houde et al. 2011*a*, 2011*b*). Based on known parental genotypes, we used an exclusion-based macro for Microsoft Excel (C. Harvie, unpublished data) to assign juveniles to their original family. Assignment success averaged 93% across all streams in the fall of 2008 (Houde et al. 2011*a*) and 90% in 2009. Many of the unassigned juveniles were likely older fish that belonged to previous release programs (Supporting Information).

Statistical Analyses

Each year of this study involved temporally independent production of gametes, independent releases of offspring, and independent sampling in the field. Survival data for both years were zero-heavy and could not be approached with simple mixed-effect models. We used a Bayesian method in R version 2.14 (R Development Core Team 2012) that allows for complex models to be fit, enables full accounting of error propagation, and permits the straightforward development of contrasts. We also developed simulations to approximate statistical power (Supporting Information).

We assessed local adaptation with the local-versus-foreign criterion (Kawecki & Ebert 2004). We used a Bayesian analysis of covariance framework to compare pure SS×SS, EE×EE, and GG×GG crosstypes in each of their local rivers with each foreign crosstype in the same river. Here, the river term is the river on which our release points (streams) were located. Juvenile mass at recapture was modeled as

$$S_i = Y_{\text{crosstype},river[i]} + \rho_{\text{MID}[i]} + U_{\text{PID}[i]} + \mu_{\text{ES}[i]} + \varepsilon_i,$$
$$\rho_{\text{MID}} \sim N(\mu_\rho, \sigma_\rho) \text{ and}$$
$$U_{\text{PID}} \sim N(\mu_U, \sigma_U), \tag{1}$$

where i is an individual data point, ε is the error, and S is the log-transformed mass at recapture. The crosstype × river interaction ($\gamma_{\text{crosstype},river}$) and the continuous egg-size covariate (μ_{ES}; centered at its mean) were treated as fixed effects. The random effects ρ_{MID} and υ_{PID} account for nonindependent variances associated with dam and sire identity, respectively. The priors for fixed effects were $N(0, 1)$ for egg size and $N(0, 2)$ for the crosstype × river interaction. Given our data, these were uninformative priors. For the random terms, the prior for each μ was a zero-mean normal prior with the variance (σ^2) estimated from the data. The variance priors were set on each standard deviation $\sigma \sim U(0,1)$ (Gelman & Hill 2007). An analogous model was developed for the analysis of survival,

$$\text{LOG}(\lambda)_i = Y_{\text{crosstype},river[i]} + \rho_{\text{MID}[i]}$$
$$+ U_{\text{PID}[i]} + \mu_{\text{ES}[i]} + \varepsilon_i + O_i, \tag{2}$$
$$\rho_{\text{MID}} \sim N(\mu_\rho, \sigma_\rho) \text{ and}$$
$$U_{\text{PID}} \sim N(\mu_U, \sigma_U),$$

where λ_i is the number of survivors in each family and follows a Poisson distribution ($\lambda_i \sim P(\lambda)$). The offset term o_i accounts for the different number of offspring in each family, and ε is a normally distributed random term [$\sim N(0, \sigma)$] added to the linear predictor to allow for overdispersion; it was given the same prior as the previous random terms. The basic model structure and priors were retained for the models outlined below.

Given that there was little evidence of local adaptation at the scale of river (see Results section), we ignored

the effect of river and examined the performance of crosstypes across all streams while accounting for stream effects (τ_{SID}). The random stream term prior for μ_τ was a zero mean with the variance (σ_τ^2) estimated from the data and was set on the standard deviation $\sigma \sim U(0, 1)$. For juvenile mass at recapture, the model was

$$S_i = \gamma_{\text{crosstype}[i]} + \rho_{\text{MID}[i]} + \upsilon_{\text{PID}[i]} + \mu_{\text{ES}[i]} + \tau_{\text{SID}[i]} + \varepsilon_i,$$
$$\tau_{\text{SID}} \sim N(\mu_\tau, \sigma_\tau). \tag{3}$$

An analogous model was developed for the survival analysis

$$\log(\lambda)_i = \gamma_{\text{crosstype}[i]} + \rho_{\text{MID}[i]} + \upsilon_{\text{PID}[i]}$$
$$+ \mu_{\text{ES}[i]} + \tau_{\text{SID}[i]} + \varepsilon_i + O_i, \tag{4}$$
$$\tau_{\text{SID}} \sim N(\mu_\tau, \sigma_\tau).$$

We compared the survival and mass estimates of all crosstypes, directly contrasted pure crosstypes with their respective inbred crosstypes, and tested for outbreeding effects by contrasting pure crosstypes with each outbred crosstype that contained any portion of alleles from the parental population. We also contrasted inbred crosstypes with each outbred crosstype that contained any portion of alleles from the inbred parental population.

We assessed the genetic basis of outbreeding effects on survival and mass at recapture with joint-scaling tests (Lynch & Walsh 1998). Crosstype means and standard errors were extracted directly from model 3 (Eq. 3) for mass at recapture and model 4 (Eq. 4) for survival (Supporting Information). We fit models with mean-only and mean-plus-additive effect, and then determined which model fit better to the crosstype means with a likelihood ratio test. We fit a model with an additive-plus-dominance effect only when the mean-plus-additive model was supported, and then we tested the additive-dominance model against the mean-additive model. We performed 12 comparisons of parental populations for survival and 12 for mass such that $\alpha = 0.05/24 = 0.002$.

Results

A total of 29,559 and 30,516 juveniles were released in 2008 and 2009, respectively. We recaptured and assigned 1522 juveniles in 2008 (5.1% of offspring released) and 1077 juveniles in 2009 (3.5% of offspring released). Mean (SE) number of juveniles captured per unit of effort for 2008 releases (0.056 juveniles × s^{-1} [0.0046]) was 35.8% higher than in 2009 (0.039 juveniles × s^{-1} [0.0065]), and 7 of the 8 streams sampled in both years had a higher capture per unit effort in 2008 (Supporting Information). All streams for which temperature data were available ($n = 6$ streams) were warmer in the summer of 2008 (mid June to mid August 2008) than in the summer of

2009; differences in growing degree days ranged from 4% to 24%. River discharge data were available for the Great Village River, and spring discharge was 34–45% greater in spring 2009 than in spring 2008 (Supporting Information).

The relationship between egg diameter and survival was positive, but the strength of this association varied markedly among years. In 2009, a 1 SD change in egg diameter was associated with a 52–120% change in survival (95% credible intervals, BCI). In 2008, a 1 SD change in egg diameter was associated with only a 3–43% change in survival. Indeed, a comprehensive supplementary analysis of egg size and survival data (Supporting Information) suggests that egg diameter explained far more variation in survival in 2009 than in 2008. Mean (SD) mass at recapture was similar for 2008 (1.87 g [0.794]) and 2009 (1.92 g [0.664]). The relationship between egg size and recapture mass was positive, and the effect was similar in 2008 (BCI = 3.3–12.0%) and 2009 (1.3–11.0%).

In Bayesian contrasts (below), we had greater statistical power to detect differences in mass than survival and a greater power to detect differences in contrasts that did not involve inbred crosstypes (Supporting Information). For contrasts of pure versus pure crosstypes (testing for local adaptation) and pure versus outbred crosstypes (testing for outbreeding effects), we were likely to correctly detect differences in mass of approximately 20% or greater and differences in survival of approximately 45% or greater (power ≈ 0.80 or greater both cases). For contrasts involving inbred crosstypes, we were likely to correctly detect differences in mass of approximately 30% or greater and differences in survival of approximately 74% or greater (power ≈ 0.80 or greater both cases). Overall, we were likely to detect moderate to large differences in mass, but differences in survival generally had to be large (approximately >45% or 74%, depending on the type of contrast) before we were likely to detect differences.

Bayesian survival contrasts detected inbreeding depression in 1 of 6 pure versus inbred comparisons. In 2009, survival of GG×GG(I) crosstypes was lower than GG×GG crosstypes (Fig. 3a). Differences in mass were observed in 2 of 6 pure versus inbred contrasts: EE×EE and SS×SS were heavier than their inbred equivalents in 2008 and 2009, respectively (Fig. 3b).

Outbreeding depression was observed in 2 of 36 pure versus outbred survival contrasts (Fig. 3a), both in 2008. Mean survival of GG×GG and SS×SS (i.e., pure parental crosstypes) was 51–68% greater than GG×SS in the Stewiacke River and 110–117% greater than GG×SS in the Great Village River (Fig. S4a). We also observed heterosis in 1 of 36 pure versus outbred survival contrasts, where EE×SS survived better than SS×SS in 2008. We observed differences in mass at recapture in 4 of 36 pure versus outbred contrasts, all in 2008 (Fig. 3b). The direction of these differences in mass was variable; outbred fish were heavier in 2 cases and lighter in 2 cases.

Inbred versus outbred contrasts revealed that outbreeding was worse for juvenile survival in 2 of 36 cases, both in 2008. Specifically, survival of GG×SS was lower than SS×SS(I) and GG×GG(I) (Fig. 3a). Inbreeding was worse for survival than outbreeding in 2 of 36 cases, both in 2009, when survival of SS×SS(I) was lower than GG×GS and SS×ES. Across both years, differences in mass were observed in 8 of 36 inbred versus outbred contrasts; inbred crosstypes were lighter in each case (Fig. 2b).

Local adaptation was not detected in either year (Fig. 4). Similarly, joint-scaling tests supported the mean-only model for performance across all population pairs (i.e., no difference among crosstypes), with 2 exceptions. There was weak support for the mean-additive model for survival of Stewiacke crosstypes in the Economy River (Table 2), which suggests a loss of local adaptation for Economy River salmon via extrinsic outbreeding depression (Supporting Information). However, the mean-only model was by far the better model after correcting for multiple comparisons. The other exception involved the survival of GG×SS in the Great Village River in 2008, where the mean-only, mean-additive, and additive-dominance models were all rejected before correcting for multiple comparisons; this suggests intrinsic outbreeding depression occurred. After correction for multiple comparisons, GG×SS survival was best described by the additive-dominance model. However, this model was only weakly supported (i.e., $p = 0.004$, $\alpha = 0.002$) and the pattern of survival across crosstypes suggests epistasis, not dominance (Supporting Information).

Discussion

One of our goals was to assess the extent of local adaptation in 3 endangered populations of Atlantic salmon. None of our Bayesian contrasts identified differences in the performance between local and foreign crosstypes (Fig. 4). Nor did joint-scaling tests suggest any strong evidence of extrinsic outbreeding depression, given that mean-additive models were never strongly supported (Table 2). However, for local adaptation contrasts, we caution that we were unlikely to detect small differences in survival (power ≈ 0.20 to detect an approximately 20% survival difference), although we were likely to detect moderate differences in juvenile size (power ≈ 0.80 to detect an approximately 20% difference). In 2008, one joint-scaling test provided weak (but nonsignificant) support for a loss of survival in Economy salmon via introgression of Stewiacke alleles (Table 2), which underlines the possibility that some degree of local adaptation may exist (also see Houde et al. 2011a). Nevertheless, no population appeared to exhibit strong or consistent patterns of local adaptation.
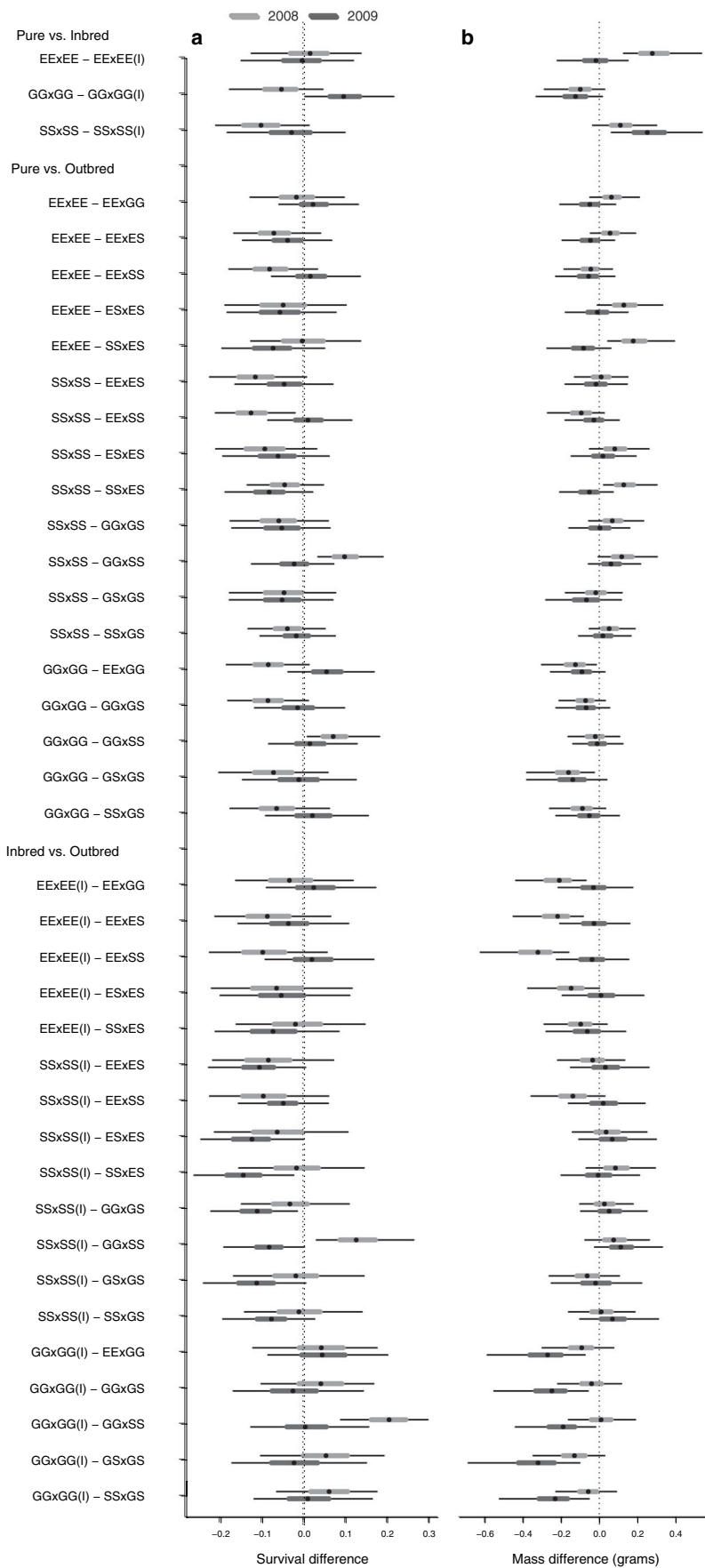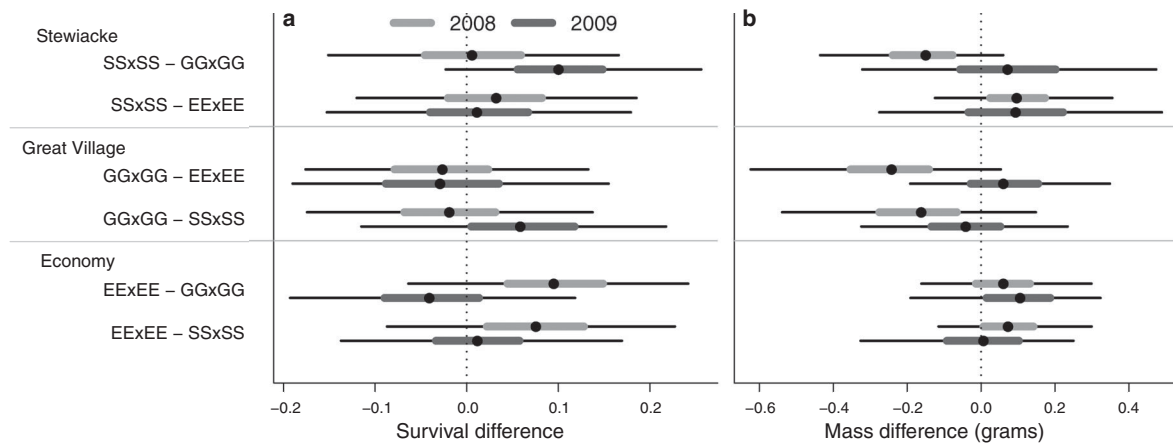
Figure 3. Pairwise contrasts for (a) survival and (b) mass at recapture for pure crosstypes versus the corresponding inbred crosstypes, pure crosstypes versus outbred crosstypes, and inbred versus outbred crosstypes. Bars are 50% credible intervals, whiskers are 95% credible intervals, and contrasts are across all streams for 2008 and 2009. Differences are expressed as the performance of the crosstypes on the left of the legend minus the performance of the crosstype on the right of the legend such that a positive difference indicates mean performance of crosstype on the left was greater. Crosstype abbreviations are defined in Fig. 2 and in the Methods section. Survival data from 2008 were obtained from Houde et al. (2011c).

Figure 4. Pairwise contrasts for (a) survival and (b) mass at recapture for all combinations of local versus foreign crosstypes in 2008 and 2009 at Stewiacke, Great Village, and Economy streams. Crosstype abbreviations are defined in Fig. 2 and in the Methods section. Bars are 50% credible intervals, and whiskers are 95% credible intervals. Stewiacke refers to contrasts performed for the Stewiacke River, where the SS×SS crosstype was local. Differences are expressed as the performance of the local crosstype minus the performance of the foreign crosstype such that a negative difference indicates that mean performance of foreign crosstype was higher. Survival data for 2008 were obtained from Houde et al. (2011c).

Table 2. Parameter estimates for joint scaling tests performed on mean estimates of survival and mass at recapture of juvenile *Salmo salar* for all combinations of parental populations (Stw, Stewiacke; Eco, Economy; Grv, Great Village) in 2008 and 2009.

Population		Survival			Mass at recapture (g)		
P1	P2	$\hat{\mu}_0$ (SE)	$\hat{\alpha}^c$ (SE)	Model[a]	$\hat{\mu}_0$ (SE)	$\hat{\alpha}^c$ (SE)	Model[a]
			2008			2008	
Eco	Stw	0.0302 (0.00252)	0.00864 (0.00419)	M[b]	1.48 (0.0403)	0.0898 (0.0684)	M
	Grv	0.0263 (0.00405)	0.00658 (0.00508)	M	1.47 (0.0656)	0.0603 (0.0912)	M
Stw	Eco	0.0297 (0.00262)	0.00272 (0.00433)	M	1.53 (0.0435)	0.00936 (0.0749)	M
	Grv	0.0191 (0.00226)	0.000365 (0.00428)	M	1.49 (0.0441)	0.0745 (0.0742)	M
Grv	Eco	0.0131 (0.00279)	−0.00192 (0.00481)	M	2.16 (0.103)	−0.211 (0.129)	M
	Stw	0.0140 (0.00183)[c]	−0.000879 (0.00438)[c]	D[d]	2.09 (0.0572)	−0.133 (0.102)	M
			2009			2009	
Eco	Stw	0.0198 (0.00216)	0.000168 (0.00367)	M	1.80 (0.0535)	−0.0782 (0.104)	M
	Grv	0.0131 (0.00279)	−0.00192 (0.00481)	M	1.66 (0.0938)	0.0903 (0.124)	M
Stw	Eco	0.0140 (0.00182)	0.00558 (0.00342)	M	1.44 (0.0592)	0.0198 (0.121)	M
	Grv	0.0148 (0.00194)	0.00150 (0.00395)	M	1.44 (0.0682)	0.0744 (0.130)	M
Grv	Eco	0.0265 (0.00402)	−0.00154 (0.00544)	M	2.07 (0.107)	0.0504 (0.127)	M
	Stw	0.0270 (0.00259)	0.00543 (0.00433)	M	1.96 (0.0580)	−0.0171 (0.103)	M

[a] *Model of best fit was a mean-only (M), mean-additive (A), or additive-dominance model (D), determined from a likelihood ratio test between M and A or A and D.*
[b] *Likelihood ratio test for A versus M: $\chi^2 = 4.3$, $p = 0.039$, $\alpha = 0.002$.*
[c] *Chi-square goodness-of-fit tests: M and A models rejected ($p < 0.001$ in both cases; $\alpha = 0.002$).*
[d] *The D model ($\hat{\delta}^c$ [SE] = -0.0118 [0.00257]) was not rejected after accounting for multiple testing ($p = 0.004$; $\alpha = 0.002$) and provides a better fit than the A model (likelihood ratio test, $\chi^2 = 21.3$, $p < 0.001$, $\alpha = 0.002$).*

We also assessed the relative conservation risks associated with inbreeding and outbreeding in our focal populations. Inbreeding decreased relative juvenile size or survival in 25% of inbred versus pure comparisons (3 of 12 contrasts), whereas an effect of outbreeding was detected in 10% of outbred versus pure comparisons (7 of 72 contrasts). Outbreeding resulted in both positive and negative effects on juvenile size and survival, relative to pure crosstypes, whereas effects of inbreeding were always realized as a decrease in size or survival of the inbred crosstype. Thus, the fitness outcome of outbreeding was difficult to predict, whereas the fitness consequences of inbreeding were always negative.

On the whole, these findings suggest that inbreeding may be worse than outbreeding for critically endangered iBoF salmon. However, direct contrasts of inbred

versus outbred crosstypes suggest that outbreeding was worse for survival in 2008, and inbreeding was worse for survival in 2009. These findings were driven by the fact that no effect of crosstype on performance was consistent from year to year (Fig. 3), and this complicates our assessment. Some of these annual differences can likely be attributed to low power, but other differences are biologically meaningful. The most compelling example is that of the GG×SS crosstype, which had lower survival than SS×SS and GG×GG in 2008 (probably as a result of epistasis [Table 2 & Supporting Information]), but there was clearly no survival difference between these same crosstypes in 2009.

It is likely that maternal effects are at least partly responsible for temporal variation in cross effects. Indeed, maternal effects can have profound influences on offspring performance in a variety of taxa, including salmonids (Einum & Fleming 1999). In the present study, the maternal effect of egg size explained an average of only 9% of the variation in offspring survival across all streams in 2008 (range 0–25% for a given stream), but egg size explained an average of 39% of this variation in 2009 (range 12–72% for a given stream) (Supporting Information). Importantly, egg size is a strong predictor of offspring fitness only when environmental quality is low (Einum & Fleming 1999), and 2009 was a year of relatively low captures per unit effort, low stream temperatures, and a relatively high water velocity in the spring. A relatively low environmental quality in 2009 likely decreased overall survival while exacerbating the importance of maternal effects on offspring fitness (Rollinson & Hutchings 2013). Thus, even though we controlled for egg size in our analyses, the strong effect of egg size in 2009 probably obscured (or made it more difficult to detect) genetic effects that may otherwise have been apparent. The lesson therefore is that environmental influences on the relationship between maternal effects and offspring fitness can complicate risk assessment in the wild.

Low-quality environments also exacerbate the severity of inbreeding depression (Fox & Reed 2011), which suggests that the strength and frequency of inbreeding depression in the wild will vary on a temporal scale. Formal comparisons of inbred versus outbred crosstypes, and of inbred versus pure crosstypes, demonstrate that inbreeding had a relatively stronger negative effect on survival in 2009 than in 2008 (Fig. 3a). It is not clear, however, whether this was a result of variation in environmental quality or a family-level effect, given that mean inbreeding coefficients for inbred crosstypes varied slightly among years (Table 1). Nonetheless, a reliable risk assessment in the wild should include a temporal component, given that variation in environmental quality can affect both the importance of maternal effects and the severity of inbreeding depression, either of which can undermine the validity of assessments that are based on cross-sectional data.

The present study also underlines logistical challenges that can be associated with risk assessments performed in the wild. For example, a comprehensive risk assessment would ideally estimate fitness over the entire lifespan of an organism. This was not possible in the present study, given the extremely low marine survival of iBoF salmon (Amiro 2003). As a result, our experiments were performed only during the juvenile stage, in which freshwater stream environments differ among populations. The disparities that we identified in juvenile mass at recapture (at an age of 4 months) probably would not influence survival during the remainder of the freshwater life stage (Carlson et al. 2008). However, these differences might have contributed to differences in lifetime reproductive success, given that juvenile size correlates with age and size at smolting, which subsequently affects survival at sea (Russell et al. 2012). In a similar vein, inbreeding and outbreeding depression may be realized at different life stages such that an exclusive focus on juveniles imposes a bias in a relative risk assessment. Specifically, inbreeding depression is often the result of viability selection during early life (e.g., Plough & Hedgecock 2011), but outbreeding depression in salmon may not be realized until adults begin homing to their natal rivers (Bams 1976). In sum, long-lived and highly mobile species are inherently difficult to study in a risk assessment framework, and biases might exist in our study owing to our exclusive focus on juveniles.

Given that it can be very difficult to accurately assess the relative risks of inbreeding and outbreeding depression in the wild and that even a sound assessment may ultimately prove untimely, what role should experiments akin to ours play in captive-breeding programs? Naturally, the model organism used herein, Atlantic salmon, exhibits a particular set of life history traits, some of which impede the facility of scientific inquiry in an inbreeding–outbreeding context (e.g., multiyear sea migration, mobile juveniles with high mortality). The answer therefore must depend in part on the study organism, its life history, and a reasonable appraisal of the logistics involved in a reliable risk assessment.

We reiterate that the low overall frequency of cross effects in the present study may reflect low statistical power. Nonetheless, our results underscore the point that an effect of inbreeding will be realized as a decrease in individual fitness, but that the fitness consequences of outbreeding are less predictable (also see Fox & Reed 2011; Frankham et al. 2011). We therefore suggest that risk assessments akin to ours can be weighted more strongly in terms of the probable outcome of outbreeding. Recently, a small but growing body of theoretical (e.g., Frankham et al. 2011; Weeks et al. 2011) and empirical research (e.g., Erickson & Fenster 2006; Pritchard et al. 2013) has questioned whether the risk to population persistence posed by outbreeding has been historically overstated. Criteria have been developed that

allow the probability of outbreeding depression to be predicted from a knowledge of factors such as taxonomic status, fixed chromosomal differences, and the number of generations in different environments (Frankham et al. 2011). This knowledge can be incorporated into existing decision trees (e.g., Weeks et al. 2011) to evaluate whether a comprehensive risk assessment is warranted. We stress that experimental evaluation of outbreeding risk will be needed in some cases. In other cases, however, it might be logistically, financially, and biologically reasonable to outbreed populations on a priori grounds (Frankham et al. 2011; Weeks et al. 2011), thereby obviating the need to test the relative risk of inbreeding and outbreeding depression.

Acknowledgments

Supporting Information

Tests for differences in the performance between offspring of wild-born and captive-born parents (Appendix S1), details of electrofishing surveys and annual differences in stream temperature and flow rate (Appendix S2), a general description of Bayesian models and analysis of power (Appendix S3), model-estimated crosstype means by river and year (Appendix S4), and egg-size analyses (Appendix S5) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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