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ARTICLE

The influence of hybridization with domesticated conspecifics on alternative reproductive phenotypes in male Atlantic salmon in multiple temperature regimes

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Abstract: Alternative reproductive phenotypes represent adaptive life-history responses to local environments. Hybridization with domesticated conspecifics exposed to selection against one of the phenotypes could affect the plasticity and incidence of alternative reproductive phenotypes within wild populations, potentially influencing individual fitness and population viability. We addressed this hypothesis by undertaking a common-garden experiment on Atlantic salmon (*Salmo salar*), a species in which males mature either as large, migratory anadromous individuals or as small, generally nonmigratory parr. Comparing one wild population and two domesticated–wild hybrids (F_1, wild backcrosses), we evaluated the incidence of parr maturity at three different temperatures. Parr maturation probability exhibited a significant quadratic relationship with body mass. Early maturation was absent in the coldest temperature treatment. Body-size maturation thresholds were higher in the warmest temperature treatment relative to the intermediate temperature treatment, resulting in a similar incidence of maturation in both treatments despite increased growth in the warmest temperature treatment. Although body-size thresholds for parr maturity did not differ between crosses, F_1 hybrids and backcrosses exhibited a lower incidence of maturity relative to wild fish (4.8%, 9.3%, and 30.1%, respectively). Changes in the incidence of alternative maturation phenotypes resulting from temperature and domesticated–wild hybridization could have negative fitness consequences for wild populations.

Résumé : Différents phénotypes reproducteurs représentent différentes réactions d'adaptation du cycle biologique au milieu local. L'hybridation avec des individus conspécifiques domestiqués exposés à une sélection visant à exclure des phénotypes précis pourrait avoir une influence sur la plasticité et la fréquence des différents phénotypes reproducteurs dans les populations sauvages, influençant ainsi potentiellement l'aptitude des individus et la viabilité des populations. Nous avons examiné cette hypothèse à la lumière d'une expérience de jardin commun visant le saumon atlantique (*Salmo salar*), une espèce chez laquelle la maturité des mâles s'observe soit chez de grands individus anadromes migrateurs ou de petits tacons généralement non migrateurs. En comparant une population sauvage et deux populations d'hybrides domestiqué–sauvage (F_1 et rétrocroisés sauvages), nous avons évalué l'incidence de la maturité à l'état de tacon à trois températures différentes. La probabilité de maturation à l'état de tacon présentait une relation quadratique significative avec la masse corporelle. Cette maturation précoce était absente dans le cas du traitement à la température la plus froide. Les seuils de taille du corps à la maturation étaient plus élevés dans le traitement à la température la plus chaude que dans le traitement à température intermédiaire, ce qui se traduisait par des incidences de maturation semblables pour les deux traitements, malgré le taux de croissance plus grand dans le traitement à la température la plus élevée. Si les seuils de taille du corps pour la maturité à l'état de tacon ne variaient pas entre les différents croisements, les hybrides F_1 et rétrocroisés présentaient toutefois une incidence de maturité plus faible que les poissons sauvages (4,8 %, 9,3 % et 30,1 %, respectivement). Des modifications de l'incidence de différents phénotypes de maturation causées par la température et l'hybridation domestiqué–sauvage pourraient avoir des conséquences négatives sur l'aptitude dans les populations sauvages. [Traduit par la Rédaction]

Introduction

Conditional alternative reproductive phenotypes represent an extreme form of phenotypic plasticity. Individuals exhibit one of two or more potential alternative phenotypes, depending on their environment and on their genotype. Fundamental to the adoption of one phenotype over another is the attainment of thresholds in continuously distributed "liability" traits (Hazel et al. 1990; Hutchings and Myers 1994; Dodson et al. 2013). These threshold "switchpoints" at which organisms adopt a particular phenotype can be genetically determined (Piché et al. 2008; Paez et al. 2011*a*), and considerable genetic differentiation for these thresholds exists both between and within populations (Aubin-Horth et al. 2006; Piché et al. 2008). Changes in extrinsic environmental factors, such as temperature, can also affect the plasticity of reproductive phenotype expression through both their impact on associated intrinsic liability traits (Prevost et al. 1992) and plastic responses for the liability threshold at which an alternative phenotype is expressed (Baum et al. 2005).

The genetic determination of conditional maturation thresholds has important consequences for wild populations that may

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interbreed with genetically distinct domesticated escapees. Since early maturation and subsequent gonadal growth are often traded off against somatic growth (Paez et al. 2011*a*; Rossignol et al. 2011), breeders might artificially select for late-maturing phenotypes through direct selection against early maturation or as an indirect (correlated) response to selection for rapid growth (Gjedrem 2000; Thorpe 2004; Debes and Hutchings 2014). Furthermore, a specific goal of many breeding programs is the reduction of genotype-by-environment interactions (Gjedrem and Baranski 2009). As a result, plasticity in maturation phenotype expression might also be reduced in domesticated populations, which could limit the capacity of offspring produced by domesticated escapees with wild individuals to plastically respond to environmental change.

The Atlantic salmon (*Salmo salar*) represents an ideal species to examine the effect of domestication on the incidence and plasticity of reproductive phenotypes. Up to 80% of males mature in fresh water, delaying or foregoing oceanic migration (Myers et al. 1986; Hansen et al. 1989) to mature much earlier (≤1–2 years of age) at an average size (15–150 g) up to one order of magnitude smaller than anadromous males (Hutchings and Myers 1988). Early maturation in salmon is influenced by body size-at-age (Piché et al. 2008; Paez et al 2011*a*), suggesting that similar gene sets may affect both traits and that body size could serve as an adequate representation or proxy of the underlying liability trait governing early maturation (Myers et al. 1986). Multiple consecutive growth-related thresholds might also govern maturation in salmon. Depending on the population, at early life-history stages both the fastest- and slowest-growing individuals in a cohort might adopt an anadromous phenotype, with those exhibiting intermediate growth adopting a resident, early maturing phenotype (Rossignol et al. 2011; Dodson et al. 2013). Reproductive phenotype expression in wild populations can also represent adaptive responses to local environmental conditions, such as temperature, oceanic survival, migration distances, and the density of other mature males (Hutchings and Myers 1994; Aubin-Horth and Dodson 2004; Piché et al. 2008).

In both regions of the northern Atlantic, domesticated escapees can account for a major portion of documented spawning salmon (Sægrov et al. 1997; Morris et al. 2008), and they can successfully interbreed with wild individuals (Bourret et al. 2011; Glover et al. 2012). Heritable reductions in the incidence and plasticity of male parr maturity through interbreeding with aquaculture escapees may result in reduced fitness of hybrid offspring relative to the wild parental population and could potentially also have negative demographic and genetic consequences for wild populations, as the presence of mature male parr can substantially increase the effective size of wild populations (Jones and Hutchings 2002; de Mestral et al. 2012; Johnstone et al. 2013).

We used a factorial genotype-by-environment experiment to determine the effect of both interbreeding with aquaculture escapees and different temperature regimes on the incidence of male parr maturity. We reared wild salmon, first generation farmed–wild hybrids, and reciprocal wild backcrosses at three temperature regimes, which allowed us to disentangle effects on male parr maturation attributable to the proportion of aquaculture alleles and the variation in growth rates accompanying different temperatures, as well as their interaction.

Materials and methods

Crosstypes

The parental Atlantic salmon were derived from two strains. The Stewiacke River, used as our "wild" study population, is an endangered Canadian population (COSEWIC 2010) that collapsed in the late 1990s and is supplemented almost entirely via a governmental captive-breeding program. Gametes for the captive-breeding program were initially obtained from individuals captured in the wild as juveniles and raised to sexual maturity (O'Reilly and Harvie 2009); subsequent offspring were released back into the wild as fry and recaptured prior to oceanic migration for continued use in the breeding program. Given that breeders were captured at an early life-history stage in the wild, males that mature as parr in the wild would be proportionately represented within the captive-breeding program. The Stewiacke River is also known to be subject to spawning intrusions by aquaculture escapees; in 1995, for example, 33% of the sampled adult salmon were escapees (Morris et al. 2008). Previous exposure to domesticated escapees prior to the establishment of the Stewiacke River population captive breeding program may have resulted in an introgression of domesticated alleles. Any effect of interbreeding with domesticated individuals found would therefore provide a conservative estimate of the effect of interbreeding with domesticated individuals; the effect of exposure to domesticated individuals in "pristine" populations may actually be greater.

The second strain used was composed of Saint John River farmed salmon descended from third generation aquaculture fish provided by the Atlantic Salmon Broodstock Development program in St. Andrews, New Brunswick, Canada. Both strains had been maintained in Dalhousie University's Aquatron research facility, Halifax, Canada, for two generations prior to the initiation of our experiment under similar laboratory conditions. Details of rearing history to maturation in the first and second generations in captivity are reported by Fraser et al. (2010) and Debes et al. (2012), respectively.

In December 2009, nine pure Stewiacke River families (wild), nine first generation (F_1) farmed–wild hybrid families, and 14 reciprocal wild backcross families (BC = wild × F_1) were generated, as detailed by Debes et al. (2013). Egg mortality did not differ significantly among crosstypes, although there was a trend for lower egg survival among domesticated dams. Reciprocal wild backcrosses were included in our design to test for the effect of farmed genes when fully introgressed into wild populations (Rhymer and Simberloff 1996). Within-crosstype families were composed primarily of full-siblings, although many half-sibling families were also generated (see online supplementary material, Table S1[1]). Maternal half-sibling families among the wild, BC, and F_1 crosstypes were prioritized for inclusion whenever possible to minimize a confounding between maternal and genotypic effects; 23 of 27 families were generated from a subset of five wild Stewiacke River females (Table S1[1]).

Rearing

Families of 250 fertilized eggs were reared in 130 L round flow-through tanks that received a constant stream of filtered dechlorinated municipal water with a mean pH of 7.26. Water temperature was monitored daily and varied among tanks by ±0.1 °C absolute difference. Multiple families were kept in each tank but were isolated using incubation boxes that permitted water flow. Embryonic development occurred in darkness, with temperatures ranging between 3.5 and 10.7 °C (mean of 5.7 °C). Tanks were monitored daily to remove mortalities and clean fungal infections. For further rearing details, see Debes et al. (2013).

When the fish reached the swim-up stage in late May, each family was placed in an individual 130 L tank with an ambient photoperiod that was maintained for the remainder of the experiment. Following the initiation of exogenous feeding (the "fry" stage), individuals were fed four times daily on dry feed (Corey Aquafeeds) with live *Artemia* supplements approximately every 4 days. Mortality after initiation of exogenous feeding was low at 6.7%. However, because of differential among-family egg mortal-

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2014-0527.

ity in the incubation stages, families were adjusted to 110 individuals in June to standardize densities.

Experimental design

On 24–28 June, 66 individuals were randomly chosen from nine families per crosstype. These individuals were anesthetized using tricaine methanesulfonate (TMS) and weighed, measured (fork length ±1 mm), and then divided evenly into six groups of 11 individuals per family. For each crosstype, nine family groups were pooled together to create six replicates in total, each containing 99 individuals (11 individuals per family, nine families for each of the three crosstypes, six replicates per crosstype, 1782 individuals in total).

On 29 June, each of the 18 replicates were randomly transferred to one of 18 temperature-controlled 130 L flow-through tanks (six tanks for each of the three temperatures); temperature was monitored daily. Tanks were cleaned regularly, and any mortality was replaced by adipose-fin-clipped "dummy fish" of the same crosstype and size (and housed under similar environmental conditions) to maintain replicate densities. Overall mortality during the experiment was low at 1.2%.

Temperature treatments

A tank-acclimation period of 10 days was permitted before exposure to the experimental temperature regimes began. Two randomly chosen tank replicates from each crosstype were exposed to one of three temperature regimes (high, medium, and low). The temperature regimes were obtained by decreasing the elevation of a natural ambient temperature curve such that the temperature followed common seasonal changes but differences between regimes were constant (Fig. S1[1]).

Optimal growth rates for Atlantic salmon parr are reported to occur between 15.6 and 20.0 °C, albeit varying between populations (Elliott and Hurley 1997; Jonsson et al. 2001). Since the optimum growth temperature for Stewiacke River parr was unknown, a high temperature regime with a maximum temperature of 20 °C (mean 17.8 °C) was chosen to obtain a high-growth environment. A medium-temperature maximum of 16 °C (mean 14.2 °C) and a low-temperature maximum of 12 °C (mean 10.6 °C) were selected to obtain intermediate and low-growth environments while maintaining a constant 4 °C temperature difference between adjacent temperature pairs. The maximum temperatures listed are idealized, however, and some variation around these numbers occurred because of technical limitations in temperature control (Fig. S1[1]).

The high treatment lies within the upper temperature limit the wild population would naturally be exposed to, where temperatures rarely exceed 22 °C (Houde et al. 2011). Data collected from streams within the same geographic region display similar patterns, with temperatures in many rarely reaching above 20 °C across 2 years (Houde et al. 2011). Once exposed to temperature regimes, individuals were fed four times daily on dry feed (Corey Aquafeeds) with a minimum of 2 h between feedings throughout the experiment, with live biweekly *Artemia* supplements.

After 168 days of exposure to the temperature regimes (13 December), all fish were euthanized using TMS over a 4-day period, measured, weighed, and frozen for later dissection to determine sex and maturation status. A fish was considered mature if it possessed white, swollen (larger than intestine), and opaque testes.

Statistical analysis

General linear mixed-effects models were used to evaluate the effect of crosstype, temperature, and their interaction on growth rate. The natural logarithms of initial and final mass were used to estimate growth rate, assuming a constant growth rate proportional to body size — that is, specific growth rate (SGR). Crosstype, temperature, and their interaction were analyzed as fixed effects, and tank identification was included as a random effects term in all models, regardless of its significance, to account for the randomization design. Time between measurements was also included as a fixed effect, such that the effect of crosstype and temperature at time 0 represented initial differences between treatments, the slope of size across time represented overall SGR, and the interactions of crosstype-by-time and temperature-by-time represented the effects of crosstype and temperature treatment, respectively, on SGR. We also included a random effect tank-by-time term to account for repeated measurements of initial and final mass within tanks, representing the error term to test environmental effects on SGR. Visual examination of model residuals detected heteroscedasticity across time; modelling separate variances for each level of time provided significantly better model fit (log-likelihood ratio test (LRT); $\chi^2_1 = 1101$, $p < 0.001$).

Data analysis was performed with the lme function in the statistical package nlme (Pinheiro et al. 2013) in R 3.0.2 (R Core Team 2013); model selection was conducted on models estimated using maximum likelihood, with final parameter estimates obtained using restricted maximum likelihood. Backwards model selection was conducted by stepwise removing nonsignificant fixed effects terms ($p > 0.05$), using Wald F tests with denominator degrees of freedom obtained using the Kenward–Roger approximation (Kenward and Roger 1997). Marginally significant fixed effects terms ($0.05 > p \leq 0.10$) were removed unless their inclusion was justified based on trends observed in prior published scientific literature. Significance of pairwise contrasts between term levels was evaluated with t tests, with p values Bonferroni-corrected for the false discovery rate to adjust for type 1 error rates.

Male parr maturation was analyzed as a binary variable (mature versus immature), using generalized linear mixed models with a binomial distribution and a logit-link function. The incidence of male parr maturation was modeled with crosstype and temperature treatment included as categorical fixed terms and the natural logarithm of final body mass (mean-centered) included as a fixed continuous covariate. All possible interactions between these effects were also tested, and tank identification was included as a random effects term. Preliminary analyses indicated that the probability of maturation generally increased with size up to a maximum, after which maturation decreased (and fish began to exhibit signs of smoltification). To account for this, a second-order polynomial for mean-centered ln mass was included in the model. Owing to a complete lack of maturation at the lowest temperature, this temperature treatment was excluded from the model.

Binary data analysis was performed with the glmer function in the statistical package lme4 (Bates et al. 2014) in R 3.0.2 (R Core Team 2013) using Laplace approximation to the likelihood. Backwards model selection was conducted by stepwise removing nonsignificant fixed effects terms ($p > 0.05$) using likelihood ratio tests, eliminating higher order terms first. Marginally significant fixed effects terms ($0.05 > p \leq 0.10$) were removed unless their inclusion was justified based on trends observed in prior published scientific literature. If a higher order term was found to be significant, all relevant lower order terms were retained. Tank identification was also included as a random effects term in all models regardless of significance to account for our randomization. Degrees of freedom calculations for pairwise comparisons of means among crosstypes using t tests were calculated based on the number of tanks, and p values were adjusted as for linear mixed-effects models.

Results

Specific growth rate

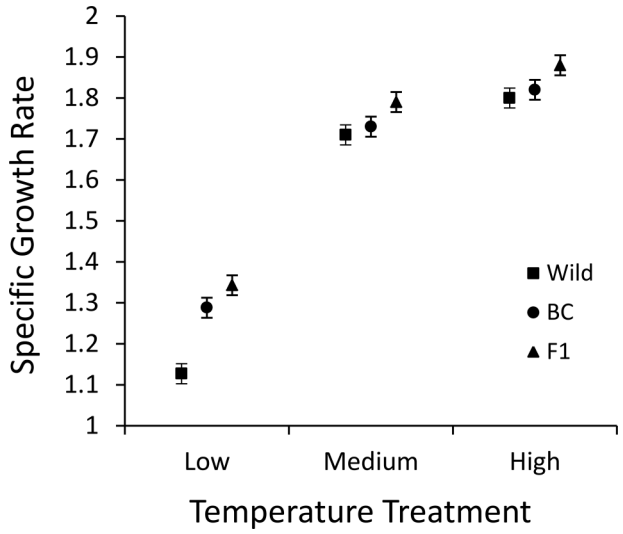
The best-fit model describing size contained temperature, crosstype, time, and the interactions between temperature and time (shown in Table 1). Temperature treatments had a significant effect upon SGR (modeled as temperature-by-time interaction; $p < 0.001$); individuals in the high temperature treatment exhib-

Table 1. Test statistics for the final model terms predicting body mass across time.

Parameter	df	*F* value	*p* value
Intercept	1, 12.5	1 277.9	<0.001
Time	1, 15.2	16 748.3	<0.001
Temperature	2, 12.5	7.4	0.008
Crosstype	2, 12.5	61.7	<0.001
Time × crosstype	2, 15.2	3.1	0.076
Time × temperature	2, 15.2	171.8	<0.001

Note: Analysis was conducted using general linear mixed models in nlme. Degrees of freedom (df) are presented as numerator, denominator.

Fig. 1. Specific growth rates of Atlantic salmon parr for each crosstype in three temperature treatments. Means with standard error for the three crosstypes are indicated by symbol types as given in the legend (F_1, first generation farmed–wild hybrids; BC, wild × F_1 hybrids).



ited increased growth relative to those in the medium temperature treatment ($t_{15.2}$ = 2.96, $p_{adj.}$ = 0.029) and low temperature treatment ($t_{12.5}$ = 17.33, $p_{adj.}$ < 0.001), and individuals grew faster in the medium temperature treatment than in the low temperature treatment ($t_{12.5}$ = 14.36, $p_{adj.}$ < 0.001; Fig. 1).

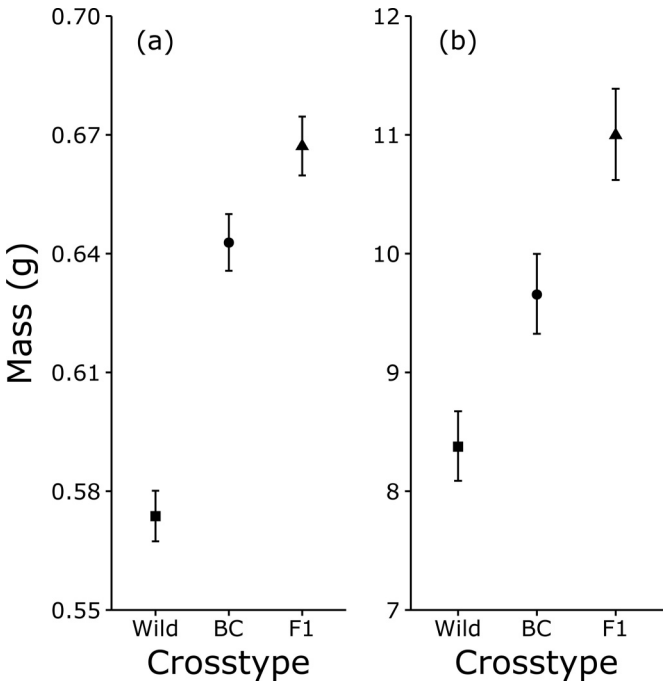
SGR across temperature treatments was only marginally different among crosstypes (p = 0.076). However, we retained this interaction term in our model because we had limited statistical power for this term (limited by the number of tanks, as we did not mark fish individually), and a previous study examining these crosstypes in more detail found a significant positive relationship between growth rate and the percentage of farmed alleles (Debes et al. 2014). A similar pattern for SGR was observed in the present experiment (F_1 > BC > wild). Removing this term from our model would therefore likely result in a false negative result, so it was retained in our final model.

Wild fish were smaller at both the start and end of the experiment than the farmed–wild F_1 hybrids ($t_{12.5}$ = 9.58, $p_{adj.}$ < 0.001 and $t_{12.5}$ = 5.51, $p_{adj.}$ = 0.001, respectively) and backcrosses ($t_{12.5}$ = 7.22, $p_{adj.}$ < 0. 001 and $t_{12.5}$ = 2.88, $p_{adj.}$ = 0.040, respectively; Figs. 2*a* and 2*b*). Backcrosses and F_1 hybrids did not exhibit significant differences in initial or final sizes ($t_{12.5}$ = 2.36, $p_{adj.}$ = 0.106 and $t_{12.5}$ = 2.64, $p_{adj.}$ = 0.063, respectively).

Maturation

No maturation occurred in the low temperature treatment irrespective of crosstype. In the medium and high temperature regimes, maturation was highest in wild salmon at 32.0% and 28.3% (respectively), intermediate in the backcross at 13.0% and

Fig. 2. Crosstype means with standard errors for initial (*a*) and final (*b*) body mass.



5.7% (respectively), and lowest in the F_1 hybrids at 6.7% and 3.0% (respectively).

The best-fit model describing early maturation included temperature, crosstype, body mass, a second-order polynomial for body mass, and an interaction between temperature and body mass as fixed effects (Table 2). Body mass had an effect upon the incidence of maturation with a highly significant second-order polynomial fit (LRT; χ^2_1 = 42.06, p < 0.001). Individual maturation probability increased with body mass up to a maximum, after which maturation probability decreased (Fig. 3).

Between the medium and high temperature treatments, no significant difference in overall maturation probability was detected (LRT; χ^2_1 = 2.33, p = 0.127), but maturation probability differed for the interaction between temperature and body mass (LRT; χ^2_1 = 4.46, p = 0.035). As a result of this interaction, the peak incidences of maturation for all crosstypes occurred at a smaller body mass in the medium temperature treatment (at 10.8 g) relative to the high temperature treatment (at 13.5 g) — that is, fitted curves for occurrences of maturation were shifted towards larger body size at the warmer temperature (Fig. 3).

Overall maturation probability differed highly significantly among the three crosstypes (LRT; χ^2_2 = 25.30, $p \leq$ 0.001; Fig. 3). We found only marginal evidence for an interaction between crosstype and body mass (LRT; p = 0.083; step 6, Table 2), so this term was subsequently dropped. For each crosstype, we predicted the maturation probability across temperature treatments (as temperature-by-crosstype interaction effects were absent) at the common size of maximum maturation probability (11.44 g). At this size, wild individuals exhibited a significantly greater incidence of maturation (50%) relative to backcrosses (17%, t_8 = 5.40, p_{adj} = 0.002) and F_1 hybrids (10%, t_8 = 5.60, p_{adj} = 0.002). Although maturation probability at this size was higher in backcrosses relative to F_1 hybrids, this trend was nonsignificant (t_8 = 1.34, p_{adj} = 0.650).

Discussion

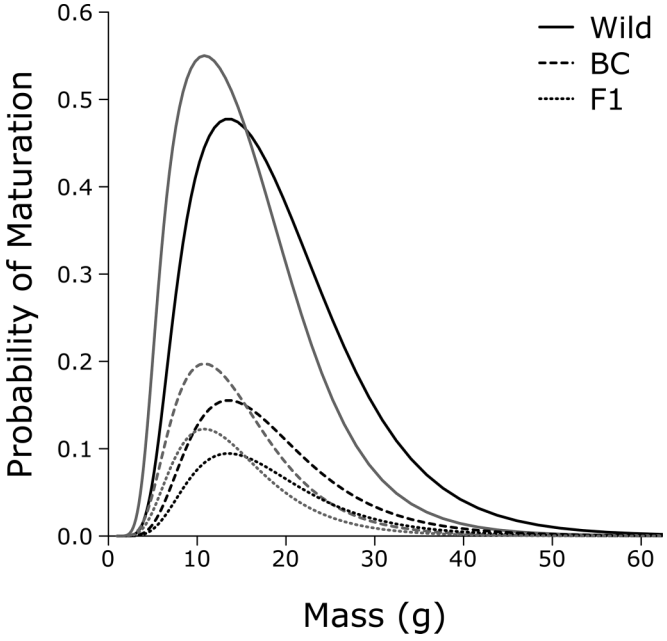
Our results indicate that interbreeding between wild and domesticated Atlantic salmon has the potential to strongly reduce the incidence of male parr maturity. Both F_1 domesticated–wild

Table 2. Results on model selection for early maturation, using log-likelihood ratio tests (LRTs).

Model No.	Description	Versus model No.	LRT	Term	χ^2	df	p
0	M + M² + T + C + T × M + C × M + C × T + C × T × M + C × M² + T × M² + C × T × M²	—	–193.97	—	—	—	—
1	M + M² + T + C + T × M + C × M + C × T + C × T × M + C × M² + T × M²	0	–193.45	C × T × M²	1.05	2	0.592
2	M + M² + T + C + T × M + C × M + C × T + C × T × M + C × M²	1	–193.97	T × M²	1.06	1	0.304
3	M + M² + T + C + T × M + C × M + C × T + C × T × M	2	–195.15	C × M²	1.30	2	0.523
4	M + M² + T + C + T × M + C × M + C × T	3	–195.23	C × T × M	0.16	2	0.921
5	M + M² + T + C + T × M + C × M	4	–197.00	C × T	3.54	2	0.171
6*	M + M² + T + C + T × M	5	–199.49	C × M	4.98	2	0.083
7	M + M² + T + C	6	–201.72	T × M	4.46	1	0.035
8	M + T + C + T × M	6	–220.52	M²	42.06	1	<0.001
9	M + M² + C + T × M	6	–202.88	T	2.33	1	0.127
10	M + M² + T + T × M	6	–214.37	C	25.30	2	<0.001

Note: Term abbreviations: M, mass; M², mass²; T, temperature; C, crosstype.
*Selected model.

Fig. 3. Relationship between the probability of maturation and mass (g). Grey lines correspond to the medium temperature treatment, black lines to the high temperature treatment. Curves for the three crosstypes are indicated by line types as given in the legend.



hybrids and wild backcrosses exhibited reduced levels of male parr maturity in age 0+ fish relative to the wild population, with overall incidences of maturation of 4.8%, 9.3%, and 30.1%, respectively. Nevertheless, all crosstypes exhibited a similar capacity to plastically respond to different growth conditions by completely suppressing maturation at the coldest temperature and increasing the size at which the probability of early maturation peaked in warmer temperatures.

Previous studies have found that maturation probability increased with size until all individuals beyond a certain size threshold exhibited maturation (e.g., Piché et al. 2008). However, because of relatively high temperatures and ad libitum feeding, the individuals in our study exhibited rapid growth, with a mean final size of 8.4 g across treatments for the pure Stewiacke River crosstype relative to 4.1 g in Piché et al. (2008). Maturation patterns in our study were therefore best modeled with a quadratic polynomial, where the probability of an individual exhibiting maturation increased up to a peak size after which the probability of maturation decreased. Beyond this size, many individuals began to display signs of smoltification (fading of parr marks and intensifying of silver coloration), which occurs as the salmon undergoes physiological changes necessary to tolerate salt water. Smoltification is initiated upon achieving a secondary growth-correlated liability threshold (Wedemeyer et al. 1980; Dodson et al. 2013). This threshold is surpassed at sizes that are typically larger than that necessary for early maturation (McCormick et al. 1998) and can supersede it in some conditions; the largest males in a particular cohort often exhibit smoltification, rather than early maturation (Paez et al. 2011*b*, Dodson et al. 2013). The size-related pattern of maturation observed in this experiment corresponds to the hypothesis that multiple developmental pathways govern maturation phenotypes in Atlantic salmon (Dodson et al. 2013).

While hybridization can lead to divergent reaction norms between hybrids and parental populations (Fraser et al. 2008; Darwish and Hutchings 2009), it can also preserve reaction norms in some traits (Morris et al. 2011). The parr body sizes at which the probability of maturation was highest did not differ between crosses. However, hybrids exhibited a reduced incidence of maturation across all body sizes in all environments, which could potentially reduce their fitness under environmental conditions in which early maturation may be advantageous. Domestication might be expected to select against an early maturation phenotype if it has associated costs (DeWitt and Scheiner 2004). Growth costs associated with early maturation (Myers and Hutchings 1986; Rossignol et al. 2011) probably result in both intentional and unintentional selection against early maturation in most breeding programs (Gjedrem 2000; Thorpe 2004). Furthermore, Atlantic salmon used in aquaculture operations exhibit early smoltification relative to their wild counterparts (Thorpe 2004) and may have been selected to initiate smoltification at a lower growth threshold, as was the case for the strain we used (Glebe 1998). Collectively, the lower incidences of maturation observed in F_1 and backcross hybrids were probably a consequence of increased growth (i.e., more males passed the size at which the probability of early maturation peaked), earlier smoltification, and the suppression of male parr maturation, all of which result from the genetic influences of their domesticated (grand-) parents.

Reduced levels of male parr maturation in the investigated domesticated–wild hybrids could be due to two factors: (*i*) direct or indirect selection against early maturity in their domesticated (grand-) parents or (*ii*) a lower natural incidence of parr maturity in the ancestral wild population from which their domesticated (grand-) parents were derived. We are unable to distinguish between these two possibilities, as the wild population used in this experiment was not the ancestral population from which the domesticated fish were derived. However, previous research comparing the wild ancestor of domesticated parents of the present study with two of its aquaculture selection lines indicated that increasing generations of domestication continuously decreased incidences of parr maturity (Debes and Hutchings 2014).

Once proportions of domesticated alleles reached levels of full introgression, the wild backcross still exhibited reduced maturation probability relative to wild fish. Therefore, the effect of domesticated–wild outbreeding on male parr maturation likely persists beyond F_1 hybrids. Backcross individuals also exhibited maturation rates that were more similar to F_1 individuals, suggesting that nonadditive genetic mechanisms might underlie divergence in male parr maturation between wild and farmed Atlantic salmon, although our low sample size of spawning adults used to generate these crosstypes precludes our capacity to rigorously test this hypothesis.

Previous investigations on natural populations have only found weak correlations between the incidence of maturation and increased growth rates due to warmer temperatures (Baum et al. 2004). In natural environments, growth-related threshold maturation sizes can shift in response to increased temperatures; this results in similar rates of male parr maturation across environments (Baum et al. 2005). Our findings experimentally confirmed this observed pattern. The size at which the probability of early maturation peaked was greater at the warmest temperature, but increased growth in this treatment maintained similar overall levels of male parr maturation relative to the medium temperature treatment.

The opportunity for growth for an individual parr is conditional upon an environment shared with other parr. Individual male parr reproductive success is also negatively associated with the total number of male parr competing for fertilization opportunities (Hutchings and Myers 1988; Jones and Hutchings 2001). Plasticity in size-associated maturation thresholds in response to growth opportunity would prevent many males in a high-growth cohort from wasting energy investing into maturation that is unlikely to provide fitness benefits due to increased mating competition (Baum et al. 2005). Alternatively, it is possible that maturation may have been determined by surpassing critical developmental thresholds (e.g., lipid reserves) early in the year that trigger maturation (Thorpe 2007). If a similar number of fish in both the medium and high temperature treatments were able to surpass this developmental cue, individuals who matured in the high temperature treatment would have reached larger sizes because of better growth opportunity, resulting in the appearance of a "shift" in the size-associated maturation thresholds.

All crosstypes in the present study maintained the capacity to respond to warmer temperature regimes by increasing the size at which the probability of early maturation peaks. If little or no fitness costs are associated with phenotypic plasticity, it should be maintained in populations that are no longer exposed to the ancestral environments in which that plasticity evolved (Ghalambor et al. 2007). Although the reduction of genotype-by-environment interactions are a goal of most breeding programs (Gjedrem and Baranski 2009), we found evidence that the plasticity of maturation thresholds in different growth environments remains preserved in hybrid domesticated–wild offspring.

Male parr from all crosstypes were also able to respond to extreme temperature changes, with maturation completely absent at colder temperatures despite many males achieving sizes comparable to mature fish in warmer conditions. Over 20% of wild males in the cold treatment achieved masses greater than 7 g, above which there was a greater than 40% chance of a male maturing in the medium temperature treatment. However, the overlap between the size of mature fish in the warmer treatments and immature male parr in the low temperature treatments may be a result of somatic growth costs associated with maturation. Maturation in male Atlantic salmon parr begins early in the life cycle, and its completion is dependent upon achieving critical growth-related or energy-store thresholds (Thorpe 2007; Dodson et al. 2013). Male parr at the low temperatures may not have achieved these early thresholds because of their reduced growth. As a result, immature male parr at low temperatures continued to invest in somatic growth, allowing them to "catch up" to or surpass the size of mature male parr in warmer treatments, whose growth rates likely declined because of costs associated with early maturation (Myers et al. 1986; Dodson et al. 2013). Alternatively, the initial rapid reduction in temperature in the coldest treatment (from 19.2 to 6.9 °C over 9 days) and the maintenance of a low thermal regime throughout may have triggered an unknown mechanism, perhaps mimicking the initiation of winter despite the presence of a photoperiod that corresponded to summer.

Conservation implications

The incidence of male parr maturation in Atlantic salmon populations can vary greatly, and these differences may reflect adaptations to local environmental conditions (Hutchings and Myers 1994). As a consequence, the observed reduction in male parr maturation in domesticated–wild hybrids could result in major genetic changes to reproductive phenotypic expression with strong potential to result in reduced hybrid fitness relative to the wild parental population. The incidence of male parr maturation can also have important demographic and genetic consequences for Atlantic salmon populations. The presence of mature male parr can increase the effective population size, as they can (*i*) balance sex ratios in female-biased anadromous spawning runs; (*ii*) decrease variance in reproductive success by increasing the proportion of individuals that contribute to a new cohort; and (*iii*) increase within-population outbreeding through between-cohort matings (Hutchings and Jones 1998; Garcia-Vazquez et al. 2001; Jones and Hutchings 2001; Saura et al. 2008; Johnstone et al. 2013).

Small populations may be particularly vulnerable to genetic input from aquaculture escapees (Hutchings 1991; Hindar et al. 2006), which is relevant to our study's highly endangered wild population, the Stewiacke River (COSEWIC 2010). Furthermore, extremely low marine-phase survival is thought to be the primary cause of the decline in the Stewiacke River population (Amiro 2003). Levels of male parr maturity may be maintained in wild populations through negative frequency-dependent selection (Hutchings and Myers 1994). Accordingly, an expected evolutionary response to increased mortality associated with oceanic migration would be an increase in the relative fitness of maturing as a male parr (Hutchings and Fraser 2008). As such, any reductions in male parr maturity in the Stewiacke River population, or in any population of conservation concern, due to interbreeding with aquaculture fish will likely result in reduced hybrid fitness relative to the wild parental population that will persist beyond the first generation and could potentially result in a depressed population growth rate.

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