

# Potential for domesticated–wild interbreeding to induce maladaptive phenology across multiple populations of wild Atlantic salmon (*Salmo salar*)

Dylan J. Fraser, C  il  n Minto, Anna M. Calvert, James D. Eddington, and Jeffrey A. Hutchings

**Abstract:** We report how aquaculture may negatively alter a critical phenological trait (developmental rate) linked to survival in wild fish populations. At the southern limit of the species range in eastern North America, the persistence of small Atlantic salmon (*Salmo salar*) populations may be constrained by interbreeding with farmed salmon that escape regularly from intensive aquaculture facilities. Using a common-garden experimental protocol implemented over an 8-year period, we show that embryos of farmed salmon and multigenerational farmed–wild hybrids (F<sub>1</sub>, F<sub>2</sub>, wild backcrosses) had slower developmental rates than those of two regional wild populations. In certain cases, our data suggest that hybrid developmental rates are sufficiently mismatched to prevailing environmental conditions that they would have reduced survival in the wild. This implies that repeated farmed–wild interbreeding could adversely affect wild populations. Our results therefore reaffirm previous recommendations that based on the precautionary principle, improved strategies are needed to prevent, or to substantially minimize, escapes of aquaculture fishes into wild environments.

**R  sum   :** Nous d  montrons comment l’aquaculture peut affecter n  gativement un trait ph  nologique essentiel (le taux de d  veloppement) li      la survie des populations sauvages de poissons.    la limite sud de l’aire de r  partition de l’esp  ce dans l’est de l’Am  rique du Nord, la persistance de petites populations de saumons atlantiques (*Salmo salar*) peut   tre restreinte par des croisements avec des saumons d’  levage qui s’  chappent r  guli  rement des installations d’aquaculture intensive.    l’aide d’un protocole exp  rimental de jardin commun maintenu sur une p  riode de 8 ann  es, nous montrons que les embryons de saumons d’  levage et des hybrides de plusieurs g  n  rations de saumons sauvages et   lev  s (r  trocroisements en nature de F<sub>1</sub> et de F<sub>2</sub>) ont des taux de d  veloppement plus lents que les poissons de deux populations r  gionales sauvages. Dans certains cas, nos donn  es indiquent que les taux de d  veloppement des hybrides sont suffisamment m  sappari  s aux conditions pr  valentes de l’environnement qu’ils auraient r  duit la survie en nature. Ceci donne    entendre que des croisements r  p  t  s entre des poissons d’  levage et des poissons sauvages pourraient affecter n  gativement les populations sauvages. Nos r  sultats nous am  nent donc    r  affirmer les recommandations ant  rieures bas  es sur le principe de pr  caution voulant que des strat  gies am  lior  es soient n  cessaires pour emp  cher, ou r  duire    un strict minimum, la fuite de poissons d’  levage vers les environnements sauvages.

[Traduit par la R  daction]

## Introduction

Unparalleled declines of many wild fish populations, coupled with growing aquaculture production, have made it critical to assess the potential risks associated with interactions between wild and escaped farmed fishes (Naylor et al. 2005). From a genetic perspective, a primary concern is the potential loss or reduction of adaptive genetic variation that may arise in wild fishes if farmed–wild interbreeding occurs. Indeed, the domestication process can elicit genetic changes in farmed fishes that are maladaptive in the wild, and recent studies support that farmed–wild hybrids may have reduced

survival (McGinnity et al. 2003; Tymchuk et al. 2007; Fraser et al. 2008) or genetically based phenotypes that likely reduce their fitness performance relative to wild fish (Einum and Fleming 1997; Fraser et al. 2010).

A more rare aspect of phenotype considered within the literature of farmed–wild interactions has been phenology. Many wild species of fishes, especially migratory ones, need to coordinate their movements and shifts in life history stages with the timing of seasonal resource availability in geographically separated areas (Crozier et al. 2008; Helfman et al. 2009). For instance, fish embryonic developmental

Received 18 December 2009. Accepted 16 July 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 16 October 2010. J21574

Paper handled by Associate Editor Eric Taylor.

**D.J. Fraser**,<sup>1</sup> Department of Biology, Concordia University, Montr  al, QC H4B 1R6, Canada.

**C. Minto**,<sup>2</sup> **A.M. Calvert**, and **J.A. Hutchings**, Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.

**J.D. Eddington**, Aquatron Laboratory, Dalhousie University, Halifax, NS B3H 4H6, Canada.

<sup>1</sup>Corresponding author (e-mail: djfraser@alcor.concordia.ca).

<sup>2</sup>Present address: Galway-Mayo Institute of Technology, Marine and Freshwater Research Centre, Dublin Road, Galway, Ireland.

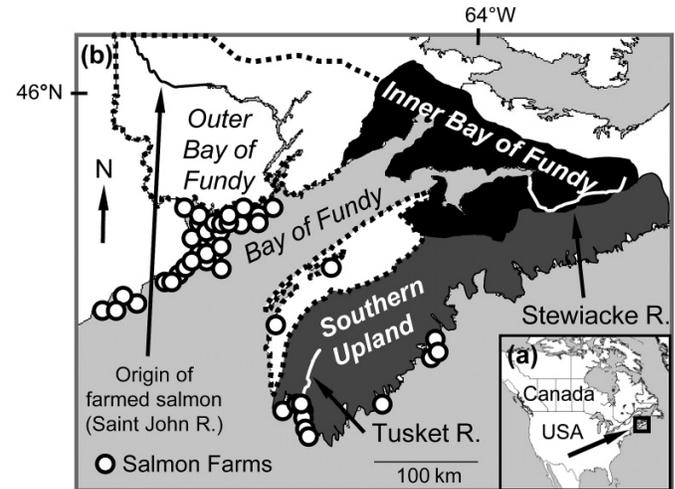
rates (i.e., the cumulative number of degree-days prior to hatch) often have a partial genetic basis and appear to be finely tuned to local environmental conditions that are optimal for growth and survival (Beacham and Murray 1987; Jensen et al. 1991; Donaghy and Verspoor 1997). As a result, if escaped farmed fishes differ from their wild counterparts in such phenological traits, farmed–wild hybrids might not synchronize their development with the timing of critical seasonal events.

We address this possibility with a consideration of embryonic developmental rates in Atlantic salmon (*Salmo salar*) populations at the southern limit of their range in the Northwest Atlantic, many of which are already endangered or declining in the USA and Canada because of a variety of human activities (US Atlantic Salmon Assessment Committee 2005; Committee of the Status of Endangered Wildlife in Canada 2006). This region also harbours intensive Atlantic salmon aquaculture. Large escapes of farmed salmon from sea cages are recurrent, with escapees entering many rivers inhabited by wild salmon, sometimes at a high rate ( $\geq 10\%$ – $20\%$  of returning adults; Morris et al. 2008). Regional escapees can spawn successfully in the wild (Carr et al. 1997) and interbreed with wild salmon (O'Reilly et al. 2006).

Embryonic developmental rates might differ genetically between farmed and wild salmon in the Northwest Atlantic for at least two, nonmutually exclusive reasons. First, farmed salmon originate chiefly from the Saint John River, a watershed of higher altitude and latitude than many regional rivers where escaped farmed salmon have been found (Morris et al. 2008) (Fig. 1). Owing to colder water and (or) longer winters, higher altitude–latitude populations often spawn earlier in autumn and have slower embryonic developmental rates than lower altitude–latitude populations (Beacham and Murray 1987, 1990; Webb and McLay 1996), although the developmental rate of the wild Saint John progenitors that generated the farmed strain is unknown. Second, artificial selection on regional farmed salmon may either intentionally or inadvertently result in genetically based changes to developmental rates from the wild state. For instance, in an analogous situation, data from cultured hatchery fish have shown that slower, genetically based developmental rates can be favoured (Reisenbichler and Rubin 1999). Additionally, available data on adult broodstock selection in fish farms and hatcheries also shows that it tends to favour those adults that spawn earlier to fill yearly production quotas (McLean et al. 2005; Bert 2007). Such earlier spawning individuals often produce offspring with slower developmental rates (Beacham and Murray 1987).

We adopted a common-garden experimental protocol to test whether, in southeastern Canada, differences existed in embryonic developmental rates among farmed salmon, wild salmon from two populations, and their multigenerational farmed–wild hybrids (i.e.,  $F_1 = \text{farmed} \times \text{wild}$ ;  $F_2 = F_1 \times F_1$ ; wild backcrosses =  $F_1 \times \text{wild}$ ). Separated by  $\approx 340$  km, the wild populations, from the Tusknet and Stewiacke rivers, were each representative of a regional group of related populations known as “Southern Upland” and “Inner Bay of Fundy”, respectively (Fig. 1). These regional groups exhibit several differences in migratory behavior, life history, and

**Fig. 1.** Maps of (a) the general study location within eastern North America and (b) the location of Atlantic salmon study populations, regional groups of wild populations, and the general location of regional salmon farms.



genetic characteristics (Committee of the Status of Endangered Wildlife in Canada 2006; Fraser et al. 2007, 2010). They have also experienced dramatic population declines since the 1990s, with the Inner Bay of Fundy now assigned an “endangered” conservation status (Fisheries and Oceans Canada 2002; Committee of the Status of Endangered Wildlife in Canada 2006). Escaped farmed salmon have been detected historically in both the Tusknet ( $\approx 1\%$ , 1998) and Stewiacke ( $\approx 33\%$ , 1995) rivers, although their presence in these and other regional rivers is likely underestimated (see Morris et al. 2008). Owing to the small size and critical conservation status of regional wild populations, it was not feasible to carry out our research in the wild.

We examined two generations of interbreeding because adverse genetic changes arising from intraspecific hybridization might not be manifested until the  $F_2$  generation when parental genes are recombined (Edmands 2007). While our study was not designed to disentangle the influence of artificial selection on developmental rates during farming from that of the farmed ancestry, our farmed salmon originated from the major strain used in regional salmon aquaculture (Saint John River). Hence, from a regional risk-assessment perspective, our study is representative both of the farmed salmon that escape into the wild and the probable farmed–wild breeding interactions that occur.

## Materials and methods

### Study populations and crosses

In 2001, unfertilized eggs and sperm were collected from sexually mature adults originating from the Tusknet River, the Stewiacke River, and the Saint John River farmed strain, which at that point had undergone four generations of artificial selection primarily for faster growth and delayed maturity (Glebe 1998) (Fig. 1). Adults represented random samples from each population or broodstock. Tusknet adults were obtained from the wild; Stewiacke adults had been collected as juveniles in the wild and subsequently raised to sexual maturity in captivity. Gametes were transferred to

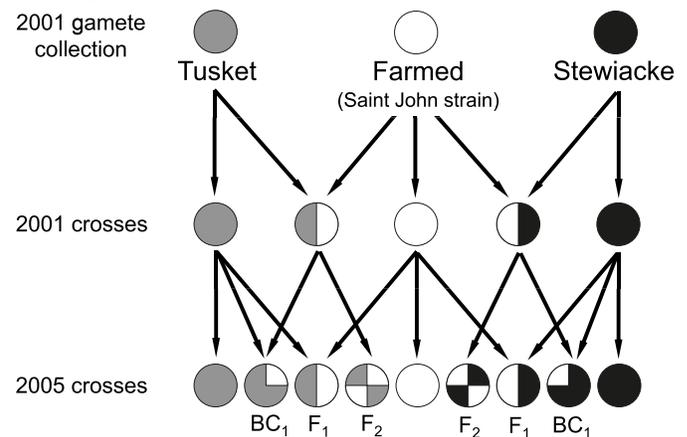
Dalhousie University's Aquatron Facility and used to generate 10 full-sibling families each of the three parental and two  $F_1$  farmed-wild hybrid crosses (i.e.,  $F_1$  farmed-Stewiacke hybrids, with parentally reciprocal families in equal numbers, and  $F_1$  farmed-Tusket hybrids with farmed females and Tusket males only; Lawlor et al. 2009; Fig. 2). Crosses generated in 2001 were raised under common environmental conditions until they attained maturity in 2005. Details of 2001–2005 rearing conditions, as well as genotyping and parentage analyses of 2005 adults back to their respective 2001 families (to avoid inbred matings in 2005 crosses) are described in Fraser et al. (2010). The 2005 adults were used to regenerate the pure and two  $F_1$  farmed-wild hybrid crosses, as well as to create two  $F_2$  farmed-wild hybrids and two farmed-wild backcrosses ( $BC_1$ ) (Fig. 2). Crosses were performed on 22, 25, and 29 November and 6 December 2005. Each cross consisted of 9–23 chiefly full-sibling families (see Supplemental Appendix S1<sup>3</sup>), except for Tusket and both  $F_2$  hybrids in which each male was crossed to two or three different females, because of logistical constraints in generating large numbers of families in these crosses with available sexually mature fish. Details on the sex of wild, farmed, or hybrid parents used to generate the multigenerational crosses ( $F_1$ ,  $F_2$ ,  $BC_1$ ) in 2005 are also found in Supplemental Appendix S1<sup>3</sup>.

### Rearing

The 2005 families comprised  $\approx 500$  eggs and were randomly allocated to one of three compartments nested within one of 60 circular 100 L tanks that received the same flow-through water source. Thus, our study made the assumption that any tank effects were equal across the crosses generated; ongoing work further suggests that tank effects at this facility have a negligible impact on developmental rates (D.J. Fraser and J.A. Hutchings, unpublished data). Compartments were of equal size, separated by equal distances, and had their bottoms and sides open and covered with a thin-mesh screen to ensure sufficient oxygenation for the eggs and hatching alevins. Under common environmental conditions (e.g., temperature  $\pm 0.1$ – $0.15$  °C, pH = 7.0), eggs were kept in the dark until hatching commenced in March 2006, and dead eggs were removed every 4 days during incubation. Temperatures were recorded daily for each tank throughout the incubation period to determine cumulative degree-days prior to hatching.

We cannot discern whether our rearing conditions might be more similar to one parental environment or the other (see McClelland and Naish 2007). Nevertheless, importantly for the study of developmental rates, our incubation temperatures approximated those to which regional wild salmon are naturally exposed annually (2–4 °C; Lacroix 1985; Houde 2009). Namely, the mean water temperature over the entire incubation period was 4.5 °C ( $\pm 0.12$  °C, standard error, SE) and water temperature was allowed to fluctuate naturally over the incubation period (between 3 and 6 °C). Water originated from a natural watershed near Dalhousie University (Pockwock Lake, at a latitude between Tusket and Stewiacke rivers). Potential rearing environment influences on developmental rates should therefore have been minimized.

**Fig. 2.** General structure of the experimental cross design and crosses generated.



Nonetheless, experimental pH (7.0) more closely resembled conditions to which farmed and Stewiacke salmon were normally exposed (pH = 6.0–6.5; Tusket pH = 4.6–5.2) (Fraser et al. 2008).

### Embryonic developmental rates

We recorded the number of individuals that hatched in each family every 24 h throughout the incubation period. Given the large number of eggs per family ( $n \approx 500$ ), digital photos were taken daily to accurately count newly hatched alevins. Owing to slight daily temperature differences between tanks (range: 0.10–0.15 °C), it was necessary to standardize the numbers of individuals hatching at any given degree-day across families. To do so, the minimum number of degree-days accumulated within 24 h over all families was used as a binning interval (4 degree-days). Then, the number of individuals hatching in each family was calculated for each 4-degree-day interval; thus, the mean cumulative degree-days to hatching for each cross could be determined for the analyses below.

### Statistical analyses

#### Mixed effects analysis

We analyzed developmental rates based on individual counts of eggs that hatched in every 4-degree-day interval. The purpose was to draw inference on whether differences in developmental rates existed among farmed, wild, and hybrid crosses. Data were nonindependent at the family level, so we chose a hierarchical mixed effects analytical framework that accounted for the within group (family) correlation when estimating differences at the cross level (Diggle et al. 2003). Letting  $Y_{ijk}$  be the cumulative degree-day to hatch for egg  $i$  in family  $j$ , cross type  $k$ , a linear mixed effects model describing the response at both the cross and family level was written as

$$Y_{ijk} = \mu_0 + \Delta\mu_k + b_j + \varepsilon_{ijk}$$

where  $\mu_0$  was the overall fixed effect mean degree-day to hatch (set to be the mean response of one of the wild populations, see below),  $\Delta\mu_k$  was the fixed-effect deviation from

<sup>3</sup>Supplementary data for this article are available on the journal Web site (<http://cjfas.nrc.ca>).

$\mu_0$  by cross  $k$ ,  $b_j$  was the random effect deviation from  $\mu_0 + \Delta\mu_k$  by family  $j$  with  $b_j$  assumed to be normally distributed  $b_j \sim N(0, \sigma_b^2)$ , and  $\varepsilon_{ijk}$  was the deviation of the individual egg from the average for family  $j$ ,  $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2)$ . The significance of the estimated  $\Delta\mu_k$  away from zero indicated whether there was a cross-difference from  $\mu_0$ , while  $b_j$  provided for the nonindependence of the response at the family level. Models were run for each of the farmed–wild crosses, i.e., one for farmed–Stewiacke crosses and one for farmed–Tusket crosses (Supplemental Appendix S2<sup>3</sup>). As an example, the model run for the farmed–Tusket crosses (equivalent for the farmed–Stewiacke comparison) was

$$Y_{ijk} = \mu_{\text{TUSK-wild}} + (\Delta\mu_k + b_j) + \varepsilon_{ijk}$$

where  $k = \{\text{F}_1 \text{ hybrid}, \text{F}_2 \text{ hybrid}, \text{BC hybrid}\}$  from the farmed–Tusket crosses, and  $b_j = \{b_1, b_1, \dots, b_{58}\}$  were the random effects per family (i.e., the total number of Tusket, farmed, and farmed–Tusket hybrid families).

### Variance decomposition

We assessed whether variance in developmental rates differed among farmed, wild, and hybrid crosses. The variance was decomposed in three ways. First, separate variances of the random effects per cross would indicate whether crosses differed in the spread of the familial response. This scenario was modeled by letting  $\sigma_b^2 = \sigma_{b_k}^2$  so that a separate random effects variance was estimated per cross  $k$ . Second, the residual variance could differ among crosses. This was modeled by decomposing the residual variance by  $\sigma_\varepsilon^2 = \sigma_{\varepsilon_k}^2$ . To check whether particular families influenced the differences of the residual variance between crosses, we thirdly placed a random effect on the residual variance so that a residual variance per cross, as well as the effect of family, could be accounted for. This was modeled using

$$\sigma_\varepsilon^2 = \sigma_{\varepsilon_k}^2 \exp(z_j)$$

where  $z_j \sim N(0, \sigma_z^2)$ . Note that the use of the exponential prevents negative estimates of the variance, while a family within a cross could have a variance that is any proportion of the overall cross residual variance.

### Inclusion of potential maternal effects

In salmonid fishes, early-life history traits can be influenced by maternal effects (e.g., Beacham and Murray 1990; Einum and Fleming 2000). Thus, when crosses differed from one another in embryonic developmental rates within a farmed–wild comparison, we carried out supplementary analyses to account for potential maternal effects (included as fixed effects). This was assessed using the subsets of families in different crosses that originated from the same mothers (a full description of these analyses is found online in Supplemental Appendix S3<sup>3</sup>). Note that owing to our cross design (Supplemental Appendix S1<sup>3</sup>), maternal effects could not be accounted for between some crosses.

### Transformations

Preliminary data plotting revealed that some families exhibited negative skew, with longer tails to the left of the hatch distribution (Supplemental Appendix S1<sup>3</sup>). Biologically, we interpreted this as a nontrivial contribution of early hatchlings in some families to the overall distribution of cu-

mulative degree-days to hatch. Though not severe (the probability density was largely symmetric), we investigated the effect of the skew on the overall results by data transformation (details online in Supplemental Appendix S4<sup>3</sup>).

Maximum likelihood was used to fit the models, which were then compared using Akaike's information criterion (Akaike 1973). The models were implemented in both AD-Model Builder RE Module (ADMB-RE; Otter Research Ltd. 2005) and SAS PROC NL MIXED (SAS Institute Inc. 2004). Maximum likelihood can produce downward bias in the standard errors of the estimates (used for the line cross analysis below), so the models were also fit via restricted maximum likelihood for comparison. Standard errors of the estimates differed negligibly between the restricted and unrestricted maximum likelihood fits. Our large sample size ensured that the maximum likelihood values tend to be unbiased in this case.

### Line cross analysis

As a final step to our analyses, the joint scaling based on least squares regression (Lynch and Walsh 1998) was used to evaluate the fit of our cumulative degree-day data in each farmed–wild comparison (i.e., cross means and variances) to a simple additive model of genetic differentiation between parental populations. No further model building involving nonadditive effects (e.g., an additive-dominance model) was conducted because the simple additive model could not be rejected (both  $\chi^2 \leq 1.54$ ,  $df = 3$ ,  $P \geq 0.67$ ). Note that the joint-scaling test assumed trait normality, which was not entirely met with our data. It was also based on a diploid model of inheritance but Atlantic salmon exhibit residual tetraploidy. Nevertheless, a suitable genetic model that can account for such a mode of inheritance currently does not exist (Fraser et al. 2008).

## Results

Embryos of farmed salmon had slower developmental rates (cumulative degree-days to hatch) than embryos from each wild population (Tusket, Stewiacke) (all  $P < 0.0001$ ; Table 1; Fig. 3; Supplemental Appendix S2<sup>3</sup>). The magnitude of this difference was greater between farmed and Tusket salmon than between farmed and Stewiacke salmon (Table 1; Fig. 3).

Hybrids (F<sub>1</sub>, F<sub>2</sub>, BC) in both farmed–wild comparisons exhibited developmental rates that were intermediate between parents and hence slower than in wild populations (Table 1; Fig. 3). A simple additive genetic model adequately explained the differences between parental and hybrid crosses (farmed–Stewiacke:  $\chi^2 = 0.71$ ,  $df = 3$ ,  $P = 0.87$ ; farmed–Tusket:  $\chi^2 = 1.54$ ,  $df = 3$ ,  $P = 0.67$ ). In particular, both F<sub>1</sub> farmed–wild hybrids differed in developmental rates from their respective wild parents, as did one second generation hybrid (farmed–Tusket backcrosses) (Table 1; Fig. 3). Removing mothers used more than once in different families within crosses did not affect the results (Supplemental Appendix S2<sup>3</sup>). Furthermore, cross differences in embryonic developmental rates remained where potential maternal effects could be accounted for (i.e., when the same mothers were used in multiple crosses) (Supplemental Appendix S3<sup>3</sup>). Familial contributions to the overall distribution of

**Table 1.** Results of the linear mixed effects analysis (with family as a random effect) to test for differences in embryonic developmental rates between crosses.

Cross	Avg. cumulative degree-days to hatch	SE	<i>t</i>	df	<i>p</i>
<b>Farmed–Stewiacke</b>					
Wild	527.62	3.39	—	—	—
Farmed	549.29	2.94	4.71	75	<0.0001
F <sub>1</sub> hybrid	535.62	2.68	1.89	75	0.0631
F <sub>2</sub> hybrid	528.43	2.19	0.21	75	0.8379
BC hybrid	527.54	2.37	-0.02	75	0.9849
<b>Farmed–Tusket</b>					
Wild	504.91	2.84	—	—	—
Farmed	549.29	2.94	11.58	56	<0.0001
F <sub>1</sub> hybrid	528.24	3.12	5.86	56	<0.0001
F <sub>2</sub> hybrid	509.68	2.87	1.27	56	0.2102
BC hybrid	511.93	3.02	1.82	56	0.0747

**Note:** The *t* statistic reflects differences from the wild population. The degrees of freedom (df) are the number of families minus two (two random effects in model — see Supplemental Appendix S2, available online at <http://cjfas.nrc.ca>). SE, standard error; BC, backcross.

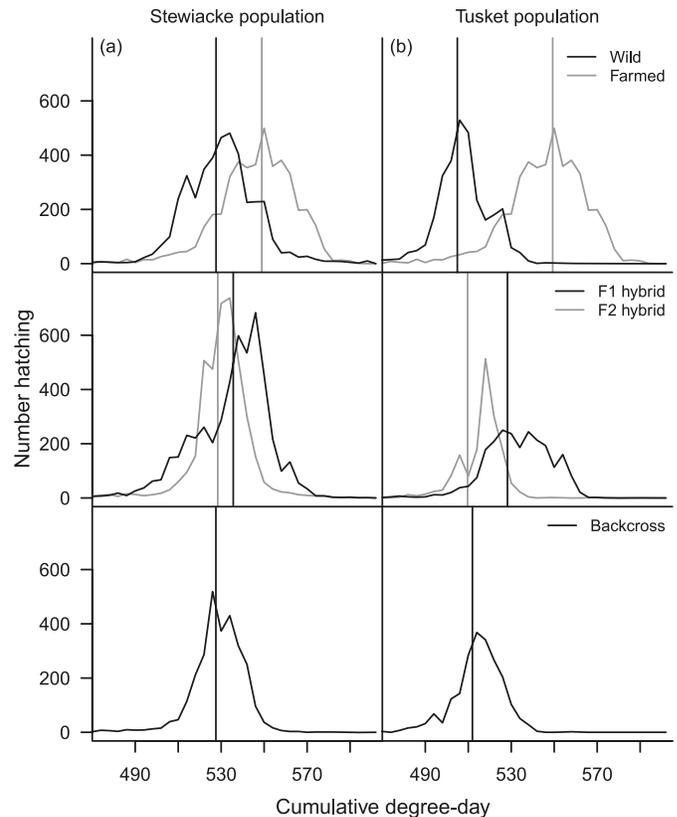
hatching times of each cross are presented in Supplemental Appendix S1<sup>3</sup>.

Finally, we found little evidence that variance in developmental rates differed between crosses. Significant differences were observed in the residual variances by cross, but once a random effect on the variance by family was included in the analysis, the differences became nonsignificant. This indicated that particular families within crosses drove the apparent differences in variance of the residuals. Incorporating separate, random effects variances on the mean hatch date in the analysis also did not result in any detectable differences among any of the crosses (Supplemental Appendices S1, S2<sup>3</sup>).

## Discussion

Our demonstration of likely heritable differences in embryonic developmental rates among Atlantic salmon populations was not unexpected based on previous studies of salmonid fishes (e.g., Beacham and Murray 1987; Jensen et al. 1991; Donaghy and Verspoor 1997). Our demonstration of genetically based phenotypic differentiation between multigenerational farmed–wild hybrids and their wild salmon counterparts is also consistent with previous studies (e.g., McGinnity et al. 2003; Tymchuk et al. 2007; Fraser et al. 2010). What distinguishes our study is the documentation of phenological trait differences in a consistent direction among farmed salmon, two wild salmon populations, and their multigenerational hybrids, within a region where farmed salmon escape repeatedly into the wild while wild salmon are severely depleted. Specifically, in the Northwest Atlantic, the farmed Atlantic salmon strain most commonly used in aquaculture had slower developmental rates than two at-risk wild Atlantic salmon populations representative of regional wild fish. In both farmed–wild comparisons studied, hybrids most likely to be produced in the wild (F<sub>1</sub>) had slower developmental rates than wild salmon (with *P* <

**Fig. 3.** The distribution of developmental rates (cumulative degree-days to hatch) for farmed–wild hybrids relative to parental crosses. (a) Stewiacke vs. farmed, (b) Tusket vs. farmed. Fitted averages of the lowest Akaike information criterion (AIC) mixed effects model, which takes family into account, are presented as vertical lines with the same line type as the distribution. Egg numbers per cross were as follows: Stewiacke (4164), Tusket (3140), farmed (4443). Farmed–Stewiacke hybrids: F<sub>1</sub> (5140), F<sub>2</sub> (8241)\*, backcross (5735)\*. Farmed–Tusket hybrids: F<sub>1</sub> (2358), F<sub>2</sub> (1734), backcross (2117). \*Egg numbers halved for the purpose of plotting.



0.05 in the farmed–Tusket comparison and *P* = 0.06 in the farmed–Stewiacke comparison). Second generation hybrids most likely to be produced in the wild (i.e., wild backcrosses; Edmands 2007) also exhibited this same trend (*P* = 0.07) in the farmed–Tusket comparison. The observed differences in mean developmental rates between regional farmed–wild hybrids and wild salmon (8–22 degree-days for F<sub>1</sub> hybrids) or between regional farmed and wild salmon (22–45 degree-days) are within or slightly greater than the range of values reported in wild-only studies of salmonids conducted at comparable spatial scales (e.g., Beacham and Murray 1987, 1990; Berg and Moen 1999).

The conservation status of regional wild salmon populations precluded experimentation in the wild. We are thus inherently restricted to making inferences about the consequences of the observed developmental rate differences beyond the laboratory. Nevertheless, the magnitude of the observed differences in mean developmental rates between regional farmed–wild hybrids and wild salmon in our study may be of biological significance in the natural environment. On average, F<sub>1</sub> hybrids would be expected to hatch 2–11 days later than wild salmon in nature, based on mean

winter incubation temperatures of 2–4 °C within regional rivers (Lacroix 1985; Houde 2009), which closely resemble the mean temperature under which our experimental research was carried out (4.5 °C). In streams, salmon hatching precedes emergence from the gravel, the point at which young alevins have exhausted the energy stores within maternally provided yolk sacs (Beacham and Murray 1987, 1990). We were unable to precisely quantify emergence times, but available data on other salmon species suggest that later-hatching salmon also emerge later (Beacham and Murray 1987, 1990). Later emergence can negatively impact alevin growth rate (Metcalf and Thorpe 1992), early survival (Einum and Fleming 2000), territory acquisition (Harwood et al. 2003), and later life-history events linked to survival (e.g., timing of smolt migration; Garcia de Leaniz et al. 2007). Importantly, such consequences may arise in nature even when emergence is delayed by only a few days (e.g., Einum and Fleming 2000). Given that regional farmed escapes are recurrent (Morris et al. 2008), and even low levels of farmed–wild gene flow can shift wild phenotypes away from the wild optimum (Ford 2002), repeated farmed–wild interbreeding could ultimately result in a reduction in the average fitness of wild individuals and a concomitant reduction in abundance of regional wild populations (Fleming et al. 2000; McGinnity et al. 2003).

Regional river temperatures can increase to 8–10 °C between the time of hatching and emergence (Lacroix 1985; Houde 2009), and so the magnitude of the difference between farmed–wild hybrids and wild salmon (in terms of numbers of days) might be offset partially by narrowing the gap in hatch time between them. Furthermore, other early life history traits linked to embryonic development rates might help to offset adverse effects of delayed hatching in farmed–wild hybrids if, relative to wild fish, such hybrids hatch or emerge at a larger size, grow faster, or exhibit more aggressive–dominant behaviours. Available data suggests that this might only occur in one of two wild populations studied. Tuskent salmon were larger (at yolk absorption), grew faster (up to 400 days after hatching), and were more aggressive than F<sub>1</sub> and backcross farmed–Tuskent hybrids (Fraser et al. 2010; Houde et al. 2010a). Regardless, farmed–wild hybrids of both wild populations exhibit a maladaptive expression of other traits important to fitness, including reduced antipredator responses (Houde et al. 2010b) and reduced acid tolerance (see Fraser et al. 2008).

We also have no evidence that F<sub>1</sub> farmed–wild hybrid alevins might still hatch at the same and presumably optimal time as wild alevins in spring because farmed females spawn earlier than wild females, or because farmed–wild hybrids inherit from their farmed parents a capacity to develop faster than wild salmon in warmer winter years. First, under common environmental conditions, males and females from all three of our parental populations (farmed, Tuskent, Stewiacke) overlapped in the dates at which they became ready to breed. Second, Darwish and Hutchings (2009) compared developmental rates between Tuskent and farmed–Tuskent backcrosses at three temperatures higher than those studied here (5.3, 9.2, and 15.4 °C) and found little evidence that backcrosses develop faster with increasing temperatures.

Finally, one of two F<sub>1</sub> hybrid crosses in our study (farmed–Tuskent) did not include parentally reciprocal fami-

lies, as wild females and farmed males were not crossed together. Such families might not exhibit as slow a developmental rate as F<sub>1</sub> hybrids composed of farmed females and wild males; a visual inspection of reciprocal familial contributions to the overall distribution of hatching times in F<sub>1</sub> farmed–Stewiacke hybrids raises this possibility (Supplemental Appendix S1<sup>3</sup>). Yet mating between wild males and farmed females, as was most represented in our two F<sub>1</sub> hybrid crosses, may be more representative of what takes place in the wild (Fleming et al. 2000).

Our results underscore the necessity for future modeling to evaluate the demographic consequences of farmed–wild interbreeding for regional wild populations, under scenarios of both recurrent and episodic intrusions of farmed escapes (Hindar et al. 2006). For instance, developmental rates of hybrids most likely to be produced in the wild (F<sub>1</sub>, wild backcrosses; Edmands 2007) were intermediate between their respective parents. Thus, overall, altered developmental rates from farmed–wild interbreeding would likely revert back to the wild state if a one-time high rate of interbreeding (e.g., through a single, large escape event) or only very low levels of interbreeding occur. Nevertheless, before a later generation fitness rebound could occur (i.e., F<sub>1</sub> and beyond), a drop in hybrid fitness in the F<sub>1</sub> generation would be expected, which might itself reduce the probability of persistence of wild populations currently at an elevated risk of extinction.

In conclusion, we have illustrated how aquaculture, a human activity undergoing rapid, worldwide expansion, may genetically alter a critical phenological event related to survival across different wild populations — in this case, populations of a migratory species, which may be particularly sensitive to phenological mismatches (Stenseth and Mysterud 2002; Crozier et al. 2008). Given the growing number of species being domesticated for human purposes (Ellstrand 2003; Hutchings and Fraser 2008; Kidd et al. 2009), our study reinforces previous recommendations for management strategies to prevent domesticated–wild interbreeding (Ellstrand 2003; Thorstad et al. 2008 and references therein) as an essential step toward retaining the appropriate genetic composition needed for phenological matching in the wild.

## Acknowledgements

Experiments undertaken with Atlantic salmon in this study comply with the requirements of the Canadian Council on Animal Care (CCAC). Thanks are extended to the Atlantic salmon broodstock program (St. Andrews, New Brunswick), Fisheries and Oceans Canada Coldbrook Biodiversity Facility (Coldbrook, Nova Scotia), and J. Lawlor for providing salmon gametes or rearing F<sub>1</sub> crosses; Dalhousie University's Aquatron Laboratory, L. Weir, A. Houde, M. Bartkowska, F. Palstra, C. Purchase, D. Hardie, T. Darwish, and L. Goodbrand for help with salmon crosses; and W. Blanchard for statistical advice. We also thank E. Taylor, P. Debes, and two anonymous reviewers for their constructive comments on the paper. Research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant to JAH and an NSERC Postdoctoral and Atlantic Salmon Federation Olin Fellowships to DJF.

## References

- Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. *In* 2nd International Symposium on Information Theory. *Edited by* B.N. Petrov and F. Csaki. Akademiai Kiado, Budapest, Hungary. pp. 267–281.
- Beacham, T.D., and Murray, C.B. 1987. Adaptive variation in body size, age, morphology, egg size, and developmental biology of chum salmon (*Oncorhynchus keta*) in British Columbia. *Can. J. Fish. Aquat. Sci.* **44**(2): 244–261. doi:10.1139/f87-034.
- Beacham, T.D., and Murray, C.B. 1990. Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. *Trans. Am. Fish. Soc.* **119**(6): 927–945. doi:10.1577/1548-8659(1990)119<0927:TESADO>2.3.CO;2.
- Berg, O.K., and Moen, V. 1999. Inter- and intra-population variation in temperature sum requirements at hatching in Norwegian Atlantic salmon. *J. Fish Biol.* **54**(3): 636–647. doi:10.1111/j.1095-8649.1999.tb00642.x.
- Bert, T.M. 2007. Ecological and genetic implications of aquaculture activities. 1st ed. Kluwer Academic Publishers Group, Dordrecht, The Netherlands.
- Carr, J.W., Anderson, J.M., and Whoriskey, F.G. 1997. The occurrence and spawning of cultured Atlantic salmon (*Salmo salar*) in a Canadian river. *ICES J. Mar. Sci.* **54**(6): 1064–1073. doi:10.1016/S1054-3139(97)80010-0.
- Committee of the Status of Endangered Wildlife in Canada. 2006. COSEWIC assessment and update status report on Atlantic salmon (*Salmo salar*) Inner Bay of Fundy populations. Committee of the Status of Endangered Wildlife in Canada, Ottawa, Ont. Available from [www.sararegistry.gc.ca/virtual\\_sara/files/cosewic/sr\\_atlantic\\_salmon\\_inner\\_bay\\_e.pdf](http://www.sararegistry.gc.ca/virtual_sara/files/cosewic/sr_atlantic_salmon_inner_bay_e.pdf) [accessed 8 May 2010].
- Crozier, L.G., Hendry, A.P., Lawson, P.W., Quinn, T.P., Mantua, N.J., Battin, J., Shaw, R.G., and Huey, R.B. 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. *Evol. Appl.* **1**(2): 252–270. doi:10.1111/j.1752-4571.2008.00033.x.
- Darwish, T.L., and Hutchings, J.A. 2009. Genetic variability in reaction norms between farmed and wild backcrosses of Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* **66**(1): 83–90. doi:10.1139/F08-185.
- Diggle, P., Heagerty, P., Liang, K., and Zeger, S. 2003. Analysis of longitudinal data. Oxford Statistical Science Series, Oxford, UK.
- Donaghy, M.J., and Verspoor, E. 1997. Egg survival and timing of hatch in two Scottish Atlantic salmon stocks. *J. Fish Biol.* **51**(1): 211–214. doi:10.1111/j.1095-8649.1997.tb02527.x. PMID: 9236102.
- Edmunds, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **16**(3): 463–475. doi:10.1111/j.1365-294X.2006.03148.x. PMID: 17257106.
- Einum, S., and Fleming, I.A. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *J. Fish Biol.* **50**(3): 634–651. doi:10.1111/j.1095-8649.1997.tb01955.x.
- Einum, S., and Fleming, I.A. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution*, **54**(2): 628–639. PMID: 10937238.
- Ellstrand, N.C. 2003. Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, Md.
- Fisheries and Oceans Canada. 2002. Atlantic salmon Maritime provinces overview for 2002. Stock Status Rep. 2002/026, Ottawa, Ont.
- Fleming, I.A., Hindar, K., Mjølnerod, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. *Proc. R. Soc. Lond. B. Biol. Sci.* **267**(1452): 1517–1523. doi:10.1098/rspb.2000.1173.
- Ford, M.J. 2002. Selection in captivity during supplementation may reduce fitness in the wild. *Conserv. Biol.* **16**(3): 815–825. doi:10.1046/j.1523-1739.2002.00257.x.
- Fraser, D.J., Weir, L.K., Darwish, T.L., Eddington, J.D., and Hutchings, J.A. 2007. Divergent compensatory growth responses within species: linked to contrasting migrations in salmon? *Oecologia* (Berlin), **153**(3): 543–553. doi:10.1007/s00442-007-0763-6.
- Fraser, D.J., Cook, A.M., Eddington, J.D., Bentzen, P., and Hutchings, J.A. 2008. Mixed evidence for reduced local adaptation in wild salmon resulting from interbreeding with escaped farmed salmon: complexities in hybrid fitness. *Evol. Appl.* **1**(3): 501–512. doi:10.1111/j.1752-4571.2008.00037.x.
- Fraser, D.J., Houde, A.S., Debes, P.V., O'Reilly, P.T., Eddington, J.D., and Hutchings, J.A. 2010. Consequences of farmed–wild hybridization across divergent wild populations and multiple traits in salmon. *Ecol. Appl.* **20**(4): 935–953. doi:10.1890/09-0694.1. PMID: 20597281.
- García de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F., Webb, J.H., Vøllestad, L.A., Villanueva, B., Ferguson, A., and Quinn, T.P. 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. *Biol. Rev. Camb. Philos. Soc.* **82**(2): 173–211. doi:10.1111/j.1469-185X.2006.00004.x. PMID: 17437557.
- Glebe, B.D. 1998. East coast salmon aquaculture breeding programs: history and future. Canadian Stock Assessment Secretariat, Res. Doc. 1998/157.
- Harwood, A.J., Griffiths, S.W., Metcalfe, N.B., and Armstrong, J.D. 2003. The relative influence of prior residency and dominance on the early feeding behaviour of juvenile Atlantic salmon. *Anim. Behav.* **65**(6): 1141–1149. doi:10.1006/anbe.2003.2125.
- Helfman, G.S., Collette, B.B., and Facey, D.E. 2009. The diversity of fishes. Wiley-Blackwell, Chichester, West Sussex, UK.
- Hindar, K., Fleming, I.A., McGinnity, P., and Diserud, O. 2006. Genetic and ecological effects of salmon farming on wild salmon: modeling from experimental results. *ICES J. Mar. Sci.* **63**(7): 1234–1247. doi:10.1016/j.icesjms.2006.04.025.
- Houde, A.S. 2009. The relative risk of inbreeding and outbreeding endangered populations of Atlantic salmon, *Salmo salar*. M.Sc. thesis, Department of Biology, Dalhousie University, Halifax, N.S.
- Houde, A.S., Fraser, D.J., and Hutchings, J.A. 2010a. Fitness-related consequences of competitive interactions between farmed and wild Atlantic salmon at different proportional representations of wild–farmed hybrids. *ICES J. Mar. Sci.* **67**: 657–667. doi:10.1093/icesjms/fsp272.
- Houde, A.S., Fraser, D.J., and Hutchings, J.A. 2010b. Reduced anti-predator responses in multi-generational hybrids of farmed and wild Atlantic salmon (*Salmo salar* L.). *Conserv. Genet.* **11**(3): 785–794. doi:10.1007/s10592-009-9892-2.
- Hutchings, J.A., and Fraser, D.J. 2008. The nature of fisheries- and farming-induced evolution. *Mol. Ecol.* **17**(1): 294–313. doi:10.1111/j.1365-294X.2007.03485.x. PMID: 17784924.
- Jensen, A.J., Johnsen, B.O., and Heggerget, T.G. 1991. Initial feeding time of Atlantic salmon, *Salmo salar*, alevins compared

- to river flow and water temperatures in Norwegian streams. *Environ. Biol. Fishes*, **30**(4): 379–385. doi:10.1007/BF02027981.
- Kidd, A.G., Bowman, J., Lesbarrères, D., and Schulte-Hostedde, A.I. 2009. Hybridization between escaped domestic and wild American mink (*Neovison vison*). *Mol. Ecol.* **18**(6): 1175–1186. doi:10.1111/j.1365-294X.2009.04100.x. PMID: 19243512.
- Lacroix, G.L. 1985. Survival of eggs and alevins of Atlantic salmon (*Salmo salar*) in relation to water chemistry of interstitial water in redds in some acidic streams of Atlantic Canada. *Can. J. Fish. Aquat. Sci.* **42**(2): 292–299. doi:10.1139/f85-037.
- Lawlor, J., Dacanay, A., Hutchings, J.A., Brown, L.L., and Sperker, S.A. 2009. Differences in pathogen resistance within and among cultured, conservation-dependent, and endangered populations of Atlantic salmon, *Salmo salar* L. *Environ. Biol. Fishes*, **84**(1): 69–78. doi:10.1007/s10641-008-9390-2.
- Lynch, M., and Walsh, B. 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, Mass.
- McClelland, E.K., and Naish, K.A. 2007. What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. *Conserv. Genet.* **8**(2): 397–416. doi:10.1007/s10592-006-9178-x.
- McGinnity, P., Prodohl, P., Ferguson, A., Hynes, R., Maoileidigh, N., Baker, N., Cotter, D., O’Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc. R. Soc. Lond. B. Biol. Sci.* **270**(1532): 2443–2450. doi:10.1098/rspb.2003.2520.
- McLean, J.E., Bentzen, P., and Quinn, T.P. 2005. Nonrandom, size- and timing-biased breeding in a hatchery population of steelhead trout. *Conserv. Biol.* **19**(2): 446–454. doi:10.1111/j.1523-1739.2005.00515.x.
- Metcalfe, N.B., and Thorpe, J.E. 1992. Early predictors of life history events: the link between first-feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar*. *J. Fish Biol.* **41**(sb Suppl A): 93–99. doi:10.1111/j.1095-8649.1992.tb03871.x.
- Morris, M.R.J., Fraser, D.J., Heggelin, Whoriskey, F.G., Carr, A.J., Hutchings, J.A., and O’Neil, S.F. 2008. Prevalence and recurrence of escaped farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers. *Can. J. Fish. Aquat. Sci.* **65**(12): 2807–2826. doi:10.1139/F08-181.
- Naylor, R., Hindar, K., Fleming, I.A., and Whoriskey, F.G. 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. *Bioscience*, **55**(5): 427–437. doi:10.1641/0006-3568(2005)055[0427:FSATRO]2.0.CO;2.
- O’Reilly, P.T., Carr, J.W., Whoriskey, F.G., and Verspoor, E. 2006. Detection of European ancestry in escaped farmed Atlantic salmon, *Salmo salar* L., in the Magaguadavic River and Chamcook Stream, New Brunswick, Canada. *ICES J. Mar. Sci.* **63**(7): 1256–1262. doi:10.1016/j.icesjms.2006.04.013.
- Otter Research Ltd. 2005. Random effects in AD Model Builder: ADMB-RE user guide. Otter Research Ltd., Sydney, B.C.
- Reisenbichler, R.R., and Rubin, S.P. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES J. Mar. Sci.* **56**(4): 459–466. doi:10.1006/jmsc.1999.0455.
- SAS Institute Inc. 2004. SAS/STAT 9.1 user’s guide. SAS Institute Inc., Cary, N.C.
- Stenseth, N.C., and Mysterud, A. 2002. Climate, changing phenology, and other life history traits: nonlinearity and match–mismatch to the environment. *Proc. Natl. Acad. Sci. U.S.A.* **99**(21): 13379–13381. doi:10.1073/pnas.212519399. PMID:12370424.
- Thorstad, E.B., Fleming, I.A., McGinnity, P., Soto, D., Wennevik, V., and Whoriskey, F. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. Norwegian Institute for Nature Research (NINA) and World Wildlife Fund Inc., Tungasletta, Norway.
- Tymchuk, W.E., Sundström, L.F., and Devlin, R.H. 2007. Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution*, **61**(5): 1225–1237. doi:10.1111/j.1558-5646.2007.00102.x. PMID:17492973.
- US Atlantic Salmon Assessment Committee. 2005. Annual report of the US Atlantic Salmon Assessment Committee. Report 17-2004 Activities, Woods Hole, Mass.
- Webb, J.H., and McLay, H.A. 1996. Variation in the time of spawning of Atlantic salmon (*Salmo salar*) and its relationship to temperature in the Aberdeenshire Dee, Scotland. *Can. J. Fish. Aquat. Sci.* **53**(12): 2739–2744. doi:10.1139/cjfas-53-12-2739.