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*Interactions between aquaculture and wild stocks:  
comparative experiences for Atlantic cod and Atlantic salmon*

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Adaptive phenotypic plasticity in Atlantic salmon and Atlantic cod and its relevance to risk assessments of aquaculture

Jeffrey A. Hutchings and Dylan J. Fraser<sup>1</sup>  
Dept Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1 CANADA

## **Abstract**

Risks to wild populations resulting from interbreeding with farmed escapees depend primarily on the probability of spawning between groups, the level of genetic differentiation between farmed and wild individuals, and the abundance of wild populations relative to conservation targets. The present work pertains to the second of these factors. In addition to the divergence that breeding designs and domestication selection can generate, genetic dissimilarities will also depend on the magnitude and type of genetic differentiation that exists among populations in the wild. Here, using common-garden experimental protocols, we describe experiments on Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*) that have documented genetic differences phenotypic plasticity. Multi-generational crosses between farmed and wild salmon have revealed (i) adaptive variability in plasticity among populations and (ii) negative fitness consequences to wild salmon resulting from interbreeding, notably from changes to developmental reaction norms. In Northwest Atlantic cod, larval survival and growth, and their plastic responses to changes in food supply and temperature, differ across spatial scales at which analyses of microsatellite DNA failed to detect population structure. Based on the Atlantic salmon research, these results are consistent with the hypotheses that (i) cod populations differ significantly in how they respond to environmental change and that (ii) these differences in plasticity are genetically based and may be a product of local adaptation. Disruption to reaction norms and adaptive phenotypic plasticity, resulting from interbreeding between wild individuals and farmed escapees, merits considerably greater attention in risk assessments of aquaculture than it has to date.

**Keywords:** phenotypic plasticity; reaction norms; farmed-wild interbreeding; common-garden experiment

Contact author: Jeffrey A. Hutchings; e-mail: [jeff.hutchings@dal.ca](mailto:jeff.hutchings@dal.ca)

<sup>1</sup>Present address: Dept Biology, Concordia University, Montréal, Québec H4B 1R6 CANADA; email: [djfraser@alcor.concordia.ca](mailto:djfraser@alcor.concordia.ca)

## Introduction

Phenotypic plasticity is the ability of a genotype to produce different phenotypes across an environmental gradient (Sultan & Stearns 2005). Plasticity can be heuristically and graphically described as a norm of reaction—a linear or nonlinear function that expresses how the phenotypic value of a trait for a given genotype changes with the environment. At the population level, reaction norms can be used to predict how individuals will respond, on average, to specific changes to an environmental variable. Genetic differences in reaction norms would reflect differences in the ability of populations to respond to environmental change and the manner in which they do so.

Assessments of the risks to wild populations resulting from genetic interactions with farmed members of the same species have tended to focus, not inappropriately, on how interbreeding might affect the average or mean expression of a particular trait by individuals in the naturally occurring population. Of equal if not greater interest is the question of whether such genetic interactions are likely to affect reaction norms of fitness-related traits in wild populations. The central question here, then, is whether interbreeding between wild and farmed fish is likely to affect the plasticity of traits associated with survival, growth, fecundity, and reproductive success, thus potentially affecting the abilities of populations to respond adaptively to temporal variability in the environment, including that imposed by climate change.

A question such as this has bearing on what may be the most important question concerning the consequences to wild populations resulting from interactions with their farmed counterparts: Does interbreeding disrupt adaptive variation in wild fish? If wild populations are not locally adapted, then interbreeding between farmed and wild fish is unlikely to affect the probability of persistence (or the extinction probability) of the wild population. Although the literature is replete with examples of *potential* local adaptation in fishes, the fact remains that adaptation is an extremely difficult thing to demonstrate unequivocally. Genetic variability in characters closely related to fitness, for example, while being consistent with, and perhaps strongly suggestive of, the hypothesis of local adaptation is not sufficient to allow one to conclude that such variability is indeed adaptive (e.g., Taylor 1991; de Leaniz et al. 2007). The alternative perspective asserts that local adaptation does not exist and that all populations of the same biological species are, more or less, equally inter-changeable, independently of the environments in which their ancestors persisted.

Here, the central objective is to provide a summary of recent research on (i) the consequences of interbreeding between wild and farmed Atlantic salmon (*Salmo salar*) and on (ii) the genetic variability in reaction norms in Atlantic salmon and Atlantic cod (*Gadus morhua*). The primary conclusions of this work can be summarized as follows: (1) interbreeding between farmed and wild Atlantic salmon will, to greater or less degrees (depending on the level of genetic variability between wild and farmed forms), negatively affect the per capita population growth rate, and fitness, of wild salmon; (2) there is genetic variability in reaction norms, and thus phenotypic

plasticity, at the population level in Atlantic cod and Atlantic salmon; and (3) there is good evidence that genetic variability in some Atlantic salmon reaction norms is adaptive.

Regarding the lessons that can be learned from risk-assessment research on farmed and wild Atlantic salmon, it is reasonable to draw the following conclusions about Atlantic cod aquaculture: (i) adaptive variability among cod populations likely exists at comparatively small spatial scales (on the order of hundreds of kilometers, possibly less in some regions); (ii) interbreeding between farmed and wild Atlantic cod will not beneficially affect wild populations; and (iii) interbreeding between farmed and wild Atlantic cod will almost certainly have negative consequences to individual fitness in the wild, although the effects on population persistence and per capita population growth may be less problematic for large stocks.

As with Atlantic salmon, it is predicted that the consequences to individual fitness and population growth rate in wild cod will depend primarily on (i) the degree of genetic differentiation between wild and farmed cod; (ii) the frequency of interbreeding between forms; and (iii) on the proportional representation of interbreeding events among all spawning episodes in the wild.

### **Research Programme on the Consequences of Interbreeding Between Farmed and Wild Atlantic Salmon**

In 2001, JAH initiated a breeding programme designed to examine the consequences of interbreeding between farmed and wild Atlantic salmon on the expression of traits closely linked to fitness. Salmon from at least two wild populations and one domesticated (farmed) population were selected for the experiments. The wild populations used in most experiments originated from Stewiacke River and Tusket River in Nova Scotia, eastern Canada (Figure 1). The farmed population is one that originated from salmon in the Saint John River, New Brunswick, and that had undergone 4 generations of domestication selection prior to the initiation of our experiments. The domesticated population constitutes the primary source of farmed Atlantic salmon in aquaculture farms in Nova Scotia and New Brunswick, the locations of which are indicated in Figure 1. Escapes by farmed salmon from these facilities occur frequently and, not uncommonly, involve very large numbers of individuals (Morris et al. 2008).

Since 2001, we have undertaken a series of common-garden experiments to document genetic differences in the average phenotypic expression of various traits, including those related to behaviour (Houde et al. 2009), disease resistance (Lawlor et al. 2009), and development (Fraser et al. in press, submitted). We have also examined the consequence of interbreeding between farmed and wild salmon on the shape of reaction norms for traits closely linked to fitness (e.g., Fraser et al. 2008; Piché et al. 2008; Darwish and Hutchings 2009).

Common-garden experiments, in which individuals from putatively different groups are reared under the same environmental conditions, provide a powerful means of assessing the genetic basis of phenotypic variation (Conover et al. 2006). In accordance with such an experimental protocol, group-level differences in the characters of interest, or their norms of reaction,

comprise evidence that these differences have a genetic and, depending on the relationship between the trait(s) and fitness, possibly adaptive basis.

## **Genetic Consequences of Interbreeding on Fitness-Related Traits**

### *Differences in gene expression*

The breeding programme produced multiple families of pure and hybrid (F1) crosses between the wild and farmed populations in 2001 (Figure 2). In 2005, F2 cross and back-crosses were also produced; F3 crosses will be produced in the autumn of 2009. At the genomic level, we have been able to document difference in gene expression between wild and farmed salmon, their F1 hybrids and back-crosses. Normandeau et al. (2009) quantified differences in gene expression from microarrays on liver tissue obtained from the previously identified farmed, wild, and farmed-wild backcrossed (i.e. F1 farmed-wild hybrid  $\times$  wild; total  $n = 50$ ) salmon raised under common environmental conditions (Figure 3). The study revealed 177 transcripts with different expression levels among the five strains. Five times more of these transcripts were differentiated between farmed parents and Tuskett backcrosses than between Stewiacke backcrosses and their farmed parents. Altered biological processes in backcrosses also differed between populations both in number and in the type of processes impacted (metabolism vs immunity). Over-dominant gene expression regulation in backcrosses varied considerably between populations (23% in Stewiacke vs 44% in Tuskett). Hence, the consequences of introgression of farm genetic material on gene expression depended on population-specific genetic architectures.

### *Differences in the average expression of survival and developmental traits in early life*

Fraser et al. (in press) have summarized a plethora of information on the various Atlantic salmon population crosses. During the embryonic stage (fertilization to first-feeding), survival differed among crosses in a common-garden experimental setting (Figure 4), although the only statistically significant difference involved F<sub>2</sub> Tuskett-Farmed hybrids, which had significantly lower survival than their parental populations. There was also a statistically significant trend for the back-crossed Tuskett-Stewiacke fish and the F<sub>2</sub> Tuskett-Stewiacke hybrids to have reduced survival relative to parental populations (Figure 4). However, these survival differences were attributable to significant differences between mothers, not crosses. Across all early life history traits, no differences in the coefficients of variation for survival were detected between crosses in each inter-population comparison.

Examining a variety of other early-life traits, length-at-hatch differed only in comparisons involving Stewiacke fish, with wild pure Stewiacke salmon being shorter than all other parents and inter-population hybrids (Figure 5). Stewiacke fish also had smaller yolk sacs relative to Tuskett pure crosses and some Tuskett-Stewiacke hybrids (Figure 5). Length-at-hatch was not correlated with yolk sac volume-at-hatch among crosses. In general, crosses with larger yolk sacs had poorer yolk sac conversion efficiencies. Thus, by the time of first feeding, length relationships between hybrids and parental populations exhibited similar trends to length-at-hatch, the notable exception being F<sub>2</sub> Tuskett-Farmed hybrids which were longer than both

parental populations. For all four traits shown in Figure 5, analyses accounting for maternal effects indicated that both cross and mother accounted for a significant amount of the variation in the data. Thus, each trait was influenced by individual maternal effects, but trait differences existed between crosses even after accounting for these effects.

#### *Human-induced maladaptation to climate change: effects of interbreeding on hatching time*

Although several traits in early life appeared not to differ among crosses (once maternal effects had been accounted for), there is strong evidence of deleterious effects on hatching time resulting from interbreeding between farmed and wild Atlantic salmon (Figure 6). Fraser et al. (submitted) examined how a human activity unrelated to climate change (aquaculture) might compound climatic adaptability in a species.

In the Northwest Atlantic, climate warming is expected at the southern limit of the salmon's range. It is anticipated that an earlier onset of spring conditions will favour wild salmon embryos with faster developmental rates to hatching. However, such an adaptive response by depleted populations of wild salmon might be hindered by interbreeding with farmed salmon that escape regularly from intensive aquaculture facilities. Again, using a common-garden experimental protocol, Fraser et al. (submitted) show that embryos of farmed salmon and multi-generational farmed-wild hybrids ( $F_1$ ,  $F_2$ , wild backcrosses) had slower developmental rates than those of two wild populations with divergent life histories (Tusket and Stewiacke populations) (Figure 6). Hybrid developmental rates were, in effect, mismatched to environmental conditions in a direction that, with repeated farmed-wild interbreeding, will almost certainly impede the adaptability of endangered wild populations to climate warming.

#### *Genetic differences in pathogen resistance between farmed and wild Atlantic salmon*

Farmed and wild Atlantic salmon in eastern Canada can differ in their resistance to disease. Comparing the farmed population with the wild Stewiacke and Tusket populations, Lawlor et al. (2009) documented significant differences in resistance to the pathogen *Listonella anguillarum*, the causative agent for vibriosis. Using a common-garden experimental protocol, and following exposure to *L. anguillarum*, salmon parr originating from the endangered Stewiacke River population experienced significantly higher mortality than both farmed parr and wild parr from Tusket River (Figure 7). Interestingly, pathogen resistance differed between sexes; males consistently experienced higher survival than females (although there was no evidence that maturity influenced pathogen resistance in male parr). If disease resistance does differ between sexes, it would seem prudent to include such an effect when evaluating the consequences of interbreeding between wild and cultured salmonids, particularly given the sex biases that have been documented for other variables that can influence probability of interbreeding, such as spawning behaviour (Fleming 1996) and dispersal (Hutchings and Gerber 2002).

## Reaction Norms and Trait Plasticity: Consequences of Interbreeding in Salmon

### *Genotype × environment interactions*

Reaction norms describe how individuals respond to environmental change. Strictly speaking, within an evolutionary context, norms of reaction pertain to responses by genotypes, the units of study when Woltereck (1909) first coined the term *Reaktionsnorm* in his work on *Daphnia*. Genotypic studies of reaction norms are not uncommon in plants, clonal organisms, and *Drosophila* (see Schlichting and Pigliucci (1998) for examples) because of the relative ease with which single genotypes can be generated and their responses to environmental change documented. However, for most sexually reproducing animals, the family or population level is the lowest level at which reaction norms can be studied.

Reaction norms inform us about the magnitude of trait plasticity, the presence of genotype×environment (G×E) interactions on the phenotypic expression of a given trait, and the extent to which the additive genetic variance ( $V_A$ ) of a trait changes with the environment. Norms of reaction that run parallel to the environmental gradient axis reflect an absence of plasticity because the trait does not change its phenotypic value with changes to the environment. Plasticity is reflected by reaction norms with non-zero slopes (Figure 8). Crossing reaction norms, indicative of G×E interaction, suggest the presence of genetic variation in plasticity. If that genetic variation is additive, then selection can produce evolutionary changes to the shapes of reaction norms.

Genotype×environment interactions can affect the degree to which alleles are subject to selection. For the reaction norms in Figure 8, the differences in phenotype among genotypes are greatest at the environmental extremes; if that genetic variation is additive, one can conclude that the  $V_A$  of the trait in question will be highest at the extremes of the environmental. By contrast,  $V_A$  will be much lower near the centre of the environmental gradient, where the reaction norms converge, because of the similarity in phenotypic values among the different genotypes. Thus, the additive genetic variation of the trait in question, and the rate at which it will respond to selection, will vary with the environment.

The hypothesis that natural selection can alter the shapes of reaction norms was examined in a comprehensive field and laboratory research programme on grayling, *Thymallus thymallus*. Haugen (2000a,bc) provided evidence of negative reaction norms between growth rate and age at maturity within four populations in south-central Norway (Figure 9). Interestingly, he found evidence of a non-plastic reaction norm for female grayling in one population, Øvre Mærrabottvatn, similar to that described for brook trout, *Salvelinus fontinalis*, in Newfoundland (Hutchings 1996). Regardless of growth rate, Øvre Mærrabottvatn grayling (OM in Figure 9) mature at age 3 yr, as compared with ranges in age at maturity of between 4 and 8 years for grayling in the other populations. Haugen (2000b) attributed this invariant reaction norm in Øvre Mærrabottvatn grayling to the significantly higher mortality experienced by individuals in this population, during both the juvenile and adult stages, relative to that in the other populations. Given the common ancestry of the five populations (Haugen 2000a,b,c), this raises

the possibility that the present invariant reaction norm observed for Øvre Mærrabottvatn grayling may represent a selective response to higher rates of mortality.

*Genetic variability in maturation thresholds for male Atlantic salmon*

The first evidence of genetic variability in reaction norms in Atlantic salmon was documented for the threshold trait that determines whether males mature as large (>1 kg) anadromous males, following a migration to sea, or as small (10-150g) parr. These alternative reproductive tactics are a product of adaptive phenotypic plasticity, such that discontinuous variation in life history depends on both genotype and the environment. Phenotypes that fall below a genetically-determined threshold adopt one tactic, while those exceeding the threshold adopt the alternative tactic. Using a common-garden experimental protocol, Piché et al. (2008) found that the growth rate at which the sneaker parr phenotype is expressed differs among pure and mixed population crosses (Figure 10). Maturation thresholds of hybrids were intermediate to those of pure crosses, consistent with the hypothesis that the life-history switch-points are heritable. Their work provided the first evidence that discontinuous reaction norms for age and size at maturity in Atlantic salmon, reflected by alternative reproductive tactics, differ genetically among populations.

*Genetic variability in reaction norms between farmed and wild backcrosses of Atlantic salmon*

Adaptive responses to environmental heterogeneity almost certainly differs among populations. This variability might be reflected by genetic variability in norms of reaction for traits closely linked to fitness. Darwish and Hutchings (2009) constructed reaction norms for several early life-history traits and compared among different crosses of salmon. Two crosses comprised second-generation, backcrosses introgressed with either farmed or wild genes; the third comprised individuals from a second-generation, pure wild cross. These crosses, where T=Tusket River population, S=Stewiacke River population, and F=farmed population, were: TT×TT (Tusket pure cross); TT×TS (Tusket×Stewiacke backcross) and TT×TF (Tusket×Farmed backcross). Using a common-garden experimental protocol, each cross was exposed to three different temperatures. Plasticity in embryonic development, growth, survival and body size was measured from fertilization up to 24 weeks of exogenous feeding.

Reaction norms differed markedly among crosses, irrespective of whether individuals interbred with those whose genes originated from another wild population or from a cultured population (Figure 11). Thus, introgression, involving individuals with comparatively few genetic differences, can significantly alter reaction norms. Darwish and Hutchings (2009) concluded that if plasticity represents an adaptive response to local environments, then changes to reaction norms resulting from interbreeding between populations are unlikely to have a beneficial effect on fitness.

*Genetic differences in compensatory growth responses between populations of Atlantic salmon*

Animals often exhibit accelerated or “compensatory” growth after periods of environmentally-induced growth depression, raising important questions about how they cope with environmental

variability. Fraser et al. (2007) tested the under-explored hypothesis that natural populations differ in their responses to restrictions in food supply and that these differences are manifested by population differences in patterns of compensatory growth.

Common-garden experiments were used to compare growth following food restriction between groups (control, treatment) of two Atlantic salmon populations (Stewiacke River, LaHave River) and their first-generation (F1) hybrids. The populations are found at similar latitudes in Nova Scotia but are characterized by differences in sea migration distance. The prediction was that long-distance migrants would better maintain growth trajectories following food restriction than short-distance migrants because they: (1) require larger body sizes to offset energetic costs of migration and (2) face greater time constraints for growth as they must leave non-breeding areas earlier to return to breeding areas.

Fraser et al. (2007) found that long-distance migrants grew faster and achieved faster compensatory growth (Figure 12), and that their overall body morphology was more streamlined (a trait known to improve swimming efficiency) than slower growing, short-distance migrants. F1 hybrids were generally intermediate in “normal” growth, compensatory growth, and body morphology. Compensatory growth responses to restrictions in food availability can differ considerably among populations and the conditions generating them are likely interconnected with selection on a suite of other traits (Fraser et al. 2007).

*Evidence of reduced local adaptation resulting from interbreeding between farmed and wild Atlantic salmon*

Interbreeding between artificially-selected and wild organisms can have negative fitness consequences for the latter. In the Northwest Atlantic, farmed Atlantic salmon recurrently escape into the wild and enter rivers where small, declining populations of wild salmon breed (Morris et al. 2008). Most farmed salmon in the region are derived from an ancestral source population that occupies a non-acidified river (Saint John River) in which pH typically ranges between 6.0 and 6.5. By contrast, many wild populations with whom escaped farmed salmon might interbreed inhabit rivers that have been acidified (pH 4.6–5.2) for several salmon generations. Should reaction norms for survival, as functions of acidity, differ genetically between farmed salmon and salmon that may have become adapted to lower levels of pH, interbreeding between forms might result in reaction norms in the offspring that are maladaptive in the wild.

Using common garden experimentation, and examining two early-life history stages across two generations of interbreeding, Fraser et al. (2008) showed that wild salmon populations inhabiting an acidified river (Tusket River) experienced higher survival at low pH (high acidity) than wild salmon from a non-acidified river (Stewiacke River), farmed salmon that originated from a non-acidified river, and F1 farmed-wild hybrids. These differences in survival were reflected by differences in reaction norms among population crosses (Figure 13). Interestingly, there was limited evidence for reduced performance in backcrosses, and F2 farmed-wild hybrids performed better or equally well to wild salmon. Thus, among first-generation hybrids (the most common offspring resulting from interbreeding between farmed and wild salmon), there is



evidence that interbreeding will negatively affect reaction norms for survival, suggesting that repeated interbreeding between farmed and wild salmon will reduce adaptive genetic variation in the wild and thereby negatively affect the persistence of depleted wild populations.

### **Conclusions Resulting From Common-Garden Experiments on Atlantic Salmon**

The various studies that have been summarized above provide some key results pertaining to the risk assessment of the consequences to wild populations resulting from genetic interactions between wild and farmed Atlantic salmon. Many of these results have been summarized by Fraser et al. (in press).

Firstly, we have detected a variety of genetically-based trait differences between a locally-derived farmed Atlantic salmon strain and divergent wild populations in the Northwest Atlantic. Secondly, trait differences between wild populations are broadly associated with their contrasting life histories in nature. Thirdly, at the spatiotemporal scale examined, many traits appeared to respond to outbreeding in a similar (additive) way. Fourthly, wild backcrossing does not completely restore hybrid trait distributions to presumably optimal wild states. Fifthly, the degree to which hybrids deviated from their parents in absolute terms increased predictably with increasing parental divergence.

It is also clear that reaction norms for a variety of traits differ genetically among salmon populations. Interbreeding with farmed salmon will almost certainly alter the shapes of reaction norms and affect the ability of individuals to respond to environmental change.

Unprecedented increases in world aquaculture production, coupled with the severe decline of many wild fish populations, have made it crucial to rigorously assess the potential risks associated with interactions between wild and farmed individuals. The work described above has contributed to this process by examining the differential effects of multi-generational farmed-wild hybridization across divergent wild populations, a key to assessing risk at larger spatiotemporal scales in fishes (Hutchings and Fraser 2008). The extent to which farmed-wild hybridization may alter the phenotypic and genetic composition of divergent wild populations is considerable, across generations and among many traits that may be of import to fitness in the wild. Although the precise consequences to the fitness of wild individuals can be expected to vary, a precautionary approach to risk assessment would acknowledge the very high probability that interbreeding with farmed individuals will have negative consequences to individual fitness, and per capita population growth, in wild populations.

### **Genetic Variability in Phenotypic Plasticity in Northwest Atlantic Cod**

#### *Introduction*

The persistence of a species depends on the resistance and resilience of its populations to anthropogenic and natural environmental perturbation. Correspondingly, risk of extinction is a function of the breadth of population responses to environmental change and of the spatial correspondence between the scale of the perturbation and the scale of adaptation. Knowledge of

the spatial scale of population structure is thus fundamental to an understanding of population dynamics, conservation biology and sustainable harvesting practices (Hutchings & Reynolds 2004; Conover et al. 2006; Drakare et al. 2006). One key question in this regard concerns the ability of fish populations to respond to environmental change; a second concerns the spatial scale at which adaptive responses to environmental change are realized. The former question depends on the level of phenotypic plasticity expressed within a population, whereas the latter is reflected by genetic differences in plasticity at the population level.

Hutchings et al. (2007) adopted a common-garden experimental protocol to examine whether there is among-population genetic variability in larval cod growth, survival and their plastic responses to variation in food and temperature.

### *Methods*

North to south, cod were sampled from: Bonavista Bay, Newfoundland (Northwest Atlantic Fishery Organization (NAFO) division 3L); Placentia Bay, Newfoundland (NAFO division 3Ps); Southern Gulf of St Lawrence (NAFO division 4T); and Western Scotian Shelf (NAFO division 4X) (Figure 14). Adult cod were captured immediately prior to the natural spawning period, transported to large tanks (12,000 or 684,000 litres in volume), and allowed to spawn undisturbed. The number of adults in each spawning group ranged between 54 and 77 individuals. The temperature in all spawning tanks was held constant at approximately 8°C.

Eggs were sampled for the common-garden experiments approximately two weeks after they were first observed in egg collectors positioned near the surface outflows of the tanks. At this time, four batches of fertilized eggs (each batch consisted of eggs spawned over two consecutive days) were collected from each population for the common-garden experiments. For each population, the number of families represented in the experiments ranged between 21 and 71, meaning that reasonable levels of genetic variability were represented in the experiments. We reared cod larvae at two temperatures (7°C and 11°C) and two food levels (1000 and 4500 prey litre<sup>-1</sup>). For each population, there were 4 replicates per treatment. The initial number of larvae per treatment was n=1200. We measured two variables: growth rate (size at 29 days post-hatch) and survival (number alive at 43 days post-hatch, i.e., metamorphosis, relative to the initial 1200 larvae in each replicate).

### *Results*

Larval growth, survival and plasticity in these traits differed among populations, based on three-way factorial analyses of variance, although the magnitude of these differences depended on the level of comparison (Table 1; Figures 15 and 16). Among all four populations, growth increased with food supply and temperature. Evidence of genetic differences in growth plasticity was revealed by significant population×temperature×food and population×temperature interactions. Differences in larval growth were also evident between warm- and cold-water populations. Larvae produced by the warm-water populations were consistently larger than their cold-water counterparts at both levels of temperature and food.

## *Conclusions*

The faster growth experienced by warm-water populations may have contributed to their higher survival at both levels of food and temperature). The influence of food and temperature on survival differed between warm- and cold-water populations, indicative of genetic differences in the shapes of their reaction norms. For example, at the lower temperature, survival of warm-water cod increased with food supply, whereas that of cold-water cod was unaffected ; at the warmer temperature, survival increased marginally with increases in food in both populations. By contrast, irrespective of food supply, temperature had no demonstrable impact on the survival of warm-water cod, whereas survival increased significantly with temperature among cold-water cod.

Larval growth rate and larval survival, and their plastic responses to food and temperature, differed across spatial scales at which microsatellite DNA failed to detect population structure (Hutchings et al. 2007). Divergent survival reaction norms indicate that warm-water populations are more sensitive to changes in food, whereas cold-water populations are more sensitive to changes in temperature.

Neither the direction nor the magnitude of demographic responses to environmental change need be the same among populations of Atlantic cod. Adaptive phenotypic plasticity can significantly influence the probability of recovery and persistence of collapsed populations by affecting their ability to respond to natural and anthropogenic environmental change. Genetic variability in the average value and phenotypic plasticity of traits fundamentally linked to fitness, at spatial scales concordant with known patterns in population structure, are consistent with the hypothesis of local adaptation in Atlantic cod.

## **How Can Studies of Farmed Atlantic Salmon Inform Risk Assessments Associated with the Farming of Atlantic Cod?**

The first major international symposium on the consequences of interactions between farmed (or cultured) and wild Atlantic salmon was sponsored by the Norwegian Directorate for Nature Management and held in Loen, Norway, in 1990 (Hansen et al. 1991). At that meeting, Hindar et al. (1991) and Hutchings (1991), along with the review by Taylor (1991), provided the first exploratory analyses of how interbreeding with farmed individuals might affect wild salmon. Their work on salmon, in conjunction with more recent theoretical and experimental studies on a variety of species, including salmon (Edmands and Timmerman 2003; Ellstrand 2003; Weir et al. 2004; Weir and Grant 2005; Mercer et al. 2006; Edmands 2007; Hutchings and Fraser 2008), suggest that the consequences to wild populations resulting from interbreeding with farmed individuals will depend on the:

- magnitude of genetic differences between wild and farmed individuals;
- mechanisms underlying genetic differences between wild and farmed fish;
- frequency of spawning intrusions by farmed fish into wild populations;
- number of farmed fishes relative to their spawning wild counterparts;
- geographical distance between the farming activity and wild populations.

At its core, however, any assessment of the risks to wild populations resulting from interbreeding with farmed individuals will depend on the degree to which individuals are locally adapted and on the spatial scale of that adaptation. It is increasingly clear that Atlantic cod differ genetically from one another at smaller spatial scales than had been previously hypothesized for marine fishes, that this variability can be manifested at a variety of genetic and phenotypic levels, and that at least some of this variability is adaptive.

Based on work undertaken on Atlantic salmon, it is highly probable that interbreeding with farmed individuals will prove deleterious to the individual fitness of wild Atlantic cod. However, this does not mean that such interbreeding will necessarily affect per capita population growth rate in cod and, thus, population/stock persistence and (or) population recovery rate. If the proportional representation of farmed cod is extremely small (just how small needs to be determined) relative to the size of the wild population, then the consequences of interbreeding to wild population persistence may be minimal. Of course, non-genetic interactions, such as disease transmission, may have more demonstrable negative effects on the persistence of wild populations, as studies on *Gyrodactylus salaris* in Norway have amply shown (Bakke et al. 1990; Johnsen and Jensen 1991).

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Table 1. Influence of population, temperature and food on larval Atlantic cod growth and survival (Hutchings *et al.* 2007).

variable	no. groups <sup>a</sup>	model term <sup>b</sup>	d.f.	<i>F</i>	<i>p</i>
growth	four	pop	3	2.38	0.108
		batch(pop)	16	10.05	<0.001
		temp	1	615.72	<0.001
		food	1	107.09	<0.001
		pop × temp	3	8.50	<0.001
		temp × food	1	8.29	0.004
		pop × temp × food	6	2.39	0.027
		residuals	599		
	two	pop	1	7.82	0.012
		batch(pop)	18	8.41	<0.001
		temp	1	728.33	<0.001
		food	1	117.40	<0.001
		temp × food	1	8.53	0.004
		residuals	608		
survival	four	food	1	12.55	<0.001
		residuals	78		
	two	pop	1	39.93	<0.001
		food	1	21.31	<0.001
		temp	1	1.75	0.190
		pop × food	1	8.68	0.004
		pop × temp	1	8.10	0.006
		residuals	74		

<sup>a</sup> Four-group comparisons included the Bonavista Bay (3L), Placentia Bay (3Ps), Southern Gulf of St Lawrence (4T) and Western Scotian Shelf (4X) populations; two-group comparisons differentiated cold-water populations (Bonavista Bay and Western Scotian Shelf) from warm-water populations (Placentia Bay and Southern Gulf of St Lawrence).

<sup>b</sup> Model terms: pop, population; temp, temperature; batch(pop), batch nested within population.



Figure 1. Location of most of the Atlantic salmon aquaculture facilities in eastern Canada (from Morris et al. 2008). The wild salmon populations described in the text are Stewiacke River and Tusknet River. The farmed salmon population originated from the Saint John River and had been subjected to four generations of domestication selection prior to the initiation of the experimental breeding programme that was initiated in 2001.

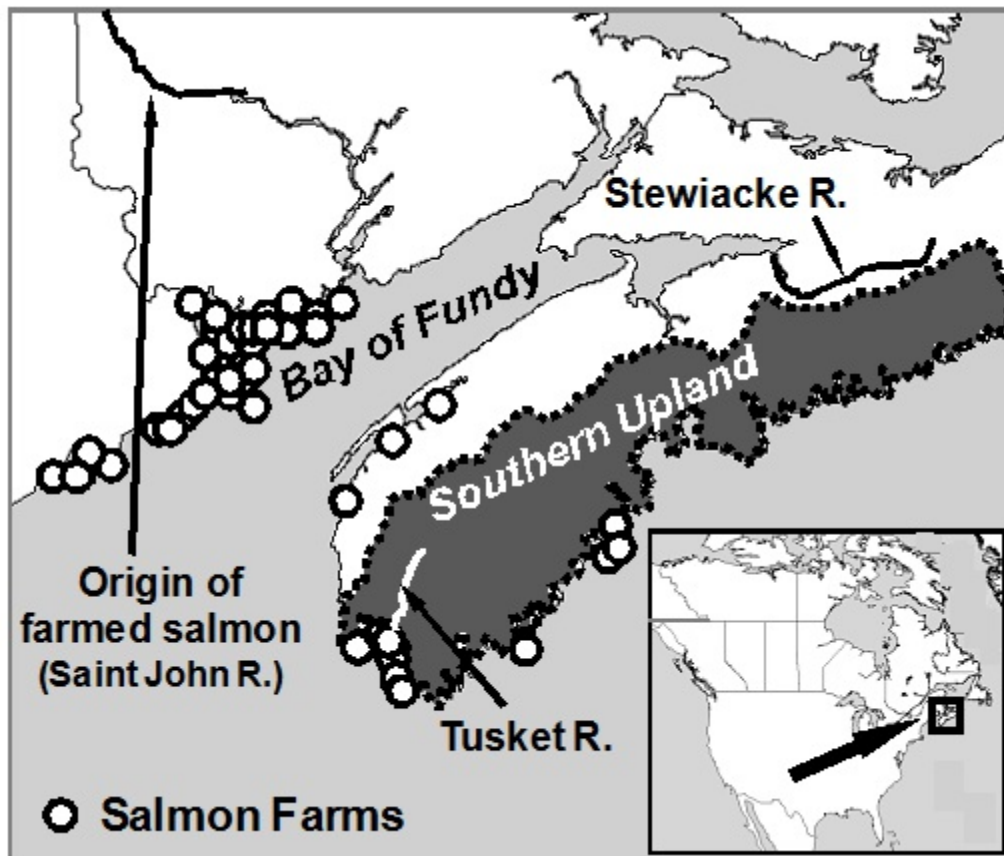


Figure 2. Breeding design for producing pure, F1, F2, and back-crosses among two wild (Stewiacke River, Tusket River) and a farmed Atlantic salmon population that had experienced four generations of domestication selection (see Figure 1 for river locations). This figure is from Fraser et al. (in press).

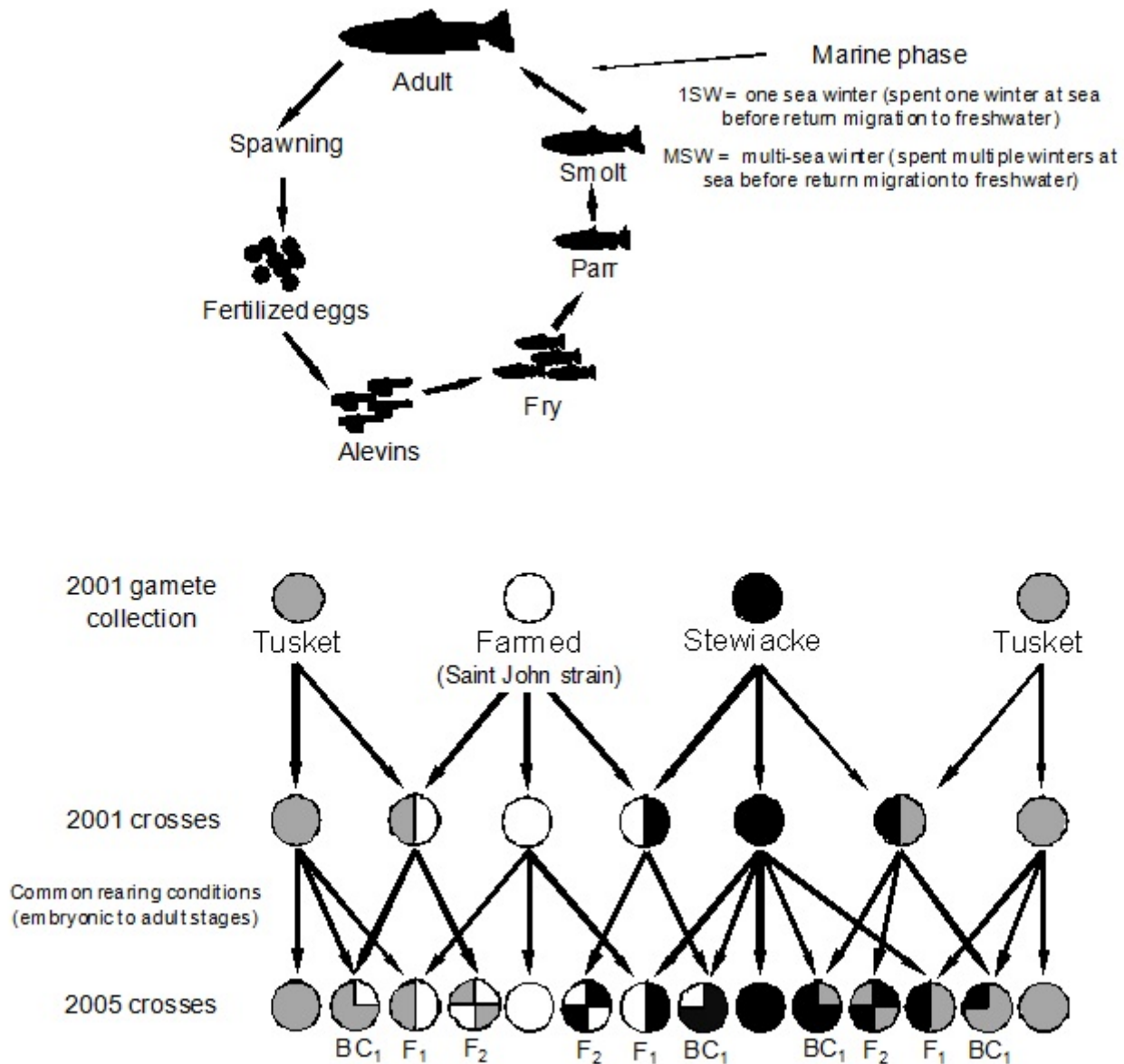


Figure 3. Gene expression differs between wild (T=Tusket, S=Stewiacke) and farmed (F) Atlantic salmon and their back-crosses (i.e., wild×farmed F1s crossed with a pure wild cross, such that BT represents the TF×TT backcross, and BS represents the SF×SS backcross). These histograms show the number of transcripts with significantly different expression in pair-wise strain comparisons (contrast, maximum  $P$ -value of 0.01). The black portions of the bars show the number of unique significant genes, while the yellow portions represent the number of repeated transcripts from the unique genes. Source: Normandeau *et al.* (2009)

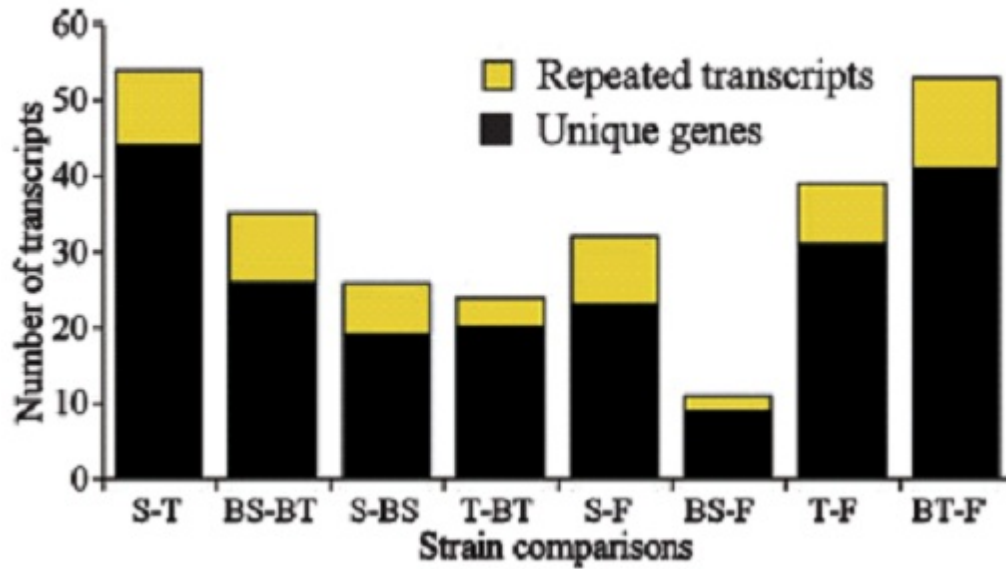


Figure 4. Boxplots of embryonic survival probabilities for different crosses of Atlantic salmon, reared in accordance with a common-garden experimental protocol. Abbreviations: TUSK and T=Tusket; STEW and S=Stewiacke; FARM and F=farmed salmon; BC=backcross. Source: Fraser *et al.* (in press)

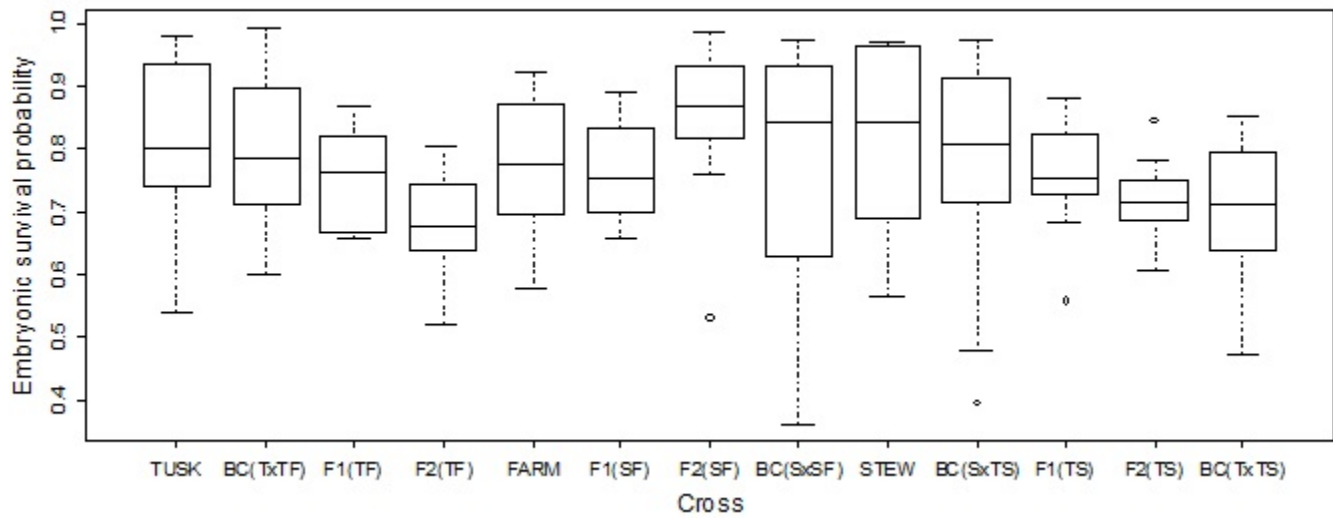


Figure 5. Boxplots of length at hatch, yolk-sac volume at hatch, yolk-sac conversion efficiency and length at first-feeding. Abbreviations: TUSK and T=Tusket; STEW and S=Stewiacke; FARM and F=farmed salmon; BC=backcross. Source: Fraser *et al.* (in press)

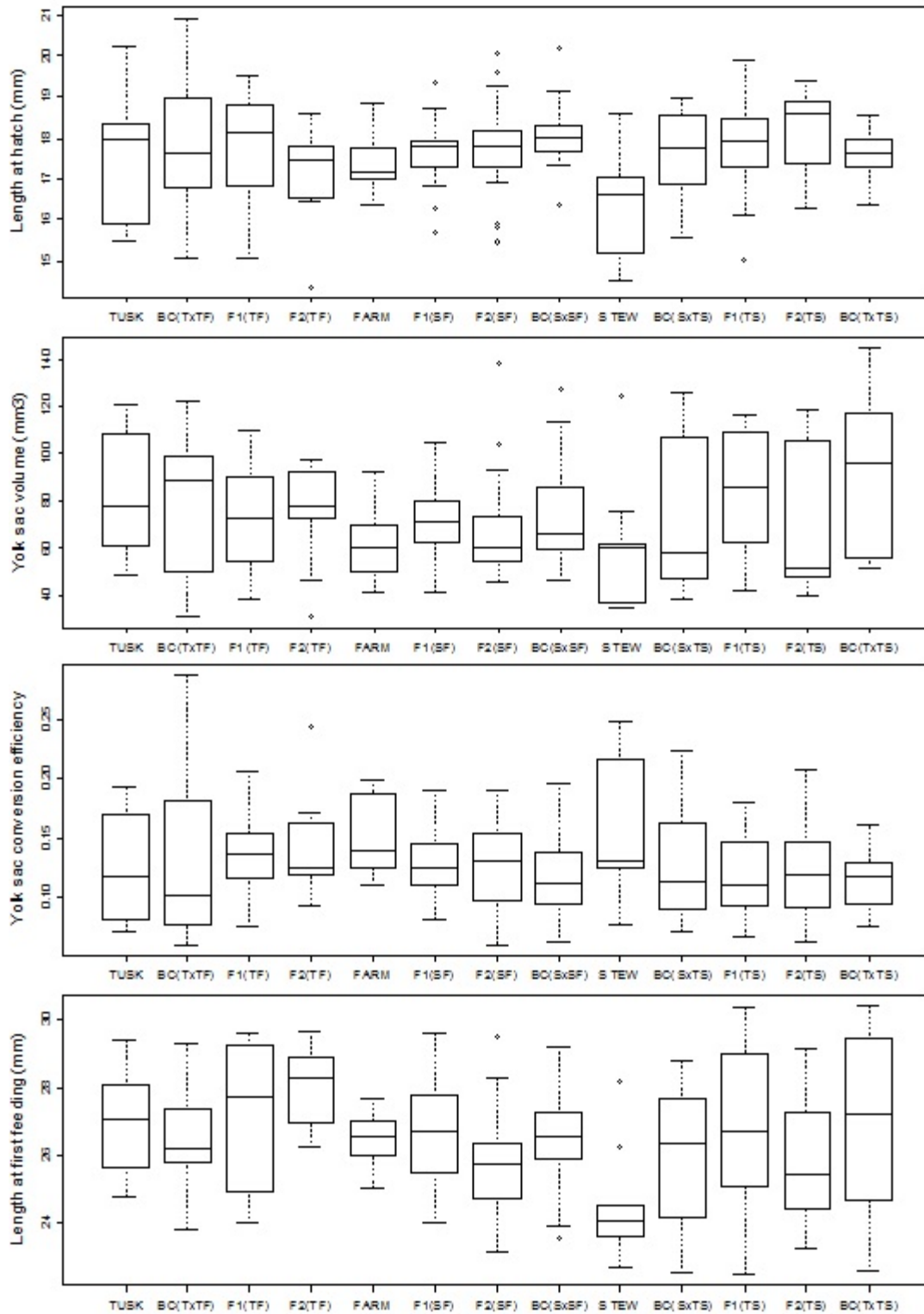


Figure 6. The distribution of developmental rates (cumulative degree-days to hatch) for farmed-wild Atlantic salmon hybrids relative to parental crosses. Left-side panel: Stewiacke vs. farmed salmon. Right-side panel: Tusket vs. farmed salmon. Fitted averages of the lowest AIC mixed effects model, that takes family into account, are presented as vertical lines with the same line type as the distribution. Egg numbers per cross were: 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. Source: Fraser *et al.* (submitted)

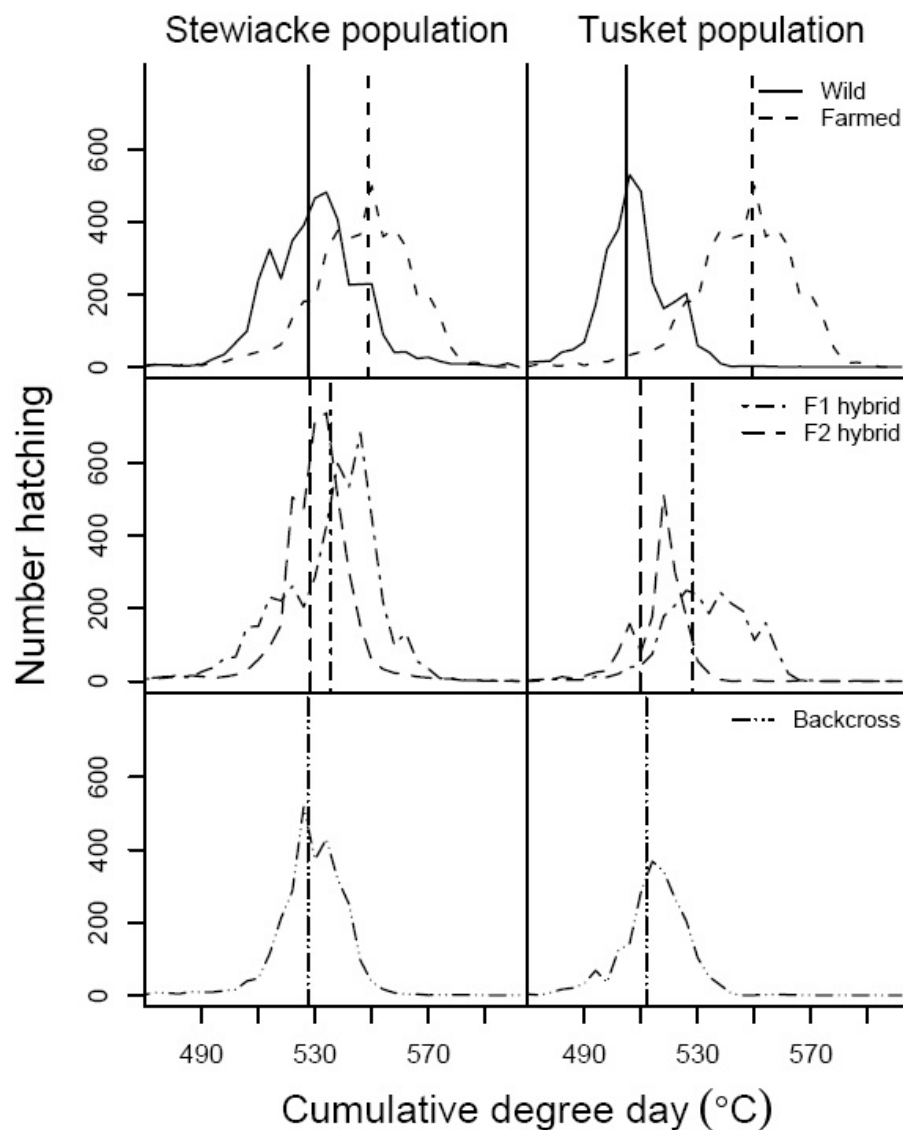


Figure 7. Mean cumulative survival probabilities (means of two replicates  $\pm$  1SE), following exposure to the pathogen *Listonella anguillarum* of Atlantic salmon parr of wild (Stewiacke and Tusket rivers) and farmed origins (females: diamonds; males: triangles). Source: Lawlor *et al.* (2009)

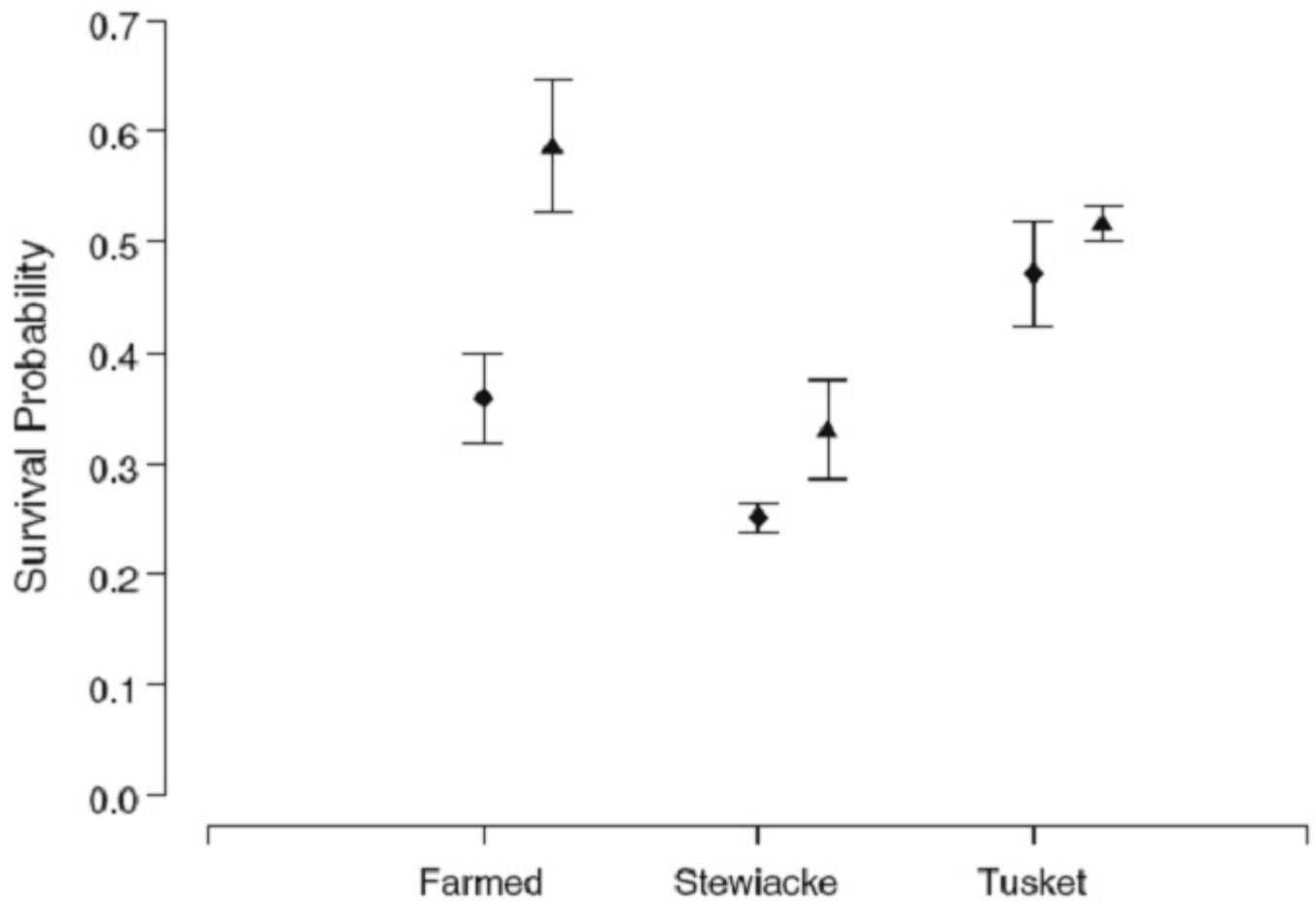


Figure 8. Norms of reaction, at the family level, for chum salmon (*Oncorhynchus keta*), as determined under experimental conditions by Beacham and Murray (1983).

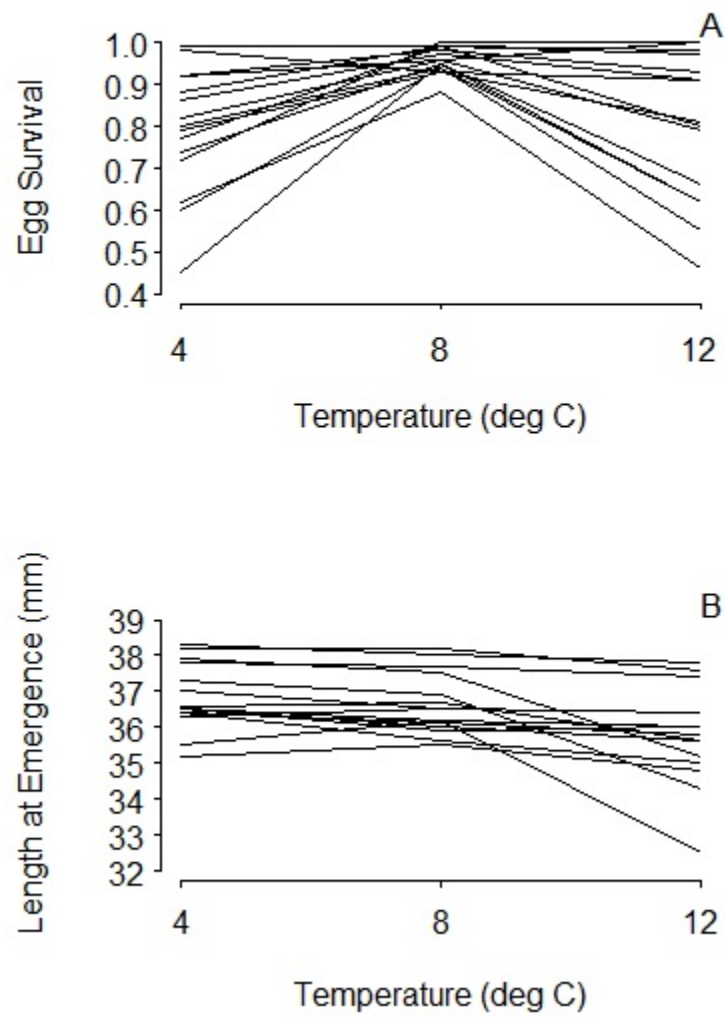




Figure 9. Genetic differences in reaction norms among wild populations of European grayling, *Thymallus thymallus*, as revealed by common-garden experimental protocols by Haugen (2000a,b,c). Norms of reaction for life history traits in grayling from five populations in south-central Norway. Reaction norms for age and size at maturity for grayling in Øvre Mærrabottvatn (OM), Lesjaskogsvatn (L), Osbumagasinet (O), Hårrtjønn (H), and Aursjøen (A); redrawn from Haugen (2000b).

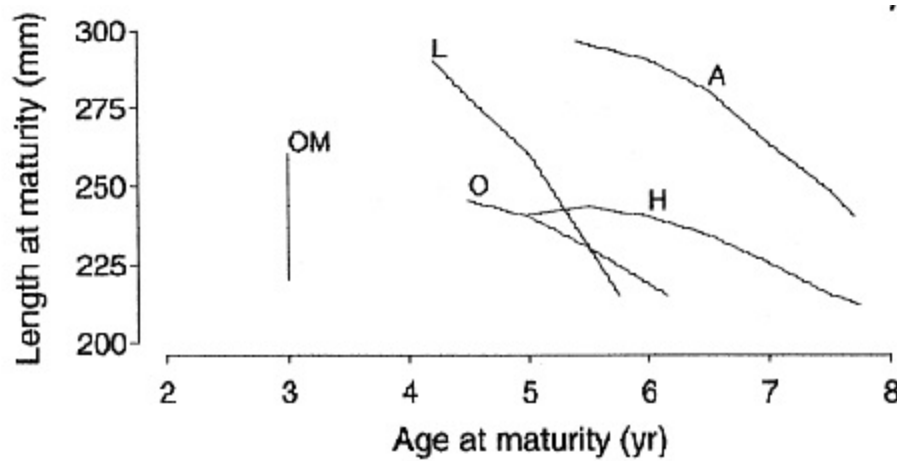


Figure 10. Genetic variability in threshold norms of reaction for maturity in male Atlantic salmon. The different curves represent fitted relationships between incidence of male parr maturity and growth rate (weight at age) for pure and hybrid population crosses, as revealed by a common-garden experimental protocol by Piché *et al.* (2008).

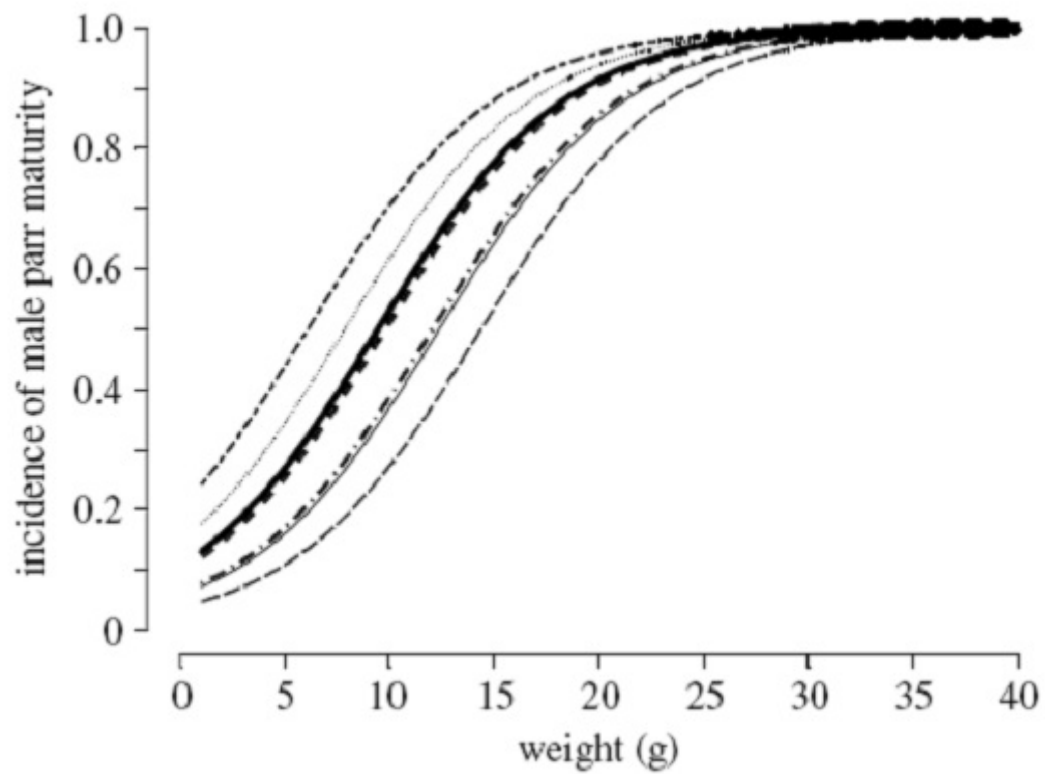


Figure 11. Norms of reaction for survival and growth of Tusket River (T) Atlantic salmon and Tusket salmon back-crossed with Stewiack River (S) salmon and farmed (F) salmon at low ( $15^{\circ}$ ), medium ( $18.5^{\circ}$ ), and high ( $22^{\circ}$ ) temperature regimes: TT $\times$ TT (solid line and squares); TT $\times$ TS (long dashes & circles); TT $\times$ TF (short dashes & triangles). Data source: Darwish & Hutchings (2009).

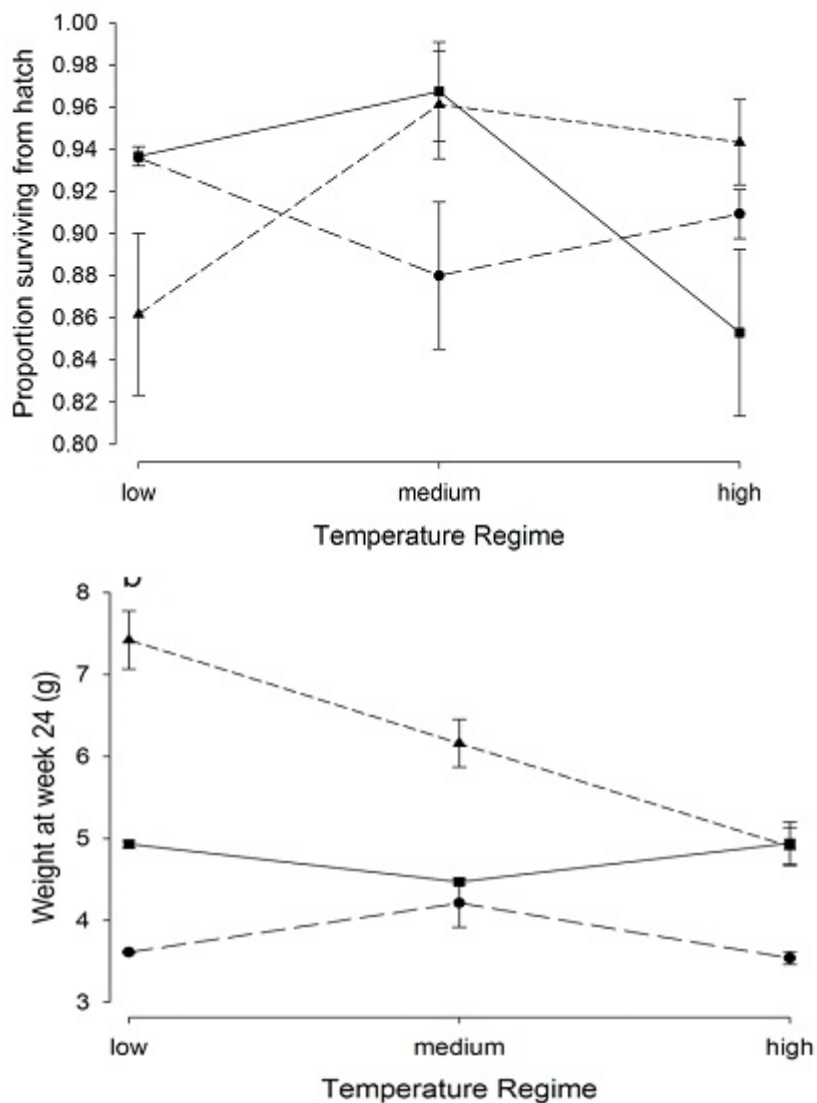


Figure 12. Differences in compensatory growth between pure and hybrid crosses of Atlantic salmon. Upper panel: weight at age (days post-emergence on the X-axis) for control fish (open symbols) and for restricted-diet fish (closed symbols). The food restriction period extended from days 27 to 65. Squares: LaHave River; circles: Stewiacke River; diamonds: F1 hybrids. Lower panel: Differences in specific growth rate among crosses. Source: Fraser *et al.* (2007)

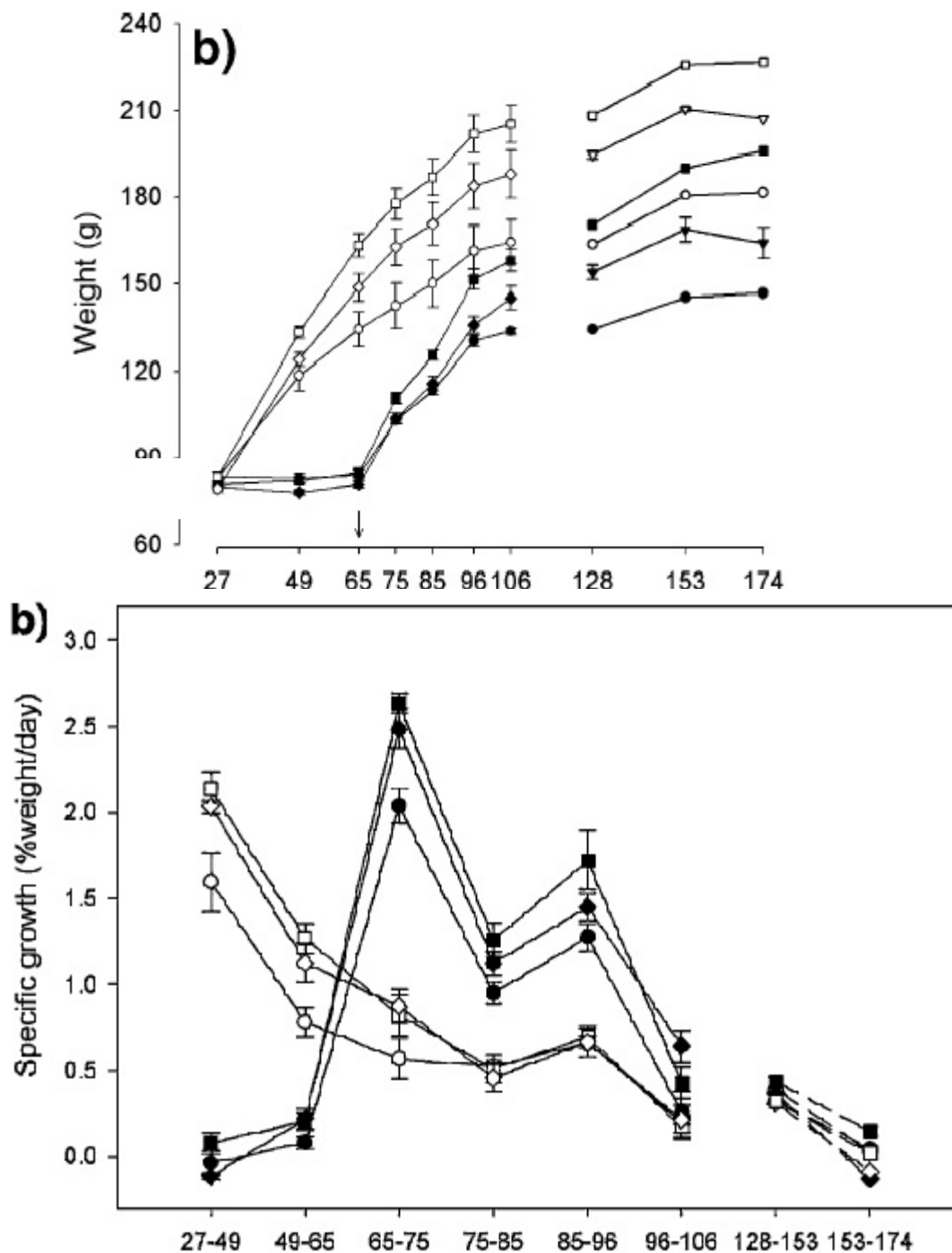


Figure 13. Norms of reaction for Atlantic salmon alevin survival, as a function of pH, for: (i) three pure crosses of salmon from Tusket River (T), Stewiacke River (S), and Farmed (F) Atlantic salmon; (ii) F1 and F2 crosses between Tusket and Farmed salmon; and (iii) a backcross (BC) involving Tusket and Farmed salmon. Data obtained from common garden experiments undertaken by Fraser *et al.* (2008).

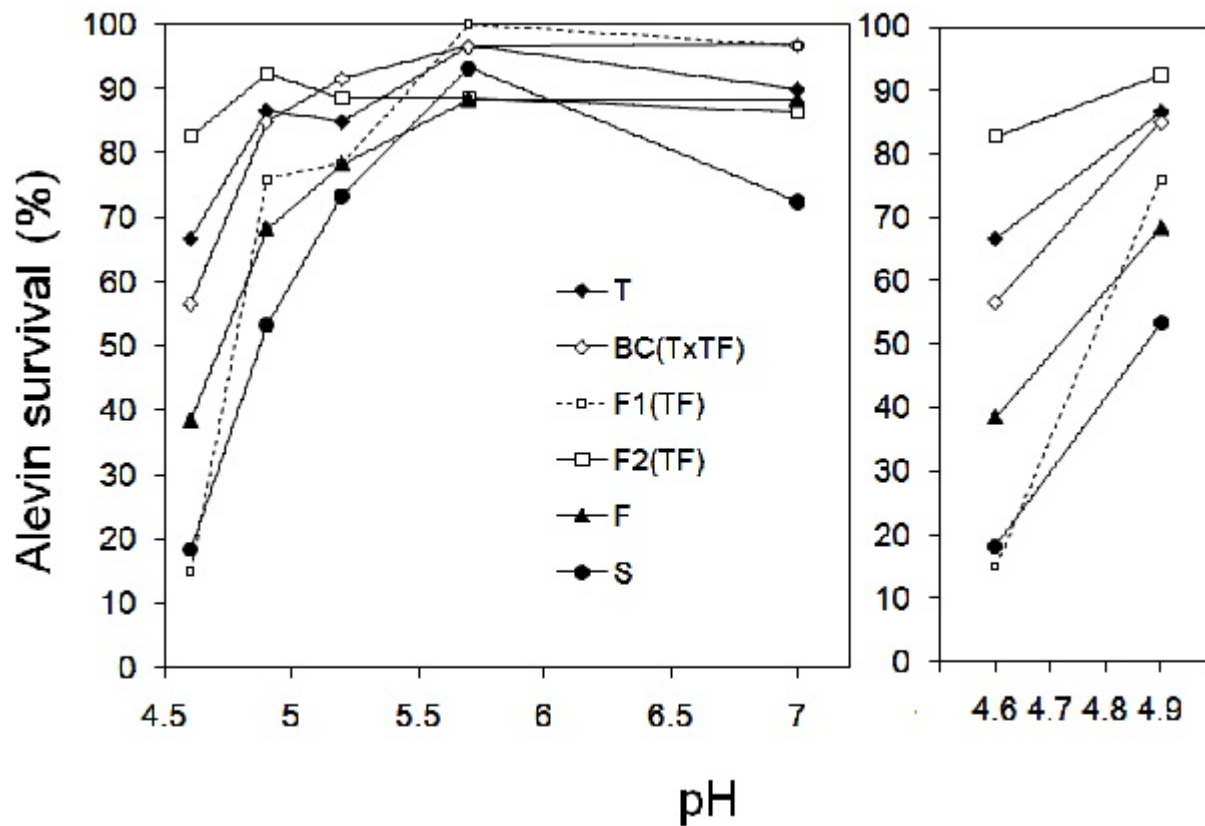


Figure 14. Study populations of Atlantic cod. (a) Sampling locations of spawning adults. (b) Depth-averaged (0-50m) water temperatures ( $\pm 1$  SE) for all available data from 1914-2003, and two months after initial spawning months (May: 3L, 3Ps, 4T; February: 4X). Source: Hutchings *et al.* (2007)

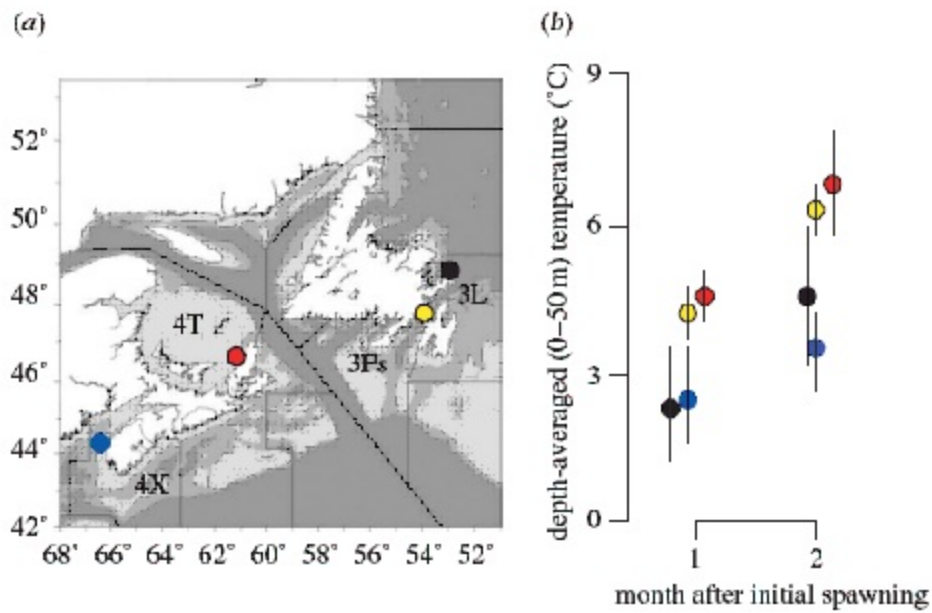


Figure 15. Norms of reaction for larval growth in Atlantic cod. The populations represented here, each by a different reaction norm, are: Bonavista Bay, northeast Newfoundland (Northwest Atlantic Fishery Organization (NAFO) division 3L; St. Pierre Bank, south Newfoundland (NAFO division 3Ps); Southern Gulf of St. Lawrence (NAFO division 4T); and Bay of Fundy (NAFO division 4X). See Hutchings *et al.* (2007) for full details.

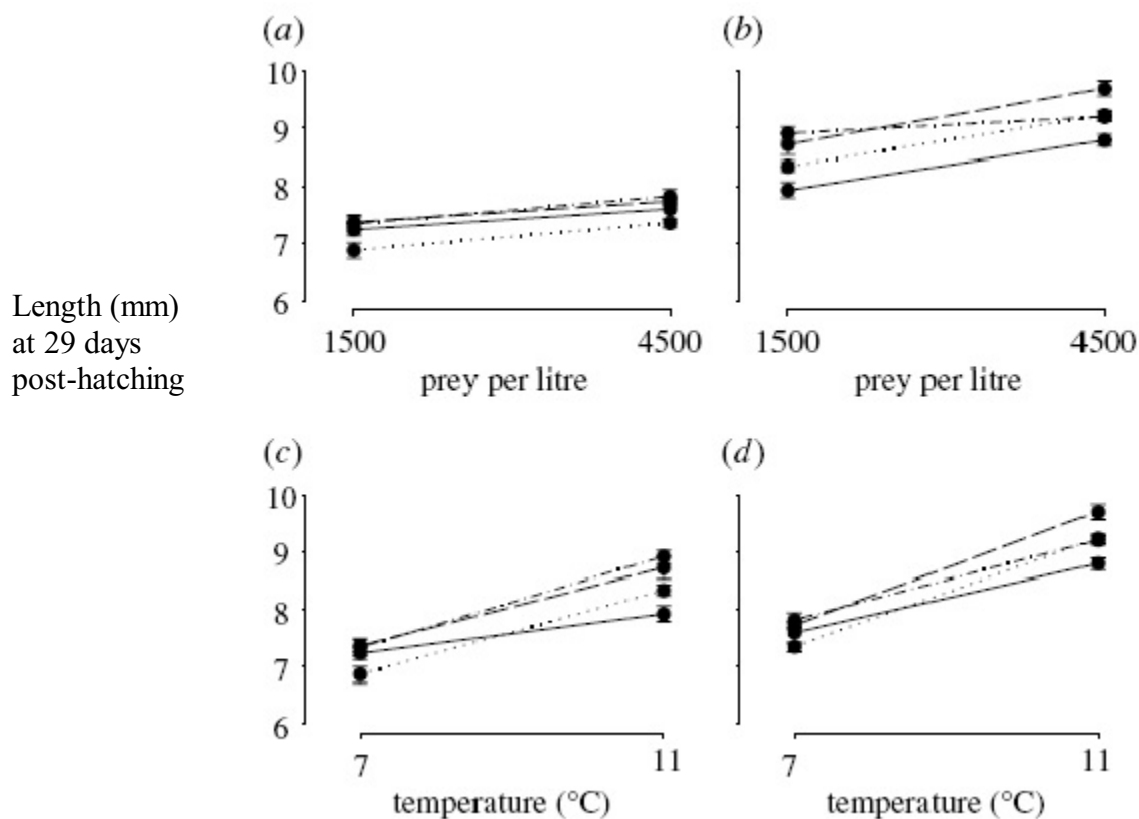


Figure 16. Norms of reaction for larval cod survival. Reaction norms for each of the four populations at (a) low and (b) high temperature and at (c) low and (d) high food supply. Solid lines, 4X cod; dotted lines, 3L cod; dashed lines, 3Ps cod; dot-dashed lines, 4T cod. See Hutchings *et al.* (2007) for full details.

