Genetically Based Effects of Domesticated-Wild Outbreeding in Atlantic Salmon

by

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Abstract

Rapid advances in the aquaculture industry pose an environmental challenge that is generated by outbreeding between escaped domesticated and wild individuals. Given that escapees genetically differ from wild individuals because of domestication and possibly by ancestry, periodic domesticated-wild outbreeding has the potential to influence fitness-related traits in wild populations. In Atlantic salmon (*Salmo salar*), the understanding of mechanisms and direction of domesticated influences are especially important because of the conservation concerns associated with many wild populations, notably in the southern parts of their North Atlantic range. My thesis investigates domestication-induced, genetically based changes during the parr stage by assessing growth, parr maturity and survival under predation for three salmon strains differing in their history of domestication, as examined in two semi-natural environments (predator present, absent). Growth and size-at-age increased with increasing generations of domestication, yet male parr maturation probability declined. Survival under gape-limited predation increased with domestication-conveyed increases in size and growth rate. Domesticated but not wild individuals exhibited stress-resistant growth in the presence of a predator. To assess mechanism and magnitudes of trait changes resulting from domesticated-wild outbreeding, a domesticated strain was crossed with a wild population (up to third-generation hybrids) and outbreeding effects were studied for different life stages, several controlled environmental laboratory conditions, and traits. Life stages included the developmental periods between egg and fry, and between immature and adult post smolts. Traits assessed included survival, yolk conversion efficiency, size-at-age, maturation probability, growth rate, mRNA transcript levels and their environmental plasticity. For many traits, both additive and non-additive genetic components in the between-population genetic architecture were revealed by cross means analyses. Furthermore, maternal outbreeding effects on early life stages were present. Altogether the results indicate that constant outbreeding effects of escapees on wild populations will increase present growth rates during all life stages and decrease early maturation probabilities for male parr and post-smolts, but by unpredictable magnitudes across hybrid generations. Maternally controlled co-adapted traits might be disrupted in hybrid mothers. Further, mixed-origin individuals might be temporarily at an advantage relative to wild individuals because of size and growth advantages and these might accelerate a wild genotypes displacement.

List of Abbreviations Used

ANOVA	Analyses of Variances
BC	Backcross
CCAC	Canadian Council on Animal Care
CI	Confidence Interval
Cor	Correlation
Cov	Covariance
D0	Wild ancestral Saint John River population salmon
D3	Domesticated salmon, three generations of selection
D5	Domesticated salmon, five generations of selection
D°	Cumulative Degree-days
DD	Domesticated salmon
ddf	Denominator degrees of freedom
df	Degrees of freedom
DFO	Department of Fisheries and Oceans
F1	First-generation hybrid
F2	Second-generation hybrid
FDR	False Discovery Rate
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
GO	Gene Ontology
iBoF	Inner Bay of Fundy
LM	Linear Model
LMM	Linear Mixed Model
Ln	Natural logarithm
LRT	Log-Likelihood Ratio Test
MCMC	Monte Carlo Markov Chain
MHC	Major Histocompatibility Complex
ML	Maximum Likelihood

NSERC	Natural Sciences and Engineering Research Council
NTU	Nephelometric Turbidity Units
PSU	Practical Salinity Units
REML	Residual Maximum Likelihood
SE	Standard Error
TMS	Tricaine Methanesulfonate
Var	Variance
WW	Wild salmon

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Chapter 1: Introduction

For many fishes, domestication is relatively recent and divergence between wild and domesticated conspecifics has developed over only a few decades and generations (Price 1984; Mignon-Grasteau *et al.* 2005). Although many fishes are farmed in ponds, hatcheries, or other closed facilities, fish farming is often conducted in separated areas of fresh or marine coastal waters, such as net-pens, from which domesticated individuals escape as a consequence of accidents or other damage (Naylor *et al.* 2005). Escapees are often in direct competition with conspecifics (reviewed by Jonsson & Jonsson 2006; Laikre *et al.* 2010), which, however, is not the focus of my thesis. The subject of my thesis starts when escapees outbreed with individuals from wild populations. From such reproduction events, domesticated-wild offspring emerge. These might be termed 'intraspecific hybrids' because of their intermediate domesticated and wild origin.

Domestication is not the only possible source of genetic divergence between a wild and a domesticated individual. Domesticated strains emerge from wild populations that might already be divergent from the wild population outbred by an escapee. Hence, divergence between domesticated and wild individuals can be caused by differences in domestication history and ancestral population divergence (Hutchings & Fraser 2008). A hybrid inherits genetically based traits from the domesticated and the wild parent and represents a genetic blend from often at least two wild populations of which one underwent changes by domestication.

Why does the genetic background of such hybrids matter? First, it has been suggested that mixing genes from divergent populations can have detrimental fitness effects, called outbreeding depression (Templeton 1986; Edmands & Timmerman 2003). Second, it has been suggested that domestication may make individuals unfit in a wild environment, given that natural selection is relaxed under domestication and that anthropogenic selection changes animals in ways that do not naturally occur (Rauw *et al.* 1998; Mignon-Grasteau *et al.* 2005). When both of these effects act simultaneously, one would think that a hybrid would have relatively low

fitness in the wild and that natural selection could readily purge wild populations of unfit genetic influences from escapees. However, this might not be the case when the numbers of escapees is large, the wild population is small, or both (Hutchings 1991; Hindar *et al.* 2006; Baskett & Waples 2013). Unfortunately, for many populations of wild Atlantic salmon (*Salmo salar*, Linnaeus, 1758), the study organism of my thesis, some level of gene flow from escapees appears likely in areas where aquaculture net-pens are located near wild populations (Carr *et al.* 1997; Skaala *et al.* 2006; Glover *et al.* 2009; Bourret *et al.* 2011).

If natural selection is unable to purge the genetic influences of domesticated on wild populations, what are the effects on wild populations? This question was raised as early as the 1980's during the emergence of large-scale aquaculture industries (ICES 1983, 1984). Since then, thirty years have passed, many wild Atlantic salmon populations have declined (Parrish *et al.* 1998; ICES 2010; COSEWIC 2011), and aquaculture production in the native range of this species has risen 254-fold from 5,300 t in 1980 to 1,346,807 t in 2011 (FAO 2013). Yet, we are still far from understanding to what extent salmon populations are adapted to their local environments, or what the genetic basis of between-population divergences is, or what exactly the consequences of fish domestication are on fitness in the wild.

In my thesis, I study possible mechanisms — genetically based traits and the genetic architecture of these traits — by which domesticated individuals might affect wild populations by outbreeding. In Chapter 2, I investigate genetically based changes in several traits as a result of different durations of domestication. In a genotype-by-environment experimental protocol associated with the presence and absence of a predator, I examine fitness-related traits during the first year of Atlantic salmon life. This study appears to be the first for this species that allows for an evaluation of domestication-induced changes for different degrees of domestication relative to its wild ancestor. I examine the domestication-induced changes in traits that are central in understanding domesticated-wild divergence: size-at-age, growth, survival under predation, and early male maturation probability. In this chapter, I also introduce the major Atlantic North American

Atlantic salmon strain, the Saint John River strain, which occurs as the domesticated representative in all chapters.

In Chapter 3, I investigate the genetic architecture between the Saint John River strain and the Stewiacke River population, the representative wild population examined in all remaining chapters, plus two generations of their hybrids. The focus here is on growth at age 2+ years, as realised under a distinct naturally occurring environmental stressor for the wild population, namely the presence of suspended sediments (Huntsman 1958). In addition, I investigate maturation frequencies and discuss how size, growth, and maturation probability might phenotypically interact, and what possible consequence this could have on wild populations as arising in combination with the detected between-population genetic architecture.

In Chapter 4, I examine differences in mRNA transcription levels between Saint John River and Stewiacke River populations, as expressed under the genotype-by-environment protocol described in Chapter 3. Here, a genotype-by-environment microarray hybridisation design allows for the exploration of differences in gene transcript levels between the two populations and first-generation hybrids, and how these three groups respond to suspended sediments on the mRNA transcript level of the gill.

In Chapter 5, I analyse developmental differences between Saint John River and Stewiacke River populations during early life until time of first feeding, and investigate the genetic architecture of several of these early expressed traits across three generations of outbreeding. This chapter focusses more than the previous chapters on genetically based effects that can only be studied when several generations of outbreeding are available. In particular, I investigate the effects of domesticated-wild outbreeding on maternal effects and how these maternal outbreeding effects affect offspring traits during early life.

In chapters 3 to 5, I use the pronoun ‘we’ rather than ‘I’ because these chapters were written with co-authors and have been published in or submitted to journals. Nevertheless, I conducted all work in wet and molecular laboratories myself, except for the parental genotyping described in chapter 5, which was fully conducted by research technician Meghan McBride, and I wrote all manuscripts and chapters. All

co-authors as named for each chapter contributed to ready-drafted manuscripts. I listed details of my contribution to each chapter in the 'Student Contributions to Manuscripts in Thesis' form that is submitted with my thesis. Copyright agreements for published or submitted articles can be found in Appendix A. For chapters 4 and 5, I omitted all or some of the supplementary material from the thesis as it was too extensive to be included. For chapters 4 and 5, direct Internet links have been added through which this missing supplementary material can be downloaded by the interested reader. When already available, Internet links to raw data files have also been provided.

Chapter 2: Influence of Domestication on Parr Maturity, Growth, and Vulnerability to Predation in Atlantic Salmon

2.1. Abstract

Domestication changes fitness-related traits that become important under domesticated-wild outbreeding. I investigated domestication-induced changes in fitness-related traits in Atlantic salmon (*Salmo salar*) under semi-natural laboratory conditions. Selection for rapid growth for 3 and 5 generations resulted in 2 and 3 times larger sizes of under-yearling parr relative to their wild ancestors. An initial size advantage and the capability to outgrow prey size more rapidly resulted in lower size-selective predation mortality for domesticated than wild individuals. No evidence could be found that domesticated individuals exhibit a more risky behaviour than wild individuals. Growth in the presence of a predator was reduced for wild but not for domesticated individuals, suggesting that domestication co-selects for predator-related stress resistance. Within-family variances in body size decreased with increasing generations of domestication which might be caused by either, or a mix of, growth depensation, changes in allele frequencies, or co-selection against environmental sensitivity. Male parr maturation probability was 10% in the wild strain, but reduced to 4% and 2% after 3 and 5 generations of domestication, respectively. Our work supports the hypothesis that male parr maturation is under growth-independent genetic control and the existence of a common domestication-independent size at highest maturation probability for under-yearlings. Together, fitness for domesticated escapees and their domesticated-wild offspring relative to wild individuals is predicted to be higher during the freshwater phase in the presence of gape-limited predators, but domesticated-wild hybrid male reproductive success might be lower under a high marine mortality.

2.2. Introduction

Domestication of animals is accompanied by genetically based changes encompassing behaviour, physiology, morphology, and life history (Kohane & Parsons 1988). These changes result from anthropogenic selection, natural selection by the domestication environment, relaxation of natural selection, and genetic drift and inbreeding in small populations (Price & King 1968; Doyle 1983; Price 1984; Kohane & Parsons 1988). As a consequence, domesticated and wild individuals differ in phenotype and genotype. It is often difficult to compare wild animals to their domesticated counterpart to study genetically based changes accompanying domestication. This is also due to uncertainty or unavailability of wild base populations for many traditionally domesticated animal species (reviewed by Price 1984; Mignon-Grasteau *et al.* 2005). Even for recently domesticated species, it is often difficult to decide what underlies differences between domesticated and wild phenotype as changes in genotype can be confounded by changes in domestication environment and genotype-by-environment interactions (Rye & Gjedrem 2005). Yet, there is interest in domestication-induced genetic changes as they reflect 'improvements' by breeding and evolution in captivity. Furthermore, when domesticated animals escape and co-exist with their wild counterpart, conservation concerns arise (Hutchings & Fraser 2008; Randi 2008; Lorenzen *et al.* 2012). This is because domesticated and wild animals can compete for common resources, and domesticated allelic combinations can enter wild populations when wild and domesticated individuals reproduce successfully.

For many traditionally domesticated animals, fitness effects on wild conspecifics are unimportant as either their wild counterparts are extinct (e.g. aurochs vs. cattle, *Bos primigenius*, Bojanus), rarely coexist (e.g. jungle fowl vs. chicken, *Gallus gallus*, Linnaeus), or domesticated escapees are unlikely to survive in the wild (many recent animal breeds; but see reviews by Randi 2008; Groeneveld *et al.* 2010). For recently domesticated animals like many fishes (Gjedrem & Baranski 2009), wild counterparts still exist (Mignon-Grasteau *et al.* 2005) and escapees are capable of

both surviving in the wild and successfully interbreeding with wild individuals. The aquaculture production of finfish is an enormously growing industry since the mid-1990's (FAO 2012), and this still emerging industry poses considerable environmental and ecological challenges. Atlantic salmon (*Salmo salar*, Linnaeus) is one of the most important aquaculture species in terms of quantity and revenue produced and its production is still increasing (FAO 2012). A major problem is that domesticated Atlantic salmon individuals escape regularly and reproduce with wild individuals (Carr *et al.* 1997; Skaala *et al.* 2006; Glover *et al.* 2012). At the same time, wild Atlantic salmon is in decline throughout much of its distribution (ICES 2010; COSEWIC 2011). Hence, there is a considerable concern of genetically based effects of escaped domesticated fish on individual fitness of remaining wild populations (Naylor *et al.* 2005; Hindar & Fleming 2007; Hutchings & Fraser 2008; Thorstad *et al.* 2008; Lorenzen *et al.* 2012; Baskett & Waples 2013).

Central to predicting effects of genetic domesticated-wild interactions on wild populations is the question how domesticated phenotypes are expressed in the wild, i.e., the plasticity of domesticated trait expression, and what the fitness of such phenotypes might be relative to wild phenotypes. A common anthropogenically selected trait in fish is growth rate or size-at-age. Increases in growth rate of 10-20% per generation of selection has been documented in many species (Gjedrem & Baranski 2009). Other phenotypic traits that correlate with growth rate often change either as a by-product or as a consequence of direct selection. Among these are physiological traits such as increases in feed conversion efficiency, age at smolting, i.e., the physiological change enabling saltwater tolerance, life-history-traits such as age at maturity (Gjerde 1984; Thodesen *et al.* 2001; Gjedrem & Thodesen 2005), and behavioural traits such as increase or decrease in aggression and schooling behaviour (reviewed by Ruzzante 1994). Furthermore, an adaptation to stress is among the first and most intense responses to domestication (Kohane & Parsons 1988). Fish grow more slowly when being stressed (McCormick *et al.* 1998) and this might result in that comparatively stress-resistant individuals are co-selected with rapid growth in domestication environments (Ruzzante & Doyle 1991).

The fitness consequences of changes in physiological and behavioural traits of fast growing domesticated fish in the wild are far from clear. Many studies suggest that fast growers are more aggressive (reviewed by Ruzzante 1994; Huntingford & Adams 2005) and take more risks under predation (Tymchuk *et al.* 2006; Houde *et al.* 2010), both of which might be caused by increased appetite associated with selection for high growth potential and also by increased or unregulated production of growth hormones in genetically modified individuals (Johnsson & Björnsson 1994; Johnsson *et al.* 1996). Some authors have suggested that rapid growth is associated with increased predation mortality, linking their results to the aforementioned hypothesised behavioural consequences arising from rapid growth (Biro *et al.* 2004; Sundström *et al.* 2004; Biro & Post 2008). Conversely, other authors suggested a higher survival of rapid growers under predation for specific life stages or environments (Sundström & Devlin 2011; Vandersteen *et al.* 2012). This view is supported as individuals with a high growth potential are able to outgrow a prey-size window more rapidly (Arendt 1997; Sogard 1997), are more competitive (Fleming & Einum 1997) and have greater potential to monopolise refuges relative to slow growers of the same age (Reinhardt & Healey 1997). As such, predation-related fitness of domesticated individuals in the wild remains a subject of current research.

In Atlantic salmon, which is usually selected for large market size (Gjedrem 2005), males can attain sexual maturity during their first year at sizes of tens of grams (Hutchings & Myers 1988) while market sizes are two magnitudes larger. Females usually reach sexual maturity for the first time after several years. In domesticated individuals, age at sexual maturation might be altered relative to wild populations. In wild and hatchery populations not selected for large market sizes, rapid growth conveys early age at maturation (Alm 1959; Hutchings 1993). Sexual maturation diverts energy from somatic to gonadal growth and this leads to a smaller size at later ages. As a consequence, breeders selecting individuals for large market size either co-select or directly select against early age-at-maturity (Gjerde 1984; Gjedrem 2005). Although it is nuisance in animal breeding, early sexual maturation in male salmon plays an important role in wild populations. The

presence of mature parr during spawning expands gene-flow across cohorts and increases effective population size (Myers *et al.* 1986; Johnstone *et al.* 2013). Taken together, the presence of early male parr maturity in wild populations might represent an important buffer against the detrimental effects resulting from high marine migration mortality and against detrimental effects resulting from small cohort sizes (de Mestral *et al.* 2012).

I studied several traits under standardised laboratory conditions in three strains of Atlantic salmon that shared equal ancestry but differed in their history of domestication. In this study, I refer to 'domesticated' or 'wild' in respect to a history of directional anthropogenic selection and will largely ignore studies and effects arising from solely inadvertent domestication selection, such as that occurring in hatcheries. I tested for among-strain differences in i) family-based growth in predator presence and absence, ii) individual early male maturity, and iii) family-based survival under predation in semi-natural environments that fall in between hatchery and natural environments to minimise bias by potential genotype-by-environment interactions. This study is the first known to me that allows for the study of different stages of domestication without confounding environmental and among-population differences with those caused by domestication while at the same time accounting for among-family variation. My study allows me to quantify domestication-induced changes in important fitness-linked traits in wild populations that are affected by domesticated-wild outbreeding.

2.3. Material and Methods

2.3.1. Strains

The Saint John River (New Brunswick, Canada; 45.267 °N, 66.067 °W) population is the source of the major Atlantic North American aquaculture strains (Wolters *et al.* 2009). I compared three strains that share 100% Saint John River ancestry but differ in their domestication history. The parents of the wild strain (D0) grew up in the Saint John River until being caught as migrating smolts (individuals prepared to enter salt water), and were raised to maturity at the

Mactaquac Biodiversity Facility, NB, but were never intentionally selected for any traits. The parents of the strain that had undergone three generations of anthropogenic selection (D3), primarily for rapid growth and for smolting at age 1 year (Glebe 1998), were held for two generations in the absence of intentional selection at the Aquatron facility at Dalhousie University, NS. The parents of the strain that underwent an estimated five generations of anthropogenic selection (D5) were derived from the same breeding programme as D3, but underwent additional generations of directional selection at aquaculture facilities of Cooke Aquaculture Inc.

On November 3 2010, the D0 strain was created at Mactaquac and shipped to Dalhousie University. On November 19th 2010, the D3 strain was created at Dalhousie University. On December 22 2010, eyed eggs from the 5th generation domesticated group (D5), which had been created between October 18 and November 4 2010 at Oak Bay hatchery, NB, were shipped to Dalhousie University. All strains were created by single pair matings of randomly selected parents either assumed to be unrelated (D0), or known to be unrelated based on pedigrees (D3 and D5).

2.3.2. Pre-Experimental Laboratory Conditions

For egg incubation, about 200 eggs from each family were incubated in two duplicate plastic compartments (13.8 cm x 17.0 cm) with mesh-covered holes (3.8 cm diameter) of which two formed one container. Twelve containers were partially submerged in each of three fibreglass troughs (60 cm x 213 cm x 30 cm). Ambient, dechlorinated municipal water was provided to each compartment from overhanging spray bars, and three air stones were evenly deployed in each trough. Until egg 'shocking' at the eyed egg stage, D0 and D3 family replicates were randomly distributed among the troughs. The D5 families were incubated up to the eyed stage at the Oak Bay hatchery in perforated plastic tubes put into tray incubators. After shocking of eggs at 320-360 degree-days (D°), all compartments were equipped with artificial turf. Due to both different fertilisation dates and an initially different incubation temperature for D5, I synchronised development in

degree-days by using a mix of ambient and heated water among the troughs (range: 3.3-8.2°C, max. difference: 4.7°C) and family duplicates were rotated to equalise cumulative degree-days (D°). One week before the predicted 100% development (time of first feeding; based on the equation of Kane (1988)), temperature was equalised among families to ambient levels. Until 100% development, the laboratory was kept in total darkness. At time of first feeding (832 D°), artificial light was set at a natural daylight cycle (corresponding to Saint John, NB). Fry were hand-fed five times daily with a mix of life *Drosophila*, *Artemia*, and *Daphnia*, and commercial starter feed of different sizes (Corey Aquafeeds Optimum). As many wild fry rejected artificial but not natural feed, this minimised bias in feed-related growth among strains. As fish grew, densities were reduced by increasing compartment numbers per family to four. On August 18nd, fish were transferred into 30 round fibreglass tanks (100 L). For each family, the previous compartment replicates were pooled and that mix was redistributing between two tanks with 35 individuals in each. Fish were kept under the same light cycle, ambient water temperature regime, and the same item mix as previously was fed reduced to three times daily. No data were recorded for these early stages as I expect a confounding of among strain differences with environmental maternal effects.

Between August 30 and September 3, all fish were anesthetised with tricaine methanesulfonate (TMS), tagged with dark blue Visible Implant Elastomer (Northwest Marine Technology, Inc., USA) at one of 15 randomly assigned family-specific subcutaneous positions, and returned to their family duplicate tanks. After a minimum recovery period of 12 days, 64 individuals from each of the 15 families (totalling 960 individuals) were, after pooling family-duplicates, randomly assigned to one of eight experimental round tanks (2 m diameter, each holding 1,130 L). To avoid a family size-by-tank bias, similar size distributions from each family were attempted to be assigned to each tank.

2.3.3. Experimental Conditions and Sampling

Parr were anesthetised and wet mass (± 0.01 g) and fork length (± 0.1 cm) were recorded immediately prior to their placement in the experimental tanks. Fish were not fed for two days prior to the measurements. It was only possible to process parr for two tanks daily such that stocking of all tanks lasted four consecutive days (Figure 2.1). Before stocking, experimental tank bottoms had been covered with natural gravel except for a ~40 cm diameter area around a central bottom drain to facilitate the removal of food remains. Each tank had been equipped with equally arranged refuges (two large hollow concrete blocks, one flat hollow concrete block, and eight hollow brick stones). Ambient, dechlorinated municipal water was provided to each tank with a spray bar 5 cm above the water surface to induce a circular current. The current in each tank was enhanced by an air-driven water jet; water depth was 35-38 cm. Photoperiod was that described previously. Feeding continued three times daily by distributing food evenly and at random across the water surface to minimise the presence of profitable territories and growth depensation (i.e. increase of among individual size variance due to competition).

Wild predators of parr (smallmouth bass, *Micropterus dolomieu*) were previously obtained by hook and line from a lake near the university (44.623°N,-

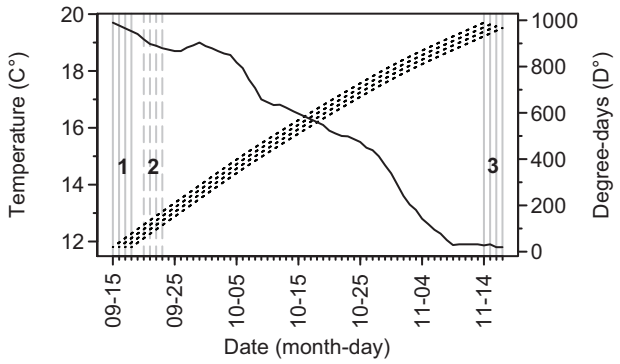

Figure 2.1: Average daily water temperature (solid black line), cumulative degree-days for each tank (dotted black lines; daily sampled tank pairs appear as one) and events. Indicated by solid horizontal grey lines are dates at which salmon size measurements were taken at start of the experiment (**1**) and at end of the experiment (**3**), whereas dates for stocking of predators are indicated by broken horizontal grey lines (**2**).

63.639°W), acclimated in single tanks (100 L) to laboratory conditions for three months, and fed daily with live earthworms and crickets. Five days after salmon stocking (Figure 2.1), one randomly assigned tank of each same-day sampled tank pair, totalling four tanks, was provided with one predator. Each predator (size range: 20-23.1 cm and 119-199 g) fit into spaces of the large blocks, but not into spaces of the small blocks and brick stones. Parr fit into all spaces and could efficiently hide and seek refuge from predation.

Between November 14 and 17 the experiment was terminated 61 days after parr stocking (56 days after predator stocking for each tank; Figure 2.1) and all fish were euthanized, using TMS. Again, feeding was discontinued two days prior to sampling. All surviving parr were successfully re-assigned to their families by tag location; size was recorded as previously described. Sex (male or female) and maturity status (mature or immature) was determined for each individual by visual inspection of the gonads following dissection. Only males had matured and the mature status was only assigned if at least one gonad contained fully ripened ('running') milt. I sampled at the end of usual spawning time (October to November; Watt & Penney 1980).

2.3.4. Statistical Analyses

2.3.4.1. Size and growth

Average size and growth in body mass or fork length was analysed using linear mixed models (LMMs). Individual measurements were transformed to their natural logarithms (Ln) as this normalised model error distributions and because geometric means were closer to population medians than arithmetic means. Also, the slope of Ln-transformed sizes across time directly represents the observed specific growth rate (SGR; Schmalhausen 1926). Cumulative degree-days, rather than calendar days, and growth are positively correlated in poikilothermic fishes. In the experiment, small but continuous among-tank differences in cumulative degree-days existed (Figure 2.1). To account for these temperature differences, I modelled *time* by using

degree-days (mean centred and divided by 100). My data did not fulfil conditions to fit a model that assumes size-dependent change in growth rate (**Supplement S2.1**).

Models were fit for mass or length with fixed effects for: each of the three strains (term *Strain*), the environmental effects of the presence or absence of a predator (term *Environment*), and the *Strain*-by-*Environment* interactions. All fixed effects also interacted with the continuous *time* covariate, such that resulting slopes represent degree-day-adjusted SGRs. To account for the completely randomised split-plot design of *Strain* within *Environment* replicates (tanks), I included the random effects term *Tank*, constituting the experimental whole plot error term, and which also accounts for among-individual correlations within tanks. As full-sibs are genetically correlated across tanks, I included the random term *Family*. In each model, the random effects term *Tank*-by-*Family* was included, constituting the experimental subplot error term and accounting for the correlation among full-sibs within each tank, i.e., for random genotype-by-environment interaction. As surviving individuals were measured twice, I accounted for the correlation arising from repeated measures at the *Tank* and the *Family* level by additionally interacting these random effects terms with the continuous *time* covariate, giving rise to a split-plot random coefficients model, and resulting in a total of six error terms. *Tank*-by-*Family*-by-*Time*, for which *Time* was taken as a factor (differentiated by the uppercase T), was modelled at the residual level.

Under this design fish individuals provide subplot replicates within each level of *Tank*-by-*Family*. However, salmon full-sibs naturally differ phenotypically and might therefore be not optimal subplot replicates (for which one wishes uniformity). Likely as a consequence of this variation, I observed negative variances for *Tank* and *Tank*-by-*Family* error terms in an initial model. In split-plot designs, negative variances can be interpreted as negative intraclass correlations (Nelder 1954). One way of dealing with negative variances is to omit the term - which is appropriate in some situations (Fletcher & Underwood 2002). However, in other situations this results in pseudoreplication (Hurlbert 1984) and may increase the type I error rate by both resulting in biased error estimates and incorrect denominator degrees of freedom (Wang *et al.* 1992). I followed the principle of

retaining all experimental error terms in my models (Nelder 1965; Brien & Demétrio 2009) and modelled the exhibited negative correlations by a compound symmetry covariance model at the residual level, where it is easiest to interpret (Molenberghs & Verbeke 2011). Lastly, I tested if heterogeneous residual variances or among-individual correlations were exhibited between the start and end of the experiment and if these differed among strains.

My LMM analyses assume normality of random effects. It has been observed that Atlantic salmon parr develop a bimodal size distribution as under-yearlings in autumn (Thorpe 1977; Bailey *et al.* 1980) and this would lead to a violation of my normality assumption and a possible bias in variances. I hence tested for deviation of unimodality for mass and length at the end of the experiment of every *Strain*-by-*Environment* combination, using Hartigan's test for unimodality, as implemented in the R package *diptest* (Maechler 2013), and obtained p-values from Monte Carlo simulations with 100,000 replicates.

2.3.4.2. Survival under predation

Survival was analysed only for data collected in the presence of a predator and was defined as the proportion of individuals at the end of the experiment relative to the number of initial individuals. Survival probability was analysed on binomial data for each level of *Tank*-by-*Family,* using a generalised linear mixed model (GLMM) with binomial residual distribution, logit link function, and using respective number of individuals as data weights. I assessed the fixed effects of *Strain* and the fixed continuous covariate of average fork length at the start of the experiment (initial *Length*, Ln-transformed, mean centred) for each level of *Tank*-by-*Family*, and the interaction of *Strain*-by-*Length*. Length was included as a predictor of survival because my predators have preferred prey sizes (Pflug & Pauley 1984) and hence parr predation susceptibility is a function of body size. Furthermore, the random effects terms *Family* and *Tank* were included to account for design and correlation as outlined for growth models. Here, *Tank* also accounts for variation in (intercepts of) survival that predator individuals might cause.

2.3.4.3. Sex proportion and male maturation probability

Sex proportion, defined as the proportion of males among all individuals, was analysed for data collected at the end of the experiment. Sex proportion was analysed on binomial data for each level of *Tank*-by-*Family,* using a model analogous to that used to analyse survival. My main interest lay in testing for the presence and differences in sex bias in the absence and presence of a predator, as such differences would be indicative of survival differences between sexes under predation, as previously suggested by behavioural studies (Dannewitz & Petersson 2001). I hence tested fixed effects of *Strain*, *Environment*, and their interaction, and also tested random effects of *Tank* and *Family* on sex proportion.

The probability of being mature, defined as the probability of each male individual of being mature, was analysed for data collected on males at the end of the experiment. Male maturation probability was analysed based on individual binary data (mature, immature) by GLMMs with a binomial residual distribution and under a logit link. Comparatively few males were mature (5.5%). Hence, with male maturation occurring as a rare event, it appeared meaningless to model maturation probability for all experimental factors. As my main interest lay in the differences among strains, and the model on sex proportions indicated no preferential predation on males (see results), I excluded the environmental factor from the model. I included *Strain* as the only fixed factorial predictor together with the continuous covariate of individual length at the end of the experiment (final *Length*, Ln-transformed, mean centred) and its quadratic term ($Length^2$). The quadratic term was included to model a body size of maximum maturation probability that accounts for the possibility that not all males above a 'threshold' size mature at this age. This model allows for examining whether maturation probability differs by elevation among strains (term *Strain*). This model represents an alternative to a previously suggested model for under-yearling parr in which differences in overall maturation probability elevations were interpreted as differences in maturation size thresholds (Piche *et al.* 2008), as has been observed for 1+ male parr (Myers *et al.* 1986).

2.3.4.4. Model fitting and hypotheses testing

LMMs for body size data were fitted with ASREML-R (Butler *et al.* 2009), using residual maximum likelihood (REML) under the Average Information algorithm in R version 2.15.2 (R Core Team 2013), which was also used as a platform for all other analyses. First, for each model, random terms were tested using likelihood ratio tests (LRTs) between nested models, interpreting $P < 0.1$ as being significant for one-sided hypotheses when testing positively constrained variances, and $P < 0.05$ as being significant for two-sided hypotheses when testing unconstrained covariances. Second, under a constant covariance structure, the fixed effects terms were tested using Wald *F*-tests with denominator degrees of freedom adjusted after Kenward and Roger (1997). Only significant fixed effects terms ($P < 0.05$) or those important to respect marginality were retained in the models.

GLMMs for sex proportion, survival, and maturation data were fitted with the R-package glmmADMB (Skaug *et al.* 2012) under Laplace approximation. Random effects were tested by LRTs between nested models. However, guidelines to methodological appropriateness for inferences about fixed effects in GLMMs are rare (Fong *et al.* 2010). I tested fixed effects after random effects by also using LRTs, again retaining only significant terms ($P < 0.05$) in the models. Overdispersion in the GLMM on binomial survival data was indicated by a ratio of deviance to degrees of freedom > 1 and I accounted for this extra variation by fitting subject random effects (i.e., *Tank*-by-*Family* effects) in addition (Browne *et al.* 2005). Lastly, as binary data-based maturation probability was extremely low (5.5%) and results might therefore be unreliable, I computed empirical confidence intervals of estimated parameters *post hoc* via Markov Chain Monte Carlo (MCMC), under a Metropolis Hastings update algorithm as implemented in glmmADMB.

2.4. Results

2.4.1. Behavioural Observations

When introduced into tanks, parr exhibited schooling behaviour in the strong current behind the spray bar. I did not observe aggression among parr throughout the study and at final sampling individuals exhibited only few hints of aggressive acts, such as damaged fins. During feeding, parr spread out and were schooling thereafter. After the introduction of the predator, schooling continued but the spreading out behaviour at feeding initially ceased. However, after several weeks differences in feeding behaviour between environments ceased, but never completely disappeared. After some time, 3 to 4 large parr in each tank appeared to have established territories in remote, current-reduced areas behind stones. Bass in all tanks remained hidden in the same large, hollow concrete block whenever observed. Predator attacks were never observed but dissection at experimental termination revealed that one bass had consumed a parr of 5 to 6 cm length.

Table 2.1: Results from hypothesis testing of fixed terms on body size. Results are based on Wald *F*-tests on model results for the response of either Ln body mass (**A**) or Ln fork length (**B**). Terms including time represent terms for specific growth rate; those without time represent terms for size. Environment is abbreviated as 'Env'. Given are degrees of freedom (df), Kenward and Roger adjusted denominator df (ddf), test-statistic (*F*) and p-value (P).

Term	df	ddf	*F*	P
A Mass				
time	1	13.8	1875	<0.001
Env	1	6.1	4.9	0.069
Env:time	1	6.3	5.7	0.054
Strain	2	12.0	43.7	<0.001
Strain:time	2	12.0	11.8	0.001
Strain:Env	2	93.9	5.6	0.005
Strain:Env:time	2	77.5	10.8	<0.001
B Length				
time	1	12.0	1842	<0.001
Env	1	6.1	5.4	0.058
Env:time	1	6.5	8.3	0.028
Strain	2	12.0	42.9	<0.001
Strain:time	2	12.1	11.5	0.002
Strain:Env	2	94.2	3.8	0.026
Strain:Env:time	2	78.1	7.5	0.001

2.4.2. Size and Growth

All strains exhibited unimodal size distributions in November (Hartigan's dip test, simulated P-range for *Strain*-by-*Environment* combinations; length: 0.199-0.717; mass: 0.534-0.948). Body mass and fork length differed among strains at both start and end of the experiment across environments (**Table 2.1, Figure 2.2**). The average-size order among strains was D0 < D3 < D5 at all times. Observed specific growth rates (SGR) across environments differed among strains in the same order as for size. For mass, neither size nor SGR differed between environments across strains. For length, size did not differ between environments but a minor indication for such differences in SGRs was obtained (**Table 2.1**). However, the interaction of *Strain*-by-*Environment* was different for size and SGR of both mass

and length (**Table 2.1**, **Figure 2.2**). This interaction was expressed in that differences in SGRs between environments were only present for the D0 strain, but for neither of the two domesticated strains (**Figure 2.2C & D**, within-strain contrasts of predicted marginal means for SGRs between environments with df approximated following Taylor (1950): D0, $t_{77.5} = 2.8$, $P = 0.006$; D3, $t_{77.5} = 0.3$, $P = 0.775$; D5, $t_{77.5} = 0.3$, $P = 0.751$). In the presence relative to the absence of a predator, the marginally predicted SGR of D0 was 19 % less for mass, and 17 % less for length.

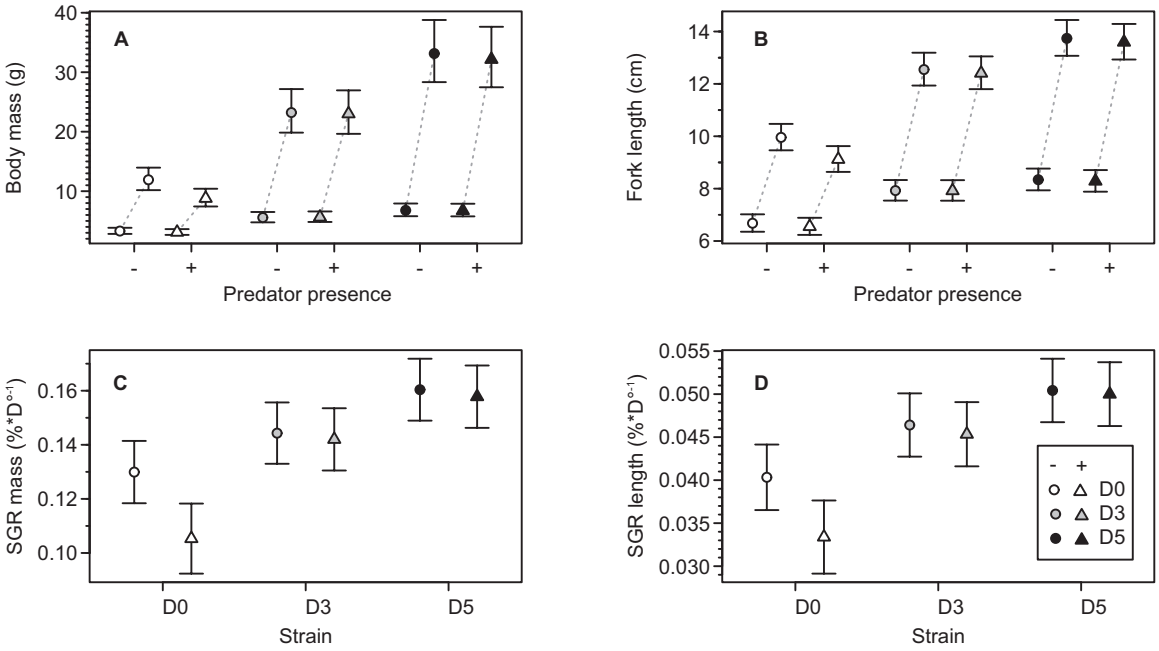

Figure 2.2: Body size and specific growth rate (SGR). Depicted are predicted retransformed marginal means for body sizes (**A** & **B**) at the start (left, lower symbol of each of the two symbols connected by the grey dotted line) and the end (right higher symbol of each of the two symbols connected by the grey dotted line) of the experiment, and corresponding SGRs (**C** & **D**) for either body mass (**A** & **C**) or fork length (**B** & **D**). Traits were recorded in either absence (circles, minus labels) or presence of a predator (triangles, plus labels) between start and end of the experiment and for each of the three strains of wild (D0, open symbols), 3rd generation domesticated (D3, grey symbols), and 5th generation domesticated Atlantic salmon (D5, black symbols). Error bars comprise approximate 95% confidence intervals.

Table 2.2: Variances and results from hypothesis testing of the covariance structure for size and growth rate. Results of significance testing are based on REML likelihood ratio tests between models with or without each term for the responses of either Ln body mass (**A**) or Ln fork length (**B**). Given is each variance (Var) or correlation (Cor), its REML estimate of the standard error (SE), the likelihood ratio test statistic (χ^2), degrees of freedom for the test (df), and the corresponding p-value (P). Variances for size terms (terms excluding *time*) are based on Ln mass (in g) or Ln length (in cm) and specific growth rate terms (terms including *time*) are based on size*100*D°$^{-1}$. Non-significant *Tank* and *Tank*-by-*time* error terms were retained as they reflected the experimental design. The correlation (ρ) characterises the residual correlation among individuals (replicates) within *Tank*-by-*Family*-by-*Time* combinations.

Term	Var or Cor	SE	X^2	df	P
A Mass					
Tank	$0.20*10^{-3}$	$0.42*10^{-3}$	0.4	1	0.551
Tank:time	$0.0089*10^{-3}$	$0.0091*10^{-3}$	2.5	1	0.115
Family	$24.6*10^{-3}$	$10.4*10^{-3}$	108.1	1	<0.001
Family:time	$0.13*10^{-3}$	$0.06*10^{-3}$	52.1	1	<0.001
Tank:Family	$5.4*10^{-3}$	$1.1*10^{-3}$	34.5	1	<0.001
D0 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	*34.8	1	<0.001
D0 residuals start	$196.4*10^{-3}$	$16.3*10^{-3}$	†48.4	6	<0.001
D0 residuals end	$308.8*10^{-3}$	$28.7*10^{-3}$	‡NA	1	NA
D3 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	44.0	1	<0.001
D3 residuals start	$161.7*10^{-3}$	$13.4*10^{-3}$	†48.4	6	<0.001
D3 residuals end	$229.9*10^{-3}$	$19.6*10^{-3}$	‡NA	1	NA
D5 $\rho_{(Tank:Family:Time:replicates)}$	-0.11	0.01	28.9	1	<0.001
D5 residuals start	$146.5*10^{-3}$	$12.0*10^{-3}$	†48.4	6	<0.001
D5 residuals end	$137.4*10^{-3}$	$11.7*10^{-3}$	‡NA	1	NA
B Length					
Tank	$0.03*10^{-3}$	$0.05*10^{-3}$	0.5	1	0.471
Tank:time	$0.00005*10^{-3}$	$0.00043*10^{-3}$	0.01	1	0.911
Family	$2.5*10^{-3}$	$1.0*10^{-3}$	105.9	1	<0.001
Family:time	$0.015*10^{-3}$	$0.007*10^{-3}$	59.3	1	<0.001

Continued on next page

Table 2.2B, continued

Term	Var or Cor	se	χ^2	df	P
Tank:Family	$0.54*10^{-3}$	$0.12*10^{-3}$	34.7	1	<0.001
D0 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	*29.2	1	<0.001
D0 residuals start	$19.2*10^{-3}$	$1.6*10^{-3}$	†,§52.2	6	<0.001
D0 residuals end	$30.5*10^{-3}$	$2.8*10^{-3}$	‡NA	1	NA
D3 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.05	§47.5	1	<0.001
D3 residuals start	$17.1*10^{-3}$	$1.4*10^{-3}$	†,§52.2	6	<0.001
D3 residuals end	$24.0*10^{-3}$	$2.0*10^{-3}$	‡NA	1	NA
D5 $\rho_{(Tank:Family:Time:replicates)}$	-0.11	0.01	32.7	1	<0.001
D5 residuals start	$15.8*10^{-3}$	$1.3*10^{-3}$	†,§52.2	6	<0.001
D5 residuals end	$12.9*10^{-3}$	$1.0*10^{-3}$	‡NA	1	NA

**Tank* variance became negative when correlation was removed; *Tank* term was omitted from reference model. †Test for homoscedastic residual variances among *Strain* levels (including a correlation among residuals within *Family*:*Tank*:*Time* levels and heteroscedastic variances for *Time*, taken as a factor, in each strain stratum). ‡Test for homoscedastic residual variances between levels of *Time*, taken as a factor, estimated for each strain separately; convergence failed due to non-estimable residual variance under a homoscedastic variance. §*Tank*:*time* variance became negative when correlation was removed; *Tank*:*time* term was omitted from reference model.

For mass and length, *Tank* variation was non-significant for both size and SGR, while among-family variation for both size and SGR and *Tank*-by-*Family* variation for size were both significant (**Table 2.2**). Size and SGR effects did not correlate for *Family* (mass: $\chi_1^2 = 0.0$, $P = 0.954$, length: $\chi_1^2 = 0.3$, $P = 0.605$) or *Tank* (mass: $\chi_1^2 = 0.0$, $P = 0.954$, length: $\chi_1^2 = 0.0$, $P = 0.896$) and both covariance parameters were therefore omitted from the models. For both traits and each strain, residuals within *Tank*-by-*Family*-by-*Time* combinations were negatively correlated (ρ = -0.11 to -0.12), indicating larger familial among-individual variation within tanks than expected. For both traits, large model-fit improvements were obtained by fitting *Strain*-specific residual variances, which exhibited a trend of decreasing residual variance with increasing domestication, and this trend was strongest at the end the experiment when residual variance was less than half in D5 relative to D0 (**Table 2.2, Supplement S2.2**).

2.4.3. Survival

In the absence of a predator, five fish out of 480 (1%) died during the experiment in two different tanks. Of these five, four were from four different wild D0 strain families, and one was from the D3 strain. An additional three dead fish (from two D0 and one D3 families) were found in the presence of a predator in three different tanks and all three fish exhibited bite marks. For these latter fish, it is impossible to distinguish between i) natural illness or death followed by attack or ii) attack followed by death. Hence, analyses of survival probability were conducted including and excluding data on these three fish for the initial count. As inferences were equal (not shown), results are reported based on data that included attacked fish in initial counts. Of 480 initially present individuals and exposed to predators for 56 days, 404 were recovered (16% were depredated).

In the GLMM on survival data, the interaction effects of *Strain*-by-*Length* on survival were non-significant ($\chi_2^2 = 3.3$, $P = 0.193$) and removed from the model. In the following model the categorical factor *Strain* and the continuous predictor *Length* were largely confounded as each term improved the model when the other term was absent (*Strain*: $\chi_2^2 = 13.1$, $P = 0.001$, *Length*: $\chi_1^2 = 12.5$, $P < 0.001$), but not in its presence (*Strain*: $\chi_2^2 = 1.1$, $P = 0.571$, *Length*: $\chi_1^2 = 0.6$, $P = 0.454$). This was most likely due to a lack of overlap in mean family body sizes between wild and domesticated strains. Only one D0 family had an initial average size similar to the smallest D3 family. Facing the inability to separate these effects, I abandoned causal simultaneous inferences on both terms (Gelman & Hill 2006) and based the fixed part of the model solely on familial fork length to explore the general predator prey-size window.

Among-family variance was non-significant in the initial model containing all fixed effects (initial model, *Family*: $\chi_1^2 = 1.5$, $P = 0.214$). Furthermore, when *Family* effects were added to the final model to test for genotypic effects without nesting the random *Family* effects within fixed *Strain* effects, among-family variance was also non-significant (final model, *Family*: $\chi_1^2 = 1.2$, $P = 0.270$) and therefore not included in any model. In the final model containing only *Length*, among-tank

variance was significant (final model, *Tank*: $\chi_1^2 = 4.4$, P = 0.037). The ratio of model deviance to degrees of freedom was 1.3, indicating overdispersion, and therefore

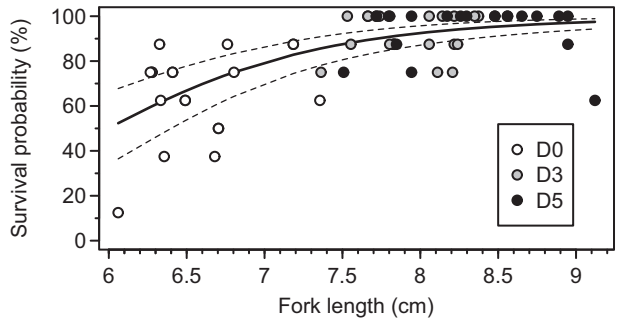

Figure 2.3: Survival probability under predation. Marginally for random tank effects predicted, retransformed survival probability as a function of retransformed fork length at the start of the experiment (thick black line) with approximate 95% confidence bands (thin dashed lines). Observed binomial data for 60 *Tank*-by-*Family* combinations have been added for each of the three strains of wild (D0, open symbols), 3rd generation domesticated (D3, grey symbols), and 5th generation domesticated Atlantic salmon (D5, black symbols).

Tank-by-*Family* random effects were fitted.

According to the final model, average survival probability increased with fork length (**Figure 2.3**) and was 89% (95% CI: 82-93%) at the overall average initial familial length of 7.6 cm. Observed overall proportion of survival for each strain (mean of final number/initial number of individuals per *Tank*-by-*Family* combinations) agreed by 0-5% with those predicted by solely average length for each strain under the model regression equation: logit^{-1}[(Ln length-2.03)*8.70+2.05]. Values, given as observed vs. predicted in %, were: D0 = 0.66 vs. 0.71; D3 = 0.92 vs. 0.92; D5 = 0.94 vs. 0.95.

2.4.4. Sex Proportion and Male Maturation Probability

Of the total 879 under-yearling parr examined at the end of the experiment in November, 455 (52%) were males. For sex proportion, both *Tank* and *Family* random effects terms were non-significant (*Tank*: $\chi_1^2 = 0.2$, P = 0.638; *Family*: $\chi_1^2 = 0.0$, P = 1.00). Furthermore, sex proportion did not differ for the interaction of *Strain*-by-*Environment* ($\chi_2^2 = 0.9$, P = 0.652) or either single effect (*Environment*:

χ_1^2= 1.2, P = 0.274; *Strain*: χ_2^2= 4.4, P = 0.110). Overall sex proportion estimated by the model was 52% and this value was not different from equality (Z = 1.01, P = 0.312; 95% CI: 48-55%). Overdispersion was not detected in any model (deviance/df = 0.97-0.99). As only males matured as under-yearlings and no significant differences in sex proportions were exhibited between environments, among strains, or for their interaction, it seemed reasonable to use data from both environments for the analyses of male maturation probability, i.e., data appeared to be unbiased by sex-specific or maturation-specific survival.

Of the 455 males, 25 exhibited fully ripened gonads (5.5%). Of these 25 mature males, 15 were from D0 (10.3% of all D0 males), seven from D3 (4.3% of all D3 males), and three from D5 (2.0% of all D5 males). The final size range of mature males was limited to 9.6-13.6 cm, while the overall male final size range was 5.6-17.6 cm. In the model on male maturation probability, the random effects term *Tank* was non-significant (LRT, χ_1^2= 0, P = 1) and removed from the model; thus, maturation probability was considered as an observational trait. The *Family* random effects term was also non-significant (LRT, χ_1^2= 0.3, P = 0.594) but retained in the model to account for genetic correlations among full-sibs, based on the *a priori* assumption of a genetic basis for maturation probability and because *Strain* effects were present.

Table 2.3: Model coefficients and means for male maturation probability as a function of Ln fork length among the three salmon strains, using the wild strain as a reference. Model coefficients are on the logit scale, and retransformed average mean maturation probabilities for each strain at the overall average fork length (11.6 cm) are given in per cent (in parentheses), each with 95% confidence interval (95% CI) obtained from either maximum likelihood estimation (ML) or post-hoc mcmc sampling (MCMC).

	*ML		MCM	
Term	**mean**	**95% CI**	**mean**	**95% CI**
D0 mean	-0.67	-1.66 to 0.32	-0.62	-1.69 to 0.19
(D0 mean)	(34%)	(16-58%)	(35%)	(16-55%)
D3 contrast	-1.52	-2.71 to -0.34	-1.59	-2.78 to -0.38
(D3 mean)	(10%)	(3-27%)	(10%)	(3-27%)
D5 contrast	-1.89	-3.47 to -0.31	-2.09	-3.78 to -0.51
(D5 mean)	(7%)	(2-27%)	(6%)	(1-24%)
Length slope	-2.3	-7.5 to 2.8	-2.6	-7.8 to 2.3
Length^2 slope	-61.1	-96.4 to -26.5	-67.5	-105.2 to -36.7

*Laplace approximation

In the final model, male maturation probability was predicted by squared final length (LRT, $\chi_1^2 = 28.2$, $P < 0.001$) and differed in elevation among strains (LRT, $\chi_2^2 = 6.9$, $P = 0.031$) at the overall mature male average fork length of 11.6 cm (**Figure 2.4**). Predicted size-frequency-corrected maturation probability at the average fork length was much lower for both domesticated strains with 7% (D5) and 10% (D3) vs. 34% in the wild strain, but all prediction errors were large (**Figure 2.4**). Model coefficients computed by Laplace approximation and those computed via *post hoc* MCMC sampling from the distribution of the model parameter values led to similar inferences (**Table 2.3**).

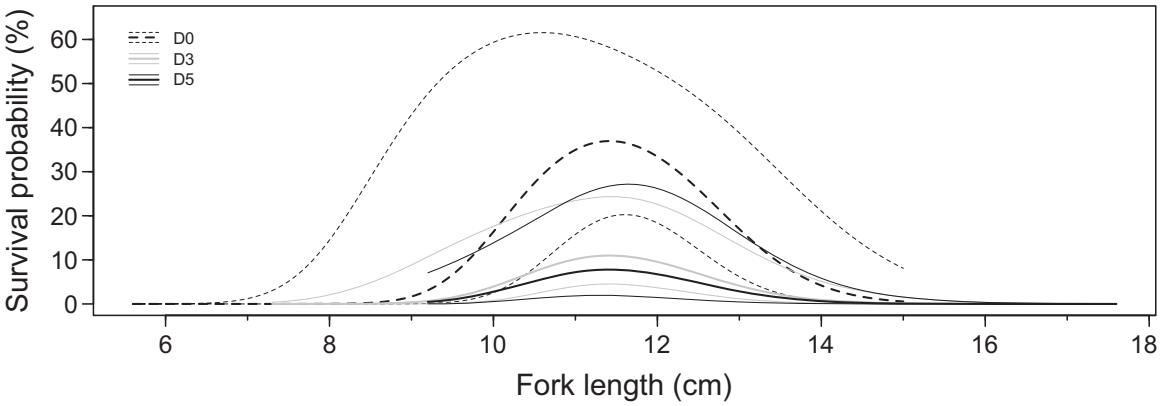

Figure 2.4: Male maturation probability. Marginally for family effects predicted, retransformed maturation probability as a function of fork length for each of the three strains with approximate 95% confidence bands. Predictions are limited to the respective observed length range for each of the three strains of wild (D0, thick dashed black line, thin dashed black intervals), 3rd generation domesticated (thick grey line, thin grey intervals), and 5th generation domesticated Atlantic salmon (D5, thick black line, thin black intervals).

2.5. Discussion

Of the traits investigated, growth rate was primarily under anthropogenic selection, but the investigated domesticated strains were also selected for rapid smolting (seawater tolerance). Increased growth rates in domesticated salmon might have led to increased survival under predation, as this trait was found to be a direct correlate of size. However, size was not the only predictor for male maturation probability and differences between wild and domesticated strains were found for size-corrected maturation probability. As such, I propose that domestication has decreased early male maturation probability, independent of size, through selection for very rapid growth (more rapid than typically observed in the wild) and possibly through selection for rapid development to the smolt stage.

2.5.1. Growth

Under semi-natural conditions, but fed *ad-libitum* and in the absence of a predator, SGRs were 1.1 or 1.3 times higher for domesticated than for wild

individuals. SGR estimates should be regarded only as approximations, not fully representing growth capacities, as trends for different relationships between growth rate and body size (**Supplement S2.1**) and different initial sizes among strains were present. SGR estimates for each strain will likely differ depending on the developmental period of growth under investigation. Nevertheless, a 2 or 3 times larger average body mass was present for domesticated relative to wild under-yearlings in November. Sexual maturation negatively affects somatic growth in male parr (Myers *et al.* 1986; Berglund 1992), but male maturation had likely little confounding influence on comparisons of growth as mature males comprised only a few per cent. Results of a previous study suggested that growth differences between wild and domesticated Norwegian Atlantic salmon would be only expressed in salt water at later ages (Fleming *et al.* 2002). In contrast, my results suggest the presence of large growth differences also for under-yearlings in fresh water. However, in the previous study, size-matched individuals were used (Fleming & Einum 1997; Fleming *et al.* 2002), whereas I used individuals fully representing size ranges. It is hence not surprising to find such disparate results between studies as the previous study might have compared the slowest growing domesticated with the fastest growing wild individuals and this might not have been representative.

Interestingly, growth rate and final size in the presence of a predator was reduced by nearly 20% in the wild strain, whereas growth of domesticated strains was unaffected. As stress resistance has been proposed to increase under domestication (Kohane & Parsons 1988; Solberg *et al.* 2012), the absence of a growth reduction in domesticated individuals might reflect some form of stress resistance towards predators. It is also possible that large individuals were less affected by predator presence than small individuals and the different responses among strains might then have been only indirectly related to domestication via rapid growth rate. Alternatively, it is possible that the fastest growing and largest wild individuals were selectively depredated in my study and growth rates of wild survivors were not reduced. This latter possibility would imply that largest, not smallest, wild individuals were depredated which conflicts with my inferences

about familial size-related survival and appears therefore unlikely. Fleming and Einum (1997) investigated the growth response to predator presence between size-matched wild and domesticated individuals and observed a trend of reduced growth only for the wild strain. Accordingly, it appears most likely that in my and the previous study wild fish were more stressed by the presence of a predator than domesticated fish and, as a consequence, exhibited slower growth.

I detected a diminishing within-family variance with increasing generations of domestication. I can exclude the possibility that this residual variation was related to predator presence or absence as this observation held true when analysed for each environment separately and a trend of increasing variance was exhibited for all strains in the presence of a predator (**Supplement S2.2**). Furthermore, I can also exclude the hypothesis that differential expression of size bimodality among strains caused differences in residual variances; bimodality was absent in all strains across environments. Growth depensation might partly explain this pattern, and variance of small wild individuals might have been increased more by competition than that of large domesticated individuals. However, a similar but weaker trend was already present at the start of the experiment before different families interacted (**Supplement S2.2**). Additive genetic variance might decrease under directional or stabilising selection (Bulmer 1971), but this would be exhibited for complex traits in large populations as diminishing variance among families, not within families. A change of within-family variance is more likely caused by allele frequency changes or allelic loss by genetic drift or inbreeding, as might be anticipated at small population sizes. Alternatively, a decreasing residual variance can reflect reduced environmental sensitivity (Hill 2004). Mass-selection is expected to have greater effects on the environmental variance than family selection (Mulder *et al.* 2007), and the breeding programme for my domesticated strains was initially based on mass-selection (Glebe 1998). However, the change of environmental variance, when not directly selected for, depends on the direction and magnitude of the genetic covariance between selected trait and environmental variance (Mulder *et al.* 2007). The presence and direction of this covariance has not yet been investigated for salmon growth and remains to be evaluated, especially given that heritability

estimates across selected generations would be affected by a diminishing environmental variance.

In growth models, residuals were negatively correlated by about 11% among full-sibs sharing a tank across all strains. Negative correlation can arise from competition, but it appears unlikely that competition was present only among full-sibs when each tank was shared with 14 times more individuals from other families. Furthermore, fish were fed *ad libitum* and I did not observe any aggressive behaviour during feeding. However, I observed that individual sizes were greatly overlapping among families and differences among individuals from different families were in many cases less than differences among full-sibs. This circumstance, in combination with a relatively low subplot replication ($n = 8$ individuals per family and tank), has likely led to the observed negative correlation among full-sibs. To avoid any inferential bias under a design with highly variable units below the whole plot replication level (represented by tanks), it might be advisable to investigate the possibility of such negative correlations whenever whole-plot (i.e., tank-) variance appears as zero or negative, and account for it.

2.5.2. Survival

According to my model, survival under predation was solely predicted by average fork length. I am unable to draw any conclusions in regard to effects of domestication on survival other than those conveyed by a larger size-at-age, as there were considerable initial differences in average sizes among strains and my predators were gape limited. However, differences in survival were not detected at the family level after adjusting for length effects. This suggests a lack of genotypic effects resulting from domestication for survival, such as differences in mortality-related behaviour, as has been previously suggested (Biro *et al.* 2004; Biro & Post 2008). In other words, if no variation for survival that is independent of length could be detected among strains and among families within and among strains, it is less likely that domestication has altered size-independent survival among strains. However, I evaluated only a few families and this certainly limits the generality of my conclusions.

Other studies of survival in relation to rapid growth-rate under predation appear to have artificially adjusted body sizes between fast and slow growing strains when investigating individuals beyond the fry stage; unfortunately the means by which these adjustments were made was not reported (Biro *et al.* 2004; Biro & Post 2008). My study was, like most similar studies, designed to be conducted on fry shortly after first feeding. Due to governmental regulations when moving eggs across provincial borders, pathogen testing had to be conducted on fry in early June to gain release from an administered quarantine-condition laboratory. Pathogen testing revealed an initially positive but unknown result and release from quarantine was delayed until end of August when the initial result was identified as being harmless. At this time, size differences among strains were already present. Different methods have been used to adjust body sizes between strains with different growth potential by either using individuals at different ages (Sundström *et al.* 2009), of matched size but equal age (Fleming & Einum 1997), or both (Tymchuk *et al.* 2009b). I decided against using size-matched individuals of the same age as individuals from the upper tail of the size distribution of a slow growing strain and those from the lower tail of a rapid growing strain are unlikely to be representative. Using individuals of different ages between strains might lead to difficult interpretations as age in Atlantic salmon is accompanied by changes in life history stages such as sexual maturation and smoltification, both of which are accompanied in changes of other correlated traits. Lastly, starving individuals to adjust sizes before the start of the experiment might bias results as fast growers might exhibit compensatory growth during the experiment, leading to confounding consequences of compensatory growth and growth rate potential. Consequential of these thoughts, I fed individuals of all strains *ad libitum* throughout and conducted studies on strains with different sizes.

As generally valid for genotypic studies with limited family sizes, family-bias might have affected my results as data are based on only five families per strain, and this made it impossible to efficiently capture within-strain variation. In comparison, previous studies assessing growth rate-related survival under predation (including studies on strains transgenic for growth hormones) were based on either unknown

numbers of families and parents (Biro *et al.* 2004; Sundström *et al.* 2004; Biro & Post 2008), similar numbers of families (4 to 5: Sundström *et al.* 2009; > 5, not further specified: Sundström & Devlin 2011), or partly higher numbers of families (49 wild vs. 5 domesticated: Vandersteen *et al.* 2012). None of these studies reported to have accounted for family effects by either using equal numbers of individuals from each family or by statistically assessing family effects on survival, although possible effects were discussed by Vandersteen *et al.* (2012). This makes it difficult to compare my results on among-familial variation in survival with other studies. Furthermore, it cannot be excluded that in other studies among-strain comparisons were confounded with among-family comparisons. As a logical consequence of the idea of the presence of selected genotypic differences among populations, inferences about genotypic effects among strains are ideally based on family means as the error term.

2.5.3. Survival and Growth

In my study, size and growth rate were positively correlated at the strain level, but not at the family level within each strain. Thus, the larger-sized domesticated strains also exhibited faster growth rates. Growth rate might have had an additional effect on survival as it provides individuals with the ability to rapidly outgrow the 'prey vulnerability' window. Hence, rapidly growing individuals have i) a higher survival probability given their larger size at the same age, and ii) outgrow the prey-size window more rapidly than slow growing individuals. Wild individuals appeared to not have taken advantage of their full growth potential in the presence of a predator and this might have made them susceptible to predation for longer periods than predicted by size and growth potential alone. Stress reduces growth and stress as conveyed by predator fear might be a function of prey body size. However, small individuals were initially also present in both domesticated strains despite their larger familial mean body sizes. If body size was the only predictor of a predation-related growth reduction, then I would have observed a growth reduction also in both domesticated strains, but this was not the case. As such, being less stressed in the presence of a predator might have influenced survival under predation

positively, even for small domesticated individuals, by allowing them to outgrow the prey size window with the same growth potential as in the absence of a predator, in contrast to small wild individuals.

In Canada, smallmouth bass of similar sizes and larger (20 to 30 cm) as used in my study (20 to 23 cm) co-occur with salmon parr of sizes comparable to those in my study (Carr & Whoriskey 2009). Based on my experimental results, bass of 20 cm are very likely to depredate salmon of 7 cm and smaller and this agrees well with previous observations in the field (Pflug & Pauley 1984). Under predation by smallmouth bass, domesticated salmon might be at an advantage as they are able to outgrow the prey window more rapidly than wild individuals, for the reasons identified above.

2.5.4. Male Maturation Probability

The detected differences for size-corrected maturation probability among strains indicated genetically based differences for this trait but I did not detect any among-family variance. The lack of significance for among-family variance does not imply that there is a lack of genetic variation as my study suffers from family number limitations in combination with a small number of successes (few mature males). For the few mature individuals observed, I did not attempt to test for a shift in body size of maximum maturation probability as a consequence of domestication. Assuming that size of maximum maturation probability was equal among strains, I conclude that domestication has significantly reduced early male maturation probability at the average body size from 34% to 10% and 7% (by 71% and 79%) after three and five generations respectively. Bailey *et al.* (1980) reported for the wild Saint John population under hatchery conditions male under-yearling maturation rates of 21% in one year, but only 3% in the following year, whereas tank densities during the second year were 40% higher. It is impossible to differentiate between environmental and genetic effects as the cause for a 7-fold difference in the previous study. It, however, stresses the necessity to account for environmental and genetic influences (family) simultaneously for a valid assessment of genetically based changes in maturation probability. I accounted for

(non-significant) among-family variation in my analyses, standardised environments among strains, and statistically evaluated all possible confounding effects and this makes us confident in drawing inferences about differences in maturation probabilities, despite the fact that male under-yearling maturation was a rare event.

Two processes during domestication might have caused a directional selection towards a lower early male maturation probability. First, sexual maturation and growth are in direct resource competition (Thorpe 2004). Hence, maturing males grow slower and might be at a net disadvantage under directional selection for rapid growth relative to immature males. Secondly, maturation and smolting (acquiring seawater tolerance) might also be negatively genetically correlated. The domesticated strains were selected for smolting as yearlings (Glebe 1998) and this might have negatively co-selected for under-yearling male maturation probability, although some under-yearling mature parr can also smolt as yearlings (Bailey *et al.* 1980). In a previous study on rapidly growing transgenic, but otherwise not selected, and slow growing wild salmon, no differences in maturation probabilities of under-yearlings were observed between maternal half-sibs (Moreau & Fleming 2012). These findings suggest that rapid growth alone does not negatively influence under-yearling male maturation probability (but it does in one year old salmon; Moreau & Fleming 2012), and this adds to the idea that a selected genetic component, independent of growth rate, accounts for the observed lower maturation probabilities in the domesticated strains (Hutchings & Myers 1994; Moreau & Fleming 2012).

Previously, it has been assumed that early maturing males are among the fastest growing individuals and that maturation takes evolutionary precedence over smoltification (reviewed by Thorpe 2004). For my wild strain, this view holds, whereas for both my domesticated strains it does not. Mature males had similar sizes across all strains and this agrees with observations on under-yearlings between rapidly growing growth hormone transgenic and slow growing wild salmon (Moreau & Fleming 2012). Mature males from my wild strain were from the upper tail of the size distribution, those of domesticated strains from its middle (D3)

or lower tail (D5), which altogether supports the presence of a size optimum rather than size or growth threshold for under-yearling male maturation probability.

Predation might have biased my results on maturation probability. Maturing male parr exhibit slower growth than immature parr (Myers *et al.* 1986; Berglund 1992) and this might have raised their predation susceptibility by outgrowing a prey-size window slower than initially same-sized immature individuals. However, I did not detect any effects of strain, predation environment, or their interaction on sex frequencies, and this indicates that males and females were equally susceptible. As only males matured, this is an indirect indication for equal susceptibility between mature and immature individuals. Furthermore, despite a lower growth rate for mature males, mature males of the small wild strain were among the largest wild individuals at the end of the experiment. Accordingly, all maturing males might have had a high survival probability as it can be assumed that they had relatively large initial sizes.

It was previously suggested that mature males might propagate introgression by domesticated-wild outbreeding (Garant *et al.* 2003). Hence, an apparent reduced male parr maturation probability by domestication might be viewed as positive from a conservation perspective as first generation-hybrid males are more likely to undertake a risky marine migration first before reproducing than wild males. However, given the presently low numbers of breeders in many wild salmon populations, and the constancy of domesticated escapee presence (Glover *et al.* 2012), it might be more likely that male parr maturation probabilities will be lowered by genetic effects from domesticated-wild outbreeding. The influence of escapees might oppose naturally regulated mechanisms of male maturation patterns, as suggested by Hutchings and Myers (1994), and lower population viability as a consequence.

In conclusion, I suggest that domestication provokes i) rapid growth rates, ii) higher survival under size-selective predation as conveyed by a large size-at-age and a larger growth potential, and iii) lower under-yearling male maturation probabilities. Lastly, iv) domestication might make individuals less prone to stress

as caused by predator presence, which adds to fully realising a growth potential in support of point ii).

2.6. Acknowledgements

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2.7. Data Accessibility

All data used for analyses are planned to be made accessible during the course of the publication of the manuscript.

2.8. Supplementary Material

2.8.1. Supplement S2.1: Testing for Parabolic Growth and Evaluating a Model for Size-Adjusted Growth Rate

Several models exist for growth rate of fishes and all rely on specific assumptions for valid inferences (Sigourney *et al.* 2008). I used the observed SGR, assuming the presence of exponential growth which might hold for studies of limited periods in young fish (Hopkins 1992). When parabolic growth is present (von Bertalanffy 1957) the use of a size-adjusted growth rate is preferred (Parker & Larkin 1959; Elliott 1975). Many ecological studies on Atlantic salmon use models and parameters obtained by extensive studies on trout and salmon by Elliott (1975) and Elliott and Hurley (1997) that unfortunately do not represent growth well in every setting (Bacon *et al.* 2004). As my study depends on valid inferences about

differences between strains, I tested for the presence of parabolic growth and whether growth-size relationships differed among strains.

Testing for Parabolic Growth

Ostrovsky (1995) outlines a simple test for the presence of parabolic growth and follows approaches that have been used for a long time in fish research (Parker & Larkin 1959). When the observed specific growth rate on the logarithmic scale (Ln*SGR*) is plotted against the body size on the logarithmic scale (Ln *W*) and the slope is negative or positive (compare Fig 4 in Elliott 1975), one can assume that the growth rate changes with body size, i.e., growth is parabolic. Parabolic growth will only be appropriately detected under constant environmental conditions (Parker & Larkin 1959; Ostrovsky 1995). Then the slope of the relationship directly contributes the coefficient (b) for a size-correction of SGR. Surprisingly, in salmonid research this coefficient appears to be rarely estimated despite its simplicity. However, the value of b appears to be insensitive for reliable body mass predictions within a large range (Iwama & Tautz 1981).

In my experiment, I tested the effects on growth rate of two constant environments (presence and absence of predation) using averaged data on 960 to 879 individuals (start to end of the experiment) from 15 different families of three different Atlantic salmon strains that were distributed across eight tanks. I could hence test for the relationships between Ln*SGR* and Ln *W* by using altogether 120 combinations of Ln*SGR* and Ln *W*: 40 combinations for each of the three strains, 60 combinations for each environment, and 20 combinations for each of the six levels of the interaction of strain and environment. As data are correlated for individuals sharing a tank and for full-sibs sharing ancestry, I accounted for this non-independence by including tank and family identifications as random effect terms in my analyses. I used a linear mixed effects model with the fixed effects terms of *Strain*, *Environment*, and the continuous predictor of Ln *W* (initial Ln body mass), and all of their interactions. I used the natural logarithm of the observed *SGR* in per cent as the response in the model. *SGR* was calculated as:

$$observed\ SGR = \frac{Ln(W_{t2}) - Ln(W_{t1})}{t2 - t1} * 100$$ **Equation 1**

for which $Ln(W_{t2})$ is the natural logarithm of body mass at end of the experiment, $Ln(W_{t1})$ is the natural logarithm of body mass at the start of the experiment (called Ln *W* above; initial Ln body mass), and *t2-t1* are the degree-days passed between start and end of the experiment. Sample sizes of individuals among the 120 combinations of *Tank*-by-*Family* differed for $Ln(W_{t2})$ due to predation mortalities. Accordingly, any model estimates in the presence of a predator are biased by the removal of individuals that contributed only to $Ln(W_{t1})$. To counteract this bias, I weighted each SGR-value by the number of contributing individuals. An initial model exhibited heteroscedasticity when examining the residuals, and this was accounted for by fitting different residuals variances to each strain what greatly improved the model (homoscedastic vs. heteroscedastic variances, REML-LRT: $\chi^2_2 = 697.5$, $P < 0.001$).

In the resulting model, the three-way interaction was non-significant (*Strain*-by-*Environment*-by-Ln *W*: $F_{2,56.7} = 0.3$, $P = 0.754$) and therefore removed. All two-way interactions in the following model were non-significant and the least significant was removed (*Environmen*t-by-Ln *W*: $F_{1,64.6} = 0.4$ $P = 0.540$). Next, *Strain*-by-Ln *W* was still non-significant ($F_{2,48.8} = 1.1$, $P = 0.355$) and therefore removed. In the last model, the main effect of Ln *W* was removed as it turned out to be still non-significant ($F_{1,74.1} = 1.0$, $P = 0.318$) and no more model terms were marginal in respect to this term. As such, I did not detect any effect of Ln *W* on *SGR*, i.e., I could not reject the null hypothesis of the absence of parabolic growth and using the observed SGR is appropriate.

Using the initial model with all fixed effects interactions, coefficients of b in the absence of a predator (and hence being unbiased for differential individual contribution caused by predation mortality) were estimated from non-significant relationships as 0.11 for the domesticated D5 strain, 0.20 for the D3, and -0.06 for the wild D0 strain. The reason why my estimates deviate so strongly from previous estimates, especially the estimate of -0.06 for the wild strain, could be numerous and I will discuss a few.

Fish for the study of Elliott (1975) and (Elliott & Hurley 1997) were obtained from hatcheries and no familial relationships or other information on genetic diversity among individuals have been reported. It might hence be possible that a much stronger relationship between LnSGR and LnW in these studies can be attributed to unaccounted kin relationships among individuals, whereas I accounted in my analyses for such non-independence, and this could explain why my relationships are messier and non-significant. Alternatively or in addition, the significant slopes b = 0.31 to 0.32 obtained by Elliott and Hurley (1997) were based on individuals whose body size differences could be mostly attributed to differences in age (spanning also a much wider size range) whereas size differences among individuals within each strain in my data are attributable to differences in past growth at the same age, and these differences between studies might be responsible for differences in the slopes and differences in variation. Lastly, I kept fish individuals together in tanks and this might have resulted in competition, although I tried to minimise any growth depensation by my husbandry practices, while Elliott and Hurley (1997) kept fish individually in aquaria. Accordingly, competition might have led to a larger divergence in growth patterns (growth depensation) in my study compared to the absence of competition in the study by Elliott and Hurley (1997).

The reason why the wild D0 strain deviated so strongly from an expected pattern with slope b =-0.06 might be partly a completely different one. For one of the five D0 families all average sizes of tank replicates were larger than those of all other D0 families and this family also grew more rapidly than expected by its initial size given it was of the wild strain. This might have 'pushed' the slope for the D0 strain to be negative. With only five families per strain it is difficult to judge if this variation in size and growth is part of the natural population variation, or if this family might have been affected by domesticated-wild outbreeding in the first or second generation. Fish farms are numerous in the area around the Saint John River and fish have escaped repeatedly in large numbers during winter storms. Genotyping individuals with the aim to test for introgression appeared to be not an option to follow as all individuals in my study share recent ancestry. However, when omitting

the suspicious family data from the analyses, the slope for the D0 strain was b = 0.04, which is positive (albeit it was far from being statistically different from zero) but still quite different from the otherwise used b = 0.31. Parker and Larkin (1959) outline further ecological and physiological reasons why slopes b can differ within a species. In fact, it was therefore suggested by Parker and Larkin (1959) to view fish growth in 'stanza' to which appropriate size adjustments for growth rate should be applied.

Size-Adjusted Specific Growth Rates for Body Mass

Following Parker and Larkin (1959), Elliott (1975) and Elliott and Hurley (1997) suggested to adjust juvenile salmon growth rates to a common size to account for an allometric scaling of the specific growth rate with body mass. According to the observed SGR, growth increments follow a constant proportion of the absolute body size, i.e., growth is exponential. Exponential growth might hold for a limited period in young fish (Hopkins 1992), but it is assumed that the ratio of anabolism to catabolism follows a non-linear function of body size (von Bertalanffy 1957) and parabolic growth is a consequence. For poikilothermic animals, the calculation of the size-adjusted growth rate is relatively straightforward under a constant temperature, or additional temperature adjustments must be made when it varies (Elliott & Hurley 1997). Under a constant temperature during the experiment, t in **Equation 1** represents time. I used cumulative degree-days for t as temperatures changed ambient-based in my experiment. Using degree-days instead of time is only an approximation that is valid for only a limited temperature range and when all individuals experienced the same temperature ranges (cf. Fig. 1 in Elliott & Hurley 1997).

The size-adjusted SGR can be obtained as:

$$adjusted\ SGR = observed\ SGR + (b * Ln(W_{average}) - b * Ln(W_{adjust}))$$ **Equation 2**

for which b is an empirically derived allometric scale coefficient (e.g., 0.31 to 0.32 for two populations of English Atlantic salmon fed *ad libitum*; Elliott & Hurley

1997), $W_{average}$ is the familial (geometric) mean mass during the experiment, and W_{adjust} is the arbitrary mass to which growth is adjusted to; I used the overall (geometric) mean mass at the start of the experiment (4.95 g) but any other value can be used. The adjustment is usually applied to individuals. As I did not have connected individual data across time but connected data for combinations of *Tank*-by-*Family*, I applied the adjustment to *Tank*-by-*Family* SGRs. This made it necessary to abandon individual mass data, as used in the analyses described in the main manuscript, and to use averages in the model on adjusted SGR instead. For each of the 120 combinations of *Tank*-by-*Family*, I calculated average observed SGR according to **Equation 1**. Then, I calculated the adjusted SGR for each *Tank*-by-*Family* combination according to **Equation 2**. Please note that this just follows the standard procedure, but that my data did not exhibit the assumed slope of 0.31 for any of my strains (as estimated by the above models that tested for parabolic growth; estimated slopes were non-significant and < 0.31). The adjusted SGR was then used directly as the response for the mixed model analyses with the fixed effects terms of *Environment*, *Strain*, and their interaction, and random effects terms of *Tank* and *Family* (see above and also the main manuscript for details). Again, *Tank*-by-*Family*-specific adjusted SGRs were weighted in the analysis by number of individual data that were used for each respective observed SGR calculation, i.e., when few individuals of a *Tank*-by-*Family* combination were left at the end of the experiment due to predation, their observed SGR was weighed less than an observed SGR that was based on an equal number of individuals at the start and end of the experiment. Note that the fixed effects when using SGR as a response instead of body weight compare to interaction effects with 'time' in the model as appearing in the main manuscript. In the model on size-adjusted SGRs, residuals also exhibited heteroscedastic patterns and as a consequence different residual variances were fitted to each strain, which greatly improved the model (homoscedastic vs. heteroscedastic variances, REML-LRT: $\chi_2^2 = 671.0$, $P < 0.001$).

Table S 2.1: Results from hypothesis testing of fixed effects on common body mass (4.95 g) adjusted SGR for body mass based on Wald *F*-tests. Given are degrees of freedom (df), Kenward and Roger adjusted denominator df (ddf), test-statistic (*F*) and p-value (P).

Term	df	ddf	*F*	P
Env	1	6.2	8.6	0.026
Strain	2	11.6	53.2	<0.001
Env:Strain	2	63.1	8.4	0.001

In the model using adjusted SGRs as a response, all fixed effects were significant (**Table S 2.1**), and this was in contrast to the model on observed SGRs for which the environmental effect on SGRs across strains did not differ. However, as the interaction effects of *Strain*-by-*Environment* were significant in both models, the inferences are equal. The major difference between both models was that under size-adjusted SGRs differences among strains were much larger with confidence interval boundaries from different strains being further apart, and that confidence intervals between SGRs of both environments showed greater overlap for the wild D0 strains (**Figure S 2.1A and B**).

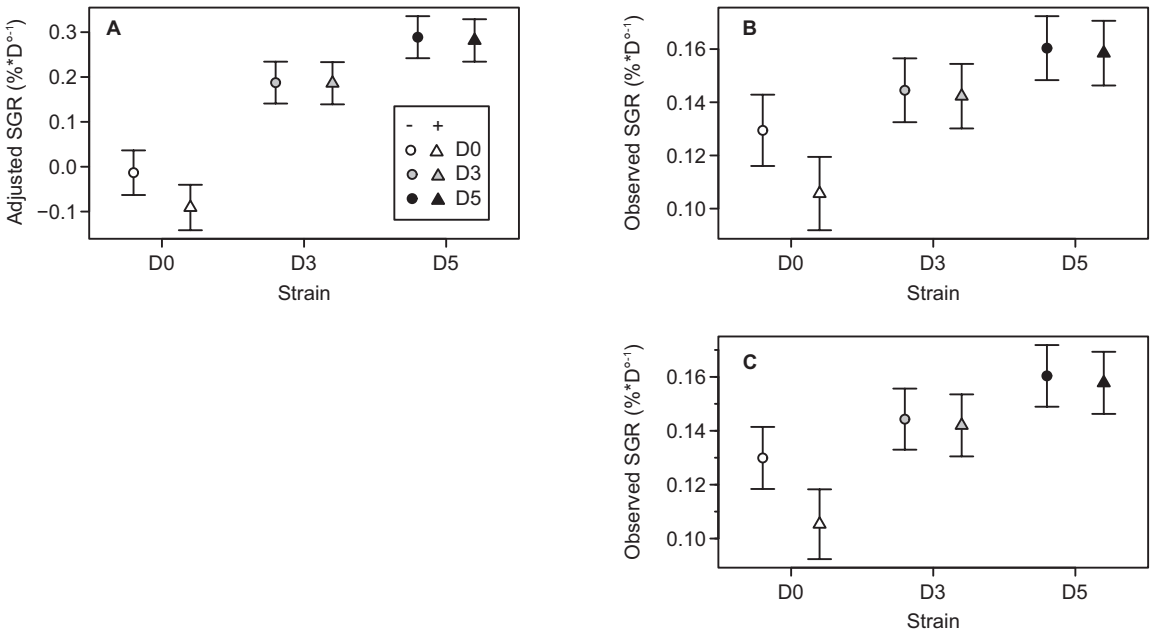

Figure S 2.1: Marginally for random effects predicted retransformed means for either to common body mass (4.95 g) adjusted SGRs (**A**) or observed SGRs (**B** & **C**). **A** and **B** are based on *Tank*-by-*Family* means for SGR, whereas **C** is based on individual body weight data. **C** is the same as in the main manuscript and here repeated to facilitate methodological comparisons. Data were recorded in either absence (circles, minus labels) or presence of a predator (triangles, plus labels) between start and end of the experiment and for each of the three strains of wild (D0, open symbols), 3rd generation domesticated (D3, grey symbols), and 5th generation domesticated Atlantic salmon (D5, black symbols). Error bars comprise approximate 95% confidence intervals. Negative adjusted growth rates do not reflect loss of body mass but are statistical artefacts resulting from the size-adjustment of SGR to the arbitrarily chosen average initial body mass.

The larger differences among strain means with smaller confidence intervals appeared to have resulted from the size-adjustment of SGRs, and this might be a problem in studies using size-adjusted growth rates as it can cause liberal results when the model assumptions and parameter estimates do not hold (as in the present case, see above). In contrast, the greater within-strain interval overlap between environments appeared to have resulted from different modelling approaches (**Figure S 2.1**: tank-by-family-by-time combinations directly constituted adjusted or observed SGRs as the response in models represented by **Figure S 2.1A**

and **B**, respectively, but tank-by-family-by-time combinations constituted the grouping for the correlated residuals for the other model with mass taken as the response in **C**). In regard to the latter methodological difference, the individual data approach based on body mass at each time point is likely closer to real differences as it makes use of sample size information for all levels of variation (at least under my REML-based mixed model approach), while the averaged data approach only makes use of individual sample size information as far as provided by my 'weights' argument.

2.8.2. Supplement S2.2: Testing for Environmental Influences on Residual Heteroscedasticity

I detected a diminishing within-family variance with increasing generations of domestication. To test for the possibility that the within-family variance (residual variance) was related to predator presence or absence, I analysed data for each environment separately. This was conducted as observed survival was greater for strains with greater residual variance; hence, I wanted to exclude the possibility that the increasing variance was simply a result of decreasing precision of the family mean estimate with decreasing numbers of survivors in a given family replicate. This test was conducted by sub-setting the data and re-running the models separately for each subset (presence or absence of predation) but otherwise as described in the main manuscript. Resulting variance estimates are presented in **Table S 2.2**. Indeed, residual variances were generally larger in the presence of a predator, but this was valid for all strains. Therefore predation cannot have caused the observed pattern, i.e. the diminishing residual variance with increasing generations of domestication was exhibited in the presence and absence of a predator.

As an alternative, growth depensation (increasing growth variance with increasing competition) might have caused the observed variance pattern among strains. Before the start of the experiment, full-sibs were only exposed to within-family competition, during the experiment all individuals were additionally exposed to competition among individuals from different families and strains. Among-strain

differences of residual variances at the start of the experiment were indeed smaller than at the end of the experiment and differences between D3 and D5 were marginal (**Table S 2.2**). However, a trend of larger residual variance for the wild D0 strain relative to both domesticated strains was already present at the start of the experiment (**Table S 2.2**). This indicates that growth depensation might have increased variances for smaller individuals (as occurring in large frequencies in D0, in intermediate frequencies in D3, and in low frequencies in D5) relatively stronger than for large individuals, but it does not explain the differences present at the start of the experiment.

Table S 2.2: Overall variances and variances separately estimated in the presence (+) or absence (-) of a predator for body size and growth rate. Estimates are for Ln body mass (**A**) or Ln fork length (**B**). Given is each variance (Var, multiplied by 10^{-3}) or correlation (Cor) and its REML estimate of the standard error (SE, multiplied by 10^{-3})). Variances for size terms (terms excluding *time*) are based on Ln mass (in g) or Ln length (in cm) and specific growth rate terms (terms including *time*) are based on size*100*$D°^{-1}$. The correlation (ρ) characterises the residual correlation among individuals (replicates) within tank-by-family-by-time combinations. NA indicates that the variance converged to zero and no estimates are available. No attempt was made to re-parameterise the models as these non-estimable variances were small and had therefore little influence on residual variances.

Term	overall		predator +		predator -	
	Var or Cor	SE	Var or Cor	SE	Var or Cor	SE
A) Mass						
Tank	0.20	0.42	NA	NA	0.30	0.58
Tank:time	0.009	0.009	0.020	0.022	0.003	0.005
Family	24.6	10.4	26.5	11.8	23.5	10.3
Family:time	0.13	0.06	0.21	0.10	0.10	0.05
Tank:Family	5.4	1.1	5.7	1.7	4.4	1.4
D0 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	-0.14	0.01	-0.11	0.01
D0 residuals start	196.4	16.3	190.6	22.7	203.8	23.7
D0 residuals end	308.8	28.7	402.8	59.1	242.2	29.0
D3 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	-0.13	0.01	-0.12	0.01
D3 residuals start	161.7	13.4	154.3	18.3	169.4	19.9
D3 residuals end	229.9	19.6	305.0	37.5	158.1	18.8
D5 $\rho_{(Tank:Family:Time:replicates)}$	-0.11	0.01	-0.10	0.02	-0.13	0.01
D5 residuals start	146.5	12.0	152.7	17.8	141.2	16.6
D5 residuals end	137.4	11.7	183.2	22.4	93.4	11.1
B) Length						
Tank	0.03	0.05	NA	NA	0.03	0.04
Tank:time	0.0001	0.0004	0.001	0.001	NA	NA
Family	2.5	1.0	2.6	1.1	2.4	1.0

Continued on next page

Table S2B continued

Term	overall		predator +		predator -	
	Var or Cor	SE	Var or Cor	SE	Var or Cor	SE
Family:time	0.02	0.01	0.02	0.01	0.01	0.01
Tank:Family	0.54	0.12	0.63	0.18	NA	NA
D0 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.01	-0.14	0.01	-0.07	0.02
D0 residuals start	19.2	1.6	18.2	2.2	20.9	2.4
D0 residuals end	30.5	2.8	38.5	5.7	26.1	3.0
D3 $\rho_{(Tank:Family:Time:replicates)}$	-0.12	0.05	-0.12	0.01	-0.10	0.01
D3 residuals start	17.1	1.4	16.1	1.9	18.4	2.1
D3 residuals end	24.0	2.0	30.4	3.7	18.4	2.1
D5 $\rho_{(Tank:Family:Time:replicates)}$	-0.11	0.01	-0.10	0.02	-0.10	0.01
D5 residuals start	15.8	1.3	15.7	1.8	16.2	1.8
D5 residuals end	12.9	1.0	16.7	2.0	9.5	1.1

Chapter 3: The Genetic Architecture between Wild and Domesticated Atlantic Salmon: Analyses of Individual Growth, Sexual Maturation, and Environmental Plasticity

This chapter is under review as:
Debes PV, Fraser DJ, Yates M, Hutchings JA (201X) The Genetic Architecture between Wild and Domesticated Atlantic Salmon: Analyses of Individual Growth, Sexual Maturation, and Environmental Plasticity.

3.1. Abstract

The genetic architecture underlying population divergence in growth and maturation has not been studied in detail despite its central importance for understanding outbreeding depression and domesticated-wild hybrid fitness. Here, using Atlantic salmon (*Salmo salar*), we studied the genetic architecture associated with domesticated-wild divergence in: i) maturation probabilities at the same age; ii) size-at-age and growth, while accounting for maturity status and sex; and iii) growth plasticity in response to environmental factors. Our work examined two populations and their multi-generational hybrids in a common experimental arrangement in which salinity and quantity of suspended sediments were manipulated to mimic naturally occurring variation. Average specific growth rates were negatively affected by suspended sediments, and differed among crosses, maturity, sex, and cross-by-maturity groups. Domesticated-wild growth divergence was equal across environments but varied among maturity groups. Collectively our results revealed both additive and non-additive outbreeding effects for growth, size-at-age, and the presence of different sex- and size-specific maturation probabilities between populations. The major implication of our work is that estimates of the genetic architecture of growth and maturation can be biased if one does not simultaneously account for temporal changes in growth and different maturation probabilities between populations. This is because traits interact differently for each

population and across generations caused by non-additive effects and a likely independent genetic control for traits. Our results emphasize the challenges to predicting morphological and life-history trait changes resulting from between-population outbreeding.

3.2. Introduction

Growth rate, the increase in body size per unit time, can vary substantially within and among populations. It can be directly or indirectly linked to fitness through life-history traits such as age-at-maturity or fecundity (Roff 1992; Stearns 2000). Rapid growth can increase survival probability and enable early reproduction but is usually traded off against later maturity with a higher fecundity (Lester *et al.* 2004). Furthermore, plasticity in growth can serve as a buffer for responding to environmental stress (Wright 1932; Schlichting & Pigliucci 1998). Consequently, growth-specific maturation phenotypes within wild populations might be shaped by local adaptation (Law 1979), or by anthropogenic selection and exploitation (Hutchings & Fraser 2008; Enberg *et al.* 2012).

Intentional or unintentional anthropogenic translocations increase outbreeding among formerly isolated populations and can result in genotypic and phenotypic changes in local populations that affect individual fitness (Rhymer & Simberloff 1996; Allendorf *et al.* 2001). A common example is the escape of domesticated aquaculture fish into environments inhabited by their wild counterparts (Naylor *et al.* 2005; Morris *et al.* 2008). Domesticated fish are normally selected for rapid growth (Gjedrem 2000). In wild or hatchery populations, increased growth rate generally leads to younger age-at-maturity (e.g., Alm 1959; Thorpe *et al.* 1983; Taranger *et al.* 2010) and a concomitant reduction in somatic growth during gamete production. Thus, to make aquaculture production economical, rapid growth and late sexual maturity are, intentionally or unintentionally, selected for in combination by many breeders (Gjedrem 2000; Thorpe 2004; Taranger *et al.* 2010). Domesticated individuals resulting from such

selection programs can exhibit rapid growth rates and attain late sexual maturity contrary to naturally exhibited patterns.

The Atlantic salmon (*Salmo salar*, Linnaeus, 1758) is among the top ten aquaculture species in terms of worldwide production, with annual production since 2009 exceeding 1 million tonnes in its native range (FAO 2013). At the same time, many wild populations are in decline and some assessed as endangered (COSEWIC 2006; ICES 2010). Linked to domesticated-wild outbreeding, widespread changes to the neutral genetic population structure of wild populations have been reported (Bourret *et al.* 2011; Glover *et al.* 2012). Domesticated fish can also introduce allelic combinations into wild populations that change presumed wild-adapted traits, such as age-at-maturity, reducing fitness as a result (McGinnity *et al.* 2003).

Such trait-linked fitness consequences are predictable across offspring generations under an additive genetic architecture between populations. This is because the average offspring phenotype in each mixed-origin generation will be as similar to the average phenotype of each parental population as the proportions of their allelic contributions. However, when a non-additive genetic architecture is present, i.e. under the prevalence of dominance or epistasis, phenotypes and associated offspring fitness in different mixed-origin generations may not be as readily predictable (Lynch 1991). Furthermore, a non-additive genetic architecture can result in initially neutral or positive fitness effects (e.g., first-generation heterosis), allowing for the propagation of domesticated allelic combinations, which are followed by negative fitness effects in later generations (Edmands 2007). Hence, a non-additive genetic architecture might bear the greatest threat to the persistence of wild populations, especially those already experiencing decline. Accordingly, the knowledge of the between-population genetic architecture is crucial in predicting trans-generational fitness consequences arising from outbreeding.

The genetic architecture of divergence in domesticated-wild growth has been investigated in several studies on fishes, but with disparate conclusions. Some studies concluded that growth between-populations has an overall additive genetic basis (Tymchuk & Devlin 2005; Tymchuk *et al.* 2006; Fraser *et al.* 2010) while

others detected non-additive genetic components for growth (McClelland *et al.* 2005; Tymchuk *et al.* 2007; Vandersteen *et al.* 2012). The presence of non-additive components is supported by transcript-level studies between domesticated and wild populations (Roberge *et al.* 2008; Normandeau *et al.* 2009; Debes *et al.* 2012) but the relationship between transcriptional and morphological phenotype are still largely unknown (Gibson & Weir 2005). Given these disparate conclusions, the genetic architecture of the differences in morphological phenotypes between domesticated and wild populations remain unknown in fishes.

Complicating matters further, results from studies of between-population genetic architecture, based on overall-population growth phenotypes, are not easily interpreted. This is especially true for indeterminate growers, such as fishes, that do not exhibit a genetically determined final size that can be analysed without confounding genotypes with other temporally acting growth-related factors. For example, between-population dominance for growth can fluctuate between populations across time (McClelland *et al.* 2005) and the genetic architecture can differ between being additive and non-additive with age and environment (Vandersteen *et al.* 2012). Incongruence in the genetic architecture among studies might be partly explained by differences in population-specific growth and maturation patterns. However, within-study incongruence is less clear and might be explained by temporally differential growth expression. Temporally changing growth can be caused by transitions between life-history stages, can differ by sex, or vary among environments (Parker & Larkin 1959; Winkelman & Peterson 1994; Gjedrem 2000; Tymchuk *et al.* 2007). Therefore, estimates of growth and its genetic architecture might have been misleading as factors other than genotype were largely unaccounted for.

It has been suggested that growth be considered as a continuous function of each individual growth trajectory (function-valued traits; reviewed by Stinchcombe *et al.* 2012) which allows for the decomposition into different growth types associated with any growth-related factor. Yet, analyses of individual-based trajectories are still rare in ecology, evolution, and genetics (Stinchcombe *et al.* 2012). This is surprising, because decomposition into growth-related factors enables

disentangling genotypic from other effects on growth, and increases inferential confidence.

The present study investigates the between-population divergence between a wild, endangered *S. salar* population and its locally occurring domesticated counterpart. Individuals of the wild population mature earlier and grow slower relative to those of the domesticated population (present study; Fraser *et al.* 2010). We describe body sizes and maturation probabilities among the two populations and three multi-generational crosses between them to investigate individual growth trajectories in response to a 2 x 2 factorial design of artificial environments (presence/absence of natural suspended sediments; two water salinities). These four controlled environmental conditions were tested because they mimic water conditions of river (fresh, clear), estuary (fresh, turbid; salty, turbid), and the sea (salty, clear), all of which are experienced by the wild population during its migration between river and sea. We decomposed differences in growth trajectories underlying environmental, life stage, and genotypic influences by using mixed model analyses. Our study aimed to provide insight into the genetic architecture associated with domesticated-wild divergence in i) maturation probabilities at the same age, ii) size-at-age and growth while accounting for maturity status and sex, and iii) growth plasticity in response to environmental factors. While we did not directly test fitness consequences of domesticated-wild outbreeding, this study allows for the prediction of potential multi-generational fitness consequences by making inferences about the detailed between-population genetic architecture of the investigated traits. We do this by adopting a novel approach that combines cross means analysis and that of individual growth trajectories.

3.3. Material and Methods

3.3.1. Populations

The wild population (WW) originated from the Stewiacke River (45.140 °N, -63.377 °W), Canada, and the domesticated population (DD) originated from the Saint John River (45.267 °N, -66.067 °W), Canada. The wild population is part of the

endangered inner Bay of Fundy (iBoF) meta-population (COSEWIC 2006). The grandparents of our WW base population were likely caught as juveniles in the wild (O'Reilly & Harvie 2009). The source-strain of the DD fish was founded in 1989 on 50 to 100 wild-caught individuals and underwent three generations of individual-based selection for rapid growth (Glebe 1998) until providing breeders for our DD base population. The parents of both parental populations and their reciprocal first-generation hybrids (F1 hybrids) were created in 2001 (Lawlor *et al.* 2008; Fig. 1) and were grown to maturity at Dalhousie University, Halifax, Canada. This laboratory-raised 2001 generation provided the parents for the 2005 fish

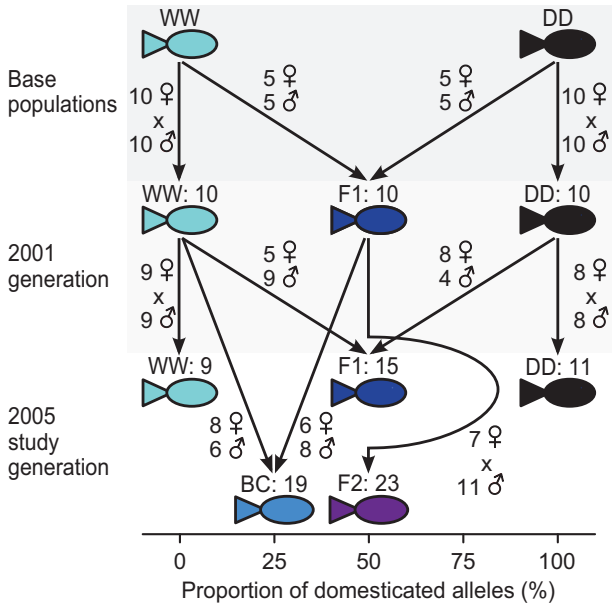

Figure 3.1: Graphical representation of the multigenerational crossing design between wild and domesticated *Salmo salar*. The number of generated families are indicated for each generation after the colon following each cross abbreviation with wild, WW (turquoise); reciprocal wild backcross, BC (light blue); reciprocal first-generation hybrid, F1 (dark blue); second-generation hybrid, F2 (violet); domesticated salmon, DD (black). Also indicated are the number of dams (Venus symbol) and sires (Mars symbol) used to generate each cross in a given generation.

generation used in this study (**Figure 3.1**).

3.3.2. Crossing Design, Pre-Experimental Conditions

In 2005, WW, DD, and F1 parents were interbred, creating the crosses of WW, DD, reciprocal F1, second-generation hybrid (F2 = F1xF1), and the reciprocal wild backcross (BC = F1xWW). Crosses were created as either full-sib families or as a mix of full-sib and half-sib families (**Figure 3.1**) and parents were re-used within and between crosses whenever possible. Inbreeding up to cousins was avoided by genotyping (see Fraser *et al.* 2010 for details). All offspring families were grown in a common laboratory. Individuals from each cross were kept from five months after initiating of feeding onwards in at least four different tanks, as described by Fraser *et al.* (2010).

3.3.3. Experimental Protocol

In July 2008, parr (freshwater individuals prior to seaward migration) were separated from smolts (individuals physiologically capable of migrating to the sea) based on external criteria (parr markings vs. silver colouration in smolts) with only smolts retained for the experiment. In September 2008, 200 randomly selected individuals from each cross were anesthetized, using eugenol, measured (wet mass $\pm$ 5 g, fork length $\pm$ 1 cm), and tagged on both sides of the head with individual alpha-numerically marked VIalpha tags (Northwest Marine Technology, USA). After a 28-day recovery period, fish were again anesthetized, identified, measured, and distributed among eight round tanks (1800 L, flow through system) with 25 fish of each of the five crosses per tank (totalling 1000 fish). For each cross, an equal size-distribution was allocated to each tank to avoid a possible cross-by-size bias among tanks. The amount of human disturbance, illumination (natural photoperiod), water quality, flow, temperature, and oxygen saturation were kept constant across tanks with daily correction-adjustments.

After a recovery period of five days, a 2 x 2 factorial arrangement of the categorical environmental factors *Salinity* and *Sediment* was randomized to the eight tanks. For *Salinity*, four of the tanks received either fresh water (level fresh: S = 0 PSU) or brackish water (level salt: S = 18 psu). For *Sediment*, the assigned tanks

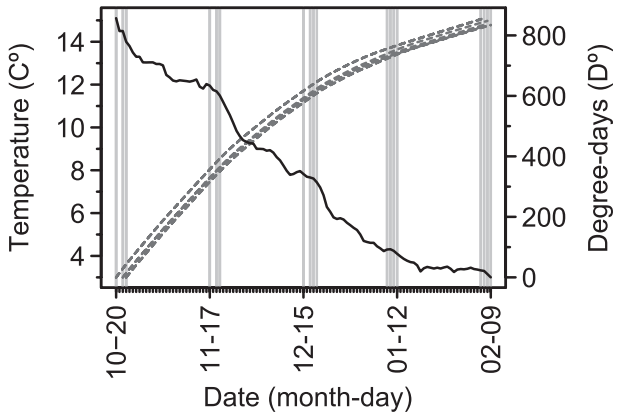

Figure 3.2: Chronology of sampling events and water temperature. Shown are average daily temperatures across all tanks (solid black line), cumulative degree-days for each tank (dotted, dark grey lines) and data for the five sampling periods (vertical light grey lines) across the duration of the growth experiment between October 2008 and February 2009.

received daily either a pulse of suspended sediments poured in by hand (level sediments: 200 mg*L^{-1} with a turbidity of 32 NTU at S = 18 psu) or clear water poured in by hand (level clear, turbidity of 0 NTU). The applied intertidal sediments were previously collected from upper mudflats of the iBoF and had been air-dried. The suspended sediment concentration applied corresponded to that naturally occurring in the iBoF (Gordon 1994), but decreased after the application with a half-time of 1.8 h. Water temperature (range: 3-15.1° C, mean: 7.8° C, **Figure 3.2**), oxygen saturation (range: 79-97%, mean: 91%), and salinity (range: 0-18.9 psu) were measured daily for each tank. Once daily after the sediment application, fish were fed *ad libitum* with four sizes of commercial pellet feed (Corey Aquafeeds, Canada) to accommodate all fish sizes. The daily feed amount, equal for each tank, was determined on the basis of fish-feeding behaviour. All individuals were anesthetized, identified, and measured as previously at 26 to 28-day intervals until the end of the experiment (**Figure 3.2**). Mortalities were replaced with same-cross individuals for which no data were recorded. At the termination of the experiment, immature and mature individuals and sex of mature individuals were readily identified by gamete stripping.

3.3.4. Statistical Analyses

Data from dead individuals those that had lost their identification tag were excluded, yielding data on 934 individuals. We first evaluated if excluded data or the (unplanned) incidences of sexual maturation by sex had the potential to cause any analytical bias by inequality of individual counts for levels of the experimental design. We analysed log-transformed (Ln) number of individuals per *Cross*-by-*Maturity* level in each tank (15 x 8 levels) for the factorial predictors of *Salinity*, *Sediment*, *Cross*, *Maturity*, and all possible interactions. A linear mixed model (LMM) was used with tank identification (*Tank*) as a random effects term. Significance ($P < 0.05$) of fixed effects terms was evaluated by conditional Wald *F*-tests, adjusted according to Kenward and Roger (1997).

3.4.4.1 Maturation Probabilities

Occurrence of sexual maturation was combined for both sexes and was taken as the probability of a binary variable (mature vs. immature) that is a function of *Cross* and baseline body length (i.e., length at the start of the experiment). Maturation in *S. salar* is usually affected by processes occurring about 6-12 months pre-dating spawning time (reviewed by Thorpe *et al.* 1998) - a time-frame which pre-dated our experimental manipulations that coincided with spawning time - but can be controlled later in males (Fjelldal *et al.* 2011). We thus also tested if maturation probability was influenced by our experimental manipulations. Generalized linear models (GLMs) with logit-link function and binomial residual distribution were used for analyses of maturation probability. In these models, we assessed influences of *Cross*, *Salinity*, and *Sediment* as fixed effects factors, baseline length (Ln-transformed, mean centred) and a corresponding squared term as fixed continuous covariates, and all possible interactions. Initially, we also tested for among-tank variation by including the random effects term *Tank* in a generalized linear mixed model (GLMM). In these models, we assessed influences of *Cross*, *Salinity*, and *Sediment* as fixed effects factors, baseline length (Ln-transformed, mean centered) and a corresponding squared term as fixed continuous covariates, and interactions of *Cross* with both length covariates. Initially, we also tested for among-tank

variation by including the random effects term *Tank* in a generalized linear mixed model (GLMM).

3.4.4.2 Effects of Cross, Maturity, and Environments on Size-at-Age and Growth

LMMs were used to investigate growth of either mass or length for all levels of the design, including interactions. The experiment was analysed as a completely randomized split plot design in which tanks represented experimental main units (main plot) to which the levels of the 2 x 2 factorial of *Salinity*-by-*Sediment* (environments) were randomized. Individuals were regarded as experimental sub units (sub plots) to which the levels of the 5 x 3 factorial of *Cross*-by-*Maturity* (genotypes) were randomized. In each growth model, the randomisation of environments and its correlation across time was accounted for by including the random effects terms *Tank* and *Tank*-by-*Time*. Mean-centred cumulative degree-days (D°, averaged across tanks for each sampling period) was used as a continuous *Time* covariate because thermal units predict growth in poikilothermic fish better than calendar days (Neuheimer & Taggart 2007) and ambient-based water temperature changed temporally (**Figure 3.2).**

Mass and length were transformed (Ln), normalising residual distributions and meeting the assumption of a linear relationship between time and size-proportional growth, and normality assumptions for the generation mean analysis. Furthermore, geometric group means were closer to original-scale group medians than arithmetic means, indicating a better representation of population means on the Ln-transformed scale.

Linear modelling of growth by the use of Ln-transformed sizes usually assumes the presence of a growth rate that is proportional to body size and that there is no change in that proportion with increasing size, age, ecological or physiological changes, i.e., it represents the observed specific growth rate (SGR). Such a constant exponential individual growth rate is likely only valid for a limited life period in young fish (Hopkins 1992). In our study, a graphical examination of growth trajectories indicated deviations from linear trends of SGRs, some of which varied across time and at several group levels of the design (see results). We were

primarily interested in comparing the linear slopes of the fixed part of the models among environmental and genotypic groups (i.e., group-specific SGRs). Therefore, we modelled non-linear growth components non-parametrically by fitting random cubic splines and random non-smooth trend deviations to all longitudinal group and unit terms (Verbyla *et al.* 1999; Welham 2009). As a result, SGR was allowed to vary among measurement intervals, and was estimated as an interval-duration weighed average for each group as an approximation to non-linear trajectories.

The model structures for among-individual (co)variances across time were chosen among 13, partly non-nested, covariance models by using the Bayesian information criterion (BIC; **Table S 3.3**). For each trait and each of the 13 models, we evaluated if modelling the (co)variances separately for each of the 15 *Cross*-by-*Maturity* levels improved the fit by using likelihood ratio tests (LRTs) between nested models. This was conducted to test *a priori* assumptions of heteroscedasticity among *Cross*-by-*Maturity* levels as a consequence of population crossing (Hayman 1958; Mather & Jinks 1982; Piepho & Möhring 2010), general population variance divergence, and population variance divergence as caused by changes of gene expression accompanying sexual maturation.

3.4.4.3 Cross Means Analysis

The between-population genetic architecture for size at mid-experimental degree-days (size-at-age) and for SGRs was investigated for both mass and length, using cross means analysis. We estimated the overall mean ($\hat{\mu}$), the diallelic additive ($\hat{d}$), and the diallelic dominant ($\hat{h}$) outbreeding effects. Further, we estimated three digenic, diallelic epistatic effects: additive-by-additive ($\hat{\imath}$), additive-by-dominant ($\hat{\jmath}$), and dominant-by-dominant ($\hat{l}$). Effects were assessed by LMMs, similar to the approach taken by Piepho and Möhring (2010) but extended to a longitudinal approach, and using effect coefficients following Hayman (1958). Results of overall growth models indicated differences in smooth and non-smooth deviations from linear growth trajectories among maturity groups. Furthermore, differences in sample sizes for Cross-by-Maturity across environments were detected (see results), so analyses were conducted separately for each level of *Maturity*. This

ensured a better representation of the covariance structures, and hence validity of the inference, and also reduced a potential bias of predicted marginal cross means by *Maturity* when averaging across environments. To allow for testing of the significances of the outbreeding effects for SGRs (= model slopes), intercepts were allowed to vary by *Cross*, and to allow testing of outbreeding effects for sizes (= model intercepts), slopes were allowed to vary by *Cross*. Estimating effects for SGR and size-at-age simultaneously resulted in conflicts because model effects for size-at-age influence those for SGR, and vice versa. We did not assess the environmental plasticity of the between-population genetic architecture for the investigated traits because no *Cross*-by-environment interactions were detected by any *Maturity*-specific model.

The fit of each outbreeding effect model was assessed by including a lack-of-fit-term (i.e., slope or intercept *Cross* term). First, we tested if $\hat{\mu}$ and $\hat{d}$, and then if $\hat{\mu}$, $\hat{d}$, and $\hat{h}$ predicted cross means, which we defined as obtaining a $P > 0.05$ for the lack-of-fit term (conditional Wald *F*-tests, adjusted as above and under which the factorial lack-of-fit term was marginal to continuous outbreeding effects terms). When all simple effects resulted in a lack-of-fit, epistatic effects were fitted (Hayman 1958). Five cross means allowed only for simultaneously fitting up to four outbreeding effects per assessed model term, leaving one degree of freedom for testing. This made it necessary to assess the fit of epistatic effects sequentially. Non-significant effects were removed from all final models.

3.4.4.4 Model Fitting and Hypothesis Testing

Analyses by GL(M)Ms were conducted under Laplace approximation to the likelihood using the R-package glmmADMB (Skaug *et al.* 2012) and analyses by LMMs were conducted under Residual Maximum Likelihood using *ASReml-R 3.0* (Butler *et al.* 2009) executed in *R 2.15.3* (R Core Team 2013).

First, the random part of each model was fitted while including all possible fixed effects terms. At this stage, we selected the among-individual covariance structure of growth models, while keeping all possible other random effects not converging to zero in the model. In the next step, for a model with chosen among-individual

covariance structure, all other random components not different from zero (LRTs; positively constrained variances $P > 0.1$, unconstrained covariances $P > 0.05$) were removed, except for both *Tank* error terms and the overall spline term. Random spline terms were tested prior to random non-smooth trend deviations (Verbyla *et al.* 1999; Welham 2009). Among-tank variance (major whole plot errors) tended to converge to zero when positively constrained and were therefore set to be unconstrained for fixed effects hypothesis testing and marginal predictions (Nelder 1954; Molenberghs & Verbeke 2011).

Second, the fixed part of each model was fitted with constant random effects terms. Non-significant fixed effects terms ($p > 0.05$; LMMs: conditional Wald *F*-tests, corrected after Kenward and Roger (1997), GLMs: LRTs) were stepwise removed, highest order first, unless their removal violated marginality (Nelder 1994). Distribution and homoscedasticity of model residuals and other random effects were validated using diagnostic plots. Tests of multiple fixed effects contrasts were conducted by Student's t-tests in which degrees of freedom were approximated as for *F*-tests and p-values were Bonferroni-adjusted.

3.4. Results

For 934 retained individuals (numbers per experimental level in supplementary **Table S 3.1**), significant differences in numbers by *Cross*, by treatments, or for their interactions were not observed (supplementary **Table S 3.2**). Omitted individual data were due to missing identifications (56 out of 1000 individuals), lack of growth (one likely sick individual), and altogether nine mortalities from all five crosses and four environments. However, maturity groups which were not under experimental control were represented by different frequencies (*Maturity*: $F_{2,56} = 48.8$, $P < 0.001$), and these additionally differed among crosses (*Cross*-by-*Maturity*: $F_{8,56} = 20.6$, $P < 0.001$). Furthermore, this differential *Cross*-by-*Maturity* representation varied across treatments (*Salinity*-by-*Sediment*-by-*Cross*-by-*Maturity*: $F_{8,56} = 2.9$, $P = 0.008$).

3.4.1. Maturation Probability

Maturation probability (mature vs. immature) was not affected by the interaction of *Cross* with length, by *Salinity* or *Sediment* environments. Furthermore, among-tank variance was not different from zero ($\chi_1^2 = 0$, p = 1). Hence, maturation probability was regarded as an observational trait and estimated with different intercepts only among levels of *Cross* and as curves that underlie a common function of fork length. Under the final model, maturation probability generally increased with length ($\chi_1^2 = 82.8$; $P < 0.001$), albeit a small increase in probability at the smallest sizes was accounted for by the squared length term ($\chi_1^2 = 12.4$, $P < 0.001$; **Figure 3.3A**). Maturation probability at the overall-baseline initial length (30.7 cm) differed among crosses ($\chi_4^2 = 121.5$, $P < 0.001$) and was higher for wild *S. salar* than for all other crosses, which had very similar maturation probabilities (**Figure 3.3A**).

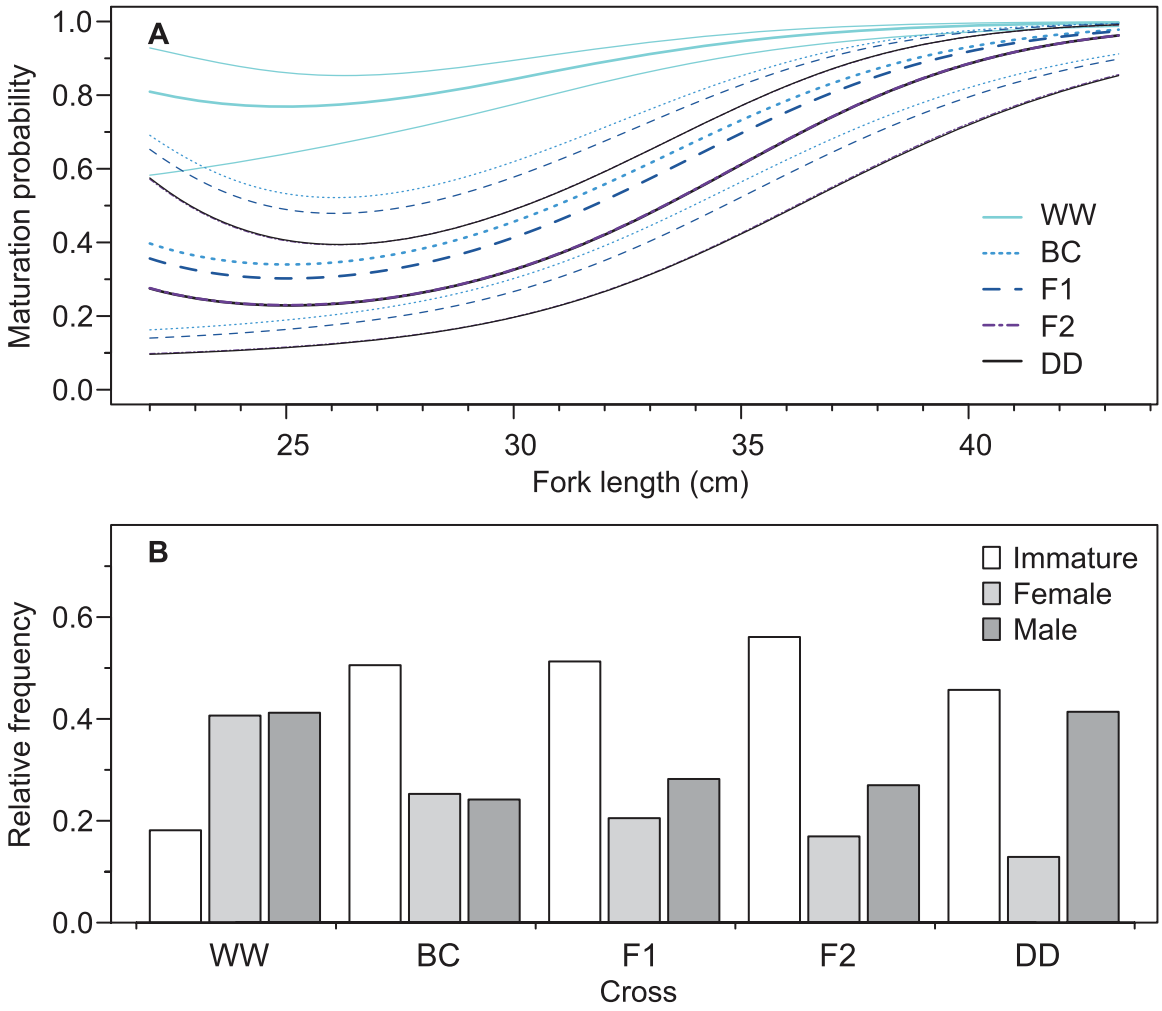

Figure 3.3: Predicted combined maturation probability and observed frequencies of maturity groups. In **A** the predicted maturation probabilities across sexes for each cross are shown as a function of retransformed fork length at the start of the experiment. Line colours and types are differentiating among the crosses as given in the key and pale lines show respective approximate 95% CI. Lines for DD and F2 have equal positions. In **B** the observed relative frequency for each of the three maturity groups is shown for each of the five crosses between wild and domesticated *S. salar*. All abbreviations are as in **Figure 3.1**.

When overall frequencies of sex among mature individuals were investigated, a sex-bias towards mature males occurred in most crosses, except for WW and BC with equal frequencies, and this bias was highest in DD (**Figure 3.3B**). Under the assumption of within-cross equality of sex frequency (including the unknown sex of immature individuals), presumed female maturation probabilities appeared to be close to additive with the highest probability in WW (**Figure 3.3B**). In contrast, the

presumed male maturation probabilities were equal in both parental populations at about 40%, whereas probabilities appeared to be 25-35% lower in all mixed-origin crosses (**Figure 3.3B**).

3.4.2. Growth Models

For all investigated among-individual covariance models, separation of the covariance structure for *Cross*-by-*Maturity* groups improved the model fit despite the 'blow up' of number of estimated parameters (LRTs; all $P < 0.001$; supplementary **Table S 3.3**). Among the 13 different covariance structures and for traits of body mass and fork length, the random coefficient model (i.e., individual intercept and slope variances plus their covariance) with additional random individual splines was selected by BIC (with 60 among-individual (co)variance parameters). In comparison, the more parameter-generous Akaike information criterion selected the third order antedependence model for mass and the unstructured model for length (210 and 225 among-individual (co)variance parameters, respectively). Residuals for RCS models were taken as identical and independent, as it is convention when using individual-based splines (Welham 2009).

Table 3.1: Retained fixed effects terms in the final models (overall maturity groups) for either body mass or fork length of crosses between wild and domesticated *Salmo salar*. Interaction terms are indicated by a connecting colon. Degrees of freedom (df) and denominator df (ddf) are given along with their *F* and p-values.

		Mass			Length		
Term	**df**	**ddf**	***F***	**P**	**ddf**	***F***	**P**
Sediment	1	4	6.4	0.065	4.5	6.4	0.052
Salinity	1	4	0.5	0.536	4.4	1.0	0.367
Cross	4	434.2	111	<0.001	422.6	199	<0.001
Maturity	2	230.1	42.1	<0.001	388.5	24.8	<0.001
Time	1	5.2	>1000	<0.001	1.8	>1000	0.001
Salinity:Cross	4	440.2	0.9	0.480	431.4	0.7	0.623
Cross:Maturity	8	232.9	1.8	0.078	233.6	1.6	0.120
Sediment:Time	1	6.4	150	<0.001	11.2	21.3	<0.001
Salinity:Time	1	6.7	0.5	0.522	9.3	0.2	0.636
Cross:Time	4	443.6	111	<0.001	390.2	58.8	<0.001
Maturity:Time	2	5.8	74.0	<0.001	108.9	547	<0.001
Salinity:Cross:Time	4	456.1	4.3	0.002	438.1	3.0	0.019
Cross:Maturity:Time	8	246.1	5.2	0.000	214.4	2.3	0.024

Among fixed effects terms, most of the higher-order interactions were non-significant and therefore removed. Remaining terms were equal between overall models for both traits (**Table 3.1**). Also, overall and *Maturity*-specific models mostly agreed with each other for fixed terms (**Table 3.1, Table 3.2**). Two exceptions were present. First, a significant *Salinity*-by-*Cross*-by-*Time* interaction in overall models of both traits was absent in all *Maturity*-specific models. Second, the significant effect for *Sediment*-by-*Time* in the overall model for length was non-significant for the female-specific model ($F_{1,7.6} = 4.6$, $P = 0.065$), and therefore removed from this model together with its non-significant intercept term.

Random effects terms modelling smooth and non-smooth deviations from linear group-level trajectories differed between the overall and the *Maturity*-specific models, and also between *Maturity*-specific models for mass or length (supplementary **Table S 3.4**, **Table S 3.5**). The estimates of among-individual (co)variances for random coefficients gave additional insight into growth

differences among crosses. The covariance between variation in individual size-at-age (intercept variance) and individual SGR (slope variance) can be expressed as a correlation (ρ). As we estimated parameters for random coefficients separately for each *Cross*-by-*Maturity* group in each model, we were able to compare ρ. For immature individuals, for which growth patterns during the study were likely least affected by maturation processes, a divergent pattern for ρ could be observed between DD and F1 crosses vs. all other crosses. For mass, DD and F1 exhibited moderate and positive correlations between size-at-age and SGR while all others did not exhibit indications for any correlation as covariances were not different from zero (**Table 3.3,** supplementary **Table S 3.4, Table S 3.5**). For length, correlations were lower and based on non-significant covariances for the former two crosses while among the latter significantly negative covariances and associated moderate and negative correlations were exhibited for BC and F2 (**Table 3.3**).

Table 3.2: Retained fixed effects terms in the final maturity-group-specific models for either body mass (**A**) or fork length (**B**) of crosses between wild and domesticated *Salmo salar*. Interaction terms are indicated by a connecting colon. Degrees of freedom (df) and denominator df (ddf) are given along with their *F* and p-values.

		immature			females			males		
Term	**df**	**ddf**	***F***	**P**	**ddf**	***F***	**P**	**ddf**	***F***	**P**
A										
Sediment	1	5.2	1.7	0.251	4.0	0.2	0.694	6.2	0.4	0.556
Cross	4	153.1	32.4	<0.001	83.3	35	<0.001	134.1	37.0	<0.001
Salinity	1	4.6	1.7	0.251	-	-	-	-	-	-
Time	1	6.7	>1000	<0.001	6.3	>1000	<0.001	6.8	928	<0.001
Sediment:Time	1	6.8	234	<0.001	6.0	47.9	<0.001	6.7	9.6	0.017
Salinity:Time	1	3.7	30.1	0.007	-	-	-	-	-	-
Cross:Time	4	140.6	74	<0.001	78.9	19.1	<0.001	133.2	30.7	<0.001
B										
Sediment	1	4.7	7.5	0.041	-	-	-	5.5	0.2	0.642
Salinity	1	4.8	0.1	0.804	-	-	-	-	-	-
Cross	4	154.4	78.7	<0.001	*83.2	59.4	<0.001	132.8	60.5	<0.001
Time	1	0.7	>1000	0.016	*9.3	520	<0.001	13.5	>1000	<0.001
Sediment:Time	1	5.3	28	0.003	-	-	-	10.8	21.3	0.001
Salinity:Time	1	6.0	6.7	0.041	-	-	-	-	-	-
Cross:Time	4	141.2	35.1	<0.001	*77	8.3	<0.001	126.1	17.2	<0.001

*The whole plot Tank error term was negative, exceeded the residual variance, and was omitted as this made the model non-estimable. Note that in the same model whole plot treatment terms were non-significant and therefore also omitted.

Table 3.3: Among-individual (co)variances (± standard errors) for size-at-age (random intercepts), specific growth rate (SGR; random slopes), and the covariance between individual size and individual SGR for immature individuals of each cross and for traits of either body mass (**A**) or fork length (**B**). For each covariance, the probability of being different from zero (P, two sided test) was approximated by likelihood ratio tests for which we also reported the test statistic (χ^2). The relationship between individual sizes and SGRs are also reported as cross-specific correlation (ρ). All variances were constrained to be positive for these tests.

Cross	Var(size)	*Var(SGR)	*Cov(size,SGR)	ρ	χ_1^2	P
A						
WW	0.082 ± 0.021	0.024 ± 0.006	0.021 ± 0.081	0.05	0.1	0.792
BC	0.086 ± 0.013	0.027 ± 0.004	-0.028 ± 0.052	-0.06	0.3	0.591
F1	0.139 ± 0.020	0.027 ± 0.004	0.209 ± 0.065	0.34	11.9	<0.001
F2	0.081 ± 0.011	0.020 ± 0.003	0.021 ± 0.066	0.03	0.1	0.739
DD	0.070 ± 0.011	0.041 ± 0.006	0.179 ± 0.062	0.34	9.7	0.002
B						
WW	0.0065 ± 0.0016	0.0026 ± 0.0007	-0.0046 ± 0.0074	-0.11	0.4	0.536
BC	0.0082 ± 0.0012	0.0014 ± 0.0002	-0.0096 ± 0.0038	-0.28	6.8	0.009
F1	0.0120 ± 0.0017	0.0013 ± 0.0002	0.0066 ± 0.0042	0.16	2.6	0.108
F2	0.0079 ± 0.0011	0.0011 ± 0.0009	0.0098 ± 0.0031	-0.33	11.9	<0.001
DD	0.0059 ± 0.0009	0.0022 ± 0.0003	0.0046 ± 0.0040	0.13	1.3	0.250

*All values for SGR and covariances have been multiplied by 100 to facilitate printing.

3.4.2.1. Effects of Environment

Effects in response to any of the factorial environmental treatments on either mass-at-age or length-at-age (age at mid-experimental degree-days) were non-significant, but significant effects on SGRs were detected. Salinity had no overall effect on SGRs of mass and length, but *Cross*-by-*Salinity* effects on SGR for both traits were detected (**Table 3.2, Figure 3.4A & C**). This was exhibited as *Salinity* effect on SGR that was different from zero only in F1 hybrids (five pairwise contrasts, F1: $t_{176.8} = 3.0$, $P_{adjusted(x5)} = 0.008$; all others: $t_{127.9 \text{ to } 154.6} = 0.3$ to 1.8, $P_{adjusted(x5)} = 0.205$ to 1) for which the overall SGR in salt water was 22% and 11% higher (mass, length) relative to fresh water. Suspended sediments had effects on SGRs of both

traits that were similar among crosses (**Table 3.2**). In the presence of suspended sediments, average SGR was 25% and 19% lower (mass, length) relative to the clear environment. However, graphical examination of average growth trajectories for treatments deviating non-parametrically from linear trajectories allowed the identification of two details (**Figure 3.4A & C**). Firstly, the presence of a response delay relative to treatment start could be identified. Secondly, the presence of an only temporary reduction in SGR in response to suspended sediments could be identified which appeared to have been shorter but stronger for mass than for

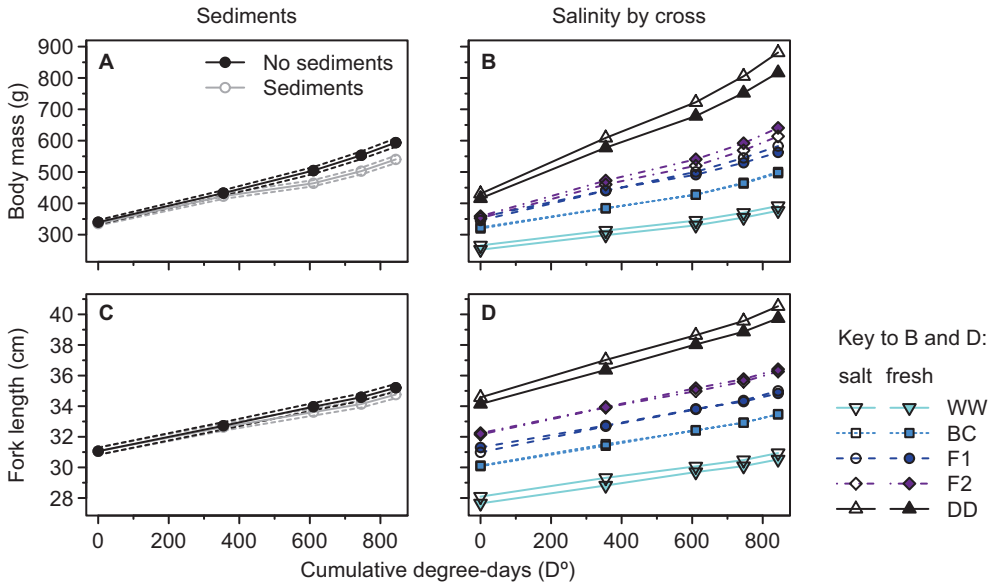

Figure 3.4: Effects of environments on growth trajectories of body mass and fork length. Marginal predictions for growth trajectories of retransformed body mass (**A**) and fork length (**C**) averaged across crosses and maturity groups is shown in the absence and presence of a daily pulse of suspended sediments, differentiated by line colour as indicated in the key of A. Dashed lines represent approximate 95% confidence intervals. Retransformed, predicted average growth for each of the five crosses is shown for retransformed body mass (**B**) and fork length (**D**) in fresh water and salt water, differentiated by colours, symbols and line types as indicated in the key. All cross abbreviations are as in **Figure 3.1**.

length (**Figure 3.4A & C**).

3.4.2.2. Effects of Cross and Maturity Group

As *Cross*-by-*Maturity* effects were detected for both size-at-age and SGR, these results are reported for the *Maturity*-specific models. The order among crosses for size-at-age and SGR of both traits was constant during the experiment with DD>F2>F1>BC>WW (**Figure 3.5**). Average size-at-age for both traits was considerably smaller for WW individuals within each maturity group than for individuals of all other crosses, except for male BCs (**Figure 3.5, Figure 3.6**). Initially, mature individuals were larger than immature individuals. Mature individuals exhibited lower SGRs than immature individuals and nearly linear average growth trajectories on the original scale. In contrast, initially smaller, immature individuals exhibited exponential-like growth trajectories on the original scale. As a consequence, patterns for initial and final sizes among maturity groups differed (**Figure 3.5**).

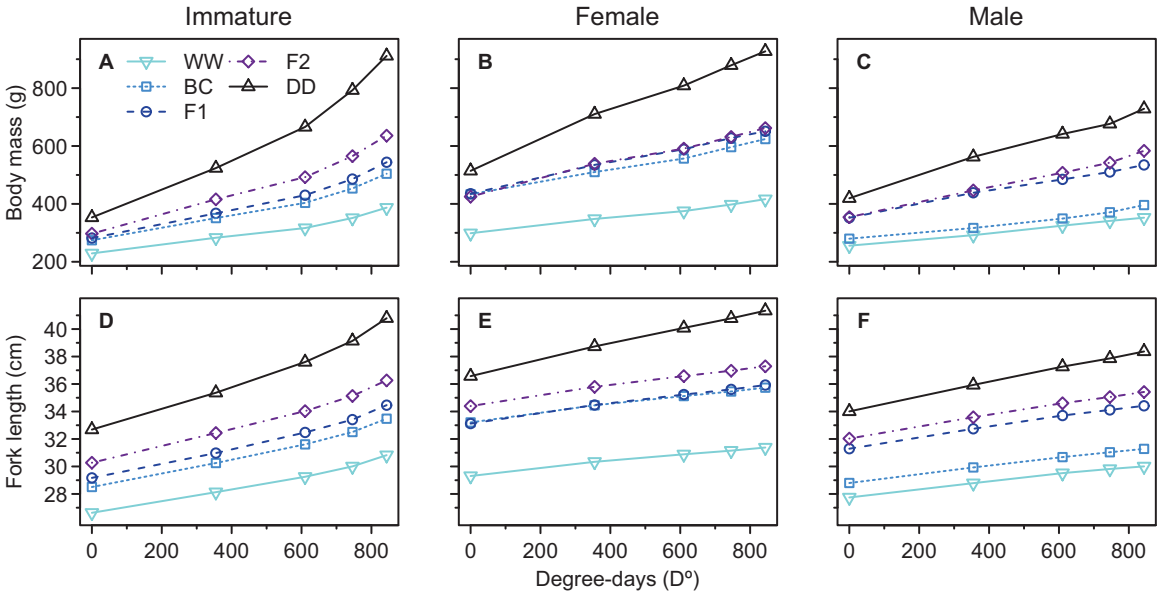

Figure 3.5: Effects of cross and maturity group on growth trajectories of body mass and fork length. Marginal predictions of growth for retransformed body mass (**A**, **B**, **C**) and fork length (**D**, **E**, **F**) averaged across all environments is shown for immature individuals (**A** & **D**), mature females (**B** & **E**) and mature males (**C** & **F**). Growth trajectories for crosses are differentiated by colours, line and symbol types as indicated in the key of **A**. All cross abbreviations are as in **Figure 3.1**.

Table 3.4: Estimated average outbreeding effects for size-at-age of body mass (Ln of g) and SGR of body mass (% $°D^{-1}$), their standard errors (SE), and their probabilities (P) for being different from zero at given denominator degrees of freedom (ddf) and associated *F*-values for either immature (**A**), female (**B**) or male (**C**) Atlantic salmon. The predicted F2 reference mean is also given for each trait.

Effect	Mean ± SE	ddf	$F_{1,ddf}$	P	Lack-of-fit
A immature					
$\hat{\mu}$ (size)	6.137 ± 0.026	-	-	-	
$\hat{d}$ (size)	-0.333 ± 0.026	94.2	167.5	<0.001	$F_{1,178}$ = 1.0,
$\hat{h}$ (size)	-0.125 ± 0.046	192.1	7.2	<0.001	P = 0.311
$\hat{\imath}$ (size)	-0.190 ± 0.073	225.6	6.8	0.010	
$\hat{\mu}$ (SGR)	0.0899 ± 0.0012	-	-	-	
$\hat{d}$ (SGR)	-0.0258 ± 0.0016	127.9	261.1	<0.001	$F_{1,160.4}$ = 0.4,
$\hat{h}$ (SGR)	-0.0118 ± 0.0023	160.7	27.0	<0.001	P = 0.517
$\hat{\imath}$ (SGR)	-0.0142 ± 0.0039	193.1	13.5	<0.001	
B female					
$\hat{\mu}$ (size)	6.295 ± 0.016	-	-	-	$F_{3,77.2}$ = 0.6,
$\hat{\imath}$ (size)	0.370 ± 0.028	64.8	177	<0.001	P = 0.618
$\hat{\mu}$ (SGR)	0.520 ± 0.0012				$F_{3,83.7}$ = 1.7,
$\hat{d}$ (SGR)	-0.0134 ± 0.0016	90.4	70.9	<0.001	P = 0.164
C male					
$\hat{\mu}$ (size)	6.082 ± 0.018	-	-	-	$F_{3,110}$ = 2.3,
$\hat{d}$ (size)	-0.334 ± 0.025	161.9	181	<0.001	P = 0.085
$\hat{\mu}$ (SGR)	0.0592 ± 0.0021	-	-	-	
$\hat{d}$ (SGR)	-0.0351 ± 0.0064	88.6	30.6	<0.001	$F_{1,96.9}$ = 1.0,
j (SGR)	-0.0229 ± 0.0065	95.7	12.5	0.001	P = 0.320
$\hat{l}$ (SGR)	-0.0073 ± 0.0024	80.9	9.6	0.003	

The effects are predicted F2 cross mean ($\hat{\boldsymbol{\mu}}$), and additive ($\hat{\boldsymbol{d}}$), dominant ($\hat{\boldsymbol{h}}$), additive-by-additive ($\hat{\boldsymbol{\imath}}$), additive-by-dominant ($\hat{\boldsymbol{\jmath}}$), and dominant-by-dominant ($\hat{\boldsymbol{l}}$) outbreeding effects.

3.4.3. Cross Means Analysis of Size and Growth

All *Maturity*-specific cross means were predicted by averaging across environments as no interaction effects with *Cross* were significant (**Table 3.2**). Cross means of immature individuals for SGRs of both traits and cross means for mass-at-age fit a combination of additive, dominant, and epistatic additive-by-additive

effects, while cross means for SGR of length fit a simpler combination of additive and dominant effects (**Table 3.4**, **Table 3.5).** These outbreeding effects were expressed as differential deviations from an additive pattern between the first and second generation of outbreeding (dominance), that additionally differed between F2 and

Table 3.5: Estimated average outbreeding effects for size-at-age of fork length (Ln of g) and SGR of body mass (% $°D^{-1}$), their standard errors (SE), and their probabilities (P) for being different from zero at given denominator degrees of freedom (ddf) and associated *F*-values for either immature (**A**), female (**B**) or male (**C**) Atlantic salmon. The predicted F2 reference mean is also given for each trait.

*Effect	mean ± SE	ddf	$F_{1,ddf}$	P	Lack-of-fit
A immature					
$\hat{\mu}$ (size)	3.509 ± 0.009	-	-	-	
$\hat{d}$ (size)	-0.121 ± 0.007	96.6	267	<0.001	$F_{1,180.4}$ = 0.4,
$\hat{h}$ (size)	-0.047 ± 0.014	190.9	11.2	0.001	P = 0.552
$\hat{\imath}$ (size)	-0.072 ± 0.022	233.2	10.8	0.001	
$\hat{\mu}$ (SGR)	0.08507 ± 0.00065	-	-	-	
$\hat{d}$ (SGR)	-0.00411 ± 0.00041	181.8	98.2	<0.001	$F_{2,152.2}$ = 0.9,
$\hat{h}$ (SGR)	-0.00107 ± 0.00031	184.2	12.1	0.001	P = 0.426
B female					
$\hat{\mu}$ (size)	3.557 ± 0.007	-	-	-	$F_{3,76.6}$ = 1.9,
$\hat{\imath}$ (size)	0.129 ± 0.008	67.2	242	<0.001	P = 0.143
$\hat{\mu}$ (SGR)	0.01007 ± 0.00046	-	-	-	$F_{3,81.1}$ = 1.9,
$\hat{d}$ (SGR)	-0.00231 ± 0.00045	126.9	26.1	<0.001	P = 0.143
C male					
$\hat{\mu}$ (size)	3.495 ± 0.007	-	-	-	$F_{3,106.1}$ = 2.3,
$\hat{d}$ (size)	-0.116 ± 0.008	162.8	215	<0.001	P = 0.089
$\hat{\mu}$ (SGR)	0.01172 ± 0.00024	-	-	-	$F_{3,113.5}$ = 0.8
$\hat{d}$ (SGR)	-0.00243 ± 0.00030	146.9	68.0	<0.001	P = 0.520

*The effects are predicted F2 cross mean ($\hat{\boldsymbol{\mu}}$), additive ($\hat{\boldsymbol{d}}$), dominant ($\hat{\boldsymbol{h}}$), additive-by-additive ($\hat{\boldsymbol{\imath}}$), additive-by-dominant ($\hat{\boldsymbol{\jmath}}$), and dominant-by-dominant ($\hat{\boldsymbol{l}}$) outbreeding effects.

BC (additive-by-additive epistasis; **Figure 3.6A, D, G, J**).

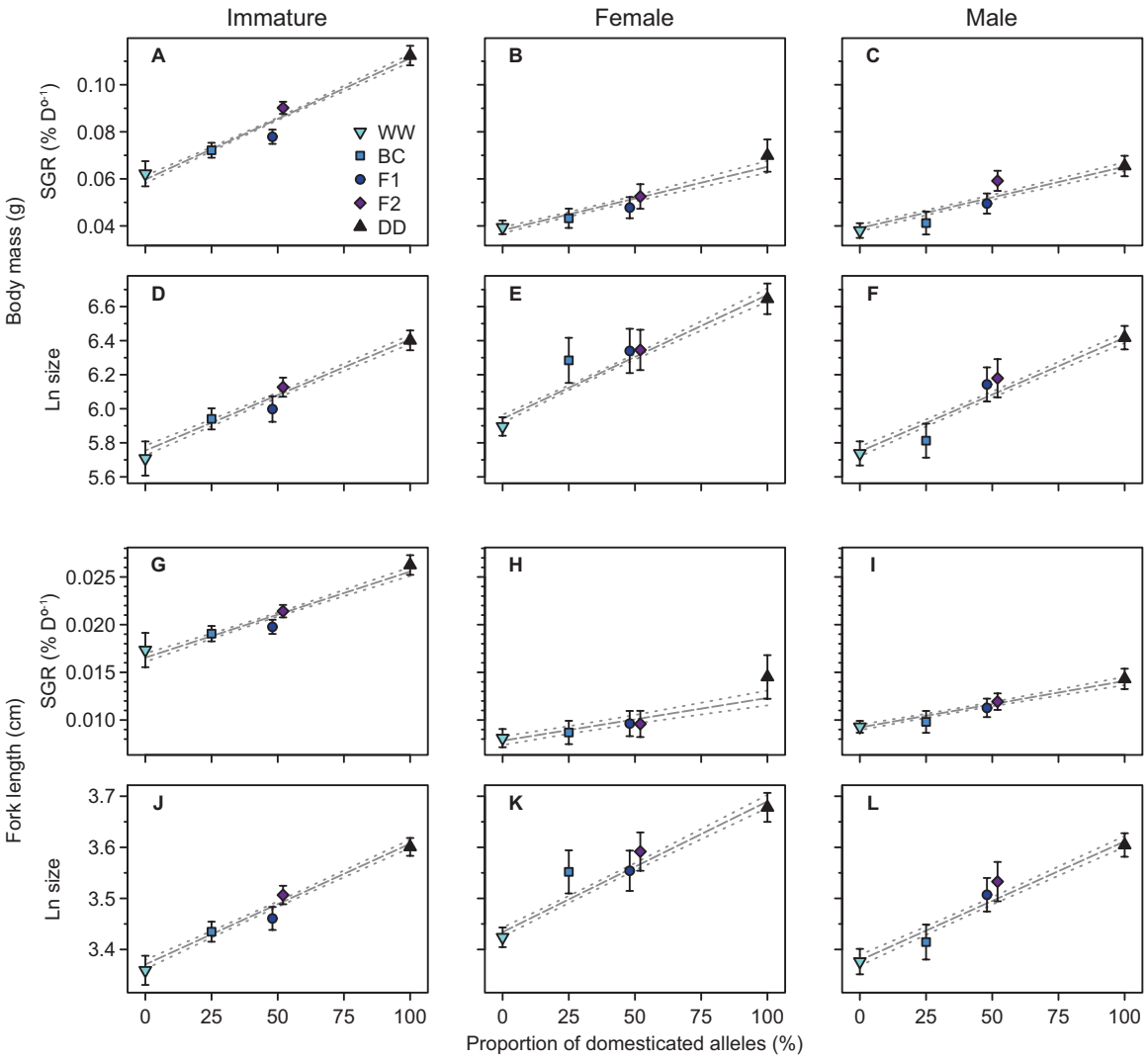

Figure 3.6: Cross means for specific growth rate (SGR) and size-at-age for the three maturity groups. Marginal predictions across environments from maturity-specific models for means of SGRs (**A**, **B**, **C**, **G**, **H**, **I**) and sizes (**D**, **E**, **F**, **J**, **K**, **L**) are depicted for body mass-at-age (**A-E**) and fork length-at-age (**F-L**). Means of crosses are differentiated by colours and symbols as indicated in the key of **A** and error bars represent approximate 95% confidence intervals. Cross means are shown separately for maturity groups of immature individuals (left column), females (middle column) and males (right column). Cross means for F1 and F2 hybrids have been off-set to improve depiction. All cross abbreviations are as in **Figure 3.1**.

Female cross means for SGRs of both traits were best explained by the additive effect, while cross means for size-at-age for both traits were best explained by the additive-by-dominant epistatic effect (**Table 3.4**, **Table 3.5**). This epistatic effect was represented by equal mean sizes among both hybrid generations and BCs (**Figure**

3.6E, K). For male individuals, cross means for size-at-age of both traits, as well as cross means for SGR of length, were best explained solely by the additive effect (Table 3.4, Table 3.5). However, male cross means for SGR of mass fit a combination of additive, epistatic additive-by-dominant and dominant-by-dominant effects (Table 3.4, Table 3.5). Despite the inferred additive effect for male sizes, a tendency of male BC size-at-age being smaller than additive could be noted (Figure 3.6E, F, K, L).

3.5. Discussion

Our results indicate the presence of both additive and non-additive outbreeding effects for specific growth rate (SGR), size-at-age, and the presence of different sex- and size-specific maturation probabilities between the domesticated and wild *S. salar* populations. Furthermore, outbreeding effects for SGR and size-at-age differed among maturity groups, and these groups occurred at different frequencies among crosses. Average population size-at-age is the sum of products of the frequency of maturity groups with their average size-at-age. As a result, studies of the genetic architecture of size that are based on mean size-at-age can be biased if effects from temporally changing growth rates among maturity groups and different frequencies of maturity groups between populations stay unaccounted for.

With regard to growth plasticity, we could not reject the null hypothesis of equal outbreeding effects for growth in different environments. Hence, with the exception of environmental growth plasticity, domesticated and wild *S. salar* are significantly different for the investigated traits of maturation probability and growth rate, and the studied populations likely diverge in allelic combinations underlying these traits. Yet, the presence of a possible effect of salinity on only F1 growth in the absence of significant differences between the parental populations remains to be evaluated.

3.5.1. Size-at-Age and Growth

Following expectations, we detected a higher size-at-age and SGR in domesticated relative to wild *S. salar*. In immature individuals, for which maturation-related growth change bias might be least, cross means for mass-at-age and for SGR of both traits, but not for length-at-age, fit a combination of additive, dominant, and additive by dominant outbreeding effects. In most fishes, isometric mass increase is roughly proportional to the cube of length increases. Accordingly, differences in mean size-at-age or growth rate are expected to be larger for mass than length and epistatic outbreeding effects in our study may have yet not been detectable for length-at-age.

The inference of epistatic effects on means agrees well with a study on variances of body mass-at-age among four *S. salar* populations that indicated equal importance of dominant and additive-by-additive variances with the additive component (Rye & Mao 1998). When taking the proportion for outbreeding effect of a given trait and rescaling for comparability ($\hat{\imath}/2$), the same non-additive effects comprise one-third and additive effects comprise two-thirds of the total effects on means in our study (omitting length-at-age). Furthermore, the single proportions agree surprisingly well between both traits for SGR and between mass SGR and mass-at-age. Our results, hence, indicate that the between-population genetic architecture for growth is made up of 66% additive and 33% non-additive effects, including a 20% total share of additive-by-additive epistatic effects.

For mature individuals in our study, incongruence between inference on size-at-age and SGR indicates the presence of inconsistencies in differences among crosses for SGR before and during the experiment. This was expected due to growth changes accompanying sexual maturation. Furthermore, there was likely an influence on cross means estimates for size-at-age of each sex through divergence in size-influenced maturation probability.

We propose that the genetic architecture of growth during limited periods, or environmental growth plasticity, should be investigated giving preference to means of growth rates and not size-at-age. Differences in size-at-age have to develop first,

might not be detectable due to larger variances for size-at-age relative to those for growth rates, and might also underlie possible past differences in growth rates, whereas growth rates during a study are representative for that period. Furthermore, environmental growth-rate responses can be detected across all sizes. These reasons may also explain why size-at-age in our crosses at younger ages appeared additive (Fraser *et al.* 2010). However, when a growth model like SGR is used that assumes linear trajectories, the linearity assumption for all predictors should be validated. Non-linearity can be easily accounted for when more than two temporal measurements are available, but this can lead to varying results with experimental duration and make between-study comparisons difficult. Such an effect is here exemplified by the presence of a delayed and only temporary SGR reduction in response to suspended sediments. Nevertheless, for immature individuals, our results show an agreement in outbreeding effects between size-at-age and SGR. This might speak for appropriateness of size-at-age data for the evaluation of genotypic effects as long as no life-history processes or environmental effects have been differing among populations.

Size-at-age is still an estimate of overall past growth and has importance for momentary individual fitness. At the same age investigated, all mixed-origin crosses were heavier and longer than wild individuals, except for mature male BCs. Under a low frequency of outbreeding, BCs are more likely to occur than F2 hybrids and effects in BC might hence be more important for population-level consequences of outbreeding and deserve special consideration. In that sense, BCs exhibited a maturity group-specific pattern of deviation from additive expectations with females being larger, males being slightly smaller, and immature individuals having a size-at-age close to expected. It remains to be evaluated if these outbreeding effects on sizes will persist in the wild under non-optimal feeding conditions and what their actual effects on individual fitness might be (discussed by Arendt 1997). Nevertheless, the mere potential for rapid growth as observed for all domesticated-wild offspring may have fitness consequences. A previous study of our crosses has shown a size-independent feeding behaviour-related reduction in anti-predator response with an increasing percentage of domesticated allelic combinations

(Houde *et al.* 2010). Faster growth potential may cause such higher risk-taking disposition due to increased appetite (Johnsson *et al.* 1996), and ultimately negatively affect fitness when predators are abundant (Biro *et al.* 2004).

3.5.2. Choice of Growth Model

Fish growth rate is known to change with age, size, and life stage. In fishes, growth is often modelled parametrically by asymptotic models. These models may represent population-averages for life-time growth where the flattening of the curve with age is caused partly by increasing frequencies of sexually mature individuals investing in gonadal rather than somatic growth (Lester *et al.* 2004) and partly due to non-linearity between body size and the ratio of anabolism to catabolism (von Bertalanffy 1957). We regard these population growth models as inappropriate for our short time, individual-based modelling approach due to the diversity of average growth trajectories for treatment, genotype, and maturity groups which are unlikely to be jointly modelled reasonably by any common parametric model (Parker & Larkin 1959).

Nonetheless, even for SGRs, it has been suggested to parametrically adjust individual fish growth rates to that of a common size for the whole population (Jobling 1983) or specifically for immature individuals in fresh water (Elliott & Hurley 1997) and this has also been followed in between-population comparisons between salinities (e.g., Fleming *et al.* 2002). The latter usage assumes that individual SGR slows down with increasing size equally across populations. The presence of different, partly opposing correlations between SGR and size among our crosses clearly violate the assumptions necessary for a common growth-rate adjustment. As a consequence, we suggest testing for differential size to SGR relationships among genotypes, environments, or life stages and to model these according to the data (similarily as suggested for 'stanza'; Parker & Larkin 1959) rather than generalizing relationships across genotypes by a common ‘correction’ which introduces a bias when correlations differ.

3.5.3. Size- and Age-at-Maturity

In nature, the fastest growing individuals typically mature first (Alm 1959; Hutchings 1993) and among-population differences might be caused by phenotypic plasticity, different genetically based maturation schedules, or both (Enberg *et al.* 2012). We were unable to test for the genetic architecture of sex-specific maturation probabilities, as we missed the sex-evaluation of immature individuals. However, when assuming equal sex ratios, the within-cross frequencies of mature females and males in relation to those of immature individuals serves as a proxy for sex-specific maturation probabilities. In that sense, our within-cross observations agreed with the common pattern that the fastest growing individuals mature first. This was contradicted by among-cross observations for females; an increasing percentage of domesticated allelic combinations resulted in an increasing size-at-age but also in a decreasing female maturation probability. This contrasts with typically observed natural patterns and strongly suggests the presence of independence for the genetic basis of growth and maturation probability between our populations. Furthermore, a different cross-means pattern in male vs. female maturation probabilities indicates sex-specificity of the genetic architecture. Such sex-specificity was somewhat expected due to sex-specific resource demands during maturation and their respective evolutionary constraints (Roff 1992; Taranger *et al.* 2010).

In mature females, we inferred an epistatic additive-by-dominant architecture for size-at-age because BCs were as large as F1 and F2 hybrids, while the latter two fit the midparental value. Hence, the strong size-deviation of solely the BC caused the pattern to be epistatic. It is possible that this pattern of size-at-age for mature females is caused by the interaction of divergence in growth rates with female size-specific maturation probability. Then, to become mature, females have to reach a population-specific size threshold that they reach with a probability that is a function of the population-specific growth rate, and whether they mature at that size is a function of the population size-specific maturation probability. Hence, it is difficult to infer which of these traits dominates this observed epistatic size-at-age pattern. However, it is possible that mere combination of additive and dominant

effects for the different correlated traits have caused the observed additive-by-dominant pattern.

Phenotypic trait interactions may have therefore had effects on the inferred genetic architecture of correlated traits. This consideration challenges the assumption that an epistatic genotype underlies an epistatic phenotype. Nevertheless, an epistatic size-at-age pattern was absent in BC males, which even exhibited an opposing trend relative to females by being smaller than expected under additivity. BCs and F2 hybrids possess 50 and 100% chromosomes, respectively, which have been recombined between populations during meiotic crossover in F1 parents. Recombination differs strongly in rates and in sites between sexes in *S. salar* (Moen *et al.* 2008) and this has the potential to create sex-specific epistasis. Furthermore, larger effects of genetic drift on X-chromosomes vs. autosomes have been suggested to play important roles in speciation (Whitlock & Wade 1995). Similar mechanisms may underlie the presence of sex-specific outbreeding effects, here primarily observed in wild-BCs where recombined chromosomes function under a wild allelic background, in contrary to the F2 hybrid.

S. salar exhibits a great plasticity in migration-maturation schedule patterns (Klemetsen *et al.* 2003), but it was surprising that smolts selected in spring (which would normally migrate to the ocean) reached sexual maturity in autumn of the same year. It may have been that mature individuals had smolted already in the previous year. However, the process of smoltification is reversible (McCormick *et al.* 2009) and smoltification and continuation of sexual maturation are not exclusive processes (Thorpe *et al.* 1998). Furthermore, these fish may have matured earlier than expected in the wild due to surplus energy available through culture conditions (Thorpe 2004). We still interpreted observed maturation schedules as genetically based threshold-differences among crosses that were shaped by common life-time environmental conditions. How far the observed *Cross*-by-*Salinity* effect on growth reflected differential acclimation abilities to saltwater under our experimental conditions was not investigated. However, cross-specific effects in the F1 cross that were different for mass and length (indicating a lower condition in salt water as

typically seen in smolts) might be an indication that this could have played an additional role in observed growth patterns.

Some of our conclusions are limited because family-bias is known to affect population-level inference (Jourdan-Pineau *et al.* 2012) and we did not account in our analyses for correlations among individuals arising from kinship. We expect that the significance of large differences in means between the populations is unlikely to change when accounted for kinship. However, the sensitive cross means analyses might have been influenced by a potential family bias or too liberal tests induced by unaccounted positive correlations among individuals and we suggest taking this additionally into account in future studies.

3.5.4. Interaction Effects from Divergence of Growth and Size- and Age-at-Maturity

Our results emphasize the challenges to predicting morphological and life-history trait change resulting from domesticated-wild outbreeding. This is because traits are correlated, such as growth rate with size-at-age and eventually age-at-maturity through maturation-size thresholds. As growth expression differs among environments and maturation probability differs for sex, so will patterns for age- and size-at-sexual maturation. In the wild, temporally changing feeding opportunities and variable environments will likely induce phenotypic plasticity for growth beyond the temporal scope of our study and this may further alter maturation schedules. Overall changes in age-at-maturity across mixed-origin offspring generations appear unpredictable because a non-additive genetic architecture underlies SGR and, at least for females, size-related maturation patterns can have opposing effects within vs. between crosses (or populations). Nevertheless, we predict that domesticated-wild outbreeding will, relative to the wild parent, increase growth potential, increase age-at-maturity, and that there might be sex-specific changes of these traits.

3.6. Acknowledgements

All work followed guidelines of the Canadian Council on Animal Care and the experiment was approved by the Dalhousie University Committee on Laboratory Animals (protocol number 08-080). We are thankful for those who indispensably helped with fish measurements: Katharina Bremer, Aimee-Lee Houde, Magdalena Bartkowska, and Laura Weir. The work was supported by Natural Science and Engineering Research Council (Canada) Discovery and Strategic grants to JAH. PVD was supported by the Patrick F. Lett Graduate Students' Assistance Bursary.

3.7. Data Accessibility

All data used for analyses are planned to be made accessible during the course of the publication of the manuscript.

3.8. Supplementary Material

Table S 3.1: Number of individuals for all factor levels of the experimental design in the three maturity groups (Maturity) between the crosses of wild (WW) and domesticated (DD) Atlantic salmon, reciprocal first- (F1) and second-generation hybrids (F2), and the reciprocal wild backcross (BC, WW x F1). Combinations of environmental factor levels for *Salinity* (levels: fresh, salt) and *Sediment* (levels: clear, sediments) are indicated by a colon. Results from an analysis of these occurrences, additionally accounting for occurrences for each tank, can be found in **Table S 3.2**.

Cross	Maturity	fresh	salt	clear	sediments	fresh:clear	fresh:sediments	salt:clear	salt:sediments	Overall
WW	immature	17	16	15	18	6	11	9	7	33
WW	female	37	37	40	34	19	18	21	16	74
WW	male	40	35	37	38	22	18	15	20	75
WW	all	94	88	92	90	47	47	45	44	183
BC	immature	49	43	45	47	27	22	18	25	92
BC	female	23	23	20	26	8	15	12	11	46
BC	male	23	21	25	19	13	10	12	9	44
BC	all	95	87	90	92	48	47	42	45	182
F1	immature	49	51	53	47	24	25	29	22	100
F1	female	17	23	16	24	8	9	8	15	40
F1	male	29	26	28	27	15	14	13	13	55
F1	all	95	100	97	98	47	48	50	50	195
F2	immature	54	52	59	47	30	24	29	23	106
F2	female	18	14	16	16	9	9	7	7	32
F2	male	24	27	20	31	9	15	11	16	51
F2	all	96	93	95	94	48	48	47	46	189
DD	immature	38	47	45	40	19	19	26	21	85
DD	female	14	10	11	13	4	10	7	3	24
DD	male	40	37	39	38	24	16	15	22	77
DD	all	92	94	95	91	47	45	48	46	186
Overall	all	472	462	469	465	237	235	232	230	934

Table S 3.2: Results for the analysis of number of individuals among all study-design term levels. The response is log-transformed (Ln) number of individuals per Cross-by-Maturity level and tank. Interactions among terms are indicated by a colon. Given for each term are degrees of freedom (df), denominator df (ddf), F-statistic (*F*) and probability (P).

Term	df	ddf	*F*	P
Intercept	1	4	>1000	<0.001
Sediment	1	4	0.7	0.439
Salinity	1	4	1.6	0.277
Cross	4	56	0.7	0.606
Maturity	2	56	48.8	<0.001
Sediment:Salinity	1	4	4.8	0.095
Sediment:Cross	4	56	0.2	0.916
Salinity:Cross	4	56	0.2	0.932
Sediment:Maturity	2	56	0.5	0.632
Salinity:Maturity	2	56	0.1	0.888
Cross:Maturity	8	56	20.6	<0.001
Sediment:Salinity:Cross	4	56	0.4	0.781
Sediment:Salinity:Maturity	2	56	2.9	0.061
Sediment:Cross:Maturity	8	56	1.5	0.181
Salinity:Cross:Maturity	8	56	0.8	0.577
Sediment:Salinity:Cross:Maturity	8	56	2.9	0.008

Among-tank stratum variance was 0.0169 with 4 df, residual stratum variance was 0.0725 with 56 df.

Table S 3.3: Results for the selection of among-individual (co)variances model structures. Models are for response of body mass (**A**) or fork length (**B**). For each (co)variance model ($Model_l$), the number of (co)variance parameters are indicated for the total (k), for among-groups (k_G), and for among-individuals (k_I). For each successfully fitted model, the AIC, BIC (both in "smaller is better" form, best model for each trait in italic) and the log residual maximum likelihood (REML) are given. Each among-individual covariance model was fitted with either homogeneous or heterogeneous parameters among the 15 Cross-by-Maturity groups. For each model, the improvement in model fit by the heterogeneous version was assessed by likelihood ratio test (LRT) for which the test statistic ($\chi 2$), degrees of freedom (df), and the approximated probability (P) are given. As the heteroscedastic models were always better as clearly indicated by LRTs, we ignored the homoscedastic versions for among-model comparisons. All models were fitted with the same fixed full structure (Sediment*Salinity*Cross*Maturity*Time) and with all possible random spline (spl(Time)) and random deviation (dev(Time)) among-group terms (spl(Time)/(Sediment*Salinity*Cross*Maturity)+dev(Time)/(Sediment*Salinity*Cross*Maturity). In the model equation asterisks indicate factor crossing (main effects plus interactions), and slashes indicates nesting (main effects to the left of the slash plus their interactions with effects to the right of the slash). Furthermore, all models were fitted with error terms for tank identification (Tank), and Tank:Time, as well as random spline and deviation terms for Tank. All random parameters that converged to zero were removed before model evaluation except for Tank and Tank:Time which were always retained to account for the splits-plot design. As models account differently for the variance across Time, parameters that converged to zero differed among models and that is the reason why k(G) differ among models.

Continued on next page

Table S 3.3 continued

Model$_I$	k	k_G	k_I	AIC	BIC	REML	k	k_G	k_I	AIC	BIC	REML	χ^2	df	*P
	Homogeneous variances for Cross-by-Maturity						Heterogeneous variances for Cross-by-Maturity								
A															
GLM	5	4	1	-4310.0	-4277.8	2160.0	19	4	15	-4548.4	-4426.4	2293.2	266.5	14	<0.001
GLMH	9	4	5	-4295.0	-4237.2	2156.5	79	4	75	-4434.7	-3927.3	2296.4	279.7	70	<0.001
CS	13	11	2	-14995.5	-14912.0	7510.8	cf	cf	30	cf	cf	cf	NA	NA	NA
CSH	16	10	6	-15012.7	-14910.0	7522.4	101	11	90	-15063.1	-14414.4	7632.6	220.4	85	<0.001
AR(1)	cf	cf	2	cf	cf	cf	cf	cf	30	cf	cf	cf	NA	NA	NA
ARH(1)	cf	cf	6	cf	cf	cf	104	14	90	-16852.7	-16184.7	8530.4	NA	NA	NA
ANTE(1)	20	11	9	-17403.9	-17275.2	8721.9	148	13	135	-17466.9	-16516.3	8881.5	319.1	128	<0.001
ANTE(2)	26	14	12	-17571.9	-17404.9	8812.0	193	13	180	-17581.5	-16341.9	8983.8	343.6	167	<0.001
ANTE(3)	28	14	14	-17573.3	-17393.5	8814.7	224	14	210	*-17582.7*	-16143.9	9015.3	401.3	196	<0.001
UN	26	11	15	-17555.7	-17388.7	8803.9	235	10	225	-17572.9	-16063.5	9021.4	435.1	209	<0.001
RC	21	18	3	-16742.9	-16608.0	8392.5	63	18	45	-16774.9	-16370.3	8450.4	116.0	42	<0.001
RCS	19	15	4	-17409.5	-17287.4	8723.7	75	15	60	-17448.3	*-16966.6*	8799.1	150.8	56	<0.001
RCSC	29	14	15	-17400.2	-17213.9	8729.1	cf	cf	225	cf	cf	cf	NA	NA	NA

Continued on next page

Table S 3.3 continued

Model$_I$	k	k_G	k_I	AIC	BIC	REML	k	k_G	k_I	AIC	BIC	REML	χ^2	df	*P
	Homogeneous variances for Cross-by-Maturity						Heterogeneous variances for Cross-by-Maturity								
B															
GLM	4	3	1	-15056.5	-15030.8	7532.2	18	3	15	-15321.5	-15205.9	7678.8	293.1	14	<0.001
GLMH	8	3	5	-15052.6	-15001.2	7534.3	78	3	75	-15208.9	-14707.9	7682.4	296.3	70	<0.001
CS	9	7	2	-28041.2	-27983.4	14029.6	38	8	30	-28220.5	-27976.4	14148.2	237.3	29	<0.001
CSH	12	6	6	-28118.1	-28041.0	14071.0	97	7	90	-28324.6	-27701.6	14259.3	376.6	85	<0.001
AR(1)	cf	cf	2	cf	cf	cf	cf	cf	30	cf	cf	cf	NA	NA	NA
ARH(1)	cf	cf	6	cf	cf	cf	cf	cf	90	cf	cf	cf	NA	NA	NA
ANTE(1)	18	9	9	-31127.0	-31011.4	15581.5	144	9	135	-31256.0	-30331.1	15772.0	381.0	126	<0.001
ANTE(2)	cf	cf	12	cf	cf	cf	187	7	180	-31431.9	-30230.9	15903.0	NA	NA	NA
ANTE(3)	cf	cf	14	cf	cf	cf	218	8	210	-31506.7	-30106.5	15971.3	NA	NA	NA
UN	28	13	15	-31428.4	-31248.6	15742.2	235	10	225	*-31527.0*	-30017.6	15998.5	512.6	207	<0.001
RC	17	14	3	-30771.2	-30662.0	15402.6	60	15	45	-30848.1	-30462.7	15484.0	162.9	43	<0.001
RCS	22	18	4	-31395.0	-31253.7	15719.5	78	18	60	-31517.1	*-31016.1*	15836.5	234.0	56	0.000
RCSC	33	18	15	-31387.0	-31175.1	15726.5	cf	cf	225	cf	cf	cf	NA	NA	NA

NA: evaluation not available, cf: convergence failed; The suffix 'H' to a model name indicates heterogeneous variances across Time; GLM: general linear model (common variance, no covariance); CS: compound symmetry (common variance, common covariance); AR(1): autoregressive order 1 (common variance, covariances decline exponentially between Time levels with increasing lag); ANTE(i): antedependence of order i (heterogeneous variances across Time, heterogeneous covariances for distance between Time levels up to lag i); UN: unstructured (heterogeneous variances across Time, heterogeneous covariances among all variances); RC: random coefficients (variance for both individual intercepts and slopes, including the covariance between intercept and slope); RCS: RC with individual splines (like RC but additional individual-based splines, independent of RC effects); RCSC: RCS with all possible covariances among three spline variances and both RC variances (like RCS but splines are additionally not independent of the remaining RC effects); *The comparisons are only approximations as among-group random effects terms varied among covariance structures and have not been tested for significance

Table S 3.4: Variance parameters for the overall and maturity-group-specific growth models of body mass. Parameters are given for the overall model (**A**), and for maturity groups of immature individuals (**B**), females (**C**), and males (**D**). A colon between terms indicates the formation of the interaction. Some terms contain splines (spl(Time)) and deviations (dev(Time)) from linear trajectories. Among-individual (co)variances are given for each cross. Crosses are abbreviated with WW, wild; F1, first-generation hybrid; F2, second-generation hybrid; and DD, domesticated Atlantic salmon. The covariance between among-individual variances for intercepts and slopes (interaction of individual with Time) is abbreviated by cov. For all among-group variance parameters, the results from REML-likelihood ratio tests (LRT) are given as test-statistic (χ^2, all with 1 degree of freedom) and probability of being different from zero (P). LRT results for among-individual (co)variance terms are reported in supplementary **Table S 3.3**. Variances were constrained to be positive.

Term	Var	SE	χ^2_1	P
A overall				
spl(Time)	7.0E-05	1.7E-04	0.2	0.643
spl(Time):Sediment	1.1E-04	1.1E-04	7.3	0.007
spl(Time):Cross:Maturity	5.3E-05	1.9E-05	52.2	<0.001
dev(Time):Maturity	1.5E-04	1.1E-04	19.4	<0.001
Sediment:Salinity:Cross:Maturity:dev(Time)	3.3E-05	1.1E-05	18.3	<0.001
*Tank	2.9E-09	1.4E-10	NA	NA
*Tank:Time	5.2E-11	2.5E-12	NA	NA
Tank:dev(Time)	7.4E-05	2.7E-05	94.3	<0.001
WW-females intercept	7.4E-02	1.2E-02		
WW-females cov(intercept, slope)	1.7E-05	3.9E-04		
WW-females:Time	1.4E-04	2.5E-05		
WW-males intercept	9.7E-02	1.6E-02		
WW-males cov(intercept, slope)	3.8E-04	4.7E-04		
WW-males:Time	1.6E-04	2.8E-05		
WW- immature intercept	8.5E-02	2.2E-02		
WW-immature cov(intercept, slope)	1.5E-04	8.3E-04		
WW- immature:Time	2.4E-04	6.4E-05		
BC-females intercept	2.1E-01	4.5E-02		

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Table S 3.4 continued

Term	Var	SE	χ_1^2	P
BC-females intercept	2.1E-01	4.5E-02		
BC-females cov(intercept, slope)	2.7E-04	9.6E-04		
BC-females:Time	1.8E-04	4.0E-05		
BC-males intercept	1.1E-01	2.4E-02		
BC-males cov(intercept, slope)	6.7E-04	8.2E-04		
BC-males:Time	2.4E-04	5.4E-05		
BC- immature intercept	8.6E-02	1.3E-02		
BC-immature cov(intercept, slope)	-2.1E-04	5.2E-04		
BC- immature:Time	2.7E-04	4.1E-05		
F1-females intercept	1.8E-01	4.0E-02		
F1-females cov(intercept, slope)	2.5E-03	1.0E-03		
F1-females:Time	1.9E-04	4.6E-05		
F1-males intercept	1.5E-01	2.8E-02		
F1-males cov(intercept, slope)	1.8E-03	8.1E-04		
F1-males:Time	2.1E-04	4.3E-05		
F1- immature intercept	1.4E-01	2.0E-02		
F1-immature cov(intercept, slope)	2.1E-03	6.6E-04		
F1- immature:Time	2.6E-04	3.9E-05		
F2-females intercept	1.2E-01	3.0E-02		
F2-females cov(intercept, slope)	1.1E-03	9.3E-04		
F2-females:Time	2.0E-04	5.4E-05		
F2-males intercept	1.6E-01	3.3E-02		
F2-males cov(intercept, slope)	-1.1E-03	8.6E-04		
F2-males:Time	2.0E-04	4.3E-05		
F2- immature intercept	8.2E-02	1.1E-02		
F2-immature cov(intercept, slope)	1.2E-04	4.1E-04		
F2- immature:Time	2.0E-04	3.0E-05		
DD-females intercept	4.7E-02	1.4E-02		
DD-females cov(intercept, slope)	1.0E-03	7.9E-04		
DD-females:Time	2.6E-04	8.2E-05		
DD-males intercept	9.6E-02	1.6E-02		
DD-males cov(intercept, slope)	1.7E-03	7.0E-04		

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Table S 3.4 continued

Term	Var	SE	χ_1^2	P
DD-males:Time	3.4E-04	5.8E-05		
DD- immature intercept	6.7E-02	1.0E-02		
DD-immature cov(intercept, slope)	1.7E-03	6.1E-04		
DD- immature:Time	4.0E-04	6.4E-05		
WW-females:spl(Time)	2.4E-04	4.5E-05		
WW-males:spl(Time)	1.6E-04	3.3E-05		
WW-immature:spl(Time)	2.0E-04	5.8E-05		
BC-females:spl(Time)	2.1E-04	4.9E-05		
BC-males:spl(Time)	2.1E-04	5.6E-05		
BC-immature:spl(Time)	2.6E-04	4.3E-05		
F1-females:spl(Time)	4.3E-04	9.8E-05		
F1-males:spl(Time)	1.6E-04	4.0E-05		
F1-immature:spl(Time)	2.1E-04	3.4E-05		
F2-females:spl(Time)	3.9E-04	1.1E-04		
F2-males:spl(Time)	2.1E-04	5.1E-05		
F2-immature):IND:spl(Time)	2.3E-04	3.8E-05		
DD-females:spl(Time)	7.3E-04	2.0E-04		
DD-males:spl(Time)	3.0E-04	5.4E-05		
DD-immature:spl(Time)	2.3E-04	4.0E-05		
Residuals	5.1E-04	2.5E-05		
B immature				
spl(Time)	4.0E-04	3.8E-04	2.9	0.089
spl(Time):Sediment	7.4E-05	8.6E-05	4.2	0.040
spl(Time):Sediment:Salinity:Cross	2.4E-05	1.3E-05	7.3	0.007
Sediment:Cross:dev(Time)	1.9E-05	1.5E-05	3.8	0.051
*Tank	NA	NA	NA	NA
*Tank:Time	NA	NA	NA	NA
Tank:dev(Time)	8.2E-05	3.4E-05	58.4	<0.001
WW intercept	8.2E-02	2.1E-02		
WW cov(intercept, slope)	2.1E-04	8.1E-04		
WW:Time	2.4E-04	6.3E-05		
BC intercept	8.6E-02	1.3E-02		

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Table S 3.4 continued

Term	Var	SE	χ_1^2	P
BC cov(intercept, slope)	-2.8E-04	5.2E-04		
BC:Time	2.8E-04	4.3E-05		
F1 intercept	1.4E-01	2.0E-02		
F1 cov(intercept, slope)	2.1E-03	6.7E-04		
F1:Time	2.7E-04	4.0E-05		
F2 intercept	8.1E-02	1.1E-02		
F2 cov(intercept, slope)	1.4E-04	4.1E-04		
F2:Time	2.0E-04	2.9E-05		
DD intercept	7.0E-02	1.1E-02		
DD cov(intercept, slope)	1.8E-03	6.2E-04		
DD:Time	4.1E-04	6.4E-05		
WWspl(Time)	2.1E-04	6.0E-05		
BC:spl(Time)	3.0E-04	4.7E-05		
F1:spl(Time)	2.4E-04	3.7E-05		
F2:spl(Time)	2.8E-04	4.3E-05		
DD:spl(Time)	2.6E-04	4.4E-05		
Residuals	4.1E-04	3.2E-05		
C females				
spl(Time):Sediment	2.6E-04	1.9E-04	12.9	<0.001
spl(Time):Cross	5.0E-05	3.4E-05	9.4	0.002
*Tank	NA	NA	NA	NA
*Tank:Time	NA	NA	NA	NA
dev(Time):Tank	7.4E-05	3.9E-05	12.4	<0.001
WW intercept	7.5E-02	1.2E-02		
WW cov(intercept, slope)	1.9E-05	3.9E-04		
WW:Time	1.4E-04	2.5E-05		
BC intercept	2.1E-01	4.5E-02		
BC cov(intercept, slope)	2.9E-04	9.5E-04		
BC:Time	1.8E-04	4.0E-05		
F1 intercept	1.7E-01	3.9E-02		
F1 cov(intercept, slope)	2.5E-03	1.0E-03		
F1:Time	1.8E-04	4.5E-05		

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Table S 3.4 continued

Term	Var	SE	χ_1^2	P
F2 intercept	1.2E-01	3.0E-02		
F2 cov(intercept, slope)	1.1E-03	9.4E-04		
F2:Time	2.1E-04	5.6E-05		
DD intercept	4.8E-02	1.4E-02		
DD cov(intercept, slope)	1.1E-03	8.0E-04		
DD:Time	2.6E-04	8.2E-05		
WWspl(Time)	2.1E-04	4.4E-05		
BC:spl(Time)	2.0E-04	4.8E-05		
F1:spl(Time)	3.9E-04	9.3E-05		
F2:spl(Time)	3.4E-04	1.0E-04		
DD:spl(Time)	7.4E-04	2.1E-04		
Residuals	6.1E-04	5.9E-05		
D males				
spl(Time):Cross	1.5E-04	7.0E-05	89.3	<0.001
Sediment:dev(Time)	1.5E-04	1.2E-04	12.4	<0.001
*Tank	NA	NA	NA	NA
Tank:Time	1.3E-06	4.3E-06	0.1	0.756
Tank:spl(Time)	3.8E-05	2.0E-05	20.9	<0.001
WW intercept	9.9E-02	1.6E-02		
WW cov(intercept, slope)	3.8E-04	4.8E-04		
WW:Time	1.6E-04	2.8E-05		
BC intercept	1.1E-01	2.4E-02		
BC cov(intercept, slope)	5.8E-04	8.1E-04		
BC:Time	2.4E-04	5.4E-05		
F1 intercept	1.4E-01	2.8E-02		
F1 cov(intercept, slope)	1.8E-03	8.4E-04		
F1:Time	2.3E-04	4.7E-05		
F2 intercept	1.6E-01	3.3E-02		
F2 cov(intercept, slope)	-1.1E-03	8.6E-04		
F2:Time	2.1E-04	4.4E-05		
DD intercept	9.7E-02	1.6E-02		
DD cov(intercept, slope)	1.7E-03	7.0E-04		

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Table S 3.4 continued

Term	Var	SE	χ_1^2	P
DD:Time	3.3E-04	5.7E-05		
WWspl(Time)	1.4E-04	3.3E-05		
BC:spl(Time)	1.8E-04	5.1E-05		
F1:spl(Time)	1.4E-04	3.9E-05		
F2:spl(Time)	1.6E-04	4.6E-05		
DD:spl(Time)	2.8E-04	5.4E-05		
Residuals	6.0E-04	4.7E-05		

*Variance was constrained to be positive and converged to zero.

Table S 3.5: Variance parameters for the overall and maturity-group-specific growth models of fork length. Parameters are given for the overall model (**A**), and for maturity groups of immature individuals (**B**), females (**C**), and males (**D**). A colon between terms indicates the formation of the interaction. Some terms contain splines (spl(Time)) and deviations (dev(Time)) from linear trajectories. Among-individual (co)variances are given for each cross. Crosses are abbreviated with WW, wild; F1, first-generation hybrid; F2, second-generation hybrid; and DD, domesticated Atlantic salmon. The covariance between among-individual variances for intercepts and slopes (interaction of individual with Time) is abbreviated by cov. For all among-group variance parameters, the results from REML-likelihood ratio tests (LRT) on the final nested models with and without each term are given as test-statistic (χ^2, all with 1 degree of freedom) and probability of being different from zero (P). LRT results for among-individual (co)variance terms are reported in supplementary **Table S 3.3**. Variances were constrained to be positive.

Term	Var	SE	χ^2_1	P
A overall				
spl(Time):Maturity	6.6E-06	4.0E-06	24.2	<0.001
spl(Time):Cross:Maturity	5.6E-07	2.7E-07	15.6	<0.001
dev(Time)	3.2E-06	5.0E-06	1.5	0.219
Sediment:Salinity:dev(Time)	2.5E-06	1.7E-06	3.0	0.083
Sediment:Cross:dev(Time)	2.3E-07	2.0E-07	2.7	0.101
Sediment:Maturity:dev(Time)	8.1E-07	6.3E-07	8.4	0.004
*Tank	NA	NA	NA	NA
Tank:lin(Time)	1.6E-08	8.0E-08	0.05	0.829
Tank:spl(Time)	7.1E-07	4.1E-07	24.6	<0.001
WW-females:spl(Time)	5.2E-06	1.1E-06		
WW-males:spl(Time)	2.6E-06	7.0E-07		
WW-immature:spl(Time)	9.9E-06	2.7E-06		
BC-females:spl(Time)	5.6E-06	1.4E-06		
BC-males:spl(Time)	4.5E-06	1.3E-06		
BC-immature:spl(Time)	9.0E-06	1.5E-06		

Continued on next page

Table S 3.5 continued

Term	Var	SE	χ_1^2	P
F1-females:spl(Time)	1.2E-05	2.7E-06		
F1-males:spl(Time)	4.0E-06	1.0E-06		
F1-immature:spl(Time)	6.4E-06	1.1E-06		
F2-females:spl(Time)	9.0E-06	2.5E-06		
F2-males:spl(Time)	2.7E-06	8.8E-07		
F2-immature:spl(Time)	7.2E-06	1.2E-06		
DD-females:spl(Time)	1.9E-05	5.2E-06		
DD-males:spl(Time)	1.1E-05	2.0E-06		
DD-immature:spl(Time)	1.2E-05	2.1E-06		
WW-females intercept	6.8E-03	1.1E-03		
WW-females cov(intercept, slope)	-3.9E-05	3.0E-05		
WW-females:Time	9.1E-06	1.6E-06		
WW-males intercept	1.1E-02	1.8E-03		
WW-males cov(intercept, slope)	-6.0E-05	3.2E-05		
WW-males:Time	6.5E-06	1.1E-06		
WW- immature intercept	6.6E-03	1.7E-03		
WW-immature cov(intercept, slope)	-5.8E-05	7.6E-05		
WW- immature:Time	2.7E-05	6.8E-06		
BC-females intercept	2.0E-02	4.3E-03		
BC-females cov(intercept, slope)	-1.5E-04	8.0E-05		
BC-females:Time	1.3E-05	2.8E-06		
BC-males intercept	1.3E-02	2.7E-03		
BC-males cov(intercept, slope)	-9.7E-05	6.6E-05		
BC-males:Time	1.4E-05	3.0E-06		
BC- immature intercept	8.2E-03	1.2E-03		
BC-immature cov(intercept, slope)	-9.5E-05	3.8E-05		
BC- immature:Time	1.4E-05	2.2E-06		

Continued on next page

Table S 3.5 continued

Term	Var	SE	χ_1^2	P
F1-females intercept	1.6E-02	3.6E-03		
F1-females cov(intercept, slope)	3.5E-05	7.3E-05		
F1-females:Time	1.3E-05	3.0E-06		
F1-males intercept	1.5E-02	2.8E-03		
F1-males cov(intercept, slope)	2.4E-05	5.7E-05		
F1-males:Time	1.1E-05	2.3E-06		
F1- immature intercept	1.2E-02	1.7E-03		
F1-immature cov(intercept, slope)	6.7E-05	4.1E-05		
F1- immature:Time	1.3E-05	2.0E-06		
F2-females intercept	1.1E-02	2.8E-03		
F2-females cov(intercept, slope)	-1.2E-04	6.7E-05		
F2-females:Time	1.1E-05	2.8E-06		
F2-males intercept	1.8E-02	3.7E-03		
F2-males cov(intercept, slope)	-1.6E-04	6.3E-05		
F2-males:Time	8.9E-06	1.9E-06		
F2- immature intercept	8.0E-03	1.1E-03		
F2-immature cov(intercept, slope)	-9.9E-05	3.1E-05		
F2- immature:Time	1.1E-05	1.6E-06		
DD-females intercept	4.9E-03	1.4E-03		
DD-females cov(intercept, slope)	7.8E-05	8.1E-05		
DD-females:Time	2.9E-05	8.7E-06		
DD-males intercept	9.3E-03	1.5E-03		
DD-males cov(intercept, slope)	4.7E-05	5.2E-05		
DD-males:Time	2.1E-05	3.5E-06		
DD- immature intercept	5.8E-03	9.0E-04		
DD-immature cov(intercept, slope)	4.3E-05	4.0E-05		
DD- immature:Time	2.2E-05	3.5E-06		

Continued on next page

Table S 3.5 continued

Term	Var	SE	χ_1^2	P
Residuals	2.0E-05	8.6E-07		
B immature				
spl(Time)	1.20E-05	2.15E-05	0.5	0.462
spl(Time):Cross	8.76E-07	6.43E-07	8.3	0.004
spl(Time):Sediment	2.57E-06	2.72E-06	11.3	<0.001
spl(Time):Salinity	1.66E-06	2.07E-06	2.7	0.100
dev(Time)	9.84E-06	1.98E-05	0.5	0.474
Salinity:Cross:dev(Time)	9.07E-07	6.70E-07	4.1	0.042
dev(Time):Tank	2.30E-06	1.16E-06	22.6	<0.001
*Tank	NA	NA	NA	NA
Tank:lin(Time)	2.33E-12	1.51E-13	1.6	0.204
WW:spl(Time)	9.31E-06	2.62E-06		
BC:spl(Time)	8.29E-06	1.47E-06		
F1:spl(Time)	5.80E-06	1.10E-06		
F2:spl(Time)	6.29E-06	1.13E-06		
DD:spl(Time)	1.07E-05	1.93E-06		
WW intercept	6.46E-03	1.63E-03		
WW cov(intercept, slope)	-4.58E-05	7.43E-05		
WW:Time	2.61E-05	6.68E-06		
BC intercept	8.17E-03	1.22E-03		
BC cov(intercept, slope)	-9.47E-05	3.82E-05		
BC:Time	1.44E-05	2.22E-06		
F1 intercept	1.20E-02	1.70E-03		
F1 cov(intercept, slope)	6.56E-05	4.16E-05		
F1:Time	1.34E-05	1.99E-06		
F2 intercept	7.88E-03	1.10E-03		
F2 cov(intercept, slope)	-9.83E-05	3.08E-05		
F2:Time	1.07E-05	1.56E-06		
DD intercept	5.88E-03	9.24E-04		
DD cov(intercept, slope)	4.60E-05	4.03E-05		
DD:Time	2.20E-05	3.48E-06		

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Table S 3.5 continued

Term	Var	SE	χ_1^2	P
Residuals	2.30E-05	1.49E-06		
C females				
spl(Time)	2.6E-06	3.7E-06	2.5	0.112
spl(Time):Tank	1.2E-06	7.1E-07	9.5	0.002
Sediment:dev(Time)	3.2E-06	3.3E-06	3.9	0.049
*Tank	NA	NA	NA	NA
Tank:lin(Time)	5.0E-07	5.3E-07	1.6	0.204
WW:spl(Time)	5.3E-06	1.1E-06		
BC:spl(Time)	6.5E-06	1.6E-06		
F1:spl(Time)	1.1E-05	2.6E-06		
F2:spl(Time)	1.0E-05	2.7E-06		
DD:spl(Time)	2.4E-05	6.3E-06		
WW intercept	6.7E-03	1.1E-03		
WW cov(intercept, slope)	-4.2E-05	3.0E-05		
WW:Time	8.9E-06	1.6E-06		
BC intercept	2.0E-02	4.3E-03		
BC cov(intercept, slope)	-1.4E-04	7.8E-05		
BC:Time	1.2E-05	2.7E-06		
F1 intercept	1.5E-02	3.5E-03		
F1 cov(intercept, slope)	4.0E-05	7.3E-05		
F1:Time	1.3E-05	3.1E-06		
F2 intercept	1.1E-02	2.8E-03		
F2 cov(intercept, slope)	-9.8E-05	6.8E-05		
F2:Time	1.1E-05	3.1E-06		
DD intercept	4.8E-03	1.4E-03		
DD cov(intercept, slope)	7.7E-05	7.9E-05		
DD:Time	2.8E-05	8.6E-06		
Residuals	1.7E-05	1.6E-06		

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Table S 3.5 continued

Term	Var	SE	χ_1^2	P
D males				
spl(Time)	1.46E-06	1.82E-06	8.6	0.003
Tank	5.04E-05	2.14E-04	0.1	0.806
*Tank:lin(Time)	NA	NA	NA	NA
Cross:dev(Time)	6.42E-07	4.92E-07	5.7	0.017
Sediment:Salinity:dev(Time)	3.24E-06	1.63E-06	61.6	<0.001
WW:spl(Time)	2.82E-06	7.07E-07		
BC:spl(Time)	5.04E-06	1.37E-06		
F1:spl(Time)	4.82E-06	1.16E-06		
F2:spl(Time)	3.02E-06	8.99E-07		
DD:spl(Time)	1.30E-05	2.29E-06		
WW intercept	1.08E-02	1.80E-03		
WW cov(intercept, slope)	-6.33E-05	3.29E-05		
WW:Time	6.55E-06	1.14E-06		
BC intercept	1.25E-02	2.72E-03		
BC cov(intercept, slope)	-9.85E-05	6.59E-05		
BC:Time	1.36E-05	3.03E-06		
F1 intercept	1.43E-02	2.77E-03		
F1 cov(intercept, slope)	1.92E-05	5.84E-05		
F1:Time	1.23E-05	2.45E-06		
F2 intercept	1.83E-02	3.67E-03		
F2 cov(intercept, slope)	-1.53E-04	6.29E-05		
F2:Time	9.05E-06	1.89E-06		
DD intercept	9.38E-03	1.53E-03		
DD cov(intercept, slope)	4.39E-05	5.17E-05		
DD:Time	2.08E-05	3.45E-06		
Residuals	1.72E-05	1.31E-06		

*Variance was constrained to be positive and converged to zero

Chapter 4: Differences in Transcription Levels among Wild, Domesticated, and Hybrid Atlantic Salmon (*Salmo salar*) from Two Environments

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4.1. Abstract

Escaped domesticated individuals can introduce disadvantageous traits into wild populations due to both adaptive differences between population ancestors and human-induced changes during domestication. In contrast to their domesticated counterparts, some endangered wild Atlantic salmon populations encounter during their marine stage large amounts of suspended sediments, which may act as a selective agent. We used microarrays to elucidate quantitative transcriptional differences among a domesticated salmon strain, a wild population, and their first-generation hybrids during their marine life stage, to describe transcriptional responses to natural sediment sediments, and to test for adaptive genetic variation in plasticity relating to a history of natural exposure or non-exposure to suspended sediments. We identified 67 genes differing in transcription level among salmon groups. Among these genes, processes related to energy metabolism and ion homeostasis were over-represented, while genes contributing to immunity and actin/myosin-related processes might also be involved in strain differentiation. Domestic-wild hybrids exhibited intermediate transcription patterns relative to their parents for two-thirds of all genes that differed between their parents; however, genes deviating from additivity tended to have similar levels to those expressed by the wild parent. Sediments induced increases in transcription levels of

eight genes, some of which are known to contribute to external or intracellular damage mitigation. Although genetic variation in plasticity did not differ significantly among groups after correcting for multiple comparisons, two genes (metallothionein and glutathione reductase) tended to be more plastic in response to suspended sediments in wild and hybrid salmon, and merit further examination as genes under natural selection.

4.2. Introduction

Gene flow from domesticated individuals can change local wild phenotypes by introducing domestication-induced traits and traits adapted only to the natural ancestral environment from which the domesticated individuals were once taken (Rhymer & Simberloff 1996; Hutchings & Fraser 2008). Consequently, when domesticated individuals escape and interbreed with wild populations, a major conservation concern is that a lower fitness of hybrids may lead to a decline of wild populations (outbreeding depression), particularly of those populations already at heightened risk of extinction (Hutchings 1991; McGinnity *et al.* 2003).

Genetically based differences between wild and domesticated individuals can arise in three principle ways. First, domesticated individuals may differ from wild individuals because the wild ancestor of the domesticated individuals experienced different natural selection pressures (Hutchings & Fraser 2008). Second, differences can arise via domestication through intentional selection (e.g. improving traits beneficial for production; Rauw *et al.* 1998), inadvertent selection (e.g. the amount and quality of space or contact with humans favours some individuals over others; Kohane & Parsons 1988), or the relaxation of natural selective pressures in the captive environment (Price & King 1968; Mignon-Grasteau *et al.* 2005). Finally, genetic drift and inbreeding can also alter the genetic make-up of domesticated strains (Price & King 1968; Taberlet *et al.* 2011). The probability and magnitude of fitness reduction in hybrids may depend on how divergent their wild and domesticated parents are and on the source of these differences. For example, as a by-product of selection for productivity, domestication can lead to changes in

metabolism, reproduction, and health that can be disadvantageous *per se* (Rauw *et al.* 1998). However, some domestication-induced behavioural changes may disappear in the wild because the genetic variation required to revert may still be present in domesticated populations (Mignon-Grasteau *et al.* 2005). In contrast, when domesticated and wild populations differ due to ancestral adaptations to their respective environments, these differences are likely to be maintained in the wild, and therefore may play a major role in environment-specific hybrid fitness.

Adaptive differences among wild populations are traditionally seen as genetic-based phenotypic differences selected for by environmental variables. However, individuals from different populations might also differ in their genetic variation for phenotypic plasticity, which may be adaptive in variable environments (Schlichting & Pigliucci 1998), even under high gene flow (Conner & Hartl 2004). This phenotypic plasticity of a trait may depend on differential gene expression (Khaitovich *et al.* 2006; Gibson 2008). Although some authors have pointed out that it is largely unknown to what extent gene expression regulation reflect local adaptation (Staubach *et al.* 2010), others have suggested that it has important evolutionary consequences (reviewed by Wray *et al.* 2003; Aubin-Horth & Renn 2009).

Fishes are useful systems in which to study the effects of domestication because the relatively recent onset of their domestication enables a meaningful comparison with their wild counterparts, unlike studies of animals with unknown, uncertain, or extinct ancestors (Mignon-Grasteau *et al.* 2005). Although several studies have examined the effects of domestication on fish gene transcription in general (Roberge *et al.* 2006; Roberge *et al.* 2008; Devlin *et al.* 2009; Normandeau *et al.* 2009; Tymchuk *et al.* 2009a; Tymchuk *et al.* 2009b; Bougas *et al.* 2010; Sauvage *et al.* 2010), none have tested for differences in gene transcription between wild and domesticated individuals relevant to a direct response to environmental variables or in regard to genetic variation in plasticity.

Wild Atlantic salmon (*Salmo salar*) may be especially affected by outbreeding with domesticated individuals because of the high frequency and magnitude of aquaculture-related escape events (Morris *et al.* 2008). Of the many fish that escape,

some spawn in rivers where wild fish co-occur (Carr *et al.* 1997), and this can result in hybrid offspring (Crozier 1993; Skaala *et al.* 2006; Bourret *et al.* 2011) that have been shown to have lower lifetime reproductive success in nature than their wild counterparts (McGinnity *et al.* 2003). Compounding the effects of this outbreeding depression is the observation that wild Atlantic salmon are in decline throughout their natural range in the north Atlantic (ICES 2010).

The Canadian inner Bay of Fundy (iBoF) salmon populations are listed as 'endangered' under the Canadian Species at Risk Act (COSEWIC 2011). This group of several river populations has been at critically low numbers since the late 1980ies, which is most likely associated with low survival during the marine phase (DFO 2010). Interestingly, many iBoF populations have been hypothesized to exhibit an unusual localized marine migration pattern that appears to be restricted to the Bay of Fundy (Huntsman 1931; Jessop 1976), rather than undertaking a long-distance migration to the waters east of Newfoundland, as favoured by most North American Atlantic salmon (Ritter 1989; COSEWIC 2006). In this bay, extreme tidal movement, erosion of surrounding red beds, and riverine input cause high amounts of suspended sediments (Yeo & Risk 1981), which can result in physiological changes, a stress-induced immune response, and mortality in salmonids (Bash *et al.* 2001). Domesticated-wild outbreeding has the potential to negatively affect remaining iBoF populations and accelerate their extirpation because domesticated salmon may have lowered general fitness in the wild and lack adaptation to locally important selective factors. Suspended sediments from the iBoF may be one of these selective factors since sediments likely affect fitness and are naturally experienced by wild salmon but not by current domesticated salmon nor by their ancestors.

Using common-garden experiments in combination with microarray technology, we compared gene transcription profiles among individuals from an iBoF Atlantic salmon population, individuals of the major eastern Canadian domesticated strain, and their first-generation hybrids. This allowed us to: i) investigate general differences in transcription patterns during the marine stage among groups (strains) and describe the transcriptional consequences of outbreeding in hybrids; ii) investigate the transcriptional response to natural suspended sediments; and iii)

test for possible adaptive genetic variation in plasticity of gene transcription in response to suspended sediments in the wild strain.

4.3. Material and Methods

4.3.1. Salmon Strains and Tank Experiment

The wild salmon used (Wild) are native to the Stewiacke River, a tributary of the Shubenacadie River on the Minas Basin, Nova Scotia, located at the head of the iBoF. Because of the confined migration of the iBoF populations and the location of the river, salmon from this river experience suspended sediments for prolonged periods repeatedly during their lives. Conversely, wild ancestors of the domesticated strain (Domesticated) were native to the Saint John River, New Brunswick, on the outer Bay of Fundy and would have undertaken a typical long-distance migration (Ritter 1989). Wild Saint John and wild Stewiacke River salmon also differ in several other life-history traits (Huntsman 1931; COSEWIC 2006; Fraser *et al.* 2010), variation at neutral genetic markers, and gene transcription profiles (Tymchuk *et al.* 2010). The domesticated strain used in this study has undergone three generations of domestication, under artificial selection intended primarily to increase growth rate (Glebe 1998), and this has also led to evolutionary changes in gene regulation

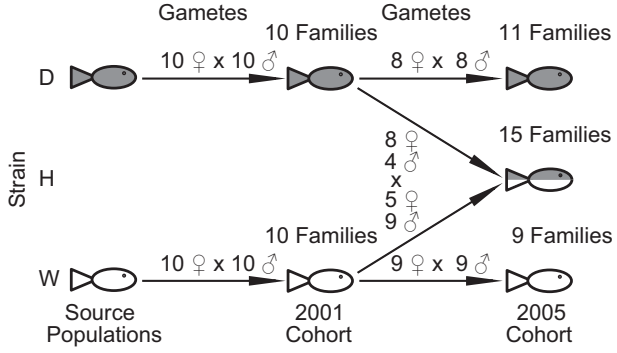

Figure 4.1: Salmon crossing design. Indicated are numbers of dams and sires from each strain (W = Wild, H = Hybrid, D = Domesticated) and each cohort crossed to generate the next cohort and numbers of families generated for each cohort.

(Roberge *et al.* 2006).

The fish used in the present study were fertilized in 2005 (details in **Figure 4.1** and in Fraser *et al.* 2010); the parents of these fish were fertilized in 2001 (details in Lawlor *et al.* 2008). Fish of both generations were hatched and raised in the Aquatron facility at Dalhousie University under similar conditions (e.g., fish density, tank type, physical parameters, and feed) each generation, thus minimising differences attributable to parental-environmental effects.

In June 2008, smolts (the salmon developmental stage involving a migration from river to sea) from the 2005 generation were separated from parr (the salmon freshwater stage), using external criteria: silver body colouration and darkened fin edges in smolts. All fish were marked by strain with visible implant elastomer tags (Northwest Marine Technology) and in July 2008 distributed among four round tanks (1790 L, flow-through system with bottom drains). To do so, all fish were measured and grouped into 5 cm size classes. Fish from each size class within each strain were distributed evenly among tanks. When distributing fish, size differences among the strains could not be accounted for as fish of both parental strains differed in average length by 23%, although all fish were of the same age. In total, each tank contained 25 fish of each of the three strains, as well as 50 fish of two additional strains not used for this study (backcrosses and second-generation hybrids) for a total of 125 fish per tank. All tanks initially received dechlorinated municipal water (before the application of sediments). During October 2008, salinity in all tanks was slowly increased to avoid a possible strong stress response due to exposing fish to the combination of sediments and salinity. This was conducted by increasingly adding ambient seawater in steps of ~5 psu every second week, until a level of ~20 psu was attained for the remaining duration of the experiment.

Intertidal sediments were collected during the summer from upper mudflats of the Minas Basin (Debert Beach, Lower Debert), air-dried (resulting in solid blocks), and manually ground to restore particle sizes, to allow for a standardised application (i.e. dry weight per litre) and facilitate re-suspension. Starting in October 2008, 358 g of the sediment powder was re-suspended in water and then poured into the middle of each of two sediment-environment tanks daily. This resulted in a pulse of 200 $mg*L^{-1}$ of suspended sediments with a turbidity of 32 NTU

(calibrated at S = 18 psu), mirroring the lower end of the natural sediment load range in the iBoF (Gordon 1994; 100 to 1000 mg*L^{-1}). The lower end was chosen because salmonids may actively avoid high sediment concentrations in the wild, as demonstrated in the laboratory (Bisson & Bilby 1982). As a procedural control, clear water was poured into the middle of the two remaining tanks. The turbidity initially dropped from 32 to 21 NTU (120 mg*L^{-1}) in the first half hour, then decreased exponentially with a half time of 1.8 hours; all sediments had dissipated after 24 hours, prior to the next application. It was technically not possible to mimic the natural tidal cycle of the Minas Basin where strong currents re-suspend sediments every 6 hours (Yeo & Risk 1981).

Unexpectedly, some fish attained sexual maturity, with a higher prevalence among wild (82%) than domesticated individuals (42%) on day 104 and 105 of the experiment. To prevent a bias in gene transcription due to maturity status and sex, only mature males were used for the subsequent analysis because they could be identified with confidence (by gently pressing along the belly towards the tail, resulting in emergent milt) and were available in sufficient numbers in all strains. To facilitate synchronisation of sampling, all excess fish (i.e., those not used in the microarray study, comprising females and immature males, backcrosses and second-generation hybrids) were removed, leaving five mature males of each of the three strains for a total of 15 fish per tank. This was done for one clear and one sediment environment tank after 104 days and for the remaining two tanks after 105 days. After an additional 16 days, fish from one clear and one sediment-environment tank were sampled within two hours (10 to 12 am); the sediment tank one hour after the sediment application (to be able to also detect short-term effects). This was repeated the following day for the remaining two tanks in reverse order with respect to treatment. Fish were not fed for two days prior to sampling. For tissue sampling, all fish of a given tank were euthanized with tricaine methanesulphonate (250 mg*L^{-1}) within 10 min of first disturbance. The left second gill arch of each fish individual was sampled in random order, wrapped in aluminium foil, flash-frozen in liquid nitrogen and kept at -80°C until RNA was extracted. Five fish from each cross and tank were sampled (totalling 10 per cross

and environment) but only randomly chosen four fish per cross from each of the four tanks were used for the subsequent analysis (eight per cross and environment). This sampling design allowed for the inference of effects caused by the environment that had not been confounded by the effect of tank.

4.3.2. Laboratory Methods

Eight fish from each of the six experimental groups were analysed, totalling 48 fish. The filaments of each gill arch were cut from frozen tissue and disrupted in tubes with steel beads and PureLink lysis buffer (Invitrogen), using a commercial shaker (QuiagenTissuLyser). RNA was extracted following the manufacturer's manual for purifying RNA from animal tissue (PureLink, Invitrogen). Subsequently, 120 µg of each sample of total RNA were treated with DNase, as outlined in Normandeau *et al*. (2009). The quality and quantity of RNA was assessed by spectrometry, using Nanodrop (Thermo Scientific) after both the extraction and the DNase treatment. Samples were processed in blocks of randomly assigned arrays to exclude methodological bias.

Reverse transcription PCR of 15 µg RNA per reaction was performed as outlined in Normandeau *et al.* (2009), but using Cy5 and Cy3 dyes with the Array50 kit (Genisphere). Because each sample was to appear on two different arrays (see next paragraph and **Figure 4.2**), the RNA for both replicates was retro-transcribed synchronously, unless arrays had to be repeated due to failures. Samples were stored at -20°C until hybridisation, which was performed as detailed in Normandeau *et al*. (2009) utilizing the Salmonid 32K microarray slide (GEO Platform GPL13225: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL13225). This microarray slide has been stepwise developed by the consortium of Genomic Research on Atlantic Salmon Project, contains also clones from Atlantic salmon gill tissue, and is composed of 27,917 Atlantic salmon and 4065 rainbow trout (*Oncorhynchus mykiss*) cDNA features (sequences) extracted from several cDNA libraries and constructed from diverse tissue-types and developmental stages of fish (Rise *et al.* 2004; von Schalburg *et al.* 2005; Koop *et al.* 2008). Of the total

31,982 features (constituting ~8800 different annotated genes), 10,482 constitute unknown genes.

The hybridisation design consisted of loops contrasting the three strains (*Strain*) within each environment and swaps contrasting the two environments (*Environment*) within each strain (**Figure 4.2**). Generally, this design is a compromise between the efficiency of detecting the main effects and their interaction (Landgrebe *et al.* 2006). Because each individual was compared once among *Strain* between *Environment* and once between *Environment* among *Strain*, dye swaps between comparisons of both main factors and for each individual were equilibrated. This resulted in a total of 48 individuals on 48 slides. A maximum of four slides were processed simultaneously and scanned directly after the dye hybridisation step, using a ScanArray scanner (PerkinElmer) at a resolution of 10 µm and 90% laser power, while manually equilibrating intensity between dyes for each slide, using the photomultiplier tube settings. All scanned images were saved in TIF format and imported into the software QuantArray (Packard BioScience) which was used to manually adjust the grid, flag bad spots, and extract the intensity data.

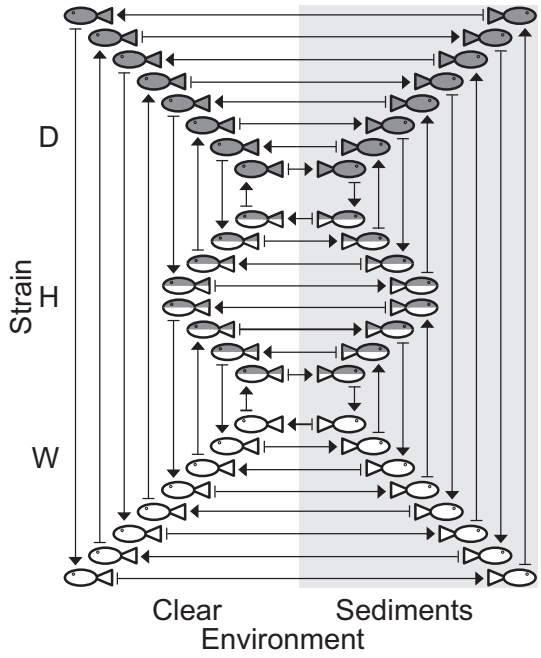

Figure 4.2: Microarray hybridisation design where every individual is represented by a fish symbol and each arrow represents one array with arrow-head and arrow-beginning for different dyes. Indicated are environments and strains (W = Wild, H = Hybrid, D = Domesticated).

4.3.3. Statistical Analyses

For each spot, the local background signal was subtracted from the mean intensity value. For the subsequent analysis, only spots were used for which i) intensity was found to be higher than the average intensity plus two standard deviations of negative control spots and ii) spots of all eight individuals of at least one of the six experimental groups fulfilled the first criterion.

The data from this subset of transcripts were analysed using the R MAANOVA package (http://churchill.jax.org/software/rmaanova.shtml). Missing data of flagged spots were replaced after their estimation using the K-nearest neighbour algorithm. Each array was corrected for spatial heterogeneity and intensity differences between dyes by using the Joint Lowess correction, followed by centralisation to correct for variations in array mean intensity. A mixed-model ANOVA was fitted to the normalised data (4483 features fulfilled the above criteria), viewing dye, *Environment*, *Strain*, and the interaction between *Strain* and

Environment as fixed effects terms and arrays and samples (individual) as random effects, and using the REML method. The statistical significance of *Strain*, *Environment* and their interaction was assessed using *F*-tests for each feature with 1000 permutations, shuffling over samples, to account for possible violations of normality and homogeneity of variances. All empirical p-values were corrected for the false discovery rate (FDR) by using the Q-VALUE package (Storey & Tibshirani 2003).

The genes that differed for *Strain* or *Environment* were tested for over-represented gene ontology (GO) categories (biological processes, cellular components, or molecular functions) by using Blast2Go (Conesa *et al.* 2005). All transcribed features were blasted with default values of the programme, using the accession numbers as given in the annotation file (available at: http://web.uvic.ca/grasp/microarray/), followed by the mapping and annotation steps in Blast2Go. A reference list was created from features with available GO terms which collapsed the 4483 transcribed features into 3056 because 803 had no gene annotation ('unknown') and GO terms could not be assigned to 624 known genes. Then, a test list was created of all features with GO annotations found to be different among strains at a FDR < 0.05. It was not possible to test for over-representation of GO terms for *Environment* at the same significance level because there were too few features. Hence, to enable this test a test list was drawn from features with GO annotation different for *Environment* at FDR < 0.2 (choosing this value arbitrarily to compromise between type 1 and type 2 errors). Both test lists were compared to the common reference list using Fisher's exact test of the Gossip package (Blüthgen *et al.* 2005) implemented in Blast2Go. Results were reduced to the most specific GO terms (i.e., the lowest level). Furthermore, genes different for transcription level among strains and between environments (at FDR < 0.05) were also manually compared in regard to common biological functions because no GO-term could be assigned for many array features using Blast2Go. This was conducted based on results obtained after using each gene as keyword on the UniProt Knowledgebase website (http://www.uniprot.org) and/or searching for articles on the ISI Web of Knowledge website (http://apps.isiknowledge.com).

For the subsequent analysis and for heatmaps depicting the main effects, intensity values of spots were first averaged across arrays for each individual and then averaged across features if representing the same gene, using the centralised intensity log2 values adjusted for technical variation (dye and array) as estimated by ANOVA. Correlations between transcription level and logarithmic body weight were tested to evaluate the possibility that differences among strains may be caused by differences in their weight. This was conducted by means of Pearson's correlations separate for each strain in order to avoid confounding strain and weight as individual weights did show little overlap between the parental strains (**Figure 4.3**).

To obtain some insight into the transcription pattern of domesticated-wild hybrids, genes differently transcribed among strains were analysed for their genetic architecture. For each gene, first pairwise contrasts depicting the additive parameter α (wild-domesticated)/2) were calculated, representing the absolute difference in intensity values between parents. This was followed by calculating contrasts for the dominance parameter δ ((wild+domesticated/2) - hybrid), representing the absolute difference of the average observed hybrid value from an expected mid-parent value under complete additivity. The ratios of δ/α with corresponding Fieller's 95% confidence intervals (CI) were calculated for features with $p < 0.05$ for both contrasts using the R-package MRATIOS (Dilba *et al.* 2012). The δ/α ratio reflects the relative difference of the hybrid value from a value under an additive genetic architecture. Values for δ/α including 95% CI greater or smaller than zero were interpreted as indicative of a non-additive genetic architecture.

4.4. Results

Of a total of 32,280 features on the array, 4483 (~14%) were detected in gill tissue of Atlantic salmon. Among the three strains of wild, domesticated, and first-generation hybrid salmon transcription levels were different for 104 features (maximum p-value of 0.0023 at FDR < 0.05; **Table S 4.1**). Nine features showed different transcription levels among individuals from environments with or without

suspended sediments (maximum p-value of 0.0001 at FDR < 0.05; **Table S 4.2**). Only the S-100P protein transcript exhibited significant differences for both terms. No feature was significantly different for the interaction of *Strain* and *Environment* after the FDR correction (FDR = 1 for each feature). However, before FDR correction, 118 features were significant for this term (p-values between 0.0007 and 0.05; **Table S 4.3**) of which two genes were also included in the set of genes that differed between the environments (see below).

4.4.1. Differences between Environments

The nine features that were transcribed at different levels between the two environments at FDR < 0.05 were collapsed into eight putative genes (**Figure 4.3**). Fold changes for these genes ranged from 1.1 to 1.4 (**Table S 4.2**). The transcription of three of the eight genes was down-regulated in fish exposed to suspended sediments. The products of these three genes are either involved in a high number of processes (ubiquitin, NAC alpha) or have no known molecular or biological function that could be linked to specific processes induced by suspended sediments (S-100P protein: Ca^{2+} -binding). The five up-regulated genes play a role in cell redox homeostasis (glutathione reductase, metallothionein), the innate immune response (complement factor B precursor), and epithelial enforcement by keratinisation and peptide cross-linking (envoplakin). The remaining feature (accession CB501822) had no significant Blast hit but showed a pattern among individuals similar to that of complement factor B and was, therefore, retained in further analyses (**Figure 4.3a**).

Under a higher FDR of 0.2, 65 features were different between environments (maximum p-value of 0.0034) of which 40 could be assigned GO terms (**Table S 4.2**). The comparison with the reference file resulted in no over-represented GO terms at a FDR < 0.05, however, the two most specific GO terms with the lowest FDR value (FDR = 0.19, maximum p-value of 0.00016; **Table S 4.4**) resembled two of the processes that were inferred manually ('glutathione-disulfide reductase activity' and 'keratinization'). The genes for complement factor B and metallothionein did not yield any GO terms.

4.4.2. Differences between Wild and Domesticated Salmon

Transcription fold changes among strains ranged from 1.1 to 3.0 (**Table S 4.1**). The 104 features with different transcription levels among the three strains represented 85 different putative genes and 19 features without Blast hits. The 85 features with known functions represented, accordingly, 67 different genes (**Figure 4.3b**), each represented by up to five features (**Table S 4.1**). Many of these genes contributed to three major processes: energy metabolism, the immune response, and actin/myosin related processes.

Many of the genes that differed among strains could be related to ATP production or its transport such as mitochondrial ATP-formation via oxidative phosphorylation (11 genes: four types of ATP synthase subunits; ADP/ATP translocase 2; cytochrome c oxidase subunits 3 and 5b; cytochrome c oxidase polypeptide VIIa; cytochrome c1, heme protein; phosphate carrier protein) or via citric cycle (three genes: malate dehydrogenase; isocitrate dehydrogenase; L-lactate dehydrogenase). Further, glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase B chain, both involved in glycolysis, and creatine kinase that catalyses storage or availability of ATP via creatine, were also differently transcribed among strains. Some genes could be associated with the innate and adaptive immune response (eight genes: two types of MHC I; MHC II; lysozyme C; myelin and lymphocyte protein; leukocyte common antigen; CD59). The third group comprised some genes involved in muscle contraction or intracellular transport via actin filaments or networks (eight genes: three types of actin; myosin regulatory light chain 2 and 2b; troponin c; cofilin-2; actin-related protein 2/3 complex subunit 3).

The results of the analysis of over-represented gene ontology (GO) categories at an FDR of 0.05 differed from manually-inferred processes. Transcripts of proteins involved in biological processes such as ‘ion transmembrane transport’, ‘proton transport’, and ‘ATP biosynthetic process’ were among the top three most specific processes being over-represented. All three over-represented categories were represented by the same genes (several subunits of the ATP-synthase and Na^+/K^+

transporting subunits alpha 1 and alpha 3; **Table S 4.4**), except for calmodulin which only contributed to the last category. Additional meaningful over-represented GO-categories included the process of 'regulation of pH' (represented by carbonic anhydrase and two Na^+/K^+ alpha polypeptides), the function 'monovalent inorganic cation transmembrane transporter activity' (mostly represented by ATP-synthase subunits), and the cellular component 'integral to plasma membrane' (**Table S 4.4**). GO-terms indicating relevance of the immune response (except for a contribution of the leukocyte common antigen and the myelin lymphocyte protein to the latter category) or actin-myosin-related structures or processes were not over-represented.

When transcription level was compared among the strains, 49 of the 86 putative genes were transcribed at a higher level in domesticated salmon (**Table S 4.7**). Among those were all genes contributing to over-represented GO categories, except for calmodulin, myelin and lymphocyte protein, and leukocyte common antigen. Genes whose products were, based on manual inference, involved in the immune response or in actin-myosin-related structures, showed a less consistent transcription pattern. Higher transcription levels in domesticated relative to wild salmon were observed for MHC class II, lysozyme C, and CD59, but lower transcription were levels observed for MHC class I, leukocyte common antigen, and the myelin lymphocyte protein. The proteins involved in actin-myosin-related structures (different types of actin, myosin regulatory light chain 2, and cofilin-2) were also up-regulated in domesticated vs. wild fish. However, components regulating actin-myosin-related processes were down-regulated, namely, troponin C regulating striated muscle contraction and actin-related protein 2/3 complex subunit 3 involved in cytoskeleton network formation regulation.

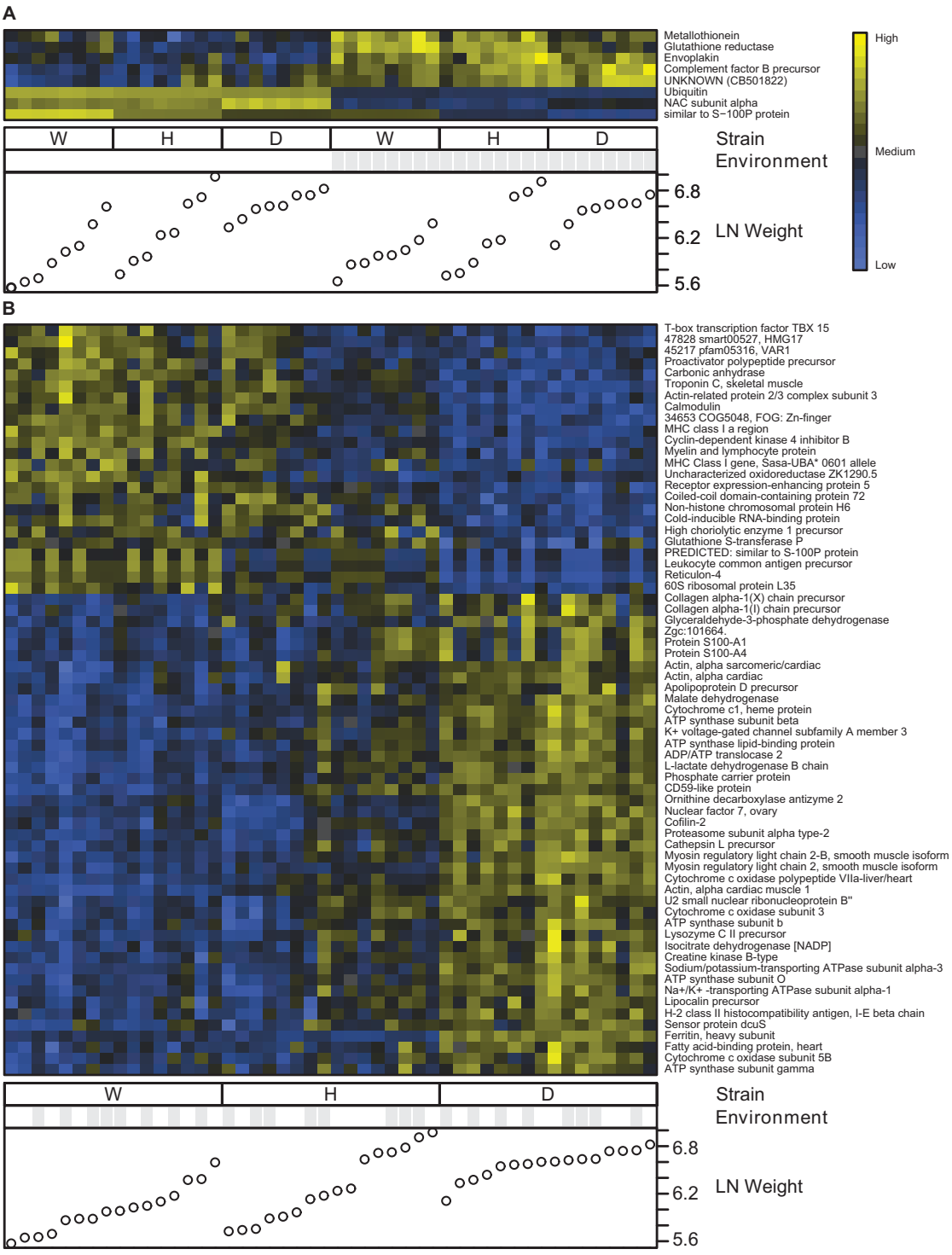

Figure 4.3: Heatmap of relative transcription levels of different transcription levels of annotated genes (FDR < 0.05) for *Environment* (**A**) and *Strain* (**B**). In addition to *Strain* (W = Wild, H = Hybrid, D = Domesticated) and *Environment* (white = no sediments, grey = suspended sediments), the natural logarithms of weight (in grams) of individuals are indicated below each heatmap. Gene names have been shortened to fit the legend.

No correlation of transcription level with logarithmic weight was detected for domesticated salmon. However, 34 of the 86 putative genes tested correlated for hybrids and 13 for wild salmon, of which eight genes were common to both (**Table S 4.7**). Remarkably, for these eight genes, the domesticated strain shows consistently higher transcription levels relative to the wild strain for positive correlations and lower transcription levels for negative correlations, each simultaneously found in

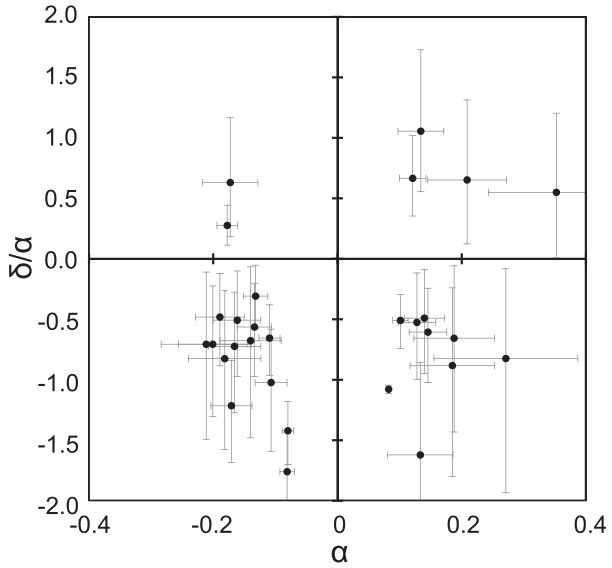

Figure 4.4: Scatter plot of the ratio of dominance (δ) and additive parameter (α) vs. additive parameter for genes with different transcription levels among strains. Only genes with non-additive transcription patterns in first-generation hybrids between wild and domesticated Atlantic salmon are shown. Error bars represent 95% confidence intervals and have been cut off for easier visualisation. Values of α larger than zero indicate higher transcription in wild salmon whereas values smaller than zero indicate higher transcription in domesticated salmon. Values of δ/α larger zero indicate hybrid values closer to domesticated salmon and values smaller than zero indicate hybrid values closer to wild salmon.

the wild and the hybrid strains (**Table S 4.7**).

4.4.3. Transcription Pattern in Domesticated-Wild Hybrids

An additive pattern of inheritance in hybrids was exhibited in 57 of the 86 differentially transcribed putative genes (δ/α not different from zero; **Table S 4.6**). In hybrids, the levels of transcripts for some genes corresponded to predicted mid-

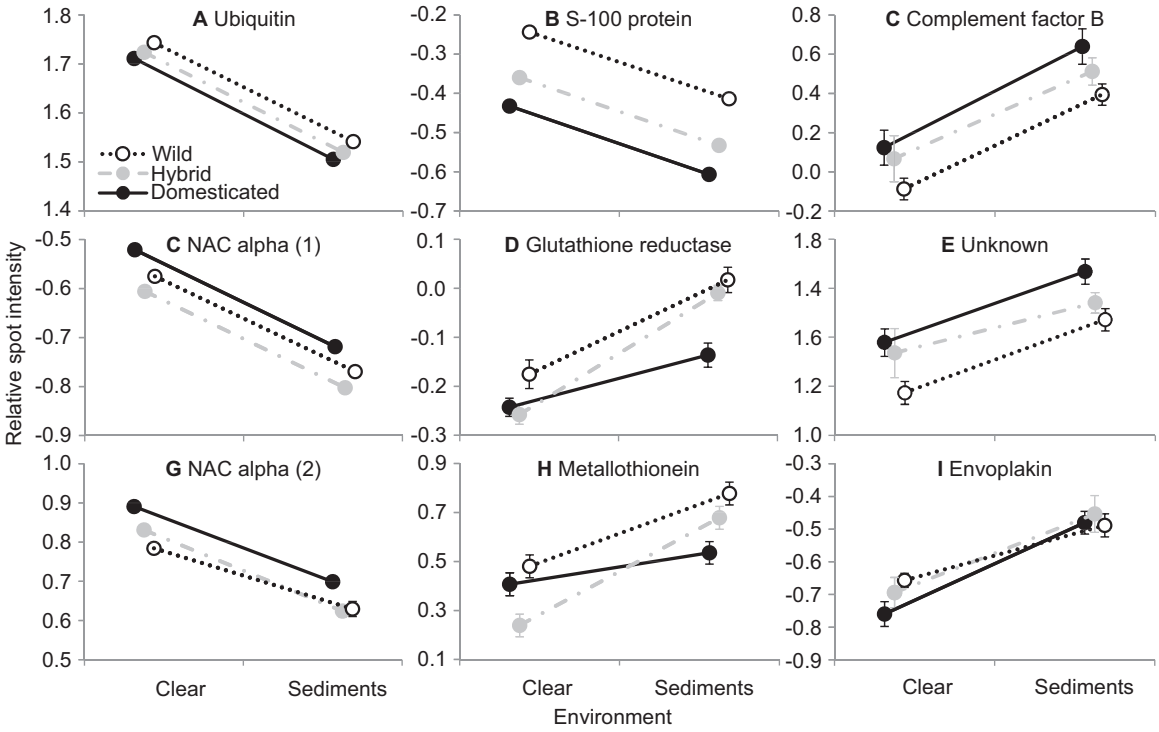

Figure 4.5: Strain-by-environment interaction plots for genes with transcript levels different between the environments (FDR < 0.05). Model corrected average relative transcription level of each experimental group is shown with 95% confidence intervals. Only E and H yielded significance for the interaction of strain-by-environment (simple P < 0.05; none were significant after FDR correction).

parent values, i.e., they exhibited a 'true' additive pattern. However, many of the genes that exhibited an averaged-additive pattern did not show intermediate levels in every hybrid individual. Instead, hybrids exhibited a mosaic pattern of similarity to either parent across individuals, and also across genes (**Figure 4.3b**). The remaining one-third of all genes different between wild and domesticated salmon deviated from an additive pattern of inheritance in hybrids and exhibited absolute values of δ/α ranging from 0.3 to 1.8 (**Figure 4.4**, **Table S 4.6**). Of all 29 deviating genes, 23 showed mean values in hybrids that were more similar to wild than to domesticated salmon (i.e. $\delta/\alpha < 0$; **Figure 4.4**).

4.5. Discussion

Our study examined (i) the degree to which gene transcription patterns differs between wild, domesticated Atlantic salmon, and their hybrids, (ii) how suspended sediments influence gene transcription, and (iii) whether wild salmon exhibit greater transcriptional plasticity. For the wild iBoF populations, it has been hypothesised that local adaptation in response to suspended sediments exists (COSEWIC 2006; Tymchuk *et al.* 2010). However, our data suggest that Minas Basin sediments did not result in significant differences in transcriptional plasticity among the salmon strains investigated. Nonetheless, it is still important to explore the data at hand in terms of its relevance to conservation, especially since statistical power to detect the strain-by-environment interaction was low. The low power, caused by a small sample size and the stringent correction we applied for multiple comparisons, may have resulted in the FDR = 1 for all features. Among the 118 features that showed strain-by-environment interaction before correcting for multiple comparisons (**Table S 4.3**) are two that exhibit differences in transcript levels between the environments (FDR < 0.05; see below): glutathione reductase and metallothionein. Both are often used as biochemical indicators in toxicological studies of fish (Atli & Canli 2008; Minghetti *et al.* 2008). Metallothionein has even shown indications of differences between fish populations in the plasticity of gene transcription response towards cadmium-exposure (Knapen *et al.* 2004). Taking this into consideration, strain-specific regulation may underlie the transcription of metallothionein and glutathione reductase. For both of these genes, differences between strains become larger in the sediment environment with a higher transcript level in wild and hybrid salmon relative to the domesticated strain (**Figure 4.5e, h**). This is concordant with the hypothesis of local adaptation to a temporarily experienced stressor through increased genetic plasticity only by the wild strain. Here, outbreeding by domesticated salmon did not negatively alter the genetically-based plastic response in first-generation hybrids since they even show

the steepest slopes between environments. It remains to be tested if a reduction of plasticity may appear in further hybrid generations.

4.5.1. Differences between Environments

Eight genes exhibited a response to suspended sediments in all salmon strains, and four of these genes may provide insight into the molecular consequences of suspended sediments. One of these genes is envoplakin, a protein found in stratified squamous epithelia. Envoplakin plays a structural role by reinforcing the plasma membrane and cell junctions (Kalinin *et al.* 2004). In fish, this epithelial type can be found enveloping the primary lamellae of the gills. Thus, the higher level of envoplakin transcript abundance in the environment containing sediments may indicate a regenerative or enforcing response to abrasive sediments, and this would be in agreement with observations of sediment-induced gill proliferation (Herbert & Merkens 1961; Sutherland & Meyer 2006). The observed increase in the transcription of complement factor B could reflect an innate immune-system response induced by the aforementioned mechanical stress or by the biological burden, such as bacteria, that sediments can carry. Possible reasons for an increase in the level of glutathione reductase and metallothionein transcripts are less clear. Both proteins are involved in protection from oxidative stress, which can have various causes including immunological challenge. However, metallothionein is also typically linked to protection from heavy metal toxicity (reviewed by Sato & Kondoh 2002). The iBoF sediments contain various metals with a higher concentration in finer particles (Loring 1979) that may be responsible for some of the differences observed.

Only eight genes responded to suspended sediments, but this modest response may have been caused by low statistical power, low sediment concentration, or infrequent sediment application (once a day). Furthermore, sampling of only mature males may have biased the results, since mature males may differ in their response from immature males or females. The observed responses in transcript levels nevertheless indicate the presence of mechanisms to mitigate external-

mechanical as well as intra-cellular damage, so our results contribute to the growing field of 'ecological annotations of genes' (Landry & Aubin-Horth 2007).

4.5.2. Differences between Wild and Domesticated Salmon

We identified quantitative transcriptional differences affecting 2.3% of all features detected in gill tissue between domesticated and wild salmon at the adult stage. In our study, the major manually-inferred biological process differing between the strains was energy metabolism, in particular ATP production via oxidative phosphorylation, which was also over-represented by the analysis of GO terms. Because the gills are the major respiratory, ion-homeostatic, and excretory organ in bony fishes (Barton 2007), a higher local energy-production may maximise capacities for those processes and contribute to faster growth. All genes associated with those processes were transcribed at a higher level in domesticated fish. A high representation of genes involved in oxidative phosphorylation among genes with high heritability for level of transcription (Roberge *et al.* 2007) indicates that they have a strong potential to respond to selection. Accordingly, over-transcription of genes related to oxidative phosphorylation observed in the present study could partly be a result of selection, as this domesticated strain has been selected for fast growth (Glebe 1998). It is also possible that any observed difference among strains may have been caused by evolutionary factors pre-dating domestication. Aside from genetic drift, ecological differences such as migration distance (see above) or water temperature of the marine feeding grounds (cf. Locarnini *et al.* 2010) may be good candidates.

Admittedly, some differences observed in the present study may be the result of a cascade of gene transcription regulation initiated by only one or few pleiotropically acting genes not surveyed that are also responsible for differences in body size. For instance, a recent study on transgenic Pacific salmon revealed that quantitative changes in transcription at numerous genes originated from the insertion of a single gene coding for growth hormone (Devlin *et al.* 2009). Although it is possible to account for the confounding effects of strain and size by investigating both size- and age-matched individuals (e.g. Tymchuk *et al.* 2009b),

this would have posed a large logistic and analytic challenge to our strain-by-environment approach that also incorporated hybrids. We argue that body size is not a predictor for all differences among strains as judged by visual (**Figure 4.3**) and statistical (**Table S 4.7**) inspection of the relationship of relative transcription level with weight. This did not result in a ubiquitous pattern of weight-transcription correlations across genes and strains. Nevertheless, those eight genes that correlated with size simultaneously in wild and hybrid salmon showed strong indications of size-dependence, although it was impossible in this study to identify weight as a cause or effect of the transcription-level differences. Furthermore, the direction of the correlation of those eight genes corresponded to the direction of the transcription-level difference between wild and domesticated salmon. As a by-product of selection in domesticated salmon, transcription variation may be reduced; which would explain why correlations were non-significant in this group. The detection of more genes correlating with size in the hybrid strain may be a side effect of their wider size-range increasing the probability to detect a correlation relative to the wild strain.

Many genes transcribed at different levels among strains are major players in the immune response. The analysis of GO terms did not corroborate this observation but may have been only of limited use because 30% of all the features transcribed in gills different between strains (at FDR < 0.05, **Table S 4.1**) did not contribute any GO terms to the analysis, with MHC class I genes and the CD59-like protein among them. A trade-off between growth and immune response in fish has been suggested on the basis of comparisons among different types of fast growing salmonids and their wild conspecifics (Tymchuk *et al.* 2009a). Under this scenario, fast growth comes at the cost of down-regulation of the immune system which is in agreement with general observations in livestock (Rauw *et al.* 1998). We found a mixed pattern, with some up- and some down-regulated genes involved in the immune response. Down-regulation of MHC class I genes, leukocyte common antigen as well as myelin and lymphocyte antigen in domesticated salmon corroborates the suggestions of Tymchuk *et al.* (2009a) and may indeed indicate a fitness reduction in domesticated salmon (under the hypothesis that a reduced transcription

translates into a reduced immune response) as the gill is a key tissue when encountering and dealing with pathogens (Haugarvoll *et al.* 2008). However, up-regulation of CD59 and MHC class II, in combination with a higher lysozyme C transcript-level in domesticated salmon opposes Tymchuk's et al. (2009a) theory. It may even explain findings of domesticated fish displaying a higher resistance towards vibriosis, a common bacterial disease in aquaculture, when compared to wild fish (Lawlor *et al.* 2008), because both latter genes are involved in the immune response towards bacteria.

Differences in MHC transcript abundance between wild and domesticated salmon might have simply resulted from allele frequency differences between ancestral populations - which are common in salmon (Landry & Bernatchez 2001; Dionne *et al.* 2007; Evans *et al.* 2010). Allele-specific microarray-spot hybridisation efficiencies may then have mimicked differences in transcription-level regulation. As another alternative explanation, a recent study found allele-specific transcription abundance of a MHC class II gene, linked to a putative *cis* regulating DNA motif (Croisetière *et al.* 2010). Under the hypothesis that polymorphisms in allele-specific regulators are adaptive, outbreeding by domesticated fish could lead to outbreeding depression by reducing fitness of hybrid offspring via changed MHC protein quality and quantity.

4.5.3. Transcription Pattern in Domesticated-Wild Hybrids

For genes exhibiting an additive inheritance, we observed a mosaic pattern of transcriptional similarity to either parent, although some genes also exhibited a 'truly' intermediate transcription pattern. Interestingly, this mirrors phenotypic observations in interspecific hybrids where some morphological characters are exhibited as intermediate and some resemble either parental species across hybrid individuals (Campton 1987). This mosaic pattern may be caused by a combination of either the correlation of transcription level with size (confined mostly to hybrids; see above), a 'random' individual-specific similarity to either parent, or gene-specific effects of non-additive transcription. Lastly, the unknown family identity of individuals, and hence parental effects, may have influenced the observed mosaic

pattern. It is, however, impossible to infer consequences of this mosaic pattern on hybrid fitness in this study.

For the one third of transcribed genes in hybrids deviating from an additive pattern, the hybrid transcription level was often closer to the level in the wild strain (23 genes of 29). This can be seen as positive from a conservation perspective, since it would probably reduce a possible negative impact of domestic-wild hybridisation in the wild. This observation is in contrast to findings in the liver of salmon juveniles reared only in fresh water where the gene transcription level of hybrid backcrosses (F1 hybrid × wild) was closer to the domesticated strain, despite a higher genetic contribution from the same wild population used in this study (Normandeau *et al.* 2009). This likely indicates the presence of tissue-specific patterns of gene transcription, or salinity-specific influence on gene regulation as previously reported in the brook charr (*Salvelinus fontinalis;* Côté *et al.* 2007). Alternatively, random differential parental contribution to hybrids between this study and Normandeau's et al. (2009) study may have caused the observed differences. A non-additive transcription level in hybrids exceeding values of either parental strain has also been observed in other salmonids (Renaut *et al.* 2009; Bougas *et al.* 2010), and is likely caused by a combination of allelic variants not found in either parental strain. Such pattern may result from non-additive effects within a locus (Rieseberg *et al.* 1999) or non-additive effects among loci involved in regulatory networks (Landry *et al.* 2007). The latter explanation may be a more likely scenario for variation in gene transcription levels.

4.5.4. Conclusion

The present study contributed the first data on gene transcription in Atlantic salmon during the pivotal marine phase that is suspected to be critical for salmon conservation (DFO 2010). It identified genes related to energy metabolism and immunity as being the major differences in gene transcription in gill tissue between wild iBoF salmon and the major prevalent aquaculture strain. Transcription patterns in first-generation hybrids appeared unpredictable and fitness consequences remain to be evaluated. Suspended sediments did induce a mostly

similar change in the transcriptional patterns of all strains. However, two genes (glutathione reductase and metallothionein) did exhibit a strain-by-environment interaction with greater plasticity exhibited by the wild strain, a finding consistent with the hypothesis that this interaction represents an adaptive response to stressful environmental conditions during a critical developmental life-stage. As such, these two genes merit further examination as genes whose expression may be evolving under the effect of natural selection.

4.6. Acknowledgements

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Project Grant awarded to J. Hutchings and L. Bernatchez. We are thankful to everyone who helped with the experimental and analytical work; especially to M. Yates, K. Bremer, D. Keiths, A. Houde, L. Weir, S. Renault, Catherine Dale, and many other members of the Bernatchez, Hutchings and Mackay lab. Further, we thank four anonymous reviewers and the editors who helped improving this manuscript. This work followed CCAC guidelines and was approved by the institutional animal care committee.

4.7. Data Accessibility

MIAME compliant data of the microarray experiment has been deposited at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with GEO accession number **GSE30555**, and is directly accessible via this link:

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30555

4.8. Supplementary Material

The following supplementary material exceeds the page length to be accommodated in this thesis and a direct download link to a .xlsx-file is:

http://onlinelibrary.wiley.com/store/10.1111/j.1365-294X.2012.05567.x/asset/supinfo/MEC_5567_sm_TableS1-S7.xlsx?v=1&s=1fc31f983c3b427e24129fcd12245ac43c1a5e0f

Table S 4.1: Features transcribed at different levels among Atlantic salmon from sediment and non-sediment environments (ANOVA permutation *F*-test, $P < 0.05$).

Supplementary Table S 4.1 exceeds the page length to be accommodated in this thesis

Table S 4.2: Features transcribed at different levels among wild, domesticated and hybrid Atlantic salmon (ANOVA permutation *F*-test, $P < 0.05$).

Supplementary Table S 4.2 exceeds the page length to be accommodated in this thesis

Table S 4.3: Features with transcription levels different for the interaction of strain-by-environment (ANOVA permutation *F*-test, $P < 0.05$).

Supplementary Table S 4.3 exceeds the page length to be accommodated in this thesis

Table S 4.4: Most specific GO terms over-represented (Fisher's exact test at FDR < 0.05) among the genes with different transcription levels among wild, domesticated and hybrid Atlantic salmon (ANOVA permutation *F*-test, FDR < 0.05).

Supplementary Table S 4.4 exceeds the page length to be accommodated in this thesis

Table S 4.5: Most specific GO terms over-represented (Fisher's exact test at FDR < 0.2) among the genes with different transcription levels between Atlantic salmon from sediment and non-sediment environments (ANOVA permutation *F*-test, FDR < 0.2).

Supplementary Table S 4.5 exceeds the page length to be accommodated in this thesis

Table S 4.6: Key variables of the analysis for non-additive gene transcription patterns by means of the δ/α ratio in hybrid salmon for genes different among wild, domesticated and hybrid Atlantic salmon (ANOVA permutation *F*-test, FDR < 0.05).

Supplementary Table S 4.6 exceeds the page length to be accommodated in this thesis

Table S 4.7: P-values and Pearson's correlation coefficients obtained from the correlation between the relative transcription level and the natural logarithmic weight for each strain for genes different among strains.

Supplementary Table S 4.7 exceeds the page length to be accommodated in this thesis

Chapter 5: Multigenerational Hybridisation and its Consequences for Maternal Effects in Atlantic Salmon

This chapter has been published as:
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5.1. Abstract

Outbreeding between segregating populations can be important from an evolutionary, conservation, and economical-agricultural perspective. Whether and how outbreeding influences maternal effects in wild populations has rarely been studied, despite both the prominent maternal influence on early offspring survival and the known presence of fitness effects resulting from outbreeding in many taxa. We studied several traits during the yolk-feeding stage in multigenerational crosses between a wild and a domesticated Atlantic salmon (*Salmo salar*) population up to their third-generation hybrid in a common laboratory environment. Using cross-means analysis, we inferred that maternal additive outbreeding effects underlie most offspring traits, but that yolk mass also underlies maternal dominant effects. As a consequence of the interplay between additive and dominant maternally controlled traits, offspring from first-generation hybrid mothers expressed an excessive proportion of residual yolk mass, relative to total mass, at time of first feeding. Their residual yolk mass was 23-97% greater than those of other crosses and 31% more than that predicted by a purely additive model. Offspring additive, epistatic, and epistatic offspring-by-maternal outbreeding effects appeared to further modify this largely maternally controlled cross-means pattern, resulting in an increase in offspring size with the percentage of domesticated allelic combinations. Fitness implications remain elusive because of unknown phenotype-

by-environment interactions. However, these results suggest how mechanistically co-adapted genetic maternal control on early offspring development can be disrupted by the effects of combining alleles from divergent populations. Complex outbreeding effects at both the maternal and offspring levels make the prediction of hybrid phenotypes difficult.

5.2. Introduction

Understanding the genetic architecture of population divergence allows for the prediction of generational trajectories of hybrids by their phenotype and relative fitness, both of which can influence adaptation, speciation, and conservation- or economical-agricultural breeding strategies (Lynch 1991; Burke & Arnold 2001; Sørensen *et al.* 2008). A particular genetic architecture, such as the presence or interplay of dominance (interaction of alleles at the same locus) and epistasis (interaction of alleles at different loci), governs the genotype-dependent trajectory of the phenotype and the mechanisms of hybrid fitness across generations (Lynch 1991).

Predicting the effects of outbreeding can be further complicated by maternal effects, defined as the maternal contribution to the offspring phenotype that can underlie both environmental and genetic effects (Räsänen & Kruuk 2007; Wolf & Wade 2009). Maternal effects act through maternal provisioning to offspring other than that generated by meiotic or cytoplasmic-derived genetic parental contribution (reviewed by Wolf & Wade 2009). Maternal effects can, at least temporarily, outweigh or interact with the offspring genotype in forming a particular phenotype (Wolf 2000). Hence, a major challenge is the disentanglement of maternal effects from environmental and direct offspring genetic effects (Willham 1980; Kruuk & Hadfield 2007).

In most studies on wild populations maternal effects are not assessed for their genetic architecture (Räsänen & Kruuk 2007), although genetic-based maternal effects are of evolutionary and ecological importance, given their role as heritable modifiers of the development and phenotype of the offspring (Mousseau & Fox

1998; Wolf & Wade 2009). Maternal effects are indirect genetic effects that are founded in an individual other than the one measured (Wolf *et al.* 1998), which might be the reason for a lack of acknowledgment that maternal effects can also be affected by outbreeding. As such, genetic maternal effects might often remain undetected unless several generations are studied (Willham 1980). Furthermore, the effect of outbreeding on maternal effects, i.e., maternal outbreeding effects, can only be studied by using hybrid dams.

Fishes of the family Salmonidae (including whitefish, trout, salmon) are suitable vertebrate study organisms for conducting studies of genetic divergence and genetic-based maternal effects. Most salmonid species occur as discrete populations isolated by strong philopatry, creating the potential for genetic differences through genetic drift and local adaptation (Fraser *et al.* 2011), and maternal effects are prevalent in this family, mostly related to egg and nest quality (Green 2008). Furthermore, this fish family is affected by a rapidly growing aquaculture industry, in addition to other anthropogenic translocations such as stocking, all of which can lead to population interbreeding and conservation-related concerns associated with outbreeding depression (Utter & Epifanio 2002). Salmonid females typically release several thousand eggs which can be divided experimentally and fertilized by multiple males, allowing for the study of outbreeding and population-specific maternal effects while controlling for individual maternal effects.

Although some studies have investigated the effects of outbreeding in early life stages of salmonids, most of these accounted only for individual maternal effects. We are aware of only two studies in which maternal between-population effects have been examined while simultaneously accounting for individual maternal variation (Houde *et al.* 2011; Aykanat *et al.* 2012). Most others, however, have ignored maternal effects by generating crosses in a non-reciprocal fashion or averaging reciprocal cross data, probably because of the logistic challenges associated with undertaking multigenerational studies. We are unaware of any study of maternal outbreeding effects in wild vertebrate populations.

We investigated several maternally influenced, fitness-related traits and their response to outbreeding in Atlantic salmon (*Salmo salar*) during the yolk-feeding

period encompassing hatch and time of first feeding, both of which are major events in the early life of fishes. Many salmonids bury their eggs in river gravel where eggs develop from which alevins hatch while relying on maternally provided egg yolk as the major source of energy and nutrients (Kamler 1992) until they emerge from the gravel and begin external feeding as fry.

We created reciprocal crosses between an endangered wild population and its major local domesticated counterpart up to their third hybrid generation. To minimize environmental maternal and environmental offspring effects, crosses were maintained in a common laboratory for three generations. We then analysed traits from 14 reciprocal crosses by using a cross-means analysis within a mixed model framework. In particular, we investigated the effect of outbreeding on maternal body size and egg size, and on offspring survival, hatch time, yolk size, and body size at both hatch and time of first feeding. Our study emphasises the potential importance of additive and non-additive maternal outbreeding effects in early life, by quantifying maternal, offspring, and epistatic offspring-by-maternal outbreeding effects for offspring trait means.

5.3. Material and Methods

5.3.1. Study Populations

Outbreeding effects were studied in crosses between endangered wild Atlantic salmon (WW) from the Stewiacke River (Nova Scotia, Canada) and domesticated salmon (DD) derived from the Saint John River population (New Brunswick, Canada). Both founder populations were provided by the Department of Fisheries and Oceans (DFO). WW salmon were caught as juveniles in the river. DD salmon were derived from 50-100 individuals of a wild-caught founder populations that had undergone three generations of selection, primarily for rapid growth (Glebe 1998). The two river populations are naturally separated by ~200 km (waterway distance) and are divergent for neutral genetic and ecological parameters (reviewed by Fraser *et al.* 2010).

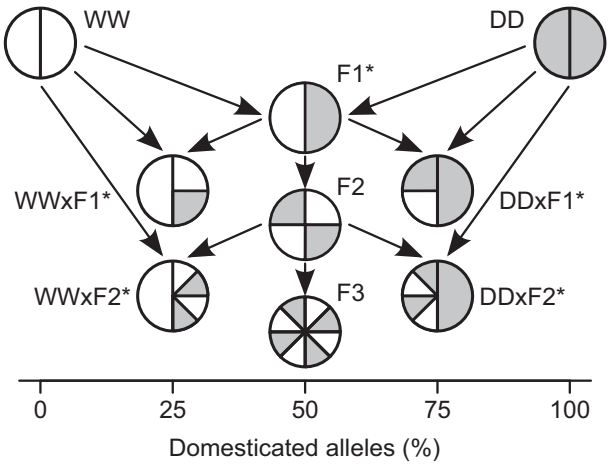

Figure 5.1: Schematic crossing design among four parental crosses of Atlantic salmon (labels beside symbols: wild, WW; domesticated, DD; first-generation hybrid, F1; second-generation hybrid, F2) that were used to create nine crosses of which five (marked by asterisks) were created in a reciprocal fashion, totalling 14 reciprocal crosses. The colours of the symbol pie charts represent percentage of alleles from WW (white) and DD (grey) and the extent of break-up of each vertically divided chart indicates the extend of relative genetic recombination between both populations.

Gametes from the founder generation were crossed in 2001, creating ten reciprocal first-generation hybrid (F1 hybrids) full-sib families (using five dams and five sires from each population) and ten full-sib families for each population. All 30 full-sib families were raised under common laboratory conditions at Dalhousie University, Halifax, Canada (details in Lawlor *et al.* 2008). In 2005, gametes from the 2001 generation were crossed to re-create parental populations and reciprocal F1 hybrids and to create second-generation hybrids (F2 hybrids = F1xF1; details in Fraser *et al.* 2010). Because only several WW families were available in 2005, a few additional WW fish from DFO were used to supplement the existing 2001 generation breeders. This might have caused a higher genetic diversity in F1 crosses than in F2 crosses of the 2005 generation. The 2005 generation was again raised under common environmental conditions (same *ad libitum* feeding regime, laboratory, water source, temperature, oxygen saturation, tank type, fish density, light intensity and regime) at Dalhousie University. In 2009, gametes from four

crosses (WW, DD, F1 and F2 hybrids) of the 2005 generation were used to create 14 reciprocal crosses (the 2009 generation; Table 5.1, Figure 5.1).

Table 5.1: Sample sizes for each of 14 crosses of the 2009 Atlantic salmon generation for initially used dams, sires, and created families (fam), and used sample sizes for each of the three developmental offspring stages for individuals, families (in parentheses), and number of dams and sires.

*Cross (♀x♂)	Initial dams x sires	Initial fam	Egg individuals (fam)	Egg dams x sires	Alevin individuals (fam)	Alevin dams x sires	Fry individuals (fam)	Fry dams x sires
WWxWW	15 x 17	32	179 (22)	11 x 13	151 (21)	11 x 13	176 (22)	11 x 13
WWxF1	15 x 13	21	126 (15)	10 x 11	112 (15)	10 x 11	118 (15)	10 x 11
WWxF2	15 x 15	21	128 (15)	11 x 11	109 (15)	11 x 11	118 (15)	11 x 11
F1xWW	15 x 13	20	133 (16)	12 x 12	113 (15)	12 x 12	125 (16)	12 x 12
F2xWW	16 x 15	20	110 (14)	11 x 11	105 (14)	11 x 11	112 (14)	11 x 11
WWxDD	15 x 13	23	143 (17)	11 x 11	116 (16)	11 x 11	135 (17)	11 x 11
F1xF1	15 x 17	30	184 (24)	12 x 17	158 (22)	11 x 15	176 (24)	11 x 15
F2xF2	16 x 15	32	211 (24)	12 x 12	183 (24)	12 x 12	192 (24)	12 x 12
DDxWW	16 x 14	23	32 (5)	4 x 5	23 (4)	4 x 4	31 (5)	4 x 4
F1xDD	15 x 12	19	112 (14)	12 x 9	102 (13)	11 x 9	109 (14)	11 x 9
F2xDD	16 x 14	20	115 (14)	11 x 10	106 (14)	11 x 10	111 (14)	11 x 10
DDxF1	16 x 13	22	12 (2)	2 x 2	11 (2)	2 x 2	16 (2)	2 x 2
DDxF2	16 x 16	22	12 (2)	2 x 2	11 (2)	2 x 2	16 (2)	2 x 2
DDxDD	16 x 17	32	59 (7)	4 x 6	39 (7)	4 x 6	50 (7)	4 x 6
Total	64 x 77	351	1556 (191)	39 x 67	1339 (184)	39 x 67	1485 (191)	39 x 67

*Cross abbreviations are wild, WW; domesticated, DD; reciprocal first-generation hybrid, F1; and second-generation hybrid, F2.

For each generation, all potential parents were tagged, fin clipped, and genotyped at three to six polymorphic microsatellite loci. This allowed the assignment of offspring to their parents by exclusion principles to avoid the crossing

of relatives to the level of second cousins, what will be termed crossing 'unrelated' parents.

5.3.2. Breeding Protocol and Laboratory Environment

For the 2009 generation, eggs from a given dam were used to create all crosses possible according to her cross (**Figure 5.1**). All 14 crosses were created in equal family numbers during each of five days (**Figure 5.2**). Each of 64 randomly selected dams was crossed to one or two randomly selected yet unrelated sires (out of a total of 77 sires) from different crosses and to two randomly selected and unrelated sires from the same cross. Crossing was accomplished by dividing stripped eggs by volume into four to eight batches of ~250 eggs each into polystyrene foam bowls followed by fertilisation. The fertilized eggs were immediately placed family-by-family into one of 354 compartments (13.8 cm x 17.0 cm). Two compartments formed one plastic container, separated by fine mesh, and each compartment had mesh-covered holes (3.8 cm diameter) on each side to permit water flow. In total, 177 plastic containers were put in groups of three into one of 59 similar 60 L round tanks.

Each tank received dechlorinated, aerated municipal water at ambient temperature by a constant flow-through system. The latter was achieved by a spray bar to induce a circular-directed water flow. Each tank was equipped with a central, circular air stone to ensure sufficient oxygen supply and within-tank temperature homogeneity. All compartments, plastic containers, and tanks were established, using the same equipment and adjustments to minimize environmental among-family variability. Tanks, suspended at two levels, were known from previous years to exhibit small but systematic daily water-temperature differences (average maximum daily difference 0.2°C), with upper level and tanks furthest from the supply being warmest. To prevent a temperature-by-cross bias, families were distributed in a stratified randomized fashion with equal proportions of families from each cross randomly distributed across each level. Temperature was measured daily for every tank (± 0.1°C; range 3.5-10.8°C; **Figure 5.2**), allowing cumulative degree-days (D°) to be calculated for each family. No replication at the family level

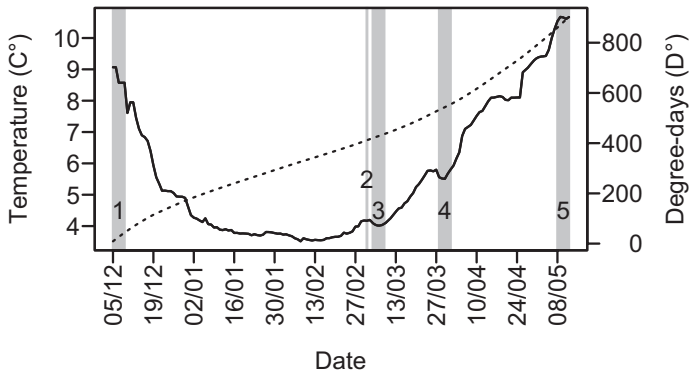

Figure 5.2: Average daily temperature (solid line) and cumulative degree-days (dotted line) for the duration of the experiment and for all families of 14 crosses of the 2009 generation between wild and domesticated Atlantic salmon. Dates for events between December 2009 and May 2010 are indicated by vertical, grey bars across the plot area with **1**, fertilisation; **2**, shocking of eggs; **3**, sampling of eyed eggs; **4**, hatch and sampling of alevins; **5**, time of first feeding and sampling of fry.

was conducted due to tank-space limitations. Eggs were maintained in the dark until the termination of the experimental work.

5.3.3. Sampling of Maternal Traits

During spawning, fork length ($\pm$ 1 cm) and body wet mass was recorded ($\pm$ 5 g) for each potential breeder. Initial numbers of fertilised eggs were counted, using photographs of each family compartment. Throughout the yolk-feeding period, opaque-turning dead eggs and dead alevins were manually removed approximately every second day to minimize the probability of fungal infection. At time of first feeding, photographs were again taken from all families and survivors counted.

At an overall average 412 D°, eggs from each family were physically stressed by heavy shaking in a bucket ('shocking') which allowed for the identification of dead eggs. During shocking, compartment bottoms were fitted with artificial turf to minimize energy loss due to alevin movement (Marr 1963). Shortly after shocking (**Figure 5.2**), eight to ten eyed eggs from each family were sampled consecutively during five days in the same order as spawned, fixed in buffered 10% formalin for 24 h, and then preserved in phosphate-buffered saline with 0.1 sodium azide until further analyses.

5.3.4. Sampling of Offspring Traits

The sampling of offspring for trait measurements occurred at two stages during the yolk-feeding period (**Figure 5.2**): larvae at 50% hatch (alevins) and unfed fry at time of first feeding (fry). After hatch commenced, the percentage of hatched alevins was estimated daily by eye for each family and, when exceeding 50%, eight (or less if unavailable) alevins were sampled, fixed, and preserved as described previously. When families spawned on the same day had reached 100% development, based on Kane (1988), eight (or less if unavailable) unfed fry were sampled from each family, fixed, and preserved as described previously.

For preserved alevin and fry, individual standard length ($\pm$ 1 mm) was recorded. The entire formalin-hardened yolk-sacs (including yolk-sac skin and oil) were precisely dissected from alevin and fry bodies and kept in individual pairs of yolk-sac and body, allowing for their separate dry mass measurements. Individual samples (including eyed eggs) were oven-dried at 60°C until no change in mass was noted in 24 h intervals and dry mass was determined ($\pm$ 0.1 mg).

5.3.5. Statistical Analyses of Maternal Traits

Differences in average fork length, body mass and (eyed) egg size among the four dam crosses (WW, DD, F1, and F2 hybrids) were examined. Length and mass (both Ln-transformed) were each assessed using a linear model (LM) with dam cross as a fixed term and common Gaussian distributed residuals. Dry mass of eyed eggs (egg size) was assessed using a linear mixed model (LMM) with dam cross as a fixed term, dam identification ('dam') nested within dam crosses (with diagonal variance structure for dam crosses) and family identification ('family') as random terms, and allowing independent strata of Gaussian distributed residuals among dam crosses. Correlations between dam traits (egg size vs. Ln fork length and Ln body mass) were tested using Pearson's product moment correlation.

5.3.6. Statistical Analyses of Offspring Traits

5.3.6.1. Survival and Incubation Period

Survival between fertilisation and time of first feeding was analysed based on the (logit-transformed) proportion of individuals surviving to the fry stage out of the initial number of eggs for each family. A LMM was used with cross as a fixed term, dam as a random term, and allowing independent strata of Gaussian distributed residuals among reciprocal offspring crosses.

Average cumulative degree-days at 50% hatch (incubation period) of families was analysed using a LMM with cross as a fixed term, dam and tank identification ('tank') as random terms, and a common Gaussian residual distribution. In this model, final number of live individuals per family ('density'), average eyed egg dry mass per family ('egg mass'), and the product of the two ('biomass') were tested for their influence on incubation period by including them as fixed continuous covariates. These and all other continuous covariates, were centred by dividing each value by the sampling-period-specific mean. These covariates were tested because they might correlate negatively with water oxygen saturation (density, biomass), or positively with total oxygen egg demand (egg mass), and both might influence hatch or development.

5.3.6.2. Cross-Means Analyses of Alevin and Fry Traits

For analyses, body length and dry mass of body and yolk for both alevins and fry were treated as six different traits to allow testing of the main genetic architecture of each trait at different times by cross means analysis. Cross means and genetic outbreeding effects were estimated for each trait, using LMMs taking into account environmental effects and kinship among individuals. The analysis followed the general LMM:

$$y = X\tau + Z_1 u_1 + Z_2 u_2 + e \qquad \textbf{Equation 3}$$

where y is a $n\ x\ 1$ vector of individual observations of a given trait, τ is a $p\ x\ 1$ vector of fixed continuous and/or categorical effects, u_1 is $q\ x\ 1$ a vector of random

effects assumed to be independent and Gaussian distributed with an overall mean of zero, u_2 is a r x 1 vector of random animal effects with correlated (co)variances based on their additive relationship matrix (see below), and e is the Gaussian distributed residual variance. X, Z_1 and Z_2 are incidence matrices relating observations y to respective effects.

Temperature and oxygen saturation are known to influence development of yolk-feeding salmon (Kamler 1992). Before assessing outbreeding effects, the influence of both environmental factors on trait means was assessed while keeping cross as a fixed term in each model (including them in of **Equation 3**). The linear influence of temperature was tested for by including the fixed covariate 'degree-days'. The influence of approximated differences in oxygen supply among families was tested for by including the fixed covariate 'density' (see above) which, however, was non-significant in all models and therefore removed.

Variation among dams (maternal environmental variance), among familial compartments (common familial environmental variance), and among tanks (common tank environmental variance) were accounted for by including identifications of 'dam', 'family', and 'tank' as random terms (in u_1 of **Equation 3**). Further, additive genetic variance among individuals as predicted by the inverse relationship numerator matrix based on the complete four-generation pedigree was accounted for by including identification of 'animal' as a random term (in u_2 of **Equation 3**). Such an animal model corrects for genetic relationships in unbalanced designs with relationship ties among individuals, and increases the accuracy of fixed parameter mean estimates and their standard errors (Komender & Hoeschele 1989).

Heterogeneous variances might be present among crosses due to segregation (Hayman 1958; Piepho & Möhring 2010) and they might be present between both parental populations. Hence, each random term was tested for heterogeneity either among the four maternal genotype levels (terms 'dam', residuals), among the nine (non-reciprocal) offspring genotype levels (terms 'family', 'animal', residuals), or among the 14 reciprocal offspring crosses (residuals). However, heterogeneity was

only observed for residuals (**Table S 5.3**). For other random terms, such a diagonal variance structure did either not improve the model fit or resulted in estimation problems for variances related to low DD dam sample sizes. As a consequence, variances for random terms other than residuals were estimated across all families.

For each of the six traits, 14 diploid outbreeding effects (based on Mather & Jinks 1982; **Table S 5.1**) were estimated by including them as fixed continuous covariates in the trait models (in τ of **Equation 3**). Effects fitted were the reference mean ($\hat{m}$), additive ($\hat{d}$), dominant ($\hat{h}$), additive-by-additive ($\hat{\imath}$), additive-by-dominant ($\hat{\jmath}$), dominant-by-dominant ($\hat{l}$), maternal additive ($\hat{d}_m$), maternal dominant ($\hat{h}_m$) maternal additive-by-maternal additive ($\hat{\imath}_m$), maternal dominant-by-maternal dominant ($\hat{l}_m$), additive-by-maternal additive ($\widehat{d.d_m}$), dominant-by-maternal additive ($\widehat{h.d_m}$), dominant-by-maternal dominant ($\widehat{h.h_m}$), and additive-by-maternal dominant ($\widehat{d.h_m}$) effects.

The model fit when including each outbreeding effect was assessed by including a lack-of-fit term (the reciprocal cross term). Any model that was non-significant for the lack-of-fit term ($P > 0.05$, Wald *F*-tests) was regarded as fitting the data. This model selection strategy for cross mean analysis was suggested by Piepho and Möhring (2010). Outbreeding effects were first tested one-by-one and then by combinations of significant effects ($P < 0.05$, Wald *F*-tests). If this resulted in several models with equal numbers of significant effects fitting the cross means, we reported all of them.

5.3.6.3. Yolk Conversion Efficiency and Yolk Percentage

Gross yolk dry mass conversion efficiency between hatch and time of first feeding (E_{yolk}) was calculated for every family with:

$$E_{yolk} = (B_f - B_a)/(Y_a - Y_f) \qquad \textbf{Equation 4}$$

in which average dry masses are: B_f, fry body; B_a, alevin body; Y_a, alevin yolk; and Y_f, fry yolk. E_{yolk} (logit-transformed) was analysed using a LMM with cross as a fixed term, dam as a random term and a Gaussian residual distribution.

Furthermore, the correlation between eyed egg size and E_{yolk} was tested using Pearson's product moment correlation.

A decrease of yolk mass and an increase in body mass between hatch and first feeding stages might also vary among crosses due to differences in yolk depletion rates. To test this, the proportion of yolk to total dry mass (logit-transformed) of all individuals were analysed by a LMM with the fixed terms of cross, stage, the interaction of cross-by-stage, and the fixed continuous covariates of degree-days at stage and density at stage. Evaluated by sequential model fitting, the random term dam-by-stage was fitted with a diagonal variance structure, permitting different among-dam variances between stages. The random term family-by-stage was fitted with an unstructured (co)variance structure permitting a covariance between familial environmental effects between stages. Gaussian residuals were allowed to have independent strata among the 28 levels of cross-by-stage.

For all models, significance ($P < 0.05$) of fixed terms (including outbreeding effects) was tested using conditional Wald-type *F*-tests with denominator degrees of freedom approximated following Kenward and Roger (1997). Significances of random terms and (co)variance structures were approximated by using Residual Maximum Likelihood (REML) log-likelihood ratio tests (LRTs) between nested models with $P < 0.1$ as the critical alpha level. Only significant effects and (co)variance structures were contained in the models except for the family term (the basic experimental unit) and the animal term (accounting for correlated individual variances) which were always retained, if applicable. Means and their standard errors were jointly obtained as marginal model predictions. Contrasts between marginal means were conducted by Student's t-tests with degrees of freedom approximated as for *F*-tests and p-values were Bonferroni-adjusted. All analyses were conducted using REML routines in *ASReml-R 3.0* (Butler *et al.* 2009).

5.4. Results

Twenty-five of all 64 dams had non-viable or poor-quality eggs (mostly DD dams) of which no or few individuals could be sampled. A necessary spawning

synchronisation might have resulted in a non-optimal spawning timing for DD dams or our laboratory conditions had population-specific effects on fertility. To exclude such potential effects from our results, only data from dams with sufficient sample size (> 3 per family) were used in the analyses.

5.4.1. Maternal Traits

Among the four dam crosses, average fork lengths were different ($F_{3,35} = 7.4$, $P < 0.001$) but differences for body masses were non-significant ($F_{3,35} = 2.8$, $P = 0.056$); WW dams were shorter (three contrasts vs. WW: $t_{13\ to\ 21} = 2.7$ to 4.1, $P_{adjusted\ (x3)} = 0.001$ to 0.043) and in their tendency lighter than other dams (**Figure 5.3a, b**). Similar average sizes were obtained with two to eight times larger sample sizes per dam cross when using data from all mature females available at spawning (supplementary **Table S 5.2**), indicating that size data on dams were representative.

Average eyed egg dry mass was different among dam crosses ($F_{3,17.9} = 6.1$, $P = 0.005$) inferred by a model that accounted for heterogeneous among-dam variances for the four dam crosses and for overall among-family variance (65-69% (6% for DD) and 1% (4% for DD) of the total variance per dam cross, respectively). Domesticated and F1 hybrid dams had similar average egg sizes that were both larger than WW dam eggs (DD vs. WW: $t_{11.5} = 3.9$, $P_{adjusted(x6)} = 0.014$; F1 vs. WW: $t_{20.4} = 3.0$, $P_{adjusted(x6)} = 0.044$); all other cross mean comparisons were non-significant ($t_{13\text{-}21.6} = 1.7$ to 2.4, $P_{adjusted(x6)} = 0.187$ to 1). WW dams had the smallest eggs and F2 hybrid dam eggs were fitting the midparental value (**Figure 5.3c**). Averaged egg size correlated positively with dam length and dam mass ($r_{length} = 0.40$, $P = 0.012$; $r_{mass} = 0.49$, $P = 0.001$).

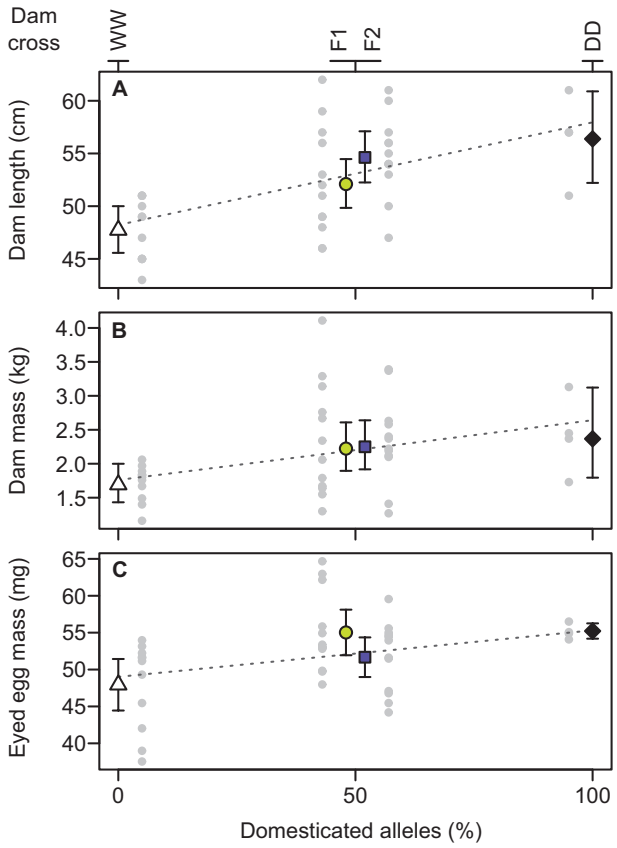

Figure 5.3: Average re-transformed fork length (**A**), re-transformed body mass (**B**), and average eyed egg dry mass (**C**) from the four 2005 generation dam crosses of wild (WW), white triangles; domesticated (DD), black diamonds; first-generation hybrid (F1), light grey circles; and second-generation hybrid (F2), dark grey squares, Atlantic salmon used to create the 2009 generation. Error bars depict approximate 95% confidence intervals. For each trait, the dam crosses have been arranged by percentage of domesticated allelic combinations. The vertically arranged grey dots beside each cross mean depict individual data (**A** & **B**) or dam mean across families (**C**).The dotted line in each panel represents the reference mean under a complete additive genetic architecture. Symbols for hybrids (at 50% domesticated allelic combinations) have been slightly off-set to improve depiction.

5.4.2. Survival and Incubation Period

Offspring survival between fertilisation and the fry stage did not differ among offspring crosses ($F_{13,12.8} = 1.2$, P = 0.388) but means varied between 12 and 54% with tendencies of lower survival for DD dam families and higher survival for families from both hybrid dam crosses (**Figure 5.4a**). Among-dam variance

accounted for 76-100% of the total variance in survival per cross, indicating little

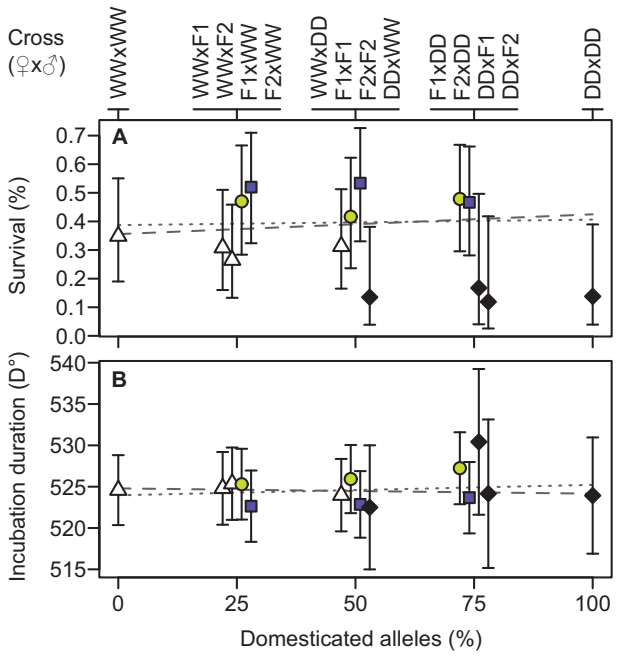

Figure 5.4: Average re-transformed percentage of surviving individuals between fertilisation and time of first feeding (**A**), and average incubation duration until 50% hatch in cumulative degree-days (**B**) for 14 reciprocal crosses of the 2009 generation between wild and domesticated Atlantic salmon. Error bars depict approximate 95% confidence intervals. The two lines in each panel represent the reference mean under either an additive (dotted line) or a maternal additive (dashed line) genetic model. Symbols for hybrids and backcrosses (at 50%, 25%, and 75% domesticated alleles) have been slightly off-set to improve depiction. Symbol colours indicate maternal cross with wild (WW), white triangles; domesticated (DD), black diamonds; first-generation hybrid (F1), light grey circles; and second-generation hybrid (F2), dark grey squares. Reciprocal crosses are indicated above the panels as dam cross by sire cross.

influence of offspring genotype.

The incubation period until 50% hatch of families lasted between 498.3 and 540.2 D° and was not associated with familial density, egg mass, or biomass ($P_{density} = 0.369$, $P_{egg\ mass} = 0.303$, $P_{biomass} = 0.982$), which suggests sufficient oxygenation and no influence of egg size on incubation period. However, among-tank variance was significant ($\chi^2_1 = 14.3$, $P < 0.001$), indicating systematic environmental

influence on degree-days until hatch and emphasizing the importance of the conducted spatial randomisation of units. Differences among offspring crosses were minor (**Figure 5.4b**) and non-significant ($F_{13,128.3} = 0.7$, $P = 0.771$). In a LMM with a fitted common intercept among all families (mean $\pm$ standard error (SE): 524.5 ± 1.1 D°), among-dam and among-tank variation accounted for 63% and 14%, respectively, of the total variance in incubation period.

5.4.3. Cross-Means Analyses of Alevin and Fry Traits

Among-dam variance was the most important variance component for the six traits of length, body dry mass and yolk dry mass of alevins and fry, comprising between 22-34% (alevin length) and 54-93% (alevin yolk mass) of the total variance per cross. Among-family variance was significant for most traits, except for alevin yolk mass, comprising between 1% (alevin yolk mass) and 9-13% (alevin length) of the total variance per cross.

Conversely, shared among-tank variance made up a small percentage of the total variance and was only significant for alevin body mass, comprising 3-6% of the total variance per cross. The additive genetic variance estimated for 'animal' varied among traits from close to zero with non-estimable error (alevin yolk and fry body mass) to 10-20% of the total variance per cross (alevin body mass). See supplementary **Table S 5.3** for further model details.

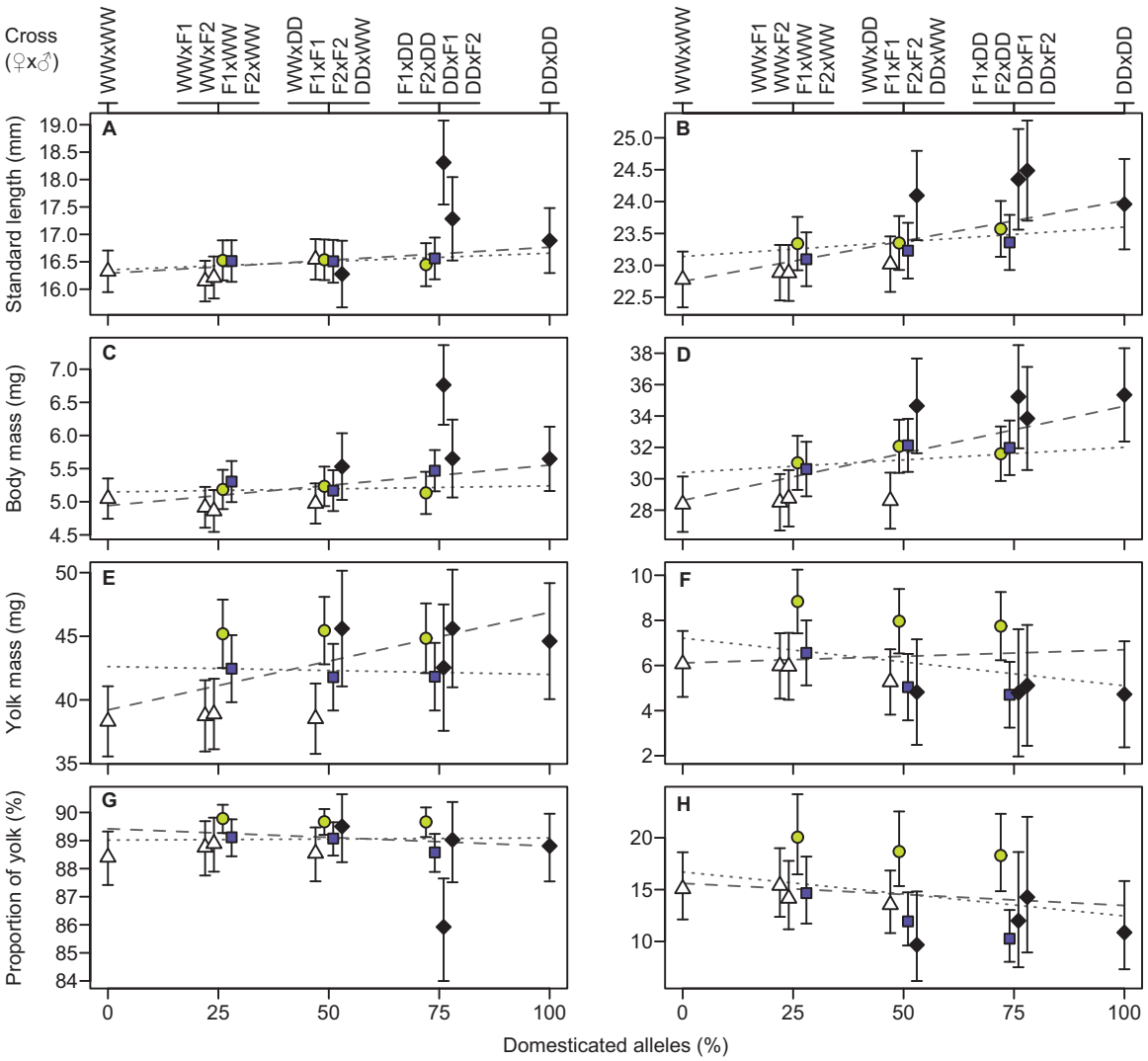

Figure 5.5: Estimated trait means with approximate 95% confidence intervals (error bars) for 14 reciprocal crosses of the 2009 generation between wild and domesticated Atlantic salmon for alevins at hatch (left panels, **A**, **C**, **E**, **G**) and fry at time of first feeding (right panels, **B**, **D**, **F**, **H**). Displayed traits are standard length (**A** & **B**), body dry mass (**C** & **D**), yolk dry mass (**E** & **F**), and proportion of yolk to total dry mass (**G** & **H**). The two lines in each panel represent the reference mean under either an additive (dotted line) or a maternal additive (dashed line) genetic model. Symbols for hybrids and backcrosses (at 50%, 25%, and 75% domesticated alleles) have been slightly off-set to improve depiction. Symbol colours indicate maternal cross with wild (WW), white triangles; domesticated (DD), black diamonds; first-generation hybrid (F1), light grey circles; and second-generation hybrid (F2), dark grey squares. Reciprocal crosses are indicated above the panels as dam by sire cross.

Alevin standard length at hatch was positively associated with degree-days (effect ± se: 0.021 ± 0.006 mm*D°-1, $F_{1,155.6} = 11.7$, P = 0.001). The common degree-day adjusted cross means were different ($F_{13,101.5} = 3.4$, P < 0.001) where the DDxF1 cross was longer than most others (**Figure 5.5**, **Table S 5.4a**). No outbreeding effect was different from zero, with the maternal additive effect having the lowest probability (P = 0.081), and no fit to the cross means could be obtained (**Table 5.2**; supplementary **Table S 5.4a**). Omitting the DDxF1 cross strongly reduced the statistical support for among-cross differences ($F_{12,92.3} = 1.9$, P = 0.050) and increased it for degree-days ($F_{1,150.4} = 13.2$, P < 0.001).

Fry standard length at time of first feeding was not associated with any tested continuous covariate. Differences among crosses were sufficiently explained by the maternal additive effect, the additive, and the additive-by-dominant effect (**Table 5.2**; **Table S 5.4b**). However, cross mean differences were non-significant ($F_{13,99.6} = 1.8$, P = 0.058) but pooled by dam cross differences among means were significant ($F_{3,38.2} = 3.1$, P = 0.042) which might support the maternal additive effect most. Increasing percentage of domesticated allelic combinations of dams resulted in increasing length of their offspring (**Figure 5.5b**).

Alevin body dry mass was positively influenced by degree-days (effect ± se: 0.026 ± 0.0053 mg* D°-1, $F_{1,154.2} = 23.6$, P < 0.001). Only the maternal additive outbreeding effect with a small effect size was significant (**Table 5.2**), but obtained under a significant lack-of-fit to the cross means ($P_{lack\text{-}of\text{-}fit} = 0.003$, supplementary **Table S 5.4c**). The degree-day adjusted cross-means pattern mirrored the inference of a maternal additive effect with the effect of increasing percentage of domesticated allelic combinations of dams resulting in increasing body mass of their offspring (**Figure 5.5c**). However, although differences among offspring cross means were significant ($F_{13,84.9} = 3.2$, P = 0.001), no indications of offspring genotype effects appeared to be present except that the DDxF1 cross was divergent from most others (**Figure 5.5c**). Omitting the DDxF1 cross resulted in a significance-loss for among-cross differences ($F_{12,85.5} = 1.6$, P = 0.118) but not for degree-days ($F_{1,153.4} = 25$, P < 0.001). Omitting the DDxF1 cross strongly reduced the statistical support for

among-cross differences ($F_{12,92.3} = 1.9$, $P = 0.050$) and increased it for degree-days ($F_{1,150.4} = 13.2$, $P < 0.001$).

Fry body dry mass was not predicted by any continuous covariate. The cross means were different ($F_{13,145.1} = 3.5$, $P < 0.001$) and best predicted by the combination of the additive with the maternal additive effect (**Table 5.2**, supplementary **Table S 5.4d**). The cross-means pattern largely followed the pattern of length at hatch and confirmed the inferred outbreeding effects (**Figure 5d**). However, the additive effect, i.e., decreasing mass with decreasing percentage of DD alleles, was small and unidirectional, exhibited in backcrosses with WW sires only.

Alevin yolk dry mass was not predicted by any continuous covariate. None of the offspring outbreeding effects were significant, but all maternal outbreeding effects and one epistatic maternal-offspring effect were (supplementary **Table S 5.4e**). Models that fit the significantly different cross means ($F_{13,86.2} = 2.2$, $P = 0.014$) included either the maternal additive, the maternal dominant, maternal dominant-by-dominant, or the epistatic offspring dominant-by-maternal dominant effect (**Table 5.2**, supplementary **Table S 5.4e**). Yolk mass among crosses largely mirrored the pattern of egg size by dam cross with the largest yolks possessed by families from DD and F1 hybrid dams (**Figure 5.5e**).However, the DDxF1 backcross exhibited a somewhat lower yolk dry mass relative to other DD dam crosses.

Fry yolk dry mass was negatively influence by degree-days (effect $\pm$ se: -0.07 $\pm$ 0.016 mg*D°$^{-1}$, $F_{1,128.8} = 21.8$, $P < 0.001$). The pattern of degree-day adjusted, different cross-means ($F_{13,108.8} = 2.5$, $P = 0.005$) was best predicted either by the

Table 5.2: Trait mean ($\hat{m}$) and outbreeding effects both with standard errors (SE), *F*-values (with degrees of freedom/denominator degrees of freedom), and p-values (P) for the best models fitted to 14 cross means for each of six traits for Atlantic salmon alevins at hatch and fry at time of first feeding. Abbreviations of outbreeding effects are used as in the text.

*Trait	$\hat{m}$ (SE)	Effect(s) (SE)	*F* (df/ddf)	P
†Alevin length	16.4 (0.1)	-	-	-
Fry length I	23.3 (0.12)	$\hat{d}$ = -0.24 (0.09)	7.7 (1/11.6)	0.017
Fry length II	23.3 (0.13)	$\hat{j}$ = -3.3 (1.6)	4.3 (1/130)	0.041
Fry length III	23.3 (0.12)	$\hat{d}_m$ = -0.5 (0.18)	8.2 (1/39.2)	0.007
‡Alevin body mass	5.3 (0.08)	$\hat{d}_m$ = -0.3 (0.11)	7.5 (1/38.8)	0.009
Fry body mass	31.7 (0.49)	$\hat{d}$ = -0.7 (0.27)	6.6 (1/9.5)	0.029
		$\hat{d}_m$ = -2.8 (0.78)	12.9 (1/39.2)	0.001
Alevin yolk mass I	43.0 (0.8)	$\hat{d}_m$ = -3.8 (1.3)	9.1 (1/36.7)	0.005
Alevin yolk mass II	40.1 (1.2)	$\hat{h}_m$ = 4.9 (1.8)	6.6 (1/37.2)	0.014
Alevin yolk mass III	40.5 (1.0)	$\hat{l}_m$ = 4.8 (1.8)	6.8 (1/37.4)	0.013
Alevin yolk mass IV	41.2 (0.91)	$\hat{h.h}_m$ = 5.4 (2.2)	6.2 (1/129.3)	0.014
Fry yolk mass I	6.3 (0.45)	$\hat{j}$ = 1.9 (0.60)	10.5 (1/150.8)	0.001
Fry yolk mass II	5.3 (0.53)	$\hat{l}_m$ = 2.9 (0.83)	12.6 (1/42.4)	<0.001
Fry yolk mass III	5.2 (0.54)	$\hat{h.h}_m$ = 5.6 (1.5)	13.9 (1/68.9)	<0.001

*Units are mm for length and mg for mass. If more than one model fit cross means, the different models for a trait are indicated by roman numerals. †For alevin length, no effect was significantly different from zero and no fit to the cross means could be obtained. ‡For alevin body mass, no significant fit to the cross means could be obtained and results are given for the model with effects significantly different from zero best fitting the cross means.

maternal dominant-by-dominant, the offspring additive-by-dominant, or the epistatic offspring dominant-by-maternal dominant effect (**Table 5.2**, supplementary **Table S 5.4f)**.These effects were mirrored by the cross-means pattern, although the additive or additive-by-dominant effect (i.e., modifying backcross means relative to maternal cross effects) was more pronounced where involving wild parents (**Figure 5.5f**). Also, the dominant-by-maternal dominant pattern (i.e., changing all F1 and F2 hybrid dam offspring means from the midparental value, but F2 hybrid dam

offspring only one fourth as much) was not recognisable for crosses from F2 hybrid dams (**Figure 5.5f**).

5.4.4. Yolk Conversion Efficiency and Yolk Percentage

Familial gross conversion efficiency did not differ among the 14 crosses ($F_{13,169} = 0.6$, $P = 0.820$, overall $E_{Yolk} \pm$ se: 0.731 ± 0.005) and among-dam variance was non-significant ($\chi^2_1 = 0$, $P = 1$). Conversion efficiency did not correlate with egg size ($r = -0.021$, $P = 0.773$).

The logit-transformed proportion of yolk to total dry mass (percentage of yolk) was negatively influenced by degree days at sampling for both stages (alevin: $F_{1,159.8} = 26.5$, $P < 0.001$; fry: $F_{1,131} = 35.2$, $P < 0.001$) but not by density (alevin: $F_{1,163.2} = 0.001$, $P = 0.944$; fry: $F_{1,172.9} = 1.4$, $P = 0.236$). Re-transformed decrease in the proportion of yolk mass with increasing degree-days was more than three times faster for fry than for alevins (effects $\pm$ se: -0.062 ± 0.030 %*D°$^{-1}$ in alevin vs. -0.221 ± 0.078 %*D°$^{-1}$ in fry). Stage-specific degree-day-adjusted percentage of yolk differed among crosses ($F_{13,128.7} = 4.2$, $P < 0.001$), between stages ($F_{1,38.4} = 3303$, $P < 0.001$), and for the interaction of cross-by-stage ($F_{13,140.8} = 2.9$, $P = 0.001$). An indication for a correlation between both stages was detected for family random effects ($\chi^2_1 = 3.0$, $P = 0.084$, $\rho = 0.31$), but not for dam random effects, indicating persistent common familial environmental influence on yolk proportion. Between the alevin and the fry stage and when averaged by dam cross, offspring from F1 hybrid dams had reduced their yolk proportion by 71%, those from WW dams by 74%, and those from both F2 and DD dams by 77%, indicating different yolk depletion rates. At hatch, most crosses had an equal average amount of around 89% yolk (**Figure 5.5g**). For fry, the pattern was more diverse and percentage of yolk among crosses differed two-fold (9-20%; **Figure 5.5h**). Most crosses showed decreasing percentages of yolk mass with increasing percentages of domesticated allelic combinations. However, all three crosses involving F1 hybrid dams had the highest percentages of yolk mass (18-20%), on average 51% larger than those of other crosses and 31% higher than predicted under an additive model (14.5%).

5.5. Discussion

We did not detect any differences in survival, hatch timing, and yolk conversion efficiency between two divergent Atlantic salmon populations. Further, even after two rounds of recombination, no effects of outbreeding on means were detected for these traits. This might suggest equality or at least compatibility of trait-specific alleles as expressed under our laboratory conditions. Nevertheless, results on other traits revealed outbreeding effects that are likely to influence offspring fitness in early life. Strong individual maternal effects were detected for most offspring traits in accordance with previous studies. While accounting for this individual among-dam variation, we detected maternal outbreeding effects on means, i.e., the differences in population-specific maternal effects and their change in hybrid dams that presumably underlie the genetic architecture of divergent alleles from both populations. Furthermore, we detected additive and non-additive outbreeding effects at the offspring genotype level and an indication of epistasis between the maternal and the offspring level. Altogether, these results render the predictability of phenotypes and their potential fitness resulting from outbreeding difficult. As we controlled for parental and offspring environments and ages, we concluded that all inferred outbreeding effects are genetically based.

5.5.1. Maternal Traits

The presence of positive correlations between female size and egg size, and between egg size and early offspring size, has been well documented in salmonids (Beacham *et al.* 1985; Ojanguren *et al.* 1996). Between the wild and domesticated populations used in the present study, large differences in maternal body size existed (see also Fraser *et al.* 2010). In our four dam crosses, egg size also generally increased with maternal body size. Nonetheless, egg size appeared to be controlled by additional mechanisms than just body size. F1 hybrid dams were shorter than F2 hybrid dams. However, shorter F1 hybrid dams produced larger eggs than longer F2 hybrid dams, albeit none of the differences between F1 and F2 hybrid dams were significant. The larger F1 than F2 dam egg size suggests the presence of a non-

additive genetic component for this trait, independent of maternal body size, which yet remains to be evaluated.

5.5.2. Maternal Outbreeding Effects

For four of six offspring traits analysed, the maternal additive outbreeding effect underlay, alone or in tandem with other effects, the cross means. This suggests that the maternal genetic additive architecture for these traits is at least partly de-coupled from the detected (dominant-by-) maternal dominant outbreeding effect for yolk mass. Yet, the maternal additive and maternal dominant outbreeding effects almost certainly had consequences for offspring traits as a result of their combination. Offspring from both F1 and F2 hybrid dams had nearly intermediate (midparental) body length and mass at both hatch and time of first feeding, but originated either from large eggs spawned by F1 hybrid dams or from intermediate-size eggs spawned by F2 hybrid dams. For offspring only from F1 hybrid dams, a presumed mismatch of large egg size and intermediate offspring size resulted in a relatively larger yolk mass. This larger percentage of yolk to total mass at both hatch and at time of first feeding was then exhibited as maternal dominant outbreeding effect.

Two different types of mismatches might explain the large residual yolk mass evident in F1 hybrid dam offspring, although the causal physiological mechanisms are unknown. Firstly, F1 hybrid dam offspring developed slower than other offspring, which would be supported by their slower yolk proportion depletion rate. Secondly, F1 hybrid dam offspring developed equally fast as other offspring but had larger residual yolks. In the first case, F1 hybrid dam offspring might reach a body size that is proportional to their egg size later in time (i.e., they might continue to grow longer than others) but also emerge later from the gravel than other crosses. In the second case, F1 hybrid dam offspring will have a body size that is smaller than predicted by their egg size and might emerge with large residual yolk at the same time as other offspring.

Unfortunately, the literature is equivocal on what represents salmon development in terms of residual yolk. Some authors use the percentage of body

mass to total mass as an index of development to predict first feeding at 97% body wet mass (Thorpe *et al.* 1984). Yet, fry in the wild might start feeding in the gravel (Gustafson-Marjanen & Dowse 1983) and early emerging fry have externally visible yolks (Garcia de Leaniz *et al.* 2000). In our study, externally visible yolks were observed on some individuals upon dissection of fry. Nevertheless, before fry sampling we observed that all offspring exhibited swim-up behaviour which is usually associated with first feeding. Lastly, degree-days for fry had no effect on body mass but a negative effect on yolk mass, making it possible that a pre-determined size had been attained and only movement caused further yolk-depletion. Hence, it is likely that all fry were fully developed. Regardless what is seen as an approximation of development, judged on residual yolk percentage, development was slower or, more likely, imbalanced between egg/yolk mass and body mass in F1 hybrid dam offspring relative to other crosses.

There are possible consequences resulting from maternal outbreeding effects for fitness-related relationships between female size and egg size, egg number and offspring size – a central concept in life history evolution (Roff 1992; Rollinson & Hutchings 2013). The phase following fry emergence has been identified as a 'critical period' because of high mortalities caused by predation and starvation (Elliott 1990; Einum & Fleming 2000). It is generally accepted that larger fry are fitter through advantages in feeding territory acquisition (Elliott 1990; Cutts *et al.* 1999), with larger sizes of only a few per cent resulting in dominance advantages (Berejikian *et al.* 1996), and predator avoidance (Mogensen & Hutchings 2012). Large eggs result in large offspring but are under evolutionary constraints because of the trade-off between egg size and egg number (Sargent *et al.* 1987). Time of emergence may also affect fitness with earlier emerging fry advantageously finding territories first (Cutts *et al.* 1999; Mogensen & Hutchings 2012), but being at a disadvantage under predation (Brännäs 1995).

In fishes, it is generally assumed that egg size is adaptive (Kamler 1992) with egg number adjusted for environmentally influenced available maternal energy (Scott 1962). Hence, maternal outbreeding effects detected in the present study have the potential to either result in smaller wild-domesticated offspring size per egg size, or

delayed emergence when hybrid dams are involved. The latter might compromise fitness more severely (Einum & Fleming 2000) and both might shift trait values in adapted populations and lead to outbreeding depression. Underdeveloped fry may also be more readily preyed upon because of reduced swimming ability (Bams 1967), but they may survive longer under food limitation by virtue of their larger residual yolk. Conversely, all domesticated dam offspring appeared to have a faster yolk depletion rate. Accordingly, predation or starvation-related fitness effects, even those resulting from simple maternal additive outbreeding effects, are likely to be environment-specific and these effects will additionally differ through maternal non-additive outbreeding effects on hybrid generation-specific phenotypes.

5.5.3. Offspring Outbreeding Effects and Epistasis

We detected offspring outbreeding effects and indications of epistatic interactions between offspring and maternal outbreeding effects. Hence, offspring traits and development are governed by the offspring genotype and possibly by interactions between maternal and offspring genotypes which both are in addition to maternal effects that are a correlate of egg size, maternal genotype, and egg-deposited factors. Outbreeding effects at the offspring level were, apart from presumed epistatic interaction with maternal effects, absent at the alevin stage but present for all traits at the fry stage. This growing influence of offspring genotype towards first feeding on means is in accordance with other studies on variances (Heath *et al.* 1999; Perry *et al.* 2004; Aykanat *et al.* 2012).

5.5.4. Remarks on Study-Related Limitations

In creating the 2005 generation, we had to obtain additional gametes from the WW source population and this might have caused a genetic heterogeneity. Yet, we did not observe a closer similarity between 2005 F1 and WW dams (contribution of new breeders in both) than between 2005 F2 and WW dams (no contribution of new breeders in F2). Furthermore, crosses generated for this study in 2009 were derived entirely from parents that had shared a life-time common laboratory environment, which likely removed previous heterogeneous environmental effects.

Low survival of offspring from domesticated dams resulted in the availability of only few DD families. Of the four DD dams from which we collected familial offspring data, two were full-sibs and two others were maternal half-sibs. This may have introduced a bias in all DD dam offspring data through, for example, their very small variation in egg size relative to other dam crosses. We cannot determine whether the small variation is a result of the domestication process (as suggested for variation in growth; Solberg *et al.* 2013) or of the close kinship among dams. However, it was not possible to account in our analyses for dam cross-specific variances (except for egg size) because of non-estimable DD dam cross variances. Hence, we had to jointly estimate among-dam variances across all families. As a result, fitted variances for DD dam offspring were likely larger than in reality and this might have resulted in conservative inferences.

We concluded that either maternal or epistatic offspring-by-maternal effects underlie alevin yolk mass, and that fry yolk mass is determined by either epistatic offspring, epistatic maternal, or by epistatic offspring-by-maternal effects. While it appears from graphical examination necessary to explain cross means by combinations of effects at the maternal and offspring level, respective cross means patterns did not agree with means predicted by each single effect. This lack of concordance might have been caused by i) too large variation for cross means to detect possible effect combinations (also because of few DD dam cross families), ii) our use of a simple diallelic model with epistasis between two loci per level, whereas quantitative traits probably underlie several loci, each with many alleles. Moreover, iii) Atlantic salmon possess many gene duplications due to a presumed family-specific genome duplication event which might render a diploid model partly inappropriate (Fraser *et al.* 2010).

5.5.5. Conclusion and Implications

We found no indications of a typical break-up of epistatic coadapted offspring genes between two divergent salmon populations. However, our work revealed a complex set of additive and non-additive effects at both the offspring and the maternal genotype level and potential epistasis between both. As a consequence,

phenotypes resulting from outbreeding between the wild and domesticated salmon examined are not readily predictable, but vary with the number of hybrid generations of both mothers and offspring, and particular environmental conditions will govern their fitness.

Our results nevertheless suggest that non-additive maternal outbreeding effects (known as maternal heterosis in animal breeding), and potentially their interaction with additive maternal and direct genetic effects, might play important roles in outbreeding-related phenomena of wild populations, such as generation-specific heterosis and outbreeding depression. The interplay of maternal additive (for offspring body size) and maternal dominant effects (for yolk size) in F1 hybrid dam offspring suggested here might be regarded as empirical evidence of the mechanistic breakdown of maternally controlled coadapted offspring traits. Such a breakdown might affect many species in which direct or indirect genetic-based maternal effects occur and its consideration is warranted in evolutionary, conservation, or economical-agricultural oriented studies. These investigations should be conducted using reciprocal breeding designs and hybrid parents to allow for the distinction between the maternal and offspring level as the origin of possible outbreeding effects.

5.6. Acknowledgements

This work was approved by the Dalhousie University Committee on Laboratory Animals and followed guidelines of the Canadian Council on Animal Care (CCAC 2005). We thank Robin Waples and an anonymous reviewer for improving this manuscript. We also thank DFO staff from Coldbrook and Mersey facilities, especially Beth Lenentine and John Whitelaw, and the Aquatron facility staff. For invaluable experimental help, we thank Matthew Yates, Katharina Bremer, Samantha Hamilton, Njal Rollinson, Aimee and Corey Houde, Dave Keith, Stephanie Mogensen. The research was supported by NSERC Strategic and Discovery Grants to JAH and by an NSERC Postdoctoral Fellowship to DJF.

5.7. Data Accessibility

All data, has been deposited at DataDryad.org (http://datadryad.org) archived as **doi:10.5061/dryad.9cs2v.** and is directly accessible via this link:

http://datadryad.org/resource/doi:10.5061/dryad.9cs2v

5.8. Supplementary Material

Some of the following supplementary material exceeds the page length to be accommodated in this thesis and a direct download link to a .xlsx-file is:

http://www.nature.com/hdy/journal/vaop/ncurrent/extref/hdy201343x6.xls

Table S 5.1: Coefficients used to estimate first order genetic outbreeding effects for six traits among 14 crosses (abbreviated dam cross by sire cross) between wild and domesticated Atlantic salmon.

*Cross	$\hat{m}$ mean	$\hat{d}$ additive	$\hat{h}$ dominant	$\hat{\imath}$ additive-by-additive	$\hat{\jmath}$ additive-by-dominant	$\hat{l}$ dominant-by-dominant	$\hat{d}_m$ maternal additive	$\hat{h}_m$ maternal dominant	$\hat{\imath}_m$ maternal additive-by-maternal additive	$\hat{l}_m$ maternal dominant by-maternal dominant	$\widehat{d.d_m}$ additive-by-maternal additive	$\widehat{h.d_m}$ dominant-by-maternal additive	$\widehat{d.h_m}$ additive-by-maternal dominant	$\widehat{h.h_m}$ dominant-by-maternal dominant
WWxWW	1	1	0	1	0	0	1	0	1	0	1	0	0	0
DDxDD	1	-1	0	1	0	0	-1	0	1	0	1	0	0	0
WWxDD	1	0	1	0	0	1	1	0	1	0	0	1	0	0
DDxWW	1	0	1	0	0	1	-1	0	1	0	0	-1	0	0
F1xF1	1	0	1/2	0	0	1/4	0	1	0	1	0	0	1/2	0
F2xF2	1	0	1/4	0	0	1/16	0	1/2	0	1/4	0	0	1/8	0
WWxF1	1	1/2	1/2	1/4	1/4	1/4	1	0	1	0	1/2	1/2	0	0
F1xWW	1	1/2	1/2	1/4	1/4	1/4	0	1	0	1	0	0	1/2	1/2
DDxF1	1	-1/2	1/2	1/4	-1/4	1/4	-1	0	1	0	1/2	-1/2	0	0
F1xDD	1	-1/2	1/2	1/4	-1/4	1/4	0	1	0	1	0	0	1/2	-1/2
WWxF2	1	1/2	1/2	1/4	1/4	1/4	1	0	1	0	1/2	1/2	0	0
F2xWW	1	1/2	1/2	1/4	1/4	1/4	0	1/2	0	1/4	0	0	1/4	1/4
DDxF2	1	-1/2	1/2	1/4	-1/4	1/4	-1	0	1	0	1/2	-1/2	0	0
F2xDD	1	-1/2	1/2	1/4	-1/4	1/4	0	1/2	0	1/4	0	0	1/4	-1/4

*WW, wild; DD, domesticated; F1, first-generation hybrids (WWxDD & DDxWW); F2, second-generation hybrids (F1xF1).

Table S 5.2: Retransformed (from natural logarithms) average fork length and total wet mass (each ± averaged standard error (SE)), at time of spawning of four year old mature females and mature males of Atlantic salmon from four crosses between a wild (WW) and a domesticated population (DD), their reciprocal first-generation hybrids (F1; WWxDD & DDxWW), and second-generation hybrids (F2; F1xF1). This table is based on sample sizes (n) including also individuals that were not used to create new crosses for the 2009 experimental generation.

	Females			Males		
Cross	n	Length (cm) ± SE	Mass (kg) ± SE	n	Length (cm) ± SE	Mass (kg) ± SE
F1	39	52.4 ± 0.71	2.2 ± 0.11	30	55.6 ± 1.06	2.4 ± 0.13
F2	40	53.4 ± 0.55	2.2 ± 0.08	40	53.3 ± 1.28	2.0 ± 0.07
DD	35	54.9 ± 0.59	2.3 ± 0.09	33	57.9 ± 0.72	2.4 ± 0.10
WW	40	46.6 ± 0.54	1.6 ± 0.06	52	46.7 ± 0.85	1.4 ± 0.05

Table S 5.3a-f: Parameters of the models predicting six trait means of 14 crosses of Atlantic salmon. 'Effect' in the header line refers to the estimated value (± standard error (SE)) of a given fixed term, or to the predicted variance of a random term. P-values (P) for fixed terms were inferred from Wald-type F-tests in which the denominator degrees of freedom (ddf) were approximated by the method of Kenward and Rogers (1997), or from log-likelihood ratio tests (LRTs) between nested models including and excluding each respective random term under constant fixed terms, and all random residuals were jointly evaluated at the overall term level. To meet assumptions of the cross means analysis that was based on these models, it was ensured that either homoscedasticity was given at the residual level for the three terms encompassing the fourteen reciprocal crosses, the nine (non-reciprocal) crosses, and the four dam crosses or heteroscedasticity was taken into account by fitting different residual strata at the most parsimonious of these three cross levels evaluated by sequentially fitted LRTs. As the exact test statistic resulting from LRTs is uncertain, for resulting p-values between 0.05 and 0.1 residual heteroscedasticity was additionally tested by using the Fligner-Killeen test of homogeneity of variances for each of the three cross levels. Given p-values for residuals are for the test vs. a model with homogenous residuals.

Supplementary Table S 5.3 exceeds the page length to be accommodated in this thesis

Table S 5.4a-f: *F*- and p-values from conditional Wald tests (incremental tests resulted in the same *F* and p-values for the lack-of-fit term) for all tested outbreeding effects and combinations of significant effects for predicting six trait means of 14 crosses of Atlantic salmon. Effects were included in the corresponding models of Supplementary Table S 5.3 after fixed environmental terms, if applicable. The order of effects, when several, gives the order for reported *F*-values (*F*) and p-values (P) corresponding to the subscripts (1 and 2); the subscript 'Lack' indicates values for the lack-of-fit term (representing the 14 cross means, fitted as last model term). Significant values ($P < 0.05$ for outbreeding effects, and $P > 0.05$ for a non-significant lack-of-fit) are given in bold. Abbreviations of outbreeding effects are used as in supplementary **Table S 5.1**.

Supplementary Table S 5.4 exceeds the page length to be accommodated in this thesis

Chapter 6: Conclusion

For domestication of Atlantic salmon, different wild populations have served as donors to establish strains throughout the area of its industrial production. Major strains were founded either by single-river populations (e.g., the currently studied major Atlantic North American Saint John River strain; Glebe 1998; Wolters *et al.* 2009) or by mixed-river populations (e.g., the major Norwegian AquaGen strains that have also been exported to many parts of the world; Thodesen & Gjedrem 2006). Accordingly, there is no such thing as the general domesticated Atlantic salmon and genetically based outbreeding effects from a domesticated strain on a wild population might be pair-specific. This is also reflected by the observation that, across strains, allelic frequencies at very different loci appear to have been changed as a result of domestication (Vasemagi *et al.* 2012), despite the suggested presence of parallel changes in transcription levels of many common genes (Roberge *et al.* 2006).

Nevertheless, the major traits selected in different breeding programmes are usually the same. Common to most breeding programmes is the goal to maximise the economy of the production and to ensure a high quality product (Gjedrem 2000; Gjedrem 2005; Thodesen & Gjedrem 2006; Gjedrem & Baranski 2009). Commonly selected traits in Atlantic salmon include rapid growth and a high feed conversion efficiency, rapid smolting, delayed maturation, but also disease resistance and high flesh quality (Friars *et al.* 1990; Friars *et al.* 1995; Gjedrem 2000; Gjedrem 2005). Accordingly, domesticated and wild Atlantic salmon might diverge most of all for these traits; paramount to all breeding programmes is the selection of rapid growth. The primary goal of a rapid growth rate in aquaculture is also reflected in a potential emergence of an industry utilising growth-hormone transgenic Atlantic salmon (Fletcher *et al.* 2004) and many of the changes observed in domesticated salmon might be paralleled in transgenic salmon (as has been suggested for coho salmon, *Oncorhynchus kisutch*; Devlin *et al.* 2009).

In my thesis, we investigated genetic based gradual changes arising from different duration of domestication in the Saint John River strain and examined

outbreeding effects between a domesticated Saint John River strain and the wild Stewiacke River population. The results of my thesis contribute new knowledge of general salmon biology and of gradual domestication effects on traits during the first year of life that are not confounded by differences in ancestry, as in most previous studies. Furthermore, we presented results on outbreeding effects of many different traits during different life-stages between hatching and sexual reproduction and these results allowed for insight into the between-population genetic architecture.

Differences in growth rate and size-at-age between domesticated and wild individuals were central to all our studies. Our results revealed that domesticated individuals grow more rapidly and were larger than wild individuals at all stages investigated, and this even included the developmental period between the egg and fry stages. Furthermore, we demonstrated the presence of large non-additive genetic components in the genetic architecture between domesticated and wild populations for morphological traits and gene transcription. The existence of non-additive effects render predicted changes in phenotype difficult. As a potential consequence, natural selection will likely not act in a linear manner on different hybrid generations as expected from their percentage of domesticated allelic combinations.

Non-additive components in the between-population genetic architecture of Atlantic salmon have not previously been detected. One exception poses indirect evidence of a lower egg survival of second-generation hybrids relative to first-generation hybrids and both parental populations (McGinnity *et al.* 2003). Lower survival in embryonic survival of second-generation hybrids could be confirmed by another study (Fraser *et al.* 2010), but was completely absent in our studied populations. In the present research, egg survival was fully determined by dam effects, i.e., by individual maternal effects, and not cross effects, whereas McGinnity *et al.* (2003) and Fraser *et al.* (2010) did not account for maternal effects on early survival. Furthermore, we investigated the only (yet) ever bred third-generation hybrid (F3) families and second-generation hybrid backcrosses between divergent domesticated and wild Atlantic salmon and could not identify any effects during

early life resulting from on-going hybridisation (and hence advanced recombination). It is hence likely that a long-feared hybrid breakdown by the breakup of coadapted genes through crossing over is absent between the studied salmon populations, at least during early life stages and under laboratory conditions. This, however, is no guarantee that such a later-generation hybrid breakdown is also absent between other populations. The pre-domestication genetic divergence between the two studied populations arose in the presence of a geographic distance between both rivers of 200 km whereas the whole distributional range spans about 7000 km from west to east (Klemetsen *et al.* 2003). For some species it has been observed that presence and severity of hybrid breakdown is a function of divergence between populations (Edmands 2002) and such scale effect remains yet to be evaluated in Atlantic salmon. A recent study suggested the presence of a unidirectional reproductive isolation between European and North American Atlantic salmon (Cauwelier *et al.* 2006), but in that study sire effects, taken as fixed effects, were confounded with cross effects so that these results are questionable and need confirmation.

The crosses used to investigate outbreeding effects had been kept in a common environment for three generations. While this removes possible environmental effects among crosses, it might have homogenised traits among crosses by inadvertent selection of the artificial environment. If this was the case, differences among crosses might even be larger than those detected.

My work is differentiated from most previous work on the between-population genetic architecture by both experimental design and statistical analyses which might have resulted in a higher precision and accuracy of trait mean estimates and, therefore, revealed non-additive outbreeding effects that might have been undetected in previous studies. As the results of my thesis stress, salmon individuals within a population, and also full-sibs within a family, exhibit a great diversity of genetically based potential phenotypes which can be further modified by environmental influences. This makes it necessary for researchers to employ a detailed breeding design, experimental design, and advanced statistical analyses to be able to reliably detect outbreeding effects of divergent wild and domesticated

populations. We tested outbreeding effects in chapters 3 and 5 on data that accounted for environmental effects, including simple and repeated measures tank effects; in chapter 5, we also accounted for correlation arising from complex kin relationships among crosses.

Previous studies on outbreeding effects in Atlantic salmon used relatively brute methodology, developed during the 1950's, which does not account for all environmental variation (Piepho & Möhring 2010); previous work also did not account for among-family variation when testing the between population genetic architecture (e.g., Fraser *et al.* 2010; Houde *et al.* 2010). Furthermore, in chapter 3 we gathered data on individual-based growth trajectories and accounted for the complex covariance structure present in longitudinal data (but were unfortunately unable to account for among-full-sib correlations), which resulted in a high detection precision of growth differences among outbred crosses in Atlantic salmon.

Throughout my thesis as a whole, I think it became clear that predictions for domestication-induced changes of traits that are often correlates of, or influenced by, growth rate or size-at-age are very difficult to make for Atlantic salmon. This is because, firstly, fishes are indeterminately growing individuals with a considerable potential of environmental plasticity and, secondly, between-population divergence for growth and some of the studied life-history traits share non-additive effects. Nevertheless, despite the presence of such complex phenotypic and genotypic interactions, results of my work demonstrate that domestication has negative effects on maturation probability of 1+ year males and that early maturation probability in post-smolt domesticated-wild females and males is much lower than would be predicted from percentages of domesticated and wild allelic combinations. This is consistent with observations of reduced male parr maturity and deferred maturation in domesticated-wild hybrids as exhibited in a natural environment (McGinnity *et al.* 2003). As such, it appears likely that domesticated-wild outbreeding effects will not only change growth potential in wild populations but also life history traits during parr and post-smolt stages. Lastly, we demonstrated that between-population divergence can have outbreeding effects on maternal effects. As far as I know, such maternal outbreeding effects had not previously been

observed in any wild populations (in contrast to crossbreeding studies in agricultural animal breeding). Yet, maternal outbreeding effects can only be identified as such with appropriate experimental and statistical design and are likely to be misinterpreted as different effects by most conventional studies.

My laboratory based results guide the way for studies to be undertaken in the wild. Presence and extend of admixture between domesticated allelic and wild gene pools and their temporal trends have been recorded for several populations (Skaala *et al.* 2006; Bourret *et al.* 2011; Glover *et al.* 2012). Yet, effects on population viability have only been modelled theoretically (Hutchings 1991; Hindar *et al.* 2006; Baskett & Waples 2013) but still remain to be evaluated empirically. However, the disentanglement between environmental and fisheries induced changes in phenotypic traits is a major challenge (Enberg *et al.* 2012) and this might also be valid for domesticated-induced evolution of wild populations as conveyed by outbreeding with escaped individuals. In contrast to fisheries-induced evolution that acts through the directional removal of certain individuals, directional selection in aquaculture is very likely much stronger as it acts by choosing individuals for reproduction and the effects of this type of selection are well known. Nevertheless, apart from genetic signatures of domesticated-wild outbreeding, to my knowledge little effort has been dedicated to estimating the effects of domesticated-wild outbreeding on phenotypic traits in wild populations (ignoring the large body of literature on non-intentionally selected hatchery populations). Instead, most studies conducted in the wild have focussed on comparing performance among domesticated, wild, and hybrid individuals (McGinnity *et al.* 1997; McGinnity *et al.* 2003; Skaala *et al.* 2012). It might hence be useful to monitor and assess temporal trends in trait changes in wild populations and investigate the correlation to temporal trends of presence and degree of domesticated-wild outbreeding. My work identified key traits differing between domesticated and wild populations of Atlantic salmon and the mechanism acting in the trait expression of their hybrids. Based on my results, examples of candidate traits to investigate would be changes in reproductive success as related to maternal outbreeding effects and as related to changes in male parr maturation probability, a lower age at smolting combined with

a smaller size at smolting, a later age at maturity combined with a larger size at maturity, as well as a lower oceanic survival resulting from a longer lasting oceanic migration. Conducting such studies in wild populations seems prudent as decline of many wild Atlantic salmon populations in Europe or North America temporally coincided with the emergence of the large scale aquaculture industries in the 1980's and 1990's (COSEWIC 2006; Ford & Myers 2008; ICES 2009) and industries are still growing on both sides of the Atlantic Ocean (FAO 2012).

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