



# Morphological consequences of hybridization between farm and wild Atlantic salmon *Salmo salar* under both wild and experimental conditions

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**ABSTRACT:** The escape of Atlantic salmon *Salmo salar* from aquaculture has been identified as a significant threat to the persistence and stability of wild salmon populations. Yet the magnitude of phenotypic impacts due to hybridization remains largely unresolved. We evaluated the phenotypic consequences of hybridization using geometric morphometrics both under natural conditions in the wild and in the laboratory using common garden experiments. Juvenile Atlantic salmon field-collected in 2015 and 2016 from 18 southern Newfoundland rivers were classified as pure wild, pure farm, or F<sub>1</sub> hybrids using genetic assignment. Overall size and shape differences between wild and farm, and wild and F<sub>1</sub> hybrid individuals were small, largely size related, and present between pure farm and other crosses. Laboratory-reared pure wild, pure farm, and F<sub>1</sub> hybrid salmon were grown in tank and semi-natural conditions. Wild fish were significantly larger than both farm and hybrid salmon at first feeding; these size differences remained at 80 d post first feeding under semi-natural conditions, but all crosses were the same size in tank conditions, and there were no differences between pure farm and hybrid individuals under either condition. Significant shape differences were present among all pairwise comparisons under tank conditions, and in semi-natural conditions, pure wild individuals differed significantly from pure farm and hybrid individuals. Our results suggest phenotypic differences observed under laboratory conditions between wild and farm×wild hybrid individuals may not be appreciable in the wild, and that significant genetic changes may occur in wild populations experiencing hybridization in the absence of obvious large phenotypic changes.

**KEY WORDS:** Hybridization · Aquaculture impacts · Atlantic salmon · *Salmo salar* · Geometric morphometrics · Common garden

## 1. INTRODUCTION

The escape of domesticated Atlantic salmon *Salmo salar* represents a significant threat to the persistence and stability of wild salmon stocks (Forseth et al. 2017, Glover et al. 2017). Domesticated salmon regularly escape aquaculture pens into the local environment (Verspoor et al. 2015, Keyser et al. 2018, Diserud et al.

2019, Glover et al. 2019) and, despite the observed reduced mating success of domesticated salmon compared to wild salmon (Fleming et al. 1996), there is widespread evidence of hybridization in the wild (Glover et al. 2013, Karlsson et al. 2016, Sylvester et al. 2018, Wringe et al. 2018). Hybrid individuals can display reduced fitness due to outbreeding depression (Fleming et al. 2000, McGinnity et al. 2003, Skaala et

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al. 2019), thus, the potential for introgression from domestic salmon into wild salmon populations is an ongoing concern (Jonsson & Jonsson 2006, Hutchings & Fraser 2008, Fraser et al. 2010). These fitness concerns and the magnitude of aquaculture production compared to wild salmon abundance have led to aquaculture escapees being identified as a central threat to the stability and persistence of wild salmon populations (Forseth et al. 2017). Although hybridization and genetic introgression between wild and escaped farmed salmon has been well documented on both sides of the North Atlantic, the biological consequences remain poorly understood, with few studies documenting the biological consequences in the wild (see Bolstad et al. 2017).

The process of domestication in fish has been shown to result in convergent phenotypic divergence from wild conspecifics; a suite of common, directional differences in phenotypic traits are brought about by domestication across many species (Wringe et al. 2016). In Atlantic salmon, domesticated individuals often display smaller heads and kypes (Wringe et al. 2016, Perry et al. 2019), greater body depth (Fleming & Einum 1997, Wringe et al. 2016), smaller rayed fins (Fleming & Einum 1997), and changes in the caudal peduncle shape (Fleming et al. 1994). Changes from the wild phenotype in these traits are likely maladaptive in the wild, as salmon morphology likely reflects local adaptations to water velocity and other environmental conditions or to mating success (Taylor 1991, Fleming et al. 1996, Jonsson 1997, Pakkasmaa & Piironen 2000). This is reflected by evidence of reduced survival of hybrid individuals both in fresh water and the marine environment (Fleming et al. 2000, Skaala et al. 2012, Sylvester et al. 2019). However, the degree to which a hybrid phenotype is shaped by selection and plasticity remains unclear. Glover et al. (2018) found that the lack of growth differences between wild and domestic salmon in natural environments is explained mostly by plasticity, in combination with growth-dependent selection. As such, the shape differences observed under aquaculture conditions may also be masked when reared in a natural environment due to the plastic response of an individual's shape to its environment (von Cramon-Taubadel et al. 2005). Improved understanding of the phenotypic effects associated with hybridization between wild and domestic salmon in the wild is needed to better predict interactions, impacts, and the consequences of escape events on wild populations.

Southern Newfoundland (Canada) is characterized by extensive Atlantic salmon aquaculture activities (DFO 2013) as well as increasingly declining wild

stocks (COSEWIC 2011, DFO 2017). Aquaculture salmon in the region, originally derived from the Saint John River, New Brunswick, differ genetically from southern Newfoundland wild salmon (Jeffery et al. 2018), and as such, escapees and hybrids have been identified genetically (Keyser et al. 2018, Wringe et al. 2018, 2019). Surveys and monitoring for aquaculture escapees regularly detect escapees in the wild both in years that do and do not have reported escape events (Keyser et al. 2018, Sylvester et al. 2018, Wringe et al. 2018). Recent work has explored the impact, in terms of the numbers of hybridization events, of a large escape event which released approximately 20 000 salmon, an amount equal to estimated abundance of wild salmon in the area (Keyser et al. 2018, Wringe et al. 2018). While the distribution and number of hybrids produced following this escape event have been investigated, the impact of hybridization on phenotype, growth, and other fitness-related traits remains unstudied.

The overall objective of this study was to explore the magnitude and nature of phenotypic (size, shape) differences resulting from hybridization between pure wild and pure farm individuals using geometric morphometrics. Specifically, we first examined shape differences among genetically identified wild, farmed, and hybrid individuals produced in the wild and collected in southern Newfoundland. Second, we used a common garden experiment to compare pure wild, pure farm, and hybrid salmon in aquaculture-like tank and semi-natural (i.e. stream channel) conditions. The comparison of individuals from field collections, common garden semi-natural, and tank experimental conditions is an exceptional opportunity and provides an exemplary gradient across which phenotypic effects may be examined. This study builds directly on previous studies exploring the prevalence of hybridization between pure wild and pure farm salmon in southern Newfoundland (Sylvester et al. 2018, Wringe et al. 2018) and differences in survival between wild, feral, and hybrid individuals in the region (Sylvester et al. 2019). Moreover, we complement a recent study exploring phenotypic differences due to hybridization in the Northeast Atlantic (Glover et al. 2018).

## 2. MATERIALS AND METHODS

### 2.1. Field sampling

Juvenile Atlantic salmon parr ( $n = 2003$ ) were caught by electrofishing in 18 rivers in Fortune Bay

and Bay D'Espoir, Newfoundland, in 2015 and 2016 (Fig. 1a; Table S1 in the Supplement at [www.int-res.com/articles/suppl/q014p085\\_supp.pdf](http://www.int-res.com/articles/suppl/q014p085_supp.pdf)). The juvenile salmon caught in southern Newfoundland were assumed to be genetically similar to their respective common garden cross type. The wild fish populations belonged to the same genetic reporting group (Jeffery et al. 2018), and the aquaculture salmon used for the common garden experiments were from the same Saint John River-derived strain that escaped into the rivers. Parr were aged based on fork length (Sethi et al. 2017), and only 0+ or 1+ individuals were analyzed due to the low number of age 2+ pure farm and hybrid individuals caught. Fin clips were taken from the right pectoral fin and stored in 95% ethanol for genotyping. Individuals were genotyped at 95 SNP loci using a custom Fluidigm EPI array (see Wringe et al. 2019 for SNP selection), following the manufacturer's protocol using 96.96 genotyping integrated fluidic circuits and read on an EP1 (Fluidigm), and analyzed using SNP genotyping analysis software (Fluidigm). Each 96-well plate contained 10 redundant samples and positive controls. Hybrid classes of

individuals were estimated using 'parallelnewhybrid' in the package 'HYBRIDDETECTIVE' with the *s* parameter (Wringe et al. 2017, 2018). 'Parallelnewhybrid' executes the program 'NEWHYBRIDS' (Anderson & Thompson 2002) in parallel utilizing multicore processors to increase the speed of analysis. 'NEWHYBRIDS' was run with a burn-in of 50 000 with 100 000 sweeps. A cut-off probability of >0.8 in a single hybrid class was used to assign individuals (Wringe et al. 2019).

## 2.2. Common garden experiment

Wild Atlantic salmon were sourced from Northeast Placentia River due to current lack of salmon aquaculture, and farmed salmon escapees and hybrids east of the Burin Peninsula (Wringe et al. 2018). Farmed salmon from the North American aquaculture strain were derived from the Saint John River, New Brunswick. Crosses were performed in 2015 and generated 44 families of 3 cross types: 20 pure Saint John River farm families, 13 one-direction  $F_1$

hybrid families (female farm  $\times$  male wild), and 11 pure Northeast Placentia wild families (Islam et al. 2020). Before being placed in the rearing environment, each fry was anesthetized using MS-222 and tagged with elastomer for individual identification. Individuals were raised in common garden experiments with pure wild (PW), pure farm (PF), and  $F_1$  hybrid ( $F_1$ ) crosses raised in semi-natural (i.e. stream) and tank conditions. For each group (PW, PF,  $F_1$ ), there were 10 individuals with 12 replicates per environment. After absorption of the yolk-sac (Day 0), fry were randomly selected from rearing tanks to be reared in either tank or semi-natural conditions. Tanks were made of Rubbermaid buckets and measured  $0.32 \times 0.24 \times 0.16$  m, while the semi-natural conditions consisted of stream tanks measuring  $1.2 \times 0.22 \times 0.15$  m with a constant flow rate of  $10\text{--}15$  cm  $s^{-1}$  and a gravel substrate. Salmon in tanks were fed salmonid starter dry feed (EWOS-Cargill) 4 times daily, and salmon in semi-natural conditions were fed live *Artemia* (1 million nauplii  $l^{-1}$ ) and frozen blood worms (Chironomidae spp., from a commer-

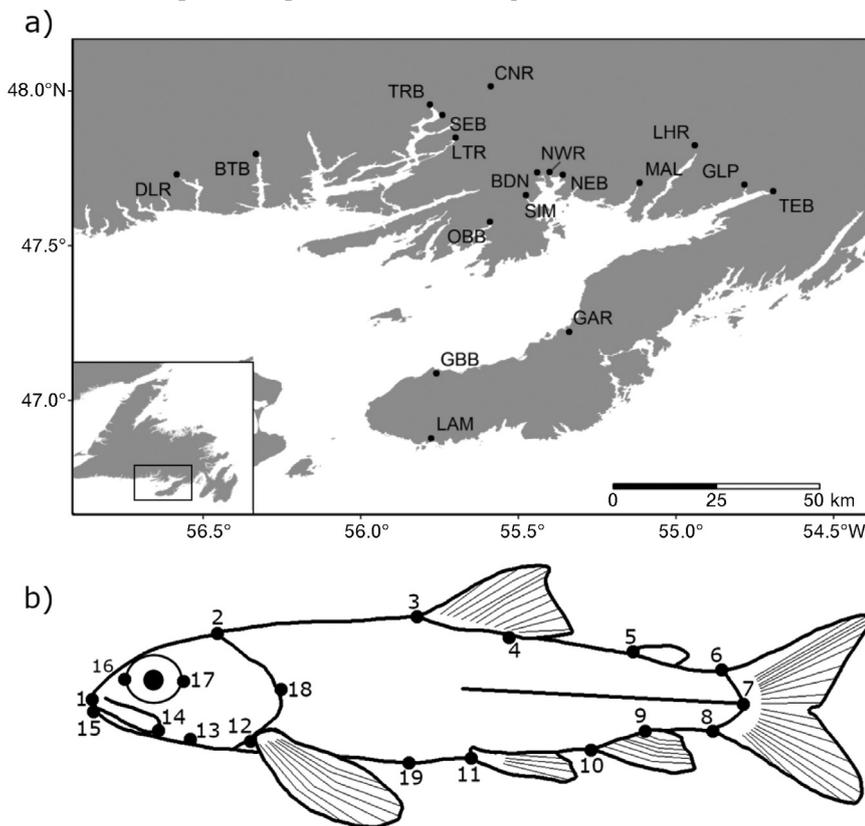


Fig. 1. (a) Sample locations in Newfoundland (Canada) rivers for 2015 and 2016 (see Table S1 in the Supplement at [www.int-res.com/articles/suppl/q014p085\\_supp.pdf](http://www.int-res.com/articles/suppl/q014p085_supp.pdf) for details) and (b) location of 19 landmarks used for geometric morphometric analysis of Atlantic salmon (see Table S2 for description of land-mark locations)

cial fish food supplier) 4 times daily. Both tank and semi-natural conditions received 12 h of light daily and had a dissolved oxygen level of  $8.03 \text{ mg l}^{-1}$ .

### 2.3. Shape analysis

Geometric morphometrics were used to quantify shape differences among wild, farm, and hybrid salmon using 19 landmarks (Table S2), from photographs of individuals on their left side (Fig. 1b). Individuals in the common garden experiment were photographed on Days 0 and 80, and all field-sampled individuals were photographed after capture. A total of 205 Day 0 individuals and 179 Day 80 individuals were analyzed after filtering photos for image quality, arching, and any other deformations (Table S3). Of the field samples genotyped, after filtering for image quality, arching, and deformations, 1356 were landmarked for geometric morphometrics. All individuals assigned to second-generation hybrid class ( $F_2$  or backcross) were removed from the analysis due to a low number of individuals caught. This left 964 samples for geometric morphometric analysis from 18 rivers aged 0+ or 1+ and assignment to PW, PF, or  $F_1$  hybrid genotype frequency classes (Anderson & Thompson 2002) (Table S1).

Landmarking was done using ImageJ (Schneider et al. 2012), and landmarks were corrected for body arching using the 'tpsUtil unbend' function along landmarks 1, 7, and 18 (Rohlf 2015). A generalized Procrustes analysis was performed using the 'geomorph' package in R v.3.5.1 (Adams & Otárola-Castillo 2013, R Core Development Team 2013), while a principal component analysis (PCA) to look at the greatest shape variation and a canonical variate analysis (CVA) to identify among group variation were performed using the package 'Morpho' (Schlager 2017). The first 2 principal components were plotted to identify areas of shape variation. To determine if overall shape differences were present, a Procrustes ANOVA was performed on the aligned shape coordinates among cross types using the package 'geomorph' (Adams & Otárola-Castillo 2013). Where significant differences were detected, pairwise differences in single morphometric variables were tested between all combinations of PW, PF, and  $F_1$  hybrids, and false discovery was accounted for using Bonferroni correction for experimental comparisons (Table S3). Centroid size was calculated as a measure of overall body size of individuals. Body depth was measured as the linear distance between landmarks 3 and 17 after Procrustes alignment. A Kruskal-Wallis ANOVA was performed on both

body depth and  $\log_{10}$  transformed centroid size to identify significant differences between PW, PF, and  $F_1$  cross types (Goodall 1991) using a pairwise Wilcoxon rank-sum test for comparisons among types. To identify differences in allometric growth, a comparison of the slopes between cross types from a shape-size regression was performed.

## 3. RESULTS

Sample sizes for wild-caught Atlantic salmon ranged from 7 to 171 per river across ages (Table S1), with an overall missing genotype frequency of 1.3%. Using a cut-off probability of 0.8 for hybrid assignment, 168 individuals were not assigned to a genotype frequency class and were removed from the analysis. For image analysis, images were filtered for jaw deformations, damage to landmark locations, and severe dorso-ventral arching. This resulted in 47 individuals being removed from tank conditions, 77 individuals being removed from semi-natural conditions, and 647 individuals removed from field samples. Note, all analyses presented below were conducted with the bending correction; for the results without bending correction, see Figs. S1–S3.

### 3.1. Geometric morphometric shape differences

Only age 1+ field-collected samples were analyzed for differences in shape because of low sample size for age 0+ individuals classified as PF. Age 1+ field samples showed overall shape differences among groups ( $df = 2$ ,  $p = 0.002$ ). Pairwise comparisons revealed that PW individuals had significant shape differences from  $F_1$  hybrid individuals, but  $F_1$  did not differ significantly from PF ( $\alpha = 0.05/3 = 0.017$ ; PF- $F_1$ ,  $p = 0.040$ ; PW- $F_1$ ,  $p = 0.001$ ; PW-PF,  $p = 0.028$ ; Table S3).

In the common garden experiment, a total of 179 and 205 individuals were sampled across the tank and semi-natural conditions, respectively (Table S4). Significant shape differences were detected among groups at both time points in tank conditions (Day 0:  $df = 2$ ,  $p = 0.001$ , Day 80:  $df = 2$ ,  $p = 0.001$ ). For both Day 0 and 80, pairwise comparisons revealed significant differences in shape among all groups ( $\alpha = 0.05/3 = 0.017$ , all  $p < 0.01$ ). In the semi-natural conditions, overall differences in shape among groups were also detected at both measurement times (Day 0:  $df = 2$ ,  $p = 0.001$ , Day 80:  $df = 2$ ,  $p = 0.001$ ). Pairwise comparisons on Day 0 found PW to differ significantly in

shape from both PF and  $F_1$ , but no significant difference in shape was detected between  $F_1$  and PF ( $\alpha = 0.05/3 = 0.017$ ; PF- $F_1$ ,  $p = 0.332$ ; PW- $F_1$ ,  $p = 0.001$ ; PW-PF,  $p = 0.001$ ). When measured on Day 80, pairwise comparisons revealed significant differences between all combinations ( $\alpha = 0.05/3 = 0.017$ ; PF- $F_1$ ,  $p = 0.001$ ; PW- $F_1$ ,  $p = 0.001$ ; PW-PF,  $p = 0.004$ ).

### 3.2. PCA

To identify major sources of shape variation, a PCA was performed on the aligned shape coordinates. The first axis for common garden and field samples explained 17.4 and 32.5% variance, respectively (Fig. 2). This axis largely corresponded with residual arching and body depth variance based on relative warps. The second axis explained 10.8–20.4% of the shape variance (Fig. 2). Based on relative warps, this axis explained body depth and head size variation

(Figs. S4–S9). While these axes explain a large proportion of the variance, the eigenvalues of these axes are  $<1$ , and there is a large degree of overlap in 95% confidence intervals among cross types.

### 3.3. CVA

In order to characterize among-group shape variation across rearing environments, a CVA was performed between PW, PF, and  $F_1$  cross types. The first axis across common garden and field conditions appears to characterize shape differences between PW and PF or  $F_1$  hybrid. This accounts for 61.9–81.9% of the between-group variation in shape (Fig. 3). The second axis characterizes shape differences between PF and  $F_1$  hybrid individuals across conditions and accounts for 38.1–18.1% variation between groups (Fig. 3). Using a leave-one-out method for cross-validation of assigning individuals to group (i.e. PW,

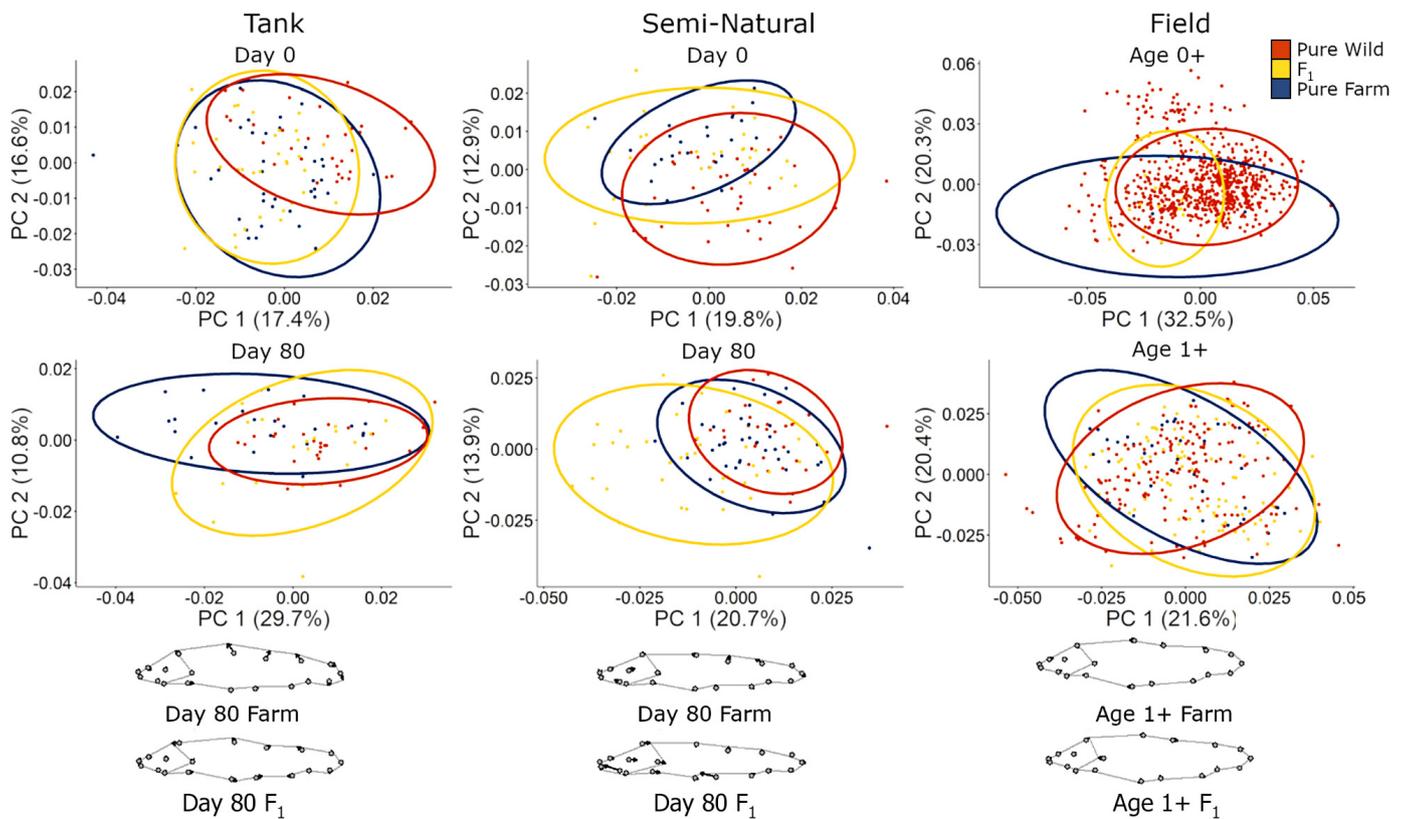


Fig. 2. Principal component analysis of landmarks after Procrustes alignment with 95% confidence ellipsis for each cross type. Experimental condition is noted above each panel, and the age group is on the x-axis. For the common garden experiment, shape is shown for Day 0 (start) and Day 80 (end) post yolk absorption. In the field component, juveniles aged 0+ (young of year) and 1+ were sampled from 18 locations (Fig. 1, Table S1). Relative differences in mean shape are also shown for the oldest ages sampled (Day 80 post yolk-sac absorption for common garden experiments and age 1+ for field samples) comparing mean shape of farm or  $F_1$  hybrids to wild salmon with 4 $\times$  magnification of shape differences for visualization. Age 0+ field-collected shape differences are not shown due to low sample size. Identification of cross type of field-sampled individuals was accomplished using genetic assignment and is described in Section 2

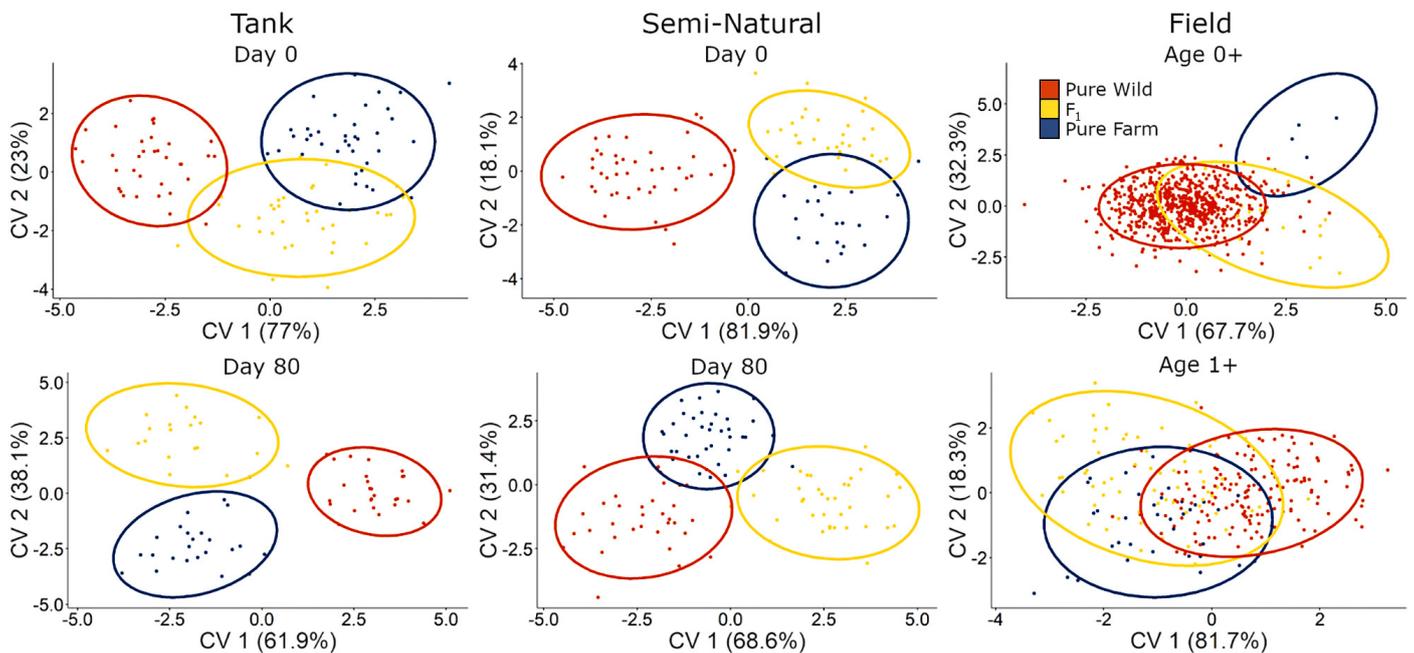


Fig. 3. Canonical variate analysis of landmarks after Procrustes alignment with 95 % confidence ellipses for each cross type. For the common garden experiment, shape is shown for Day 0 (start) and Day 80 (end) post yolk absorption. Juveniles aged 0+ (young of year) and 1+ were sampled from field locations (18 southern Newfoundland rivers; Fig. 1). Identification of cross type of field-sampled individuals was accomplished using genetic assignment and is described in Section 2

PF,  $F_1$ ) based on shape resulted in 65.8% accuracy identifying field age 1+ individuals and 96.6% accuracy in field age 0+ individuals (Table S5). In the common garden conditions, the cross-validation accuracy ranged from 74.1 to 79.6% (Table S5). The majority of the misassignment to group occurred between  $F_1$  and PF, except in age 0+ field samples, where the majority of individuals were assigned as PW. See Figs. S4–S9 for mean shape of each group.

### 3.4. Body depth ANOVA

The mean shape comparison and relative warps along PC1 in the PCA both suggested that differences in body depth were among the major contributors to overall shape differences between groups. ANOVAs were performed on the inter-landmark distance between landmarks 3 and 19 (Fig. 1b), separately by age and rearing condition. When reared in tank conditions, significant differences in body depth were detected on Day 80, but not Day 0 (Day 0:  $\chi^2 = 0.602$ ,  $p = 0.740$ ; Day 80:  $\chi^2 = 14.8$ ,  $p < 0.001$ ). PF and  $F_1$  individuals had a significantly deeper body depth than pure wild ( $\alpha = 0.05/3 = 0.017$ ; PF- $F_1$ ,  $p = 0.407$ ; PW- $F_1$ ,  $p = 0.022$ ; PW-PF,  $p < 0.001$ ), the mean body depth of  $F_1$  hybrids was intermediate to PW and PF

(PF = 0.12773,  $F_1$  = 0.12616, PW = 0.12171). In both semi-natural (Day 0:  $\chi^2 = 0.341$ ,  $p = 0.843$ ; Day 80:  $\chi^2 = 3.7$ ,  $p = 0.155$ ) and field conditions (Age 1:  $\chi^2 = 0.253$ ,  $p = 0.881$ ), there were no significant differences between the body depth of cross types (Fig. 4).

### 3.5. Size ANOVA

To compare overall size differences among groups, ANOVAs were performed between cross types using centroid size as a measure of overall individual size. Centroid size is the square root of the sum of squared distances from the centroid to each landmark. In field salmon samples, PW individuals were significantly larger than  $F_1$  individuals at age 0+, but there the sample size was insufficient to evaluate PF individuals (Fig. 5). By age 1+, significant differences in size were evident among cross types ( $\chi^2 = 10.7$ ,  $p = 0.005$ ), with PF being significantly larger than  $F_1$  and PW (PF- $F_1$ ,  $p = 0.0011$ ; PW- $F_1$ ,  $p = 0.356$ ; PW-PF,  $p = 0.0056$ ). On Day 0 in the common garden experiment, PW in both tank ( $\chi^2 = 41.5$ ,  $p < 0.001$ ; PF- $F_1$ ,  $p = 0.700$ ; PW- $F_1$ ,  $p < 0.001$ ; PW-PF,  $p < 0.001$ ) and semi-natural conditions ( $\chi^2 = 36.3$ ,  $p < 0.001$ ; PF- $F_1$ ,  $p = 0.360$ ; PW- $F_1$ ,  $p < 0.001$ ; PW-PF,  $p < 0.001$ ) had a greater centroid size than  $F_1$  and PF. After 80 d, there

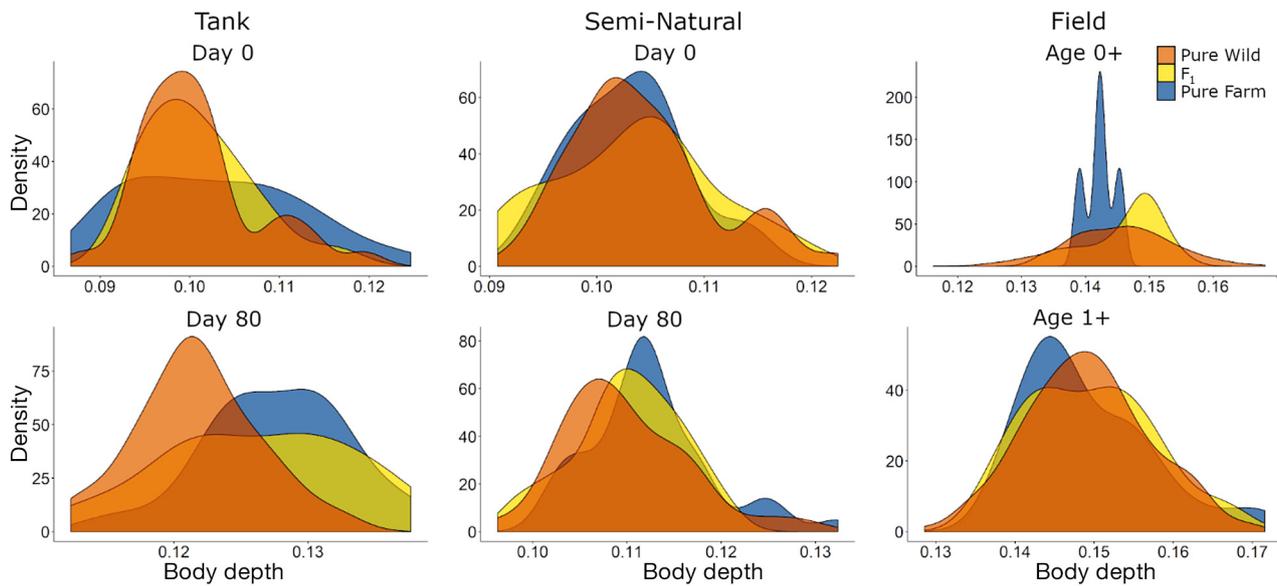


Fig. 4. Body-depth measurements using inter-landmark distance between landmarks 3 and 17. Landmarks are as illustrated in Fig. 1b. Individuals were measured on Day 0 (start) and Day 80 (end) post yolk absorption for the common garden experiment. Field salmon aged 0+ and 1+ were sampled from 18 rivers in southern Newfoundland as shown in Fig. 1. Identification of cross type of field sampled individuals was accomplished using genetic assignment and is described in Section 2

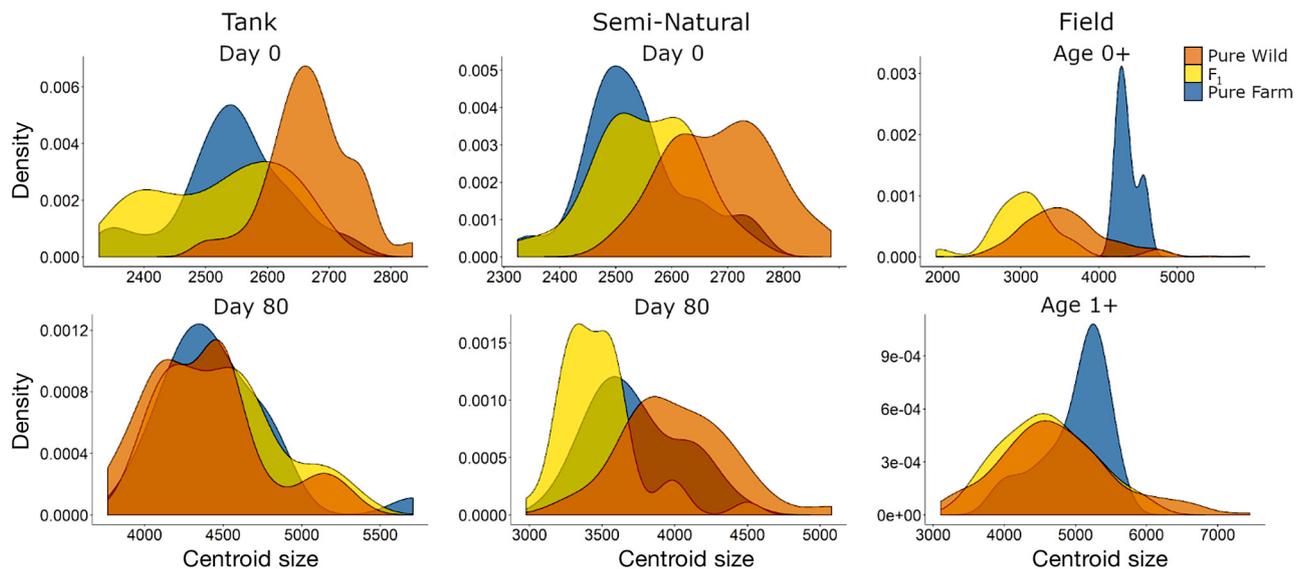


Fig. 5. Centroid sizes of juvenile salmon calculated using 19 landmarks for each sample (see Fig. 1b). The y-axis represents the distribution of individuals for each group of that size. Measurements for the common garden experiment were taken on Day 0 (start) and Day 80 (end) post yolk absorption. Juveniles aged 0+ and 1+ were sampled from southern Newfoundland rivers (Fig. 1a, Table S1). Field-captured/collected pure farm salmon aged 0+ have a small sample size and were not evaluated statistically, but are presented for completeness. Identification of cross type of field-sampled individuals was accomplished using genetic assignment and is described in Section 2

were no differences between groups in tank conditions ( $\chi^2 = 1.62$ ,  $p = 0.445$ ). In semi-natural conditions, PW were still significantly larger than PF and F<sub>1</sub> ( $\chi^2 = 36.4$ ,  $p < 0.001$ ; PF–F<sub>1</sub>,  $p < 0.001$ ; PW–F<sub>1</sub>,  $p < 0.001$ ; PW–PF,  $p < 0.001$ ), and PF were significantly larger than F<sub>1</sub>.

### 3.6. Shape–size regression slopes comparison

To determine if differences in shape were related to differences in allometry between groups, a regression of centroid size on shape was performed. In the common garden tank conditions, there were no significant

differences in the slope of shape and size among cross types on both Day 0 ( $p = 0.472$ ,  $R^2 = 0.0547$  explained by size) and Day 80 ( $p = 0.286$ ,  $R^2 = 0.0752$  explained by size). In the common garden semi-natural conditions (Day 0:  $p = 0.015$ ,  $R^2 = 0.0769$  explained by size; Day 80:  $p = 0.119$ ,  $R^2 = 0.0752$  explained by size), only Day 0 was significant, indicating that different cross types have different allometric growth, but this is not present by Day 80. In field samples ( $p = 0.071$ ,  $R^2 = 0.0268$  explained by size), there were no significant differences in the shape–size interaction slopes among cross types. This indicates that the shape differences present are not solely the result of allometric growth.

#### 4. DISCUSSION

The escape of aquaculture Atlantic salmon represents a significant threat to the persistence and stability of wild populations (Forseth et al. 2017), yet the magnitude of phenotypic impacts due to hybridization remains largely unresolved (but see Bolstad et al. 2017). Here we evaluated the shape and size phenotypic consequences of hybridization using geometric morphometrics in both common garden experiments and following field escape events. Our results suggest significant variation across settings and that the phenotype (i.e. shape and size) of pure farm and wild × farm hybrid Atlantic Salmon parr in the wild has a strong plastic component. As the environmental conditions in this study approached a wild setting (i.e. from aquaculture-like tank conditions, to a semi-natural environment, to natural rivers), differences among cross types (e.g. PW, PF,  $F_1$ ) decreased and the size and shape converged. These results build on recent work documenting a lack of, or limiting, phenotypic differences among wild and farm crosses in the wild, supporting a significant role for phenotypic plasticity (Glover et al. 2018) and highlighting the role that plasticity may play in masking genetic changes associated with introgression between wild and farm escaped Atlantic salmon.

##### 4.1. Differences in size

In general, differences in size between PW and PF offspring decreased over time under both experimental and field conditions, potentially reflecting differing influences of maternal contributions, phenotypic plasticity, and selection in the wild. Interestingly, initial sizes differed significantly between PW and PF or  $F_1$  individuals in the common garden experiments

(tank and semi-natural), but by Day 80, differences were absent under tank conditions. The initial differences are likely due to the maternal effects of egg size (Einum & Fleming 2000), and this is subsequently overcome by the increased growth of aquaculture offspring under tank conditions as shown elsewhere (Solberg et al. 2013). If the experiment were carried further, then it is likely that the farm individuals would grow to a larger size than wild individuals in tank conditions. Similarly, the difference in size between PW and PF offspring decreased over fish age in the field setting, and this convergence of size among field-collected individuals could be a result of either phenotypic plasticity and/or selection via growth-potential mortality (e.g. Glover et al. 2018)

Farmed Atlantic salmon have been found to outgrow wild salmon severalfold when raised in hatchery conditions, while hybrid individuals are often intermediate in size (Harvey et al. 2016a,b,c, Glover et al. 2018). This difference in growth is maintained under different feeding regimes (Harvey et al. 2016b), feed type (Harvey et al. 2016c), and salmon density (Harvey et al. 2016a). The increase in farm salmon growth rate in aquaculture conditions is the main trait selected for in breeding programs (Gjedrem 2000) and is a result of increased growth hormone production (Fleming et al. 2002). Harvey et al. (2016a) reported that farm individuals outgrew wild individuals in semi-natural conditions, contrasting our results. However, in that study, fry were fed commercial feed *ad libitum* as opposed to live feed as done in our study, and the fry were of Norwegian aquaculture origin, which have been domesticated for approximately twice as many generations as North American aquaculture salmon (Gjedrem et al. 1991, Islam et al. 2021). A previous study has shown that limiting commercial feed resulted in reduced growth rate differences between farm and wild salmon (Solberg et al. 2013), and using natural food in semi-natural conditions resulted in no growth differences after 6 wk (Solberg et al. 2020). Our results reinforce previous conclusions that differences in farm salmon growth rates compared to wild individuals are a result of domestication (Fleming et al. 2002), but also that the response is still plastic, and as the environment approaches a natural river, the differences in size may be reduced.

##### 4.2. Shape

The differences observed in shape between PW, PF and  $F_1$  Atlantic salmon were subtle, most pronounced

under artificial conditions and reduced or absent among the wild-collected samples. Previous studies have shown salmon phenotype to be plastic, with shape differences in the head and fin lengths reported between wild salmon reared in a hatchery compared to those reared in a river (Blanchet et al. 2008). Similarly, differences in head shape have been observed between wild Atlantic salmon caught in a river compared to hatchery-reared salmon (Fleming et al. 1994, Solem et al. 2006). Phenotypic differences between wild and hatchery individuals has also been observed in coho salmon *Oncorhynchus kisutch* (Swain et al. 1991, Hard et al. 2000), chinook salmon *O. tshawytscha* (Busack et al. 2007, Tiffan & Connor 2011), and rainbow trout *O. mykiss* (Pulcini et al. 2014), as well as a suite of other fish species (Wringe et al. 2016). However, while phenotypic differences between individuals reared in artificial and wild conditions are often detected, when farmed individuals are reared in natural rivers longer-term, these phenotypic differences become reduced or absent over time (Fleming et al. 1994), supporting conclusions made here that phenotypic variation in Atlantic salmon shape is subject to significant phenotypic plasticity and heavily influenced by environmental conditions experienced.

#### 4.3. Plasticity

Our results demonstrate that both size and shape of PW and PF Atlantic salmon may display significant divergence under artificial (i.e. aquaculture) settings, but in more natural conditions, size and shape may converge. This plasticity in phenotypic response matches the results of Glover et al. (2018), who studied the growth potential of Atlantic salmon in Norway. Glover et al. (2018) found no differences between farm and wild smolts reared in a river and exposed to natural selection, but when transferred to aquaculture-like tank conditions, the farm salmon started outgrowing the wild salmon at a maximum ratio of ~1:1.8. Farm salmon have been shown to outgrow wild salmon even further under standard hatchery conditions by a ratio of ~1:4.91–5.15 (Solberg et al. 2013), illustrating that a combination of plasticity and selection explains why no differences were detected between these groups in the river (Glover et al. 2018). This is consistent with our results that revealed different phenotypic outcomes in tank and semi-natural conditions at Day 80. The plasticity of the juvenile salmon phenotype allows for rapid phenotypic changes over short time periods (Pakkasmaa & Piironen 2000).

This can result in wild salmon shifting towards the hatchery phenotype when reared in hatchery conditions and domestic salmon shifting towards the wild phenotype in more complex environments (von Cramon-Taubadel et al. 2005), a phenomenon also observed in domestic brown trout *Salmo trutta* raised in the wild (Sánchez-González & Nicieza 2017). However, there remains a genetic component to shape and size that can manifest in significant phenotypic variation among wild salmon from different populations when raised in simple hatchery conditions (Sheehan et al. 2005), even though fish from the same populations may converge in phenotype when raised in more complex environments (von Cramon-Taubadel et al. 2005, Glover et al. 2018).

#### 4.4. Limitations

Attempts to control for dorso-ventral arching may have biased estimates of significant shape variation in regions of the body where these differences are present (Valentin et al. 2008). However, individuals with severe arching were removed prior to analysis, there were no changes in significance of the results with or without the utilization of the arching correction, and it is unlikely either method would be biased in the severity affecting one group. After Procrustes superimposition, any error in the position of landmarks due to this arching would be distributed across all the landmarks, making identification of single landmarks with higher measurement error difficult (Fruciano 2016). The combination of measurement error with small differences in highly variable traits could have contributed to inferred convergence in shape within experiments. However, measurement error of this type is unlikely to influence our conclusions of convergence in size across cross types.

Camera lens distortions can also lead to systematic variances in the landmark positions that can result in artificial clustering (Collins & Gazley 2017). To mitigate this potential problem, the same camera and setup were used to photograph individuals within the field and common garden experiments, and specimens were photographed in the center of the image. These steps should mitigate any error caused by camera lens distortions and would be unlikely to change any conclusions from our study.

It is also possible that natural selection may be contributing to convergence observed among the cross types in the wild. This could occur through farm or hybrid individuals that diverged from the optimal phenotype and suffered reduced survivorship or

being assigned to different ages due to significantly increased or decreased growth. Moreover, different rivers may provide differing selective or growth regimes, thereby reducing evidence for a consistent across-river trend. Nonetheless, the consistent trends we detected in the common garden experiments and the results of Glover et al. (2018) clearly support a strong role for phenotypic plasticity in the patterns they observed.

#### 4.5. Conclusions

We used a combination of a common garden experiment and field sampling to evaluate genetic and environmental contributions to juvenile salmon shape in wild Atlantic Salmon hybridizing with aquaculture-escaped individuals. Our results indicate the presence of significant phenotypic plasticity in the shape and size of salmon, with evidence of convergence under natural river environments, and show that the typical phenotypic differences between PW, PF, and F<sub>1</sub> fish occur in an aquaculture setting. Our results are consistent with those of Glover et al. (2018), who found that the faster growth of farm salmon compared to wild fish was reduced when reared in a natural river, but when they were transferred to a tank environment, the increased growth trait characteristic of farm salmon reappeared. The results of our semi-natural experiment and those of Glover et al. (2018) support the hypothesis that not all of the size and shape convergence between farm and wild salmon is due to size- or shape-biased selection (genetics), but that there is a plastic component. While we focused solely on shape and size phenotypes, other components of phenotype such as behavior (Islam et al. 2020) and hatch timing (Hamoutene et al. 2017) may also be important in hybrid salmon fitness but were not examined here. This characterization of the biological consequences of hybridization between wild and farm salmon is increasingly important as the process of domestication continues, wild populations decline, and there are more opportunities for contact between wild and aquaculture salmon (Keyser et al. 2018, Sylvester et al. 2018).

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#### LITERATURE CITED

- ✦ Adams DC, Otárola-Castillo E (2013) Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol Evol* 4:393–399
- Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* 160:1217–1229
- Blanchet S, Páez DJ, Bernatchez L, Dodson JJ (2008) An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): implications for supportive breeding programs. *Biol Conserv* 141:1989–1999
- ✦ Bolstad GH, Hindar K, Robertsen G, Jonsson B and others (2017) Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. *Nat Ecol Evol* 1:0124
- ✦ Busack C, Knudsen CM, Hart G, Huffman P (2007) Morphological differences between adult wild and first-generation hatchery Upper Yakima River spring chinook salmon. *Trans Am Fish Soc* 136:1076–1087
- ✦ Collins KS, Gazley MF (2017) Does my posterior look big in this? The effect of photographic distortion on morphometric analyses. *Paleobiology* 43:508–520
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada) (2011) COSEWIC assessment and status report on the Atlantic salmon *Salmo salar* in Canada. COSEWIC, Ottawa
- ✦ DFO (Fisheries and Oceans Canada) (2013) Recovery potential assessment for the South Newfoundland Atlantic salmon (*Salmo salar*) designatable unit. Science Advisory Report 2012/007. Canadian Science Advisory Secretariat. <https://waves-vagues.dfo-mpo.gc.ca/Library/40573412.pdf>
- ✦ DFO (2017) Stock assessment of Newfoundland and Labrador Atlantic salmon – 2016. Science Advisory Report 2017/035. Canadian Science Advisory Secretariat. [https://publications.gc.ca/collections/collection\\_2017/mpo-dfo/Fs70-6-2017-035-eng.pdf](https://publications.gc.ca/collections/collection_2017/mpo-dfo/Fs70-6-2017-035-eng.pdf)
- ✦ Diserud OH, Fiske P, Sægrov H, Urdal K and others (2019) Escaped farmed Atlantic salmon in Norwegian rivers during 1989–2013. *ICES J Mar Sci* 76:1140–1150
- ✦ Einum S, Fleming IA (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* 54:628–639
- Fleming IA, Einum S (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. *ICES J Mar Sci* 54:1051–1063
- ✦ Fleming IA, Jonsson B, Gross MR (1994) Phenotypic divergence of sea-ranched, farmed, and wild salmon. *Can J Fish Aquat Sci* 51:2808–2824
- ✦ Fleming IA, Jonsson B, Gross MR, Lamberg A (1996) An experimental study of the reproductive behaviour and suc-

- cess of farmed and wild Atlantic salmon (*Salmo salar*). *J Appl Ecol* 33:893–905
- Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. *Proc R Soc B* 267:1517–1523
- Fleming IA, Agustsson T, Finstad B, Johnsson JI, Björnsson BT (2002) Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). *Can J Fish Aquat Sci* 59:1323–1330
- Forseth T, Barlaup BT, Finstad B, Fiske P and others (2017) The major threats to Atlantic salmon in Norway. *ICES J Mar Sci* 74:1496–1513
- Fraser DJ, Houde ALS, Debes PV, O'Reilly P, Eddington JD, Hutchings JA (2010) Consequences of farmed–wild hybridization across divergent wild populations and multiple traits in salmon. *Ecol Appl* 20:935–953
- Fruciano C (2016) Measurement error in geometric morphometrics. *Dev Genes Evol* 226:139–158
- Gjedrem T (2000) Genetic improvement of cold-water fish species. *Aquacult Res* 31:25–33
- Gjedrem T, Gjøen HM, Gjerde B (1991) Genetic origin of Norwegian farmed Atlantic salmon. *Aquaculture* 98:41–50
- Glover KA, Pertoldi C, Besnier F, Wennevik V, Kent M, Skaala Ø (2013) Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. *BMC Genet* 14:74
- Glover KA, Solberg MF, McGinnity P, Hindar K and others (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: status of knowledge and unanswered questions. *Fish Fish* 18:890–927
- Glover KA, Solberg MF, Besnier F, Skaala Ø (2018) Cryptic introgression: evidence that selection and plasticity mask the full phenotypic potential of domesticated Atlantic salmon in the wild. *Sci Rep* 8:13966
- Glover KA, Urdal K, Næsje T, Skoglund H and others (2019) Domesticated escapees on the run: the second-generation monitoring programme reports the numbers and proportions of farmed Atlantic salmon in >200 Norwegian rivers annually. *ICES J Mar Sci* 76:1151–1161
- Goodall C (1991) Procrustes methods in the statistical analysis of shape. *J R Stat Soc B* 53:285–321
- Hamoutene D, Perez-Casanova J, Burt K, Lush L, Caines J, Collier C, Hinks R (2017) Early life traits of farm and wild Atlantic salmon *Salmo salar* and first generation hybrids in the south coast of Newfoundland. *J Fish Biol* 90:2271–2288
- Hard JJ, Berejikian BA, Tezak EP, Schroder SL, Knudsen CM, Parker LT (2000) Evidence for morphometric differentiation of wild and captive reared adult coho salmon: a geometric analysis. *Environ Biol Fishes* 58:61–73
- Harvey AC, Juleff G, Carvalho GR, Taylor MI and others (2016a) Does density influence relative growth performance of farm, wild and F<sub>1</sub> hybrid Atlantic salmon in semi-natural and hatchery common garden conditions? *R Soc Open Sci* 3:160152
- Harvey AC, Solberg MF, Glover KA, Taylor MI, Creer S, Carvalho GR (2016b) Plasticity in response to feed availability: Does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon *Salmo salar* parr? *J Fish Biol* 89:1754–1768
- Harvey AC, Solberg MF, Troianou E, Carvalho GR and others (2016c) Plasticity in growth of farmed and wild Atlantic salmon: Is the increased growth rate of farmed salmon caused by evolutionary adaptations to the commercial diet? *BMC Evol Biol* 16:264
- Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. *Mol Ecol* 17:294–313
- Islam SS, Wringe BF, Bradbury IR, Fleming IA (2020) Behavioural variation among divergent European and North American farmed and wild Atlantic salmon (*Salmo salar*) populations. *Appl Anim Behav Sci* 230:105029
- Islam SS, Wringe BF, Bøe K, Bradbury IR, Fleming IA (2021) Early-life fitness trait variation among divergent European and North American farmed and Newfoundland wild Atlantic salmon populations. *Aquacult Environ Interact* 13:323–337
- Jeffery NW, Wringe BF, McBride MC, Hamilton LC and others (2018) Range-wide regional assignment of Atlantic salmon (*Salmo salar*) using genome wide single-nucleotide polymorphisms. *Fish Res* 206:163–175
- Jonsson B (1997) A review of ecological and behavioural interactions between cultured and wild Atlantic salmon. *ICES J Mar Sci* 54:1031–1039
- Jonsson B, Jonsson N (2006) Cultured Atlantic salmon in nature: a review of their ecology and interaction with wild fish. *ICES J Mar Sci* 63:1162–1181
- Karlsson S, Diserud OH, Fiske P, Hindar K (2016) Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. *ICES J Mar Sci* 73:2488–2498
- Keyser F, Wringe BF, Jeffery NW, Dempson JB, Duffy S, Bradbury IR (2018) Predicting the impacts of escaped farmed Atlantic salmon on wild salmon populations. *Can J Fish Aquat Sci* 75:506–512
- McGinnity P, Prodöhl P, Ferguson A, Hynes R and others (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc R Soc B* 270:2443–2450
- Pakkasmaa S, Piironen J (2000) Water velocity shapes juvenile salmonids. *Evol Ecol* 14:721–730
- Perry WB, Solberg MF, Besnier F, Dyrhovden L and others (2019) Evolutionary drivers of kype size in Atlantic salmon (*Salmo salar*): domestication, age and genetics. *R Soc Open Sci* 6:190021
- Pulcini D, Russo T, Reale P, Massa-Gallucci A, Brennan G, Cataudella S (2014) Rainbow trout (*Oncorhynchus mykiss*, Walbaum) develop a more robust body shape under organic rearing. *Aquacult Res* 45:397–409
- R Core Development Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rohlf FJ (2015) TpsUtil. Version 1.64. Department of Ecology and Evolution, State University of New York, Stony Brook, NY. [www.sbmorphometrics.org/soft-utility.html](http://www.sbmorphometrics.org/soft-utility.html)
- Sánchez-González JR, Nicieza AG (2017) Phenotypic convergence of artificially reared and wild trout is mediated by shape plasticity. *Ecol Evol* 7:5922–5929
- Schlager S (2017) Morpho and Rvcg — shape analysis in R: R-Packages for geometric morphometrics, shape analysis and surface manipulations. In: Zheng G, Li S, Székely G (eds) *Statistical shape and deformation analysis. Methods, implementation and applications*. Academic Press, p 217–256
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675
- Sethi SA, Gerken J, Ashline J (2017) Accurate aging of juvenile salmonids using fork lengths. *Fish Res* 185:161–168

- ✦ Sheehan TF, Kocik JF, Cadrin SX, Legault CM, Atkinson E, Bengtson D (2005) Marine growth and morphometrics for three populations of Atlantic salmon from eastern Maine, USA. *Trans Am Fish Soc* 134:775–788
- ✦ Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. *Can J Fish Aquat Sci* 69:1994–2006
- ✦ Skaala Ø, Besnier F, Borgstrøm R, Barlaup BT and others (2019) An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. *Evol Appl* 12:1001–1016
- ✦ Solberg MF, Zhang Z, Nilsen F, Glover KA (2013) Growth reaction norms of domesticated, wild and hybrid Atlantic salmon families in response to differing social and physical environments. *BMC Evol Biol* 13:234
- ✦ Solberg MF, Robertsen G, Sundt-Hansen LE, Hindar K, Glover KA (2020) Domestication leads to increased predation susceptibility. *Sci Rep* 10:1929
- ✦ Solem Ø, Berg OK, Kjosnes AJ (2006) Inter- and intra-population morphological differences between wild and farmed Atlantic salmon juveniles. *J Fish Biol* 69:1466–1481
- ✦ Swain DP, Riddell BE, Murray CB (1991) Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. *Can J Fish Aquat Sci* 48:1783–1791
- ✦ Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Bradbury IR (2018) Migration effort and wild population size influence the prevalence of hybridization between escaped farmed and wild Atlantic salmon. *Aquacult Environ Interact* 10:401–411
- ✦ Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC and others (2019) Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. *Evol Appl* 12:705–717
- ✦ Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* 98:185–207
- Tiffan KF, Connor WP (2011) Distinguishing between natural and hatchery Snake River fall chinook salmon sub-yearlings in the field using body morphology. *Trans Am Fish Soc* 140:21–30
- ✦ Valentin AE, Penin X, Chanut JP, Sévigny JM, Rohlf FJ (2008) Arching effect on fish body shape in geometric morphometric studies. *J Fish Biol* 73:623–638
- ✦ Verspoor E, McGinnity P, Bradbury IR, Glebe B (2015) The potential direct and indirect genetic consequences for native Newfoundland Atlantic salmon from interbreeding with European-origin farm escapes. *Res Doc* 2015/030. Canadian Science Advisory Secretariat. <https://waves-vagues.dfo-mpo.gc.ca/Library/359696.pdf>
- ✦ von Cramon-Taubadel N, Ling EN, Cotter D, Wilkins NP (2005) Determination of body shape variation in Irish hatchery-reared and wild Atlantic salmon. *J Fish Biol* 66:1471–1482
- ✦ Wringe BF, Purchase CF, Fleming IA (2016) In search of a 'cultured fish phenotype': a systematic review, meta-analysis and vote-counting analysis. *Rev Fish Biol Fish* 26:351–373
- ✦ Wringe BF, Stanley RRE, Jeffery NW, Anderson EC, Bradbury IR (2017) HYBRIDDETECTIVE: a workflow and package to facilitate the detection of hybridization using genomic data in R. *Mol Ecol Resour* 17:e275–e284
- ✦ Wringe BF, Jeffery NW, Stanley RRE, Hamilton LC and others (2018) Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. *Commun Biol* 1:108
- ✦ Wringe BF, Anderson EC, Jeffery NW, Stanley RRE, Bradbury IR (2019) Development and evaluation of SNP panels for the detection of hybridization between wild and escaped Atlantic salmon (*Salmo salar*) in the western Atlantic. *Can J Fish Aquat Sci* 76:695–704

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