



Common-garden comparison of relative survival and fitness-related traits of wild, farm, and hybrid Atlantic salmon *Salmo salar* parr in nature

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ABSTRACT: When escapee farmed Atlantic salmon *Salmo salar* interbreed with wild fish, the introgression of maladaptive genes can lower wild population productivity and alter key life history traits. To date, only a few European studies have compared wild, farm, and hybrid salmon under common conditions in the wild, isolating the influence of genetics on survival and fitness-related traits. Here, we examined the performance of experimentally derived Atlantic salmon fry from 4 cross types (wild, farm, and reciprocal F₁ hybrids) during the first summer of growth at 3 locations in southern Newfoundland. Overall survival was high, with the cross type rank order consistent across sites (mean percent recaptured: wild-mother hybrids 26.2% ≈ wild 26.0% > farm 19.2% > farm-mother hybrids 12.8%). Wild fish were smaller than wild-mother hybrids and farm fish, though differed less in size from farm-mother hybrids. At 2 out of 3 sites, wild-mother hybrids were larger than wild and farm-mother hybrid fish but had only a small size advantage over farm fish. Shape differences were small and mainly related to body depth, with the largest differences between wild and farm fish. Wild-mother hybrids had fewer parr marks than other cross types at a single site, and though differences in the size of marks were minimal, farm fish tended to have the narrowest marks. Overall, these results show that genetic differences exist for fitness-related traits among wild, farm, and hybrid juveniles, even over short temporal scales and under favourable environmental conditions, and may contribute to patterns of reduced farm-mother hybrid and feral farm survival in the wild.

KEY WORDS: Aquaculture escapes · Farm–wild hybridization · Introgression · Juvenile performance

1. INTRODUCTION

Since its beginning in the late 1960s, the domestication of Atlantic salmon *Salmo salar* has subjected farm fish to directional selection, inadvertent domestication selection, and relaxed wild natural selection pressures over multiple generations (reviewed by Glover et al. 2017). As a result, farm fish differ from wild fish both genetically (e.g. Skaala et al. 2005, Karlsson et al. 2011, Besnier et al. 2015, Wringe et al.

2019) and phenotypically for a variety of traits such as growth, morphology, behaviour, age at maturity, and reproductive success (e.g. Fleming & Einum 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019). In the wild, Atlantic salmon are characterized by significant adaptive diversity in response to their local environments (Taylor 1991, Garcia de Leaniz et al. 2007, Fraser et al. 2011), and the hybridization of escaped farm fish with wild fish can erode this local adaptation and lead to a reduc-

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tion in fitness (McGinnity et al. 2003, Baskett et al. 2013, Sylvester et al. 2019, Skaala et al. 2019) and population productivity (Fleming et al. 2000, Bradbury et al. 2020). Therefore, investigating differences in fitness-related traits as well as differences in survival itself between wild, farm, and hybrid salmon is key to determining and managing the impacts of escaped farm salmon on wild salmon populations.

Generally, field and laboratory studies indicate that farm Atlantic salmon typically grow faster than wild conspecifics (i.e. Glover et al. 2009, 2018, Solberg et al. 2013b, Harvey et al. 2016b, Skaala et al. 2019). This may be advantageous for farm parr in competition with wild parr, as faster-growing farm fish may displace slower-growing wild fish from suitable habitat during the parr stage in the wild (Fleming et al. 2000, McGinnity et al. 2003). However, faster growth may also be a liability for farm fish in the wild, with evidence for selection against such faster-growing individuals (e.g. growth-potential mortality; Solberg et al. 2020) in addition to the lower risk-aversion of farm fish (e.g. Fleming & Einum 1997). Thus, the influence of growth on survival appears to depend on the specific nature of the ecological interactions between cross types, as well as the environment in which they live.

Differences in morphology among wild, farm, and hybrid Atlantic salmon (Fleming et al. 1994, Fleming & Einum 1997, Solem et al. 2006) may also be indicative of differential adaptation and survival capabilities. For example, differences in head size may be reflective of different feeding/foraging environments (Solem et al. 2006), while fin sizes may be reflective of the water velocities experienced by each cross type in their respective environments (Riddell & Leggett 1981), and body shape may be a result of selection pressures necessitating a certain level of swimming performance (Fleming & Einum 1997). In addition, differences in number and contrast of parr marks (the dark vertical bands on the sides of parr) between wild and hatchery strains may impact the juveniles' abilities to camouflage against the streambed and thus avoid predators (Culling et al. 2013). Given these implications, the degree of morphological differentiation between interacting cross types has the potential to contribute to differences in performance and, ultimately, survival.

To date, just a few studies have quantified survival and phenotypic differences among wild, farm, and hybrid juveniles in the wild, and all have been done in Europe (Einum & Fleming 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019). In general, these studies show that farm juveniles

typically have lower survival compared with their wild conspecifics, while hybrid survival is generally intermediate to the 2 pure crosses (McGinnity et al. 1997, Fleming et al. 2000, Skaala et al. 2012, 2019). However, since Atlantic salmon populations tend to be highly differentiated genetically between the eastern and western Atlantic (King et al. 2001, Lehnert et al. 2019, 2020), the nature of wild–farm interactions in these European studies may not be directly applicable to North American farm–wild interactions. It is crucial to characterize differences between farm, wild, and hybrid salmon at local scales since the extent to which farm introgression has an impact on a wild population seems often to be dependent on factors such as the demographics of the wild population in question and its original relatedness to the invading farm stock (e.g. Heino et al. 2015, Wringe et al. 2018).

This study aimed to assess differences in survival, growth, and morphology of North American first-feeding Atlantic salmon fry from 4 cross types (wild, farm, and reciprocal F_1 hybrids) during the first summer of growth in multiple tributaries of a natural river on the south coast of Newfoundland. Specifically, the 4 main objectives were to compare among all cross types within each study site (1) odds of recapture, (2) recapture size (weight and length) and condition, (3) external morphology, and (4) parr marks. The overarching goal was to examine differences among these 4 cross types while isolating the influence of genetics on these traits by subjecting fish to common environmental conditions within each of 3 study sites throughout the experimental period. The use of 3 replicate experimental sites/tributaries allowed for investigation as to whether farm, wild, and hybrid fish differ from one another in their traits by the same degree in different environments. This study extends existing research on wild, farm, and hybrid Atlantic salmon performance in the wild in southern Newfoundland (Wringe et al. 2018) to better inform predictions of population responses to escaped farm salmon in Atlantic Canada (e.g. Keyser et al. 2018, Sylvester et al. 2019, Bradbury et al. 2020).

2. MATERIALS AND METHODS

2.1. Cross, release, and recapture

Between 28 November and 21 December 2017, 4 different crosses of Atlantic salmon *Salmo salar* were generated: 8 families of wild offspring from wild parents of the Garnish River, 6 families of farm offspring

from parents of the Saint John River farm strain, and 7 and 6 families of the reciprocal F_1 hybrids of the above-mentioned farm and wild parents (denoted farm-mother hybrids [F ϕ hyb] and wild-mother hybrids [W ϕ hyb], respectively). The Garnish River system is located on the Burin Peninsula on the south coast of Newfoundland, emptying into Fortune Bay (mouth of river: 47.2353, -55.3443) and is near an area of intensive Atlantic salmon aquaculture (Fig. 1). The Saint John River (New Brunswick) strain of Atlantic salmon is, to date, the only farm strain used in Atlantic Canada commercial aquaculture operations. Families from farmed mothers were spawned earlier (28 November–7 December) than those from wild mothers (7–21 December) due to a difference in timing of spawning-readiness of these females. Numbers of parents used in crosses were $n = 3$ (farm mothers and fathers, respectively), $n = 9$ (wild fathers), and $n = 10$ (wild mothers). The relatively small number of farm parents used in the crosses reflects the number of available fish that matured that year. Parents of each cross were fin-clipped, with samples stored in anhydrous ethanol for later use in parentage assignment of offspring. A sample of 10 unfertilized eggs from each female was retained and subsequently weighed and photographed for size measurements to account for potential associated maternal effects.

Embryos were incubated in Heath trays on ambient water at the Ocean Sciences Centre of Memorial University (St. John's, Newfoundland and Labrador, Canada), where ambient water temperature was monitored daily (range: 3–8°C), and dead embryos were removed every 4–5 d. Shortly after emergence (i.e. first feeding), juveniles were pooled (22 May 2018) by cross type and transferred to 470 l flow-through circular holding tanks (0.9 m diameter \times 0.5 m high). Pooled families within a cross type were roughly equal in terms of numbers of fry, with all families estimated to have at least 100 fry (except for one family from each of farm, wild, and W ϕ hyb, respectively). Juveniles were raised in ambient water and fed a combination of *Artemia* and salmonid starter dry feed (crumbles: 0.5 g, caloric content: 55% protein and 15% fat; EWOS-Cargill) for 1 mo, followed by only dry feed until release. Release occurred on 11 July

2018 at 3 tributary sites of the Garnish River (Fig. 1). Environmental variation was not quantified among the release sites as it was not a focus of the study; rather, the interest was in replicability of findings across different environments. Prior to release, all fry were adipose fin-clipped (anaesthetized using MS-222 at a dose of 50 mg l⁻¹ buffered with an equal amount of sodium bicarbonate) to distinguish them from wild fish upon later recapture. In addition, fish to be released at Site 2 were photographed. A total of 2000 juveniles were to be released at each site; however, since some fry died during transportation to the sites, the actual numbers released were 1932 at Site 1, 1980 at Site 2, and 1972 at Site 3. Numbers of all 4 cross types released at each site were approximately equal, though the W ϕ hyb group had ~50 more fish and the wild group ~50 fewer fish than the farm and F ϕ hyb groups at each site. Fry were released at 4 locations approximately 50 m apart at each site. Animal use was approved by the Memorial University of Newfoundland Institutional Animal Care Committee (IACC) following Canadian Council of Animal Care (CCAC) guidelines, under protocol number 18-01-IF.

Recapture occurred from 2–5 October 2018 using multiple pass electrofishing. For Site 3, a single electrofishing unit (LR-24 Backpack Electrofisher; Smith Root) was used; for Sites 1 and 2 (which had wider channels), 2 electrofishing units were used on opposite sides of the channel. The electrofishing

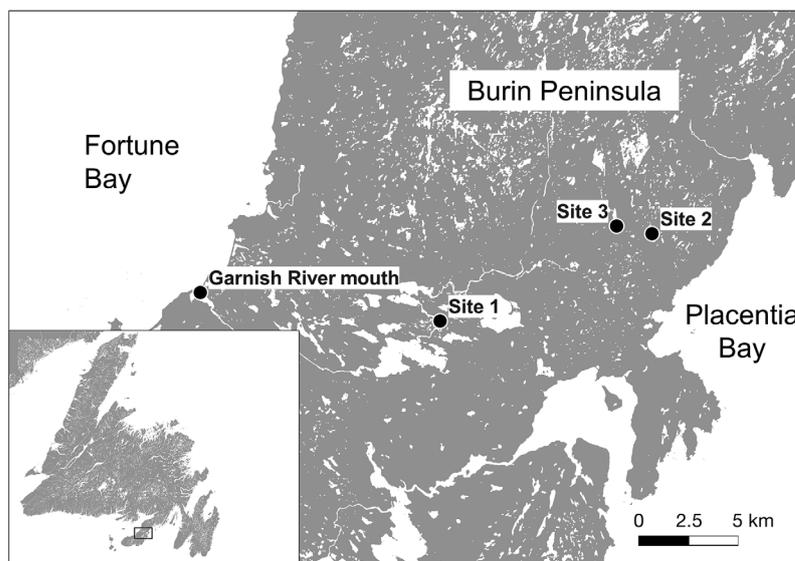


Fig. 1. The 3 tributary study sites of the Garnish River, Newfoundland, Canada, used for the release and recapture experiment with Atlantic salmon. Inset: the island of Newfoundland; box: general study area. Map created in QGIS; map style created in Mapbox; data by OpenStreetMap under ODbL

units were set at 550 V and 60 Hz, with a duty cycle of 25%. Recapture began approximately 150–200 m downstream from the first release points, over which the first ~20 m no fish were recaptured (Sites 1 and 2), or at a culvert downstream from the first release point (Site 3). Electrofishing continued upstream until a natural barrier was reached (Site 3) or when no experimental individuals were recaptured over approximately 25 m (Sites 1 and 2). Sites 2 and 3 were each sampled on 2 different days, while Site 1 was sampled on only one day due to logistical constraints.

Recaptured fish were kept alive until processing, which occurred approximately 2 h following the end of electrofishing each day. Fish were euthanized using MS-222 (400 mg l⁻¹ buffered with an equal amount of sodium bicarbonate) and immediately photographed (Sony Alpha a5000) on the left side using a photo stand (Kaiser Reprokid digital copy stand) set at a fixed height. A size and colour standard (X-Rite Colorchecker passport) was photographed for each set of photos (i.e. at the beginning of each sampling session). All fish were then weighed (± 0.01 g). The caudal fin was clipped and stored in anhydrous ethanol for later parentage analysis.

2.2. Genetic analysis

Parentage analysis was performed using caudal fin tissue samples to assign recaptured individuals to family, and therefore either wild, farm, or one of the 2 hybrid groups. Parentage analysis was done using a panel of 31 microsatellite loci with a total of 277 alleles (multiplex panel 1a from Bradbury et al. 2018), which are a subset of a larger panel of 101 loci for the Atlantic salmon genome in Atlantic Canada. Of these 31 loci, 25 were ultimately used for parentage assignment, with 6 original loci excluded due to either a high percentage of missing offspring genotypes or a high estimated allelic dropout rate. The 25 loci used included 2 with a tetranucleotide repeat sequence and 23 with a trinucleotide repeat sequence and 10–13 repeats. All loci had ≥ 4 alleles, with an average of 8.4 alleles locus⁻¹ over the entire panel (Bradbury et al. 2018). Additional information on locus-specific primers, repeat motifs, and chromosome numbers can be found in Table S1 of Bradbury et al. (2018).

DNA was extracted using the DNeasy 96 Blood and Tissue Kit (QIAGEN), following the manufacturer's protocol for Purification of Total DNA

from Animal Tissues. Microsatellite loci were PCR-amplified following the protocol described by Zhan et al. (2017). Sequencing was run on an Illumina MiSeq and scored using MEGASAT software (Zhan et al. 2017). Each individual fish was assigned back to its family (and therefore also its cross type) using the software program COLONY (Jones & Wang 2010). Test trials for COLONY included genotype data for all unique samples, parents, within-plate redundants, and cross-plate controls, to ensure the assignment sensitivity and accuracy of COLONY given the set of input parameters used. The final run included only unique samples (i.e. no redundants or controls).

2.3. Image analysis

Fork length measurements were taken from recapture photos of all fish at each site using ImageJ software version 1.52a (Schneider et al. 2012). In addition, 200 photos taken of fish pre-release were also measured for fork length, for a total of 50 per cross type. Care was taken to follow the lateral line of the fish's body, to account for any body arching when present. Condition factor at recapture was calculated as the residuals taken from the regression of $\ln(\text{recapture weight})$ on $\ln(\text{recapture length})$ (Bolger & Connolly 1989, Wootton 1998).

For morphology analysis, landmarks were selected as in Fig. 2 and digitized using ImageJ software. All photos of fish were visually pre-screened for excess body curvature caused by fish positioning, lopsidedness, or other positioning factors that would cause problems with analysis (no recaptured fish had visible spinal deformities). Ultimately, 734 fish were landmarked, comprising 166 from Site 1, 290 from Site 2, and 278 from Site 3, of which 191 were wild, 163 were farm, 102 were F ϕ hyb, and 278 were W ϕ hyb fish. Landmarks were saved as x–y coordinates. In addition to measurements using landmarks, 120 fish (10 site⁻¹ per cross type) were selected for pectoral fin length measurements, which were taken manually (due to variation in fin positioning) in ImageJ.

For parr mark measurements, 10 fish from each site \times cross type combination were chosen randomly for analysis (total: 120 fish). Parr marks were counted manually, and each mark was measured width-wise across its widest point parallel to the lateral line and lengthwise perpendicular to the lateral line from the lateral line to the bottom of the mark. Measurements of marks were performed using ImageJ.



Fig. 2. Location and numbering of morphometric landmarks used for generalized Procrustes analysis of Atlantic salmon. Landmarks are a subset of those described by Winans (1984): (1) point of snout on upper jaw, (2) most posterior point of maxillary, (6) origin of dorsal fin, (8) anterior attachment of dorsal membrane from caudal fin, (9) base of middle caudal rays, and (10) anterior attachment of ventral membrane from caudal fin; in addition to a subset of those described by Fleming et al. (1994): (3) most anterior point of eye orbit, and (7) point directly below anterior dorsal fin origin on ventral body surface. Two additional landmarks were also included: (4) most posterior point of eye orbit, and (5) most posterior point of operculum

2.4. Statistical methods

2.4.1. Models for recapture, weight, and parr marks

All statistical analyses were performed in R version 4.0.2 (R Core Team 2020). The probability of recapture is the product of 2 probabilities: the probability of survival to time of recapture and the probability of being encountered at time of recapture (Skalski et al. 2009). However, since the design of this experiment did not allow for the separation of these 2 probabilities, probabilities and odds of recapture were used as estimates of survival here. Since the recapture (survival) data is presence–absence in form, a generalized linear model with binomial family and logit link was used for the analysis, with odds of recapture as the response.

For comparisons of release length, a linear model was used for analysis with cross type included as a factor. Recapture length and condition factor were also analyzed using linear models. All recapture size models included site and cross type as factors in addition to mean egg weight (for each mother \times cross type \times site combination) as a covariate and all possible interaction terms. Assumptions of normality and homoscedasticity of residuals were assessed by visual examination of residual-fit plots and normal Q–Q plots of residuals, and assumptions were met for all models ultimately used. As diagnostic plots indicated a general linear model was not a good fit for recapture weight data, a generalized linear model with the gamma family (identity link) was used since the gamma model family is also appropriate for positive continuous data. The analysis of the parr mark

data used linear models to test for the effects of site and cross type on (1) number of parr marks, (2) mean width of marks, and (3) mean length of marks below the lateral line. Parr mark length and width were ln transformed before model analysis, and ln-transformed standard length was included as a covariate. Finally, the linear model for pectoral fin length (ln transformed) also included standard length (ln transformed), site, and cross type.

Although we do report p-values for model parameters, based on the recommendations of Wasserstein et al. (2019) we do not use the terms ‘statistically significant’ or ‘non-significant’ (or indeed, ‘difference vs. no difference’) with the p-value of 0.05 serving the delineator of this dichotomy. Instead, the relative evidence for the data given the models and their parameter estimates was assessed using likelihood ratios (LRs), with assessments of relative magnitude of evidence given with LR = 8 indicating ‘strong’ evidence, and LR = 32 for ‘quite strong’ evidence (Royall 1997) (though note that these designations are not meant to be ‘cut-offs’ or confer a decision-making rule). In this paper, all LRs greater than 8 will be referred to as ‘strong’ evidence. LRs give the likelihood of the data given 2 different/competing models (Glover & Dixon 2004); that is, the likelihood of the data given a model including a specific variable vs. given a model lacking it. For generalized linear models, LRs were calculated using the change in deviance (e.g. Christensen 1990) from an analysis of deviance on the given generalized linear model. For linear models, LRs were calculated using the sums of squares and were corrected for number of model parameters as in Glover & Dixon (2004). Finally, the

'emmeans' package (Lenth 2020) was used on the results of the respective models to calculate pairwise differences in effect sizes and their confidence intervals, approximated as \pm twice the standard error of the effect size estimate.

2.4.2. Morphology analysis

Raw landmark coordinates were corrected for fish body arching using 'tpsUtil' (Rohlf 2015) and the 'unbend' function along landmarks 1, 5, and 9 (Fig. 2). Following correction for arching, coordinates were aligned using a generalized Procrustes analysis (Gower 1975, Rohlf & Slice 1990) to provide Procrustes coordinates using the 'geomorph' package in R (Adams et al. 2020). Subsequently, a Procrustes ANOVA was performed on the aligned coordinates using the 'procD.lm' function to test for differences in overall shape between sites and cross types. The Procrustes ANOVA was performed using a residual randomization permutation method, with 9999 permutations. Site and cross type were included as fixed factors, and centroid size was included as a covariate to test for differences in allometry between groups. All possible interaction terms were also initially included. Pairwise differences in mean shape between crosses were then compared within each site using the 'pairwise()' function in the package 'RRPP' (Collyer & Adams 2018, 2020), with the distance statistic being the length of vectors between least-squares mean vectors for shape.

3. RESULTS

3.1. Survival (recapture)

In 2018, a total of 1284 fish were recaptured, and 1242 were successfully genotyped and assigned parentage. The LR results from the binomial generalized linear model of the recapture data indicated strong evidence for the inclusion of site ($LR = 2.31 \times 10^{27}$, $p < 0.001$), cross type ($LR = 2.60 \times 10^{25}$, $p < 0.001$), and the interaction term ($LR = 439.44$, $p = 0.058$) in the model (Table S1 in the Supplement at www.int-res.com/articles/suppl/q014p035_supp.pdf). Given the evidence for the site \times cross type interaction term, comparisons of the odds of recapture among the 4 cross types were subsequently performed separately within each site. The probabilities of recapture (not shown) for all cross types were higher at Sites 2 and 3 than at Site 1, which is not surprising given that Sites 2 and 3 were sampled twice while Site 1 was only sampled once.

The W ϕ hyb and wild cross types had the highest percentages recaptured across all sites (26.2 and 26.0%, respectively), followed by pure farm (19.2%), and then F ϕ hyb (12.8%) (Fig. 3A). However, only certain cross type pairs had consistently large differences from one another in their recapture odds across all 3 sites (Fig. 3B). Wild and W ϕ hyb had nearly identical odds of recapture at all sites. W ϕ hyb and wild had higher odds of recapture than F ϕ hyb at all 3 sites, and W ϕ hyb also had higher odds than farm at 2 out of 3 sites. On the other hand, differences in recapture odds for wild vs. farm were slightly less than that of W ϕ hyb vs. farm, with error bars overlapping the 1:1 odds ratio line at 2 out of 3 sites (i.e. indicating a result of no difference in recapture odds for these pairs was also reasonably likely).

3.2. Release length

There was strong evidence for the effect of cross type on length at release ($F_{3,196} = 5.53$, $LR = 147.6$, $p = 0.001$). However, differences were very small overall, with the largest mean difference in release length between any cross type being 2.03 mm (equivalent to only 6.3% of mean release length for all cross types; see Fig. 4b).

3.3. Size at recapture

There was insufficient evidence for an effect of cross type on egg weight ($LR = 0.49$, $p = 0.081$), with a mean (\pm SD) egg weight from farm mothers of 94.0 ± 19.3 mg and that of wild mothers, 91.6 ± 11.5 mg. However, results of the linear model indicated there was strong evidence for an effect on mother identity nested within cross type on egg weight ($LR = 8.53 \times 10^{39}$, $p < 0.001$). Therefore, mean egg weight for each individual mother was included as a covariate in the models for recapture weight, length, and condition.

There was strong evidence for an effect of mean egg weight ($LR = 68.75$, $p < 0.001$), site ($LR = 4.97 \times 10^{11}$, $p < 0.001$), and cross type ($LR = 212.41$, $p < 0.001$) on offspring recapture weight, as well as for the interactions of mean egg weight \times cross type ($LR = 82.11$, $p < 0.001$), and site \times cross type ($LR = 23.78$, $p < 0.001$) (Table S2). For offspring length, there was also strong evidence for an effect of mean egg weight ($LR = 1.63 \times 10^{10}$, $p < 0.001$), site ($LR = 4.69 \times 10^{68}$, $p < 0.001$), and cross type ($LR = 1.64 \times 10^{18}$, $p < 0.001$), as well as for the egg weight \times cross type interaction ($LR = 9.25 \times 10^{18}$, $p < 0.001$) and the site \times cross type interaction

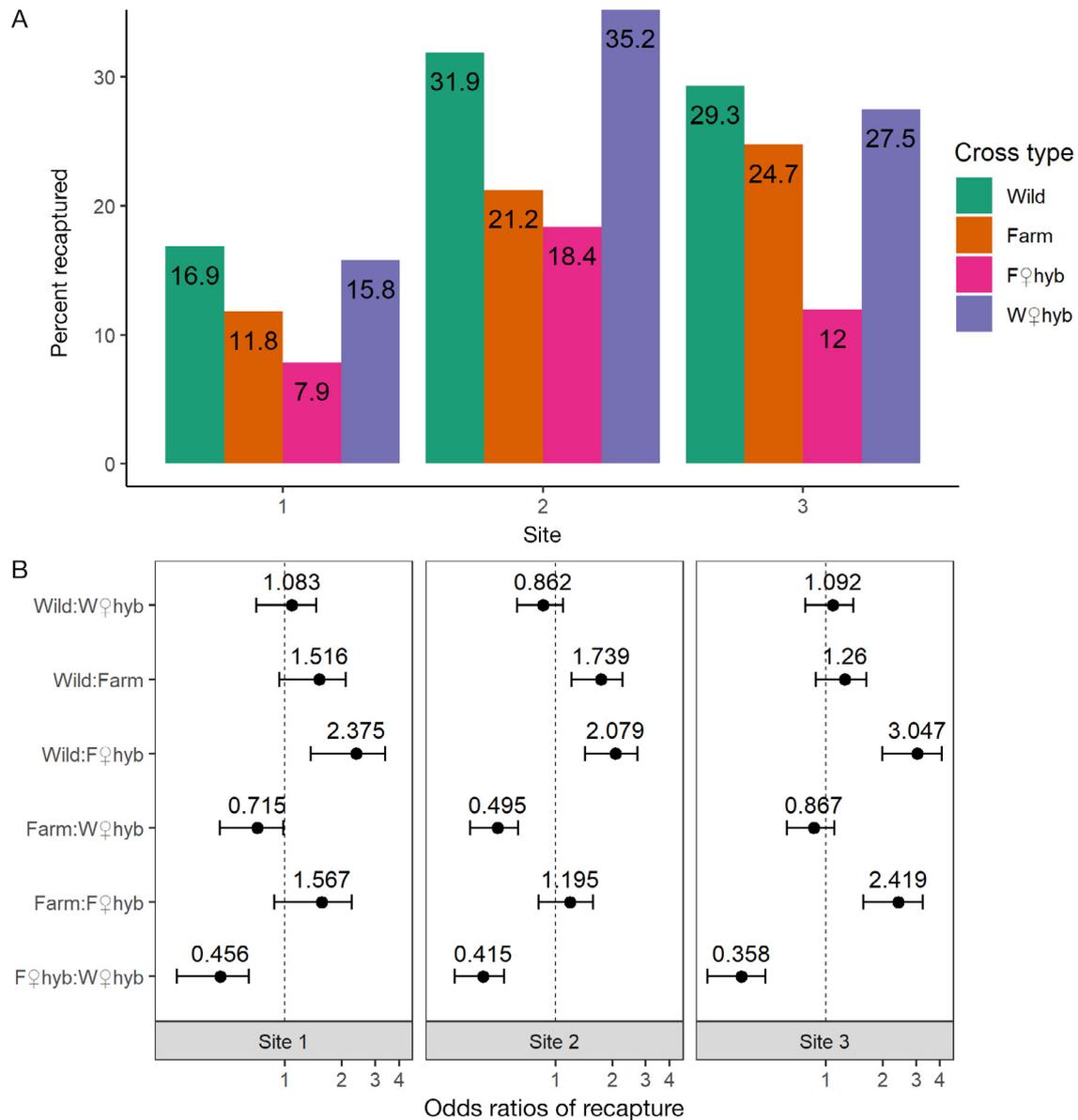


Fig. 3. (A) Percentages of Atlantic salmon recaptured by cross type and site and (B) odds ratios of recapture for pairwise cross type combinations at each site. Error bars in (B) represent ± 2 SE of the odds ratio estimates. Odds ratios and SE were back-transformed from the logit scale. F♀hyb: farm-mother hybrid; W♀hyb: wild-mother hybrid

($LR = 1.37 \times 10^5$, $p < 0.001$) (Table S3). Given the evidence for the site \times cross type interaction term, pairwise comparisons in mean recapture weights and lengths among cross types were subsequently assessed within each site separately. These comparisons were made at the grand mean value for egg weight (93.2 mg), thereby taking into account the effect of egg weight on recapture size.

At the grand mean egg weight value, all cross types were their smallest (weight and length) at Site 1 and largest at Site 3 (Figs. 5A & 6A, Table S4). Wild individuals were smallest on average consistently

across all 3 sites. At Sites 2 and 3, W♀hyb were largest, followed by farm and then F♀hyb. However, at Site 1, the order of cross types from largest to smallest was farm > F♀hyb > W♀hyb > wild.

The largest pairwise differences in recapture weight and length between cross types occurred at Site 3, except for the wild: F♀hyb pairing, for which the largest difference was at Site 1 (Figs. 5B & 6B). W♀hyb and farm were consistently larger than wild across all sites. On the other hand, farm and W♀hyb were similar in size at all sites (and error bars overlapped zero difference in recapture size), as were

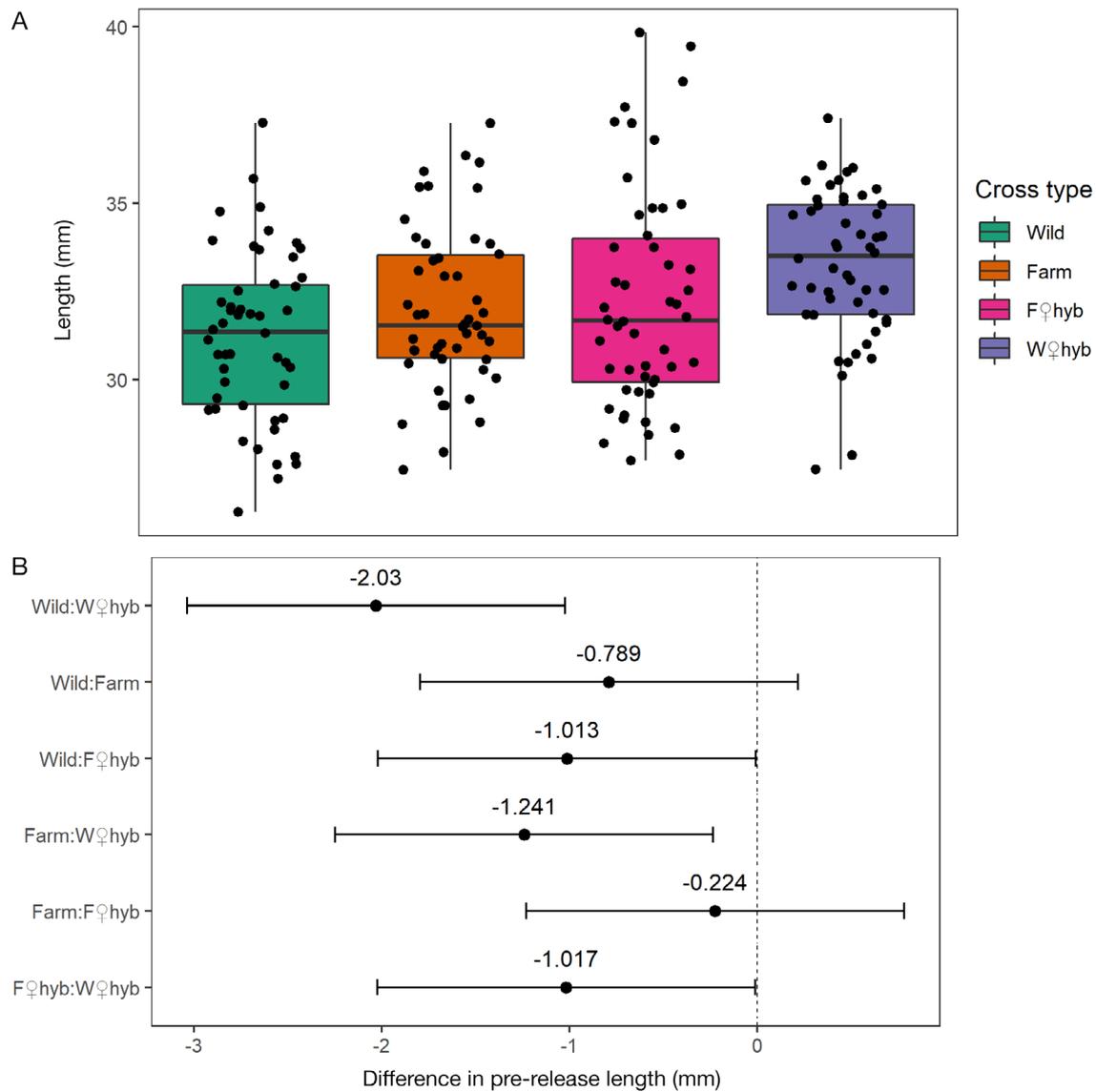


Fig. 4. (A) Boxplots of Atlantic salmon release length \times cross type. Lines in boxplots represent median value, upper and lower hinges represent third and first quartiles (respectively), and upper and lower whiskers extend to furthest value no more than 1.5 times the inter-quartile range above and below the upper and lower hinges (respectively). (B) Pairwise differences in mean release length for all cross types. Error bars on pairwise differences represent ± 2 SE of the difference estimate

wild vs. F♀hyb at Sites 2 and 3, F♀hyb vs. W♀hyb at Site 1, and farm vs. F♀hyb at Sites 1 and 2.

3.4. Condition factor at recapture

There was strong evidence for an effect of site (LR = 9.83×10^{15} , $p < 0.001$), cross type (LR = 3.40×10^7 , $p < 0.001$), and all interactions except egg weight \times site on condition factor (Fig. 7A, Table S5). Pairwise differences were very similar for each cross type pair

at Sites 2 and 3, with wild having lower condition than all other cross types and farm having higher condition than W♀hyb (Fig. 7B). By contrast, the ± 2 SE bars for all pairwise comparisons at Site 1 spanned zero difference in condition factor.

3.5. Morphology

Initially, a linear model with centroid size, site, cross type, and all possible interaction terms was run on the Procrustes coordinates. The LR for centroid size

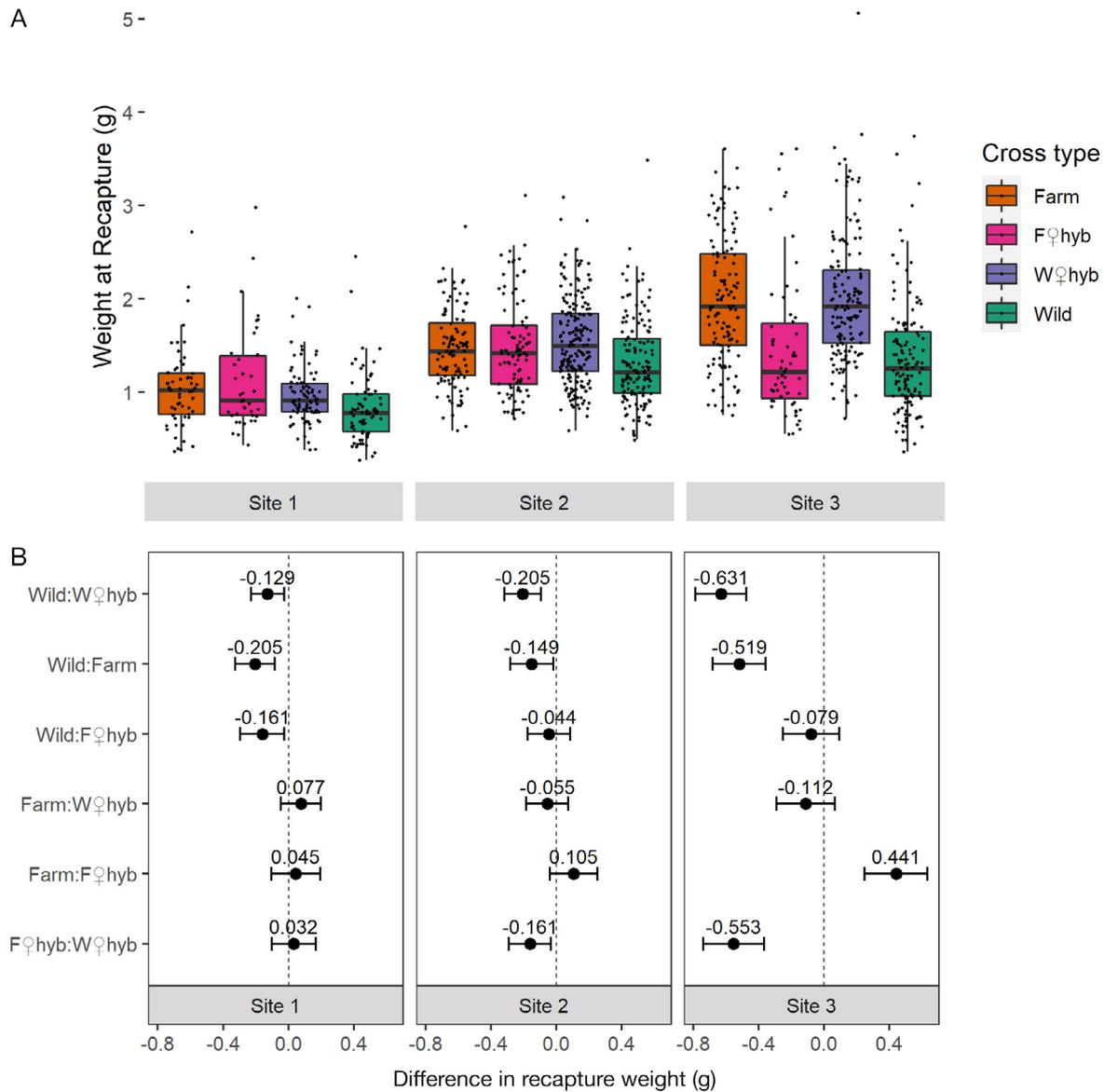


Fig. 5. (A) Weights of each Atlantic salmon at recapture for each cross type \times site pairing, and (B) pairwise differences in mean recapture weight for each cross type by site pairing. Boxplot parameters in (A): lines: median values; upper and lower hinges: third and first quartiles (respectively); upper and lower whiskers extend to furthest value no more than 1.5 times the interquartile range above and below the upper and lower hinges (respectively). Error bars on pairwise differences in (B) ± 2 SE of the difference estimate

($F_{1,727} = 85.71$, $LR = 2.27 \times 10^{14}$, $p < 0.001$), site ($F_{2,727} = 35.42$, $LR = 3.00 \times 10^{12}$, $p < 0.001$), and cross type ($F_{3,727} = 27.78$, $LR = 9.26 \times 10^{15}$, $p < 0.001$) were >100 , and these terms were thus selected for a reduced model (all other terms had LRs < 1) (Table S6). LRs for the effects of centroid size, site, and cross type provided strong evidence for their inclusion in the reduced model; however, the size of their effects on shape was small. Centroid size explained 8.9% of the variation in the data, site explained 6.3%, and cross type explained 8.6% (Table S6). Pairwise comparisons

of distances between least-squares means of overall shape were made between cross types and between sites (Table 1), after accounting for centroid size. For cross type comparisons, distances between means were largest for farm vs. wild and smallest for farm vs. F♀hyb; for sites, distances were largest between Sites 1 and 2 and smallest between Sites 2 and 3. The largest cross type differences appeared to be for landmarks associated with body depth, with wild having smaller depths than other cross types; however, once again it is important to note that overall differences were small.

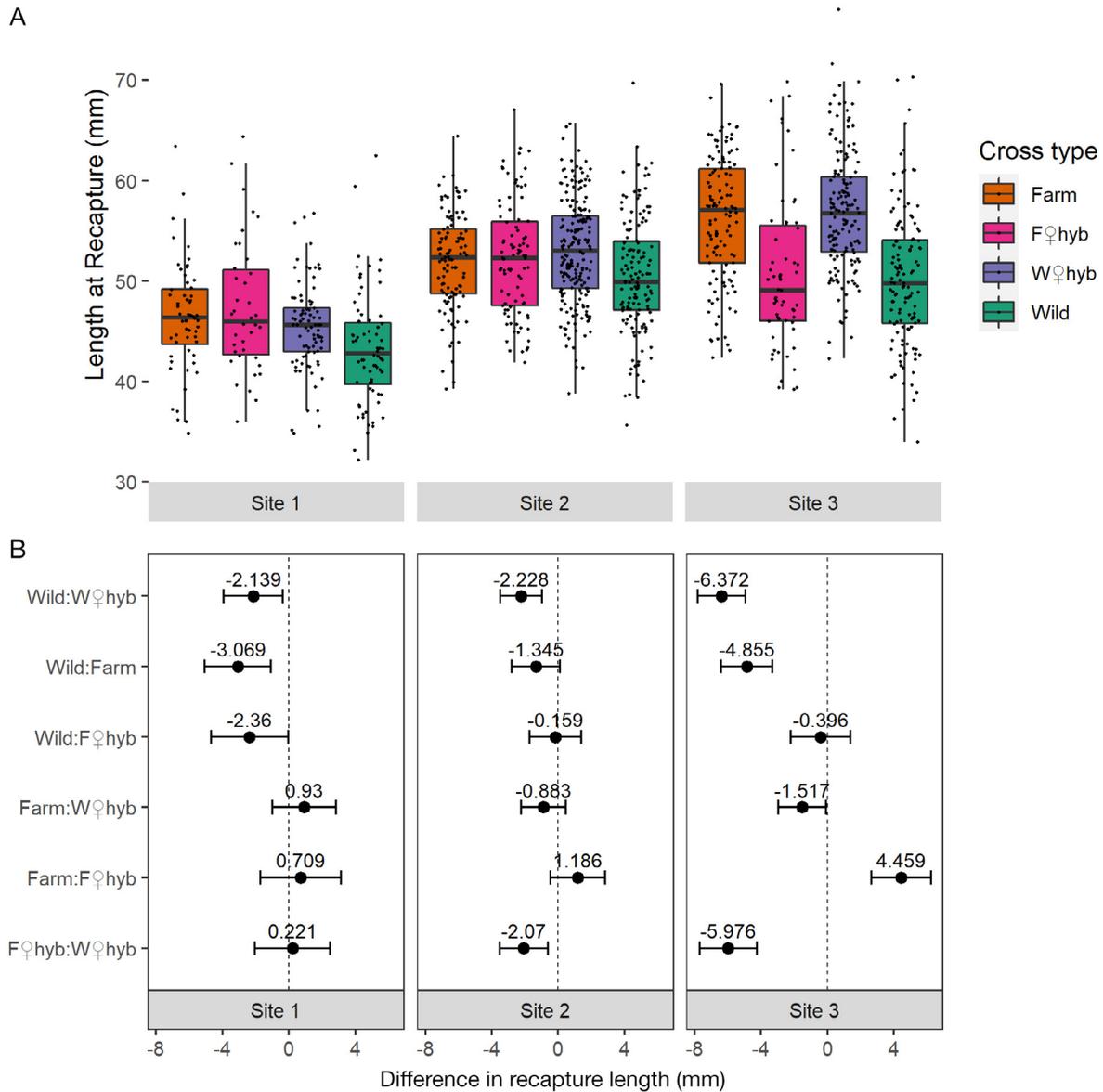


Fig. 6. Same as Fig. 5, but for (A) lengths of each Atlantic salmon at recapture for each cross type by site pairing, and (B) pairwise differences in mean recapture length for each cross type by site pairing

For pectoral fin length, after accounting for the effect of fish standard length ($F_{1,96} = 483.4$, $LR = 7.04 \times 10^{35}$, $p < 0.001$), there was strong evidence for an effect of site on this trait ($F_{2,96} = 19.5$, $LR = 2.19 \times 10^6$, $p < 0.001$), but insufficient evidence for an effect of cross type or any interactions ($LR < 1$, $p > 0.1$). Predicted pectoral fin lengths were back-calculated from ln-transformed emmeans predictions: 11.59 mm (Site 2), 10.48 mm (Site 3), and 10.38 mm (Site 1). All site predictions were calculated at the grand mean fish standard length of 43.8 mm (which was also back-calculated from the ln-transformed mean output by the model).

3.6. Parr mark number and size

There was insufficient evidence for the standard length covariate term ($F_{1,97} = 3.26$, $LR = 1.7$, $p = 0.074$) as well as the site term and its interactions with standard length ($LR < 1.0$) on number of parr marks. However, there was evidence for the cross type term ($F_{3,97} = 2.53$, $LR = 16.3$, $p = 0.062$) as well as its interaction with site ($F_{6,97} = 1.93$, $LR = 263.6$, $p = 0.083$) on parr mark number. Therefore, pairwise comparisons of mean numbers of marks between cross types were conducted separately within each site. The largest overall pairwise differences occurred

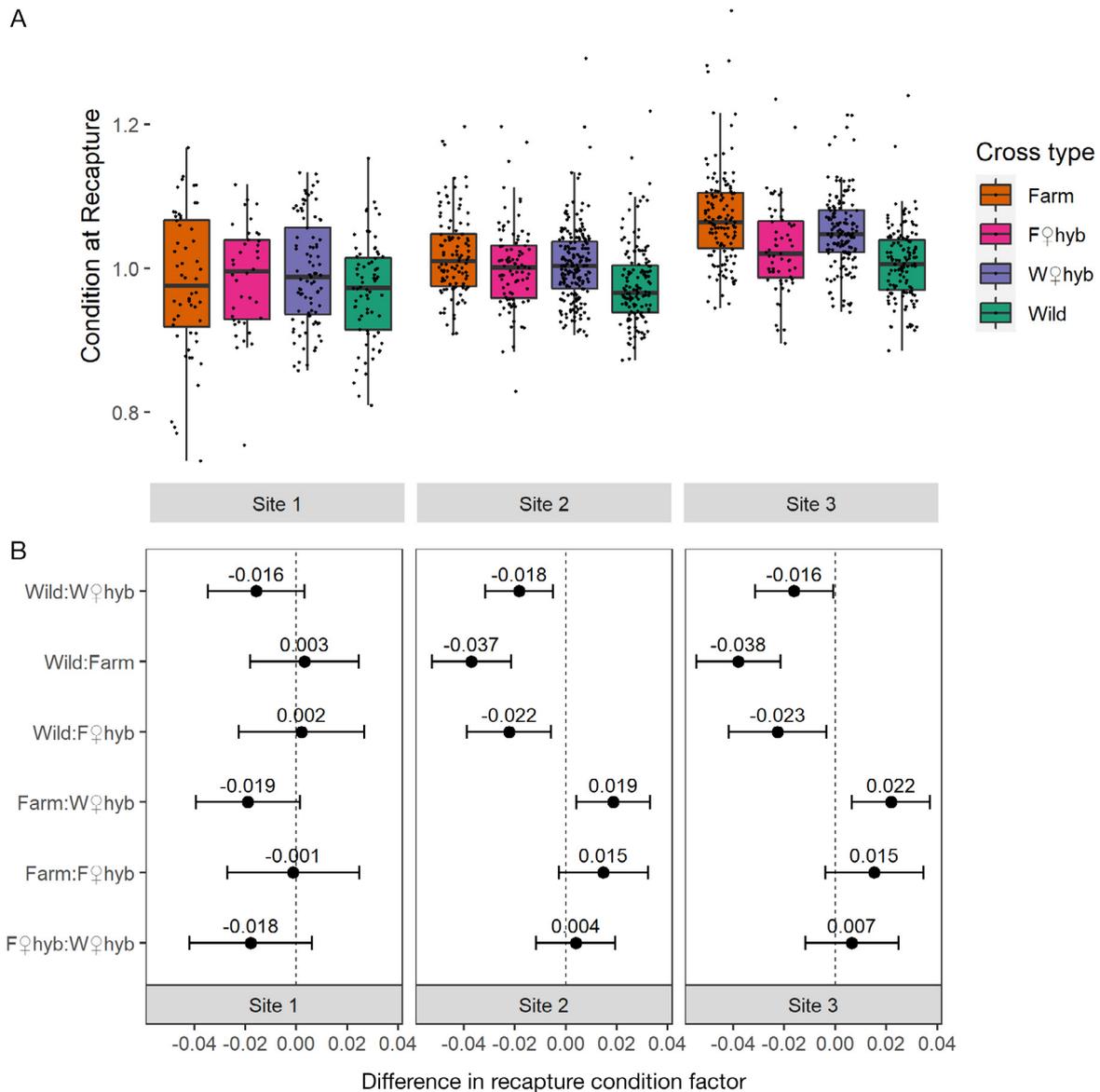


Fig. 7. Same as Fig. 5, but for (A) condition of each Atlantic salmon at recapture for each cross type by site pairing, and (B) pairwise differences in mean condition for each cross type by site pairing

at Site 3, with W♀hyb having fewer parr marks than all 3 of the other crosses (differences all >1.7 marks; Fig. 8A, Table S7). In contrast, differences for all other pairs were <1.2 marks, with their 2*SE marks spanning zero difference in number of marks.

There was strong evidence for an effect of standard length and site on mean mark length and width (Table 2). The largest differences in mean mark length at the grand mean of standard length (44.7 mm; back-calculated from ln-transformed grand mean length output by emmeans function) were at Site 1 for F♀hyb :W♀hyb and farm: W♀hyb (Fig. 8B). However, difference in size of marks for all pairs was

small overall, with error bars for difference estimates spanning zero difference across all sites. One consistent result across all sites though was that farm fish tended to have narrower marks than the other cross types (Fig. 8C, Table S7).

4. DISCUSSION

We found a consistent pattern of relative cross type survival across multiple study sites, with survival higher for parr from wild mothers than those from farm mothers. We also found differences in size at

Table 1. Distances (d) between least-squares means for pairwise cross type and site comparisons of fish shape in Atlantic salmon, as well as the upper 95% confidence limit (UCL) of the distribution generated through the resampling process, and the number of standard deviations the distance is away from the mean distance generated through the resampling procedure (Z)

Comparison	d	UCL	p	Z
Cross type				
Farm:F ϕ hyb	0.00447	0.00354	0.005	3.125
Farm:W ϕ hyb	0.0112	0.00280	0.001	16.993
Farm:Wild	0.0149	0.00304	0.001	21.206
F ϕ hyb:W ϕ hyb	0.00945	0.00327	0.001	11.485
F ϕ hyb:Wild	0.0123	0.00349	0.001	14.610
W ϕ hyb:Wild	0.00529	0.00268	0.001	6.811
Site				
1:2	0.00968	0.00278	<0.001	14.495
1:3	0.0135	0.00281	<0.001	20.650
2:3	0.00603	0.00237	<0.001	9.723

recapture among cross types, with environment also influencing patterns of growth. Morphology differences existed between sites and cross types, though very small in general. Overall, our results extend previous findings of proportional change in cross type abundances in southern Newfoundland (Wringe et al. 2018, Sylvester et al. 2019), as well as size and shape differences (Perriman et al. 2022) among offspring following a large aquaculture escape event in 2013, thereby contributing additional population-specific data to the body of research on wild–farm interactions.

4.1. Survival

We observed significant cross type-related differences in recapture odds that were consistent across a range of environmental conditions. In line with previous work, we saw that wild offspring had higher recapture/survival than farm offspring (McGinnity et al. 1997, 2003, Fleming et al. 2000, Skaala et al. 2012, 2019). While our results for hybrid relative survival did not follow the generally observed cross type survival trend (wild > hybrids > farm), it is important to note that some previous studies have reported results of relative survival at the cohort and/or family level that do not agree with the overall cross type pattern, and our results do correspond to some of these findings. For example, McGinnity et al. (2003) found a fairly high farm:wild relative survival value (farm ~84% of wild) for one 0+ cohort, which falls within the range of our farm:wild percent recaptured

estimates (farm: 66–85% of wild). In addition, results which correspond to our finding of high W ϕ hyb survival include McGinnity et al. (1997), who found W ϕ hyb had the highest overall survival for one cohort of 0+ parr and spring smolts, and Skaala et al. (2012), who found that for one experimental cohort, W ϕ hyb fish had essentially the same relative survival as pure wild. Though the relatively small numbers of parents (especially of farmed origin) used in our study may not reflect within-cross type diversity to the same degree as in studies using greater numbers of families, our results nevertheless emphasize the potential for certain families to over- or under-perform relative to their overall cross type. In particular, instances of higher-than-expected hybrid success may be critical given that most escaped farm fish that breed do so with wild rather than other farm fish, meaning hybrid offspring are more likely to occur than pure feral farm offspring (e.g. Fleming et al. 1996, 2000). Therefore, it is the relative performance of hybrid offspring that will be especially important for predicting future impacts of escapees on wild populations.

It should be expected that differences in temporal scale between previous studies and this one would contribute to differences in relative recapture results. Results of studies that quantified freshwater survival at the smolt stage (Skaala et al. 2012, 2019) reflect patterns of survival over a longer period of time and a broader range of environmental conditions (perhaps most notably, winter) than those experienced by our fish. In addition, studies that began at earlier life stages would have had early-life (i.e. egg, larval) mortality, and potentially parental spawning success influence their 0+ recapture numbers, while our study looks exclusively at relative survival over the first summer of growth and controls for these factors. For example, compared with our results, the larger proportion of F ϕ hyb 0+ recaptures relative to W ϕ hyb found by Fleming et al. (2000) (who began their study at the breeding stage) appeared to be largely due to F ϕ hyb being the more likely of the 2 F $_1$ hybrid groups given differential parental spawning success (Fleming et al. 1996). In addition, the fact that we found a smaller farm:wild survival differential than McGinnity et al. (1997) (farm: 51–53% of wild) and Fleming et al. (2000) (farm: ~70% of wild), could be because our study does not reflect mortality during the egg and larval stages, which these 2 other studies do.

Factors other than cross type, such as egg size, may also be an important influence on the relative survival of juvenile Atlantic salmon, and the influence of egg size may vary among populations and studies. Skaala

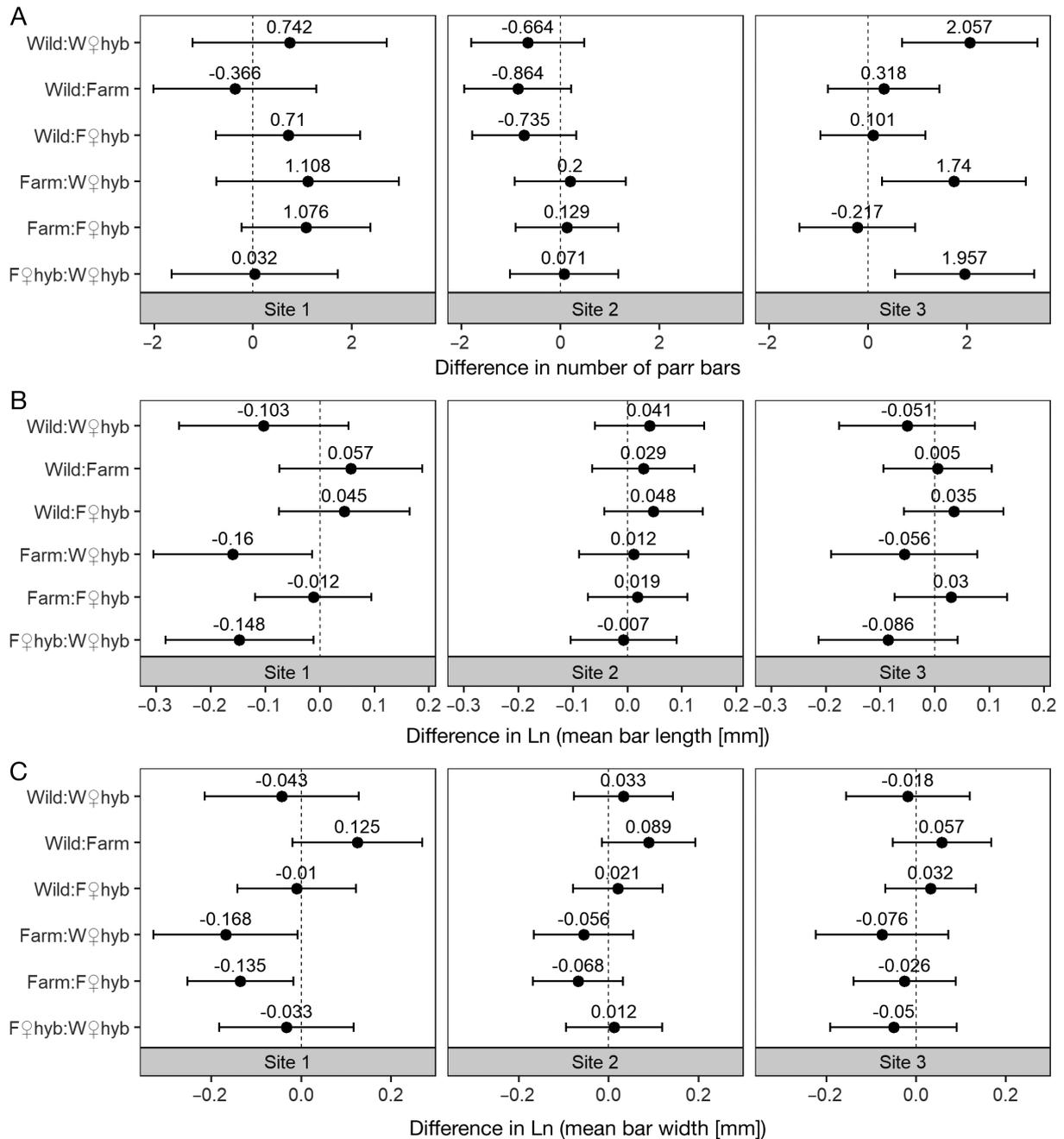


Fig. 8. Differences in (A) mean number of parr marks; (B) mean $\ln(\text{parr mark length})$ below lateral line; and (C) mean $\ln(\text{parr mark width})$ for each cross type pair of Atlantic salmon at each site. Error bar: ± 2 SE of the difference estimate. Differences were calculated at the grand mean of standard length (45.20 mm)

et al. (2012), whose farm eggs were slightly larger than those from wild fish, found that when the effect of egg size was controlled for, farm juveniles had lower survival overall from egg to smolt stage than both wild and hybrids; however, when it was not controlled for, the families with the highest and lowest overall survival were both farm in origin. On the other hand, Skaala et al. (2019) used farm eggs that were

smaller than wild, and found that while egg-smolt survival of farm fish was lower than that of wild overall, it was relatively higher after controlling for egg size than before. In our study, it was not possible to include egg weight as a covariate in the survival model since the number of fry from each mother initially released was unknown. However, there was substantial variation in egg weight among mothers, though

Table 2. ANOVA results of the linear model for mean Atlantic salmon parr mark length below lateral line, and mean mark width by site and cross type. The standard length covariate was ln transformed prior to model analysis. LR: likelihood ratio

Source	df	Ln(parr mark length)					Ln(parr mark width)				
		SS	MS	<i>F</i>	<i>p</i>	LR	SS	MS	<i>F</i>	<i>p</i>	LR
Standard length	1	4.600	4.400	487.24	<0.001	6.74×10 ³⁹	5.376	5.376	467.870	<0.001	4.1×10 ³⁷
Site	2	0.159	0.079	8.392	<0.001	377.404	0.275	0.137	11.958	<0.001	7078.48
Cross type	3	0.004	0.002	0.155	0.927	0.043	0.089	0.030	2.567	0.059	1.902
Standard length × site	2	0.009	0.004	0.463	0.631	0.153	0.020	0.010	0.884	0.416	0.248
Standard length × cross type	3	0.083	0.028	2.932	0.037	2.539	0.083	0.028	2.420	0.071	1.327
Site × cross type	6	0.008	0.001	0.133	0.992	0.0006	0.015	0.003	0.219	0.970	0.001
Standard length × site × cross type	6	0.088	0.015	1.556	0.168	0.030	0.053	0.009	0.771	0.594	0.002
Residuals	97	0.916	0.009				1.115	0.012			

not between farm and wild mothers on the whole (farm egg weight mean: 94.0 ± 19.3 mg; wild egg weight mean: 91.6 ± 11.5 mg), so this could very well mean that egg weight played a role in family-specific survival and simply remains undetectable here.

It is important to note that the nature of the experimental design meant that some factors potentially affecting performance were not able to be accounted for. Firstly, differences in timing of mother spawning-readiness resulted in families being spawned over approximately a 3 wk period, with families from farm mothers being spawned slightly earlier than those from wild mothers. However, there was still overlap in spawning timing for some farm-mother and wild-mother families, and the relatively strong performance of the later-spawned Wϕhybs suggests that mother spawning time did not greatly influence juvenile performance while in-river. In addition, while fry were released into the river sites early in their development, the life stages prior to release were likely to have some of the highest mortality rates—for example, McGinnity et al. (1997) found that differential survival between wild and farmed juveniles was greatest from the eyed-egg stage to the first summer. Therefore, our results of relative survival may have been different than if juveniles had experienced wild conditions beginning from hatch or the egg stage. We also could not account for the potential effects of early hatchery rearing on subsequent juvenile performance in the wild. Indeed, recent work has found that hatchery rearing can induce epigenetic changes that may result in reduced survival and fitness in captive-reared fish (e.g. Le Luyer et al. 2017). While the common-garden design of this study means that all cross types experienced similar effects due to hatchery rearing, epigenetic effects from early hatchery rearing could nevertheless have impacted the performance of all fish compared to if they had lived their whole lives in the wild.

Overall, more work is needed to investigate cross type differences in the North American wild to ultimately determine if the patterns of relative survival we observed here are broadly representative of North American populations. To date, North American studies comparing performance of farm, wild, and hybrid juveniles in the lab have found varying results in comparison to European studies. Hamoutene et al. (2017) found that North AmeFϕhyb, while potentially the most likely of the F₁ hybrids to occur (Fleming et al. 1996), had the highest mortality rates at the egg and fry stages in a lab environment, similar to our findings for relative survival of this cross type in the wild. However, other North American lab studies have found that relative survival of F₁ hybrids compared to parent populations can be environment-dependent (Fraser et al. 2008), therefore a broader survey of North American rivers/populations would be prudent and beneficial. Nevertheless, in general, our results corroborate previous research globally showing that fish from farm mothers tend to have lower survival than those from wild mothers, and differences from previously established general patterns that we see are reflective of the need for more population-specific work in order to account for this variability.

4.2. Size

We did not find evidence that larger size consistently confers higher survival for cross types as a group (though it is important to note that we were not able to investigate a link between individual size at recapture and survival within cross type groups). While Wϕhyb were the largest cross type at Sites 2 and 3 and had the highest relative survival overall, this link between larger size and higher survival did not extend to the other cross types. In particular, wild parr having the second-highest survival but being

smallest at all 3 sites suggests that being small does not automatically confer a relative survival disadvantage over the first summer. Even if wild parr were being displaced from beneficial habitat by larger farm fish (Fleming et al. 2000, McGinnity et al. 2003), it does not seem to have been overly detrimental during this time period. In addition, the lack of evidence for larger size of farm fish conferring higher survival is also consistent with results of previous studies finding evidence for at least some selection against fast-growing farm fish in the wild (e.g. Glover et al. 2018), as larger farm fish may be bolder and therefore more risk-prone than their wild counterparts (Fleming & Einum 1997, Islam et al. 2020, Solberg et al. 2020). Ultimately, our results suggest that relative size rankings of cross types cannot be used as a reliable predictor of their relative survival across a range of environmental conditions.

The result of wild parr being smallest on average across all sites is consistent with results from similar European studies (e.g. Einum & Fleming 1997, Fleming & Einum 1997, Fleming et al. 2000, Glover et al. 2009, Solberg et al. 2013b, Harvey et al. 2016). In contrast, while a few recent studies on Newfoundland Atlantic salmon juveniles have found wild fish to be largest at certain early life stages, it is likely that a cross type-based maternal egg size effect contributed to these results, and likely did not play a role in our study. For example, Perriman et al. (2022) found wild fish to be largest overall at first-feeding and 80 d later in a semi-natural environment as well as being larger than hybrids at age 0+ in the wild. Hamoutene et al. (2017) found wild to be largest at hatch in a hatchery environment, and Islam et al. (2021) found wild fish were longer than farmed (though not heavier) at first feeding in a lab environment. Authors of all 3 of these studies attributed the larger relative size of their wild juveniles to the fact that the eggs they used from wild females were larger than those from farmed females. In contrast, in our study there was little difference in average egg size of wild and farmed mothers, therefore wild juveniles as a group would not be expected to have this particular size advantage initially (and indeed, wild juveniles were also the smallest cross type at release). Overall, our size results for the pure crosses are in line with results of previous research, demonstrating that farmed fish tend to outgrow wild across a range of environmental conditions, though to a lesser extent in more challenging growth conditions (reviewed by Glover et al. 2018).

A potentially notable result we report is the apparent size advantage of Wϕhyb observed at 2 sites,

since hybrids have typically been found to be intermediate in size between pure crosses (e.g. Einum & Fleming 1997, Fleming & Einum 1997, Fleming et al. 2000, Glover et al. 2009, Solberg et al. 2013a, Harvey et al. 2016). Nevertheless, our observations are supported by the results of Skaala et al. (2012), who found that for a cohort using hybrids from wild mothers, the hybrids were indeed the largest cross type as smolts, while in 2 other cohorts using hybrids from farm mothers, the hybrids were either intermediate to the pure crosses or smallest as smolts. There is often variability in growth among populations of the same cross type, and certain populations under certain environmental conditions may over- or underperform relative to their overall cross type (e.g. Harvey et al. 2016); therefore, it is possible that the particular parental crosses used in our study resulted in a Wϕhyb strain that was a particularly good performer under specific conditions (i.e. Sites 2 and 3). Alternatively, since Wϕhyb were also the largest cross type at release, their relative size at recapture could be reflective of their initial size; though since farm and Fϕhyb fish outgrew Wϕhyb at Site 1, initial size is likely to be only a partial influencer of recapture size and/or only an important factor under certain environmental conditions.

4.3. Morphology

Though wild Atlantic salmon populations have been found to be genetically distinct from one another (e.g. Fraser et al. 2011, Bourret et al. 2013), the degree to which variation in morphology is related to genetics vs. environment is not yet clear. Our results indicate that there is evidence for effects of both environment and cross type on body morphology, with the largest differences in shape being for landmarks associated with body depth; however, overall shape differences were small. Fleming & Einum (1997) also found that tank-reared Norwegian farmed juveniles had deeper bodies than tank-reared wild juveniles did, as did Perriman et al. (2022) for tank-reared Newfoundland juveniles at 80 d post-first-feeding. Aquaculture selection for greater weight in salmonids has potentially resulted in a corresponding increase in body depth (Gjerde & Schaeffer 1989), and the aquaculture environment may 'release' farmed fish from the natural selection pressures for a more streamlined body that is often associated with stronger swimming performance (Fleming & Einum 1997). These genetic-based morphological differences between cross types may be

less expressed phenotypically in the wild vs. culture (e.g. Solem et al. 2006), and instances of morphological convergence among cross types with increasing time spent in a wild environment have also been observed (e.g. Fleming et al. 1994, Von Cramon-Taubadel et al. 2005, Perriman et al. 2022, results which are in line with our observations of small overall differences in the wild. In addition, the lack of evidence for a cross type effect but strong evidence for a site effect on pectoral fin length suggests that environment plays a stronger role in shaping this trait than does genetics, perhaps through differences in flow characteristics of the 3 sites. Overall, our results suggest that any genetic differences in morphological traits between the wild and farmed populations we used are not strongly expressed in a wild environment, at least over the first summer.

4.4. Parr marks

Our results show strong evidence for an effect of cross type as well as a site \times cross type interaction on number of parr marks, corroborating previous studies showing that both genetics and environment play a role in determining parr mark numbers. Juvenile salmon in different environments are likely to experience selection for certain parr mark patterns because this trait influences their ability to camouflage against the riverbed and thus hide from predators (Donnelly & Dill 1984, Culling et al. 2013). Boulding et al. (2008) found 3 quantitative trait loci that influenced parr mark numbers, a finding which was supported by the results of their common-garden experiment finding different numbers of marks on fish from different genetic backgrounds raised in a common environment. Since we found that W ϕ hyb had the fewest parr marks and outgrew the other cross types by the largest margin at Site 3, it is possible that they had an environment-specific genetic advantage for parr mark number (i.e. enabling better camouflage, allowing more energy to be spent on feeding and less on predator avoidance). At the other 2 sites, cross type differences in parr mark number were minimal, despite W ϕ hyb remaining the cross type with highest recapture odds. This suggests that the influence of parr mark number on overall survival is not particularly strong, or perhaps this trait interacts with others in varying ways under different conditions to influence survival.

On the other hand, for parr mark size (length and width), there was a lack of evidence for a cross type effect but strong evidence for a site effect, suggesting that plasticity plays a larger role than genetics for

this trait. Indeed, Jørgensen et al. (2018) found that the environment played a larger role in determining spot pigmentation patterns on Atlantic salmon than did genetics (though the latter did still have an effect). In general, our results for parr mark number and size were quite variable, and pairwise differences between cross types were small. We observed the largest pairwise cross type differences for parr mark size at Site 1 (where pure farm fish had narrower marks than both hybrids), which was also the site with the lowest growth overall. This perhaps suggests that selection for parr mark size (and thus camouflage ability) operates more strongly under more challenging growth conditions, potentially due to trade-offs in energy allocation for feeding vs. predator avoidance.

5. CONCLUSIONS

Overall, our results showed that there are differences among farmed, wild, and hybrid Atlantic salmon parr for fitness-related traits and survival even at small temporal scales under favourable environmental conditions. We showed that plasticity for these traits exists within cross types, with differing relative performance in terms of size and morphology at replicate study sites. The fact that the pattern of relative survival was consistent across sites but was not obviously linked to patterns in any of the fitness-related traits suggests that other traits (unexamined here) may differ among cross types and influence survival, or perhaps multiple traits combine to have a cumulative effect on survival. Ultimately, as the first North American wild-environment study of its kind, this work could serve as a lead-in for additional research on these specific populations in the wild, especially to further investigate potentially critical findings such as the possibility of wild-mother hybrid vigour. Such research provides key data for these populations in areas of high aquaculture activity and will help to inform predictive modeling of escapee impacts and subsequent management decisions.

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