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Quantifying heritable variation in fitness-related traits of wild, farmed and hybrid Atlantic salmon families in a wild river environment

Authors:

Thomas E. Reed[1], Paulo Prodöhl[2], Rosaleen Hynes[2], Tom Cross[1], Andy Ferguson[2], Philip McGinnity[1]

Affiliations:

[1] School of Biological, Earth & Environmental Sciences, University College Cork, Cork, Ireland.

[2] Institute for Global Food Security, School of Biological Science, Medical Biology Centre, Queen's University, Belfast, Northern Ireland.

Corresponding author details:

Philip McGinnity, School of Biological, Earth & Environmental Sciences, University College Cork, Cork, Ireland. Email: P.McGinnity@ucc.ie Phone: +353 98 42317

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Abstract

Farmed fish are typically genetically different from wild conspecifics. Escapees from fish farms may contribute one-way gene flow from farm to wild gene pools, which can depress population productivity, dilute local adaptations and disrupt coadapted gene complexes. Here we reanalyse data from two experiments (McGinnity *et al.*, 1997, 2003) where performance of Atlantic salmon (*Salmo salar*) progeny originating from experimental crosses between farm and wild parents (in three different cohorts) were measured in a natural stream under common garden conditions. Previous published analyses focussed on group-level differences but did not account for pedigree structure, as we do here using modern mixed-effect models. Offspring with one or two farm parents exhibited poorer survival in their first and second year of life compared with those with two wild parents and these group-level inferences were robust to excluding outlier families. Variation in performance among farm, hybrid and wild families was generally similar in magnitude. Farm offspring were generally larger at all life stages examined than wild offspring, but the differences were moderate (5-20%) and similar in magnitude in the wild versus hatchery environments. Quantitative genetic analyses conducted using a Bayesian framework revealed moderate heritability in juvenile fork-length and mass and positive genetic correlations (>0.85) between these morphological traits. Our study confirms (using more rigorous statistical techniques) previous studies showing that offspring of wild fish invariably have higher fitness and contributes fresh insights into family-level variation in performance of farm, wild and hybrid Atlantic salmon families in the wild. It also adds to a small, but growing, number of studies that estimate key evolutionary parameters in wild salmonid populations. Such information is vital in modelling the impacts of introgression by escaped farm salmon.

Keywords: introgression, hybridisation, outbreeding depression, fitness, salmonid, aquaculture

Introduction

Intentional releases from hatcheries or unintentional escapes from aquaculture facilities can lead to genetic introgression between captive and wild fish populations where interbreeding occurs. Commercial farming of Atlantic salmon (*Salmo salar*) has increased dramatically over the past few decades, raising concerns over the genetic and ecological impacts on native populations (Naylor *et al.*, 2005). Escapes from open net-pen culture facilities regularly occur, either via chronic low-level 'leakage' or acute events (e.g. storms) that release thousands of fish at one time (Naylor *et al.*, 2005). Many wild Atlantic salmon stocks are currently severely depleted (ICES, 2010) and in some regions farm escapees can account for a third or more of salmon caught at sea (Hansen *et al.*, 1999) or on the spawning grounds (Fiske *et al.*, 2006). A range of studies have demonstrated that escaped farm salmon can successfully spawn in the wild (Fleming *et al.*, 1996) and hence may contribute one-way gene flow from farm to wild gene pools (Clifford *et al.*, 1998; Skaala *et al.,* 2006; Glover *et al.*, 2012, 2013).

Farmed Atlantic salmon are often genetically different from wild conspecifics due to geographical origin, founder effects (Skaala *et. al.*, 2004), and especially domestication selection and genetic drift in captivity. For example, artificial selection for economically desirable traits such as faster growth and delayed maturity has been applied to many farm strains (Gjøen and Bentsen, 1997; Gjedrem 2000). The domestication process can also lead to rapid genetic changes in farm populations as a result of unintentional selection on non-target traits, for example increased aggression, higher risk-taking and altered feeding behaviours (Einum and Fleming, 1997; Fleming *et al.*, 2002; Houde *et al.*, 2010), or as a result of relaxed selection and genetic drift due to propagation with limited number of broodstock (Lynch and O'Hely, 2001).

In the wild, salmon populations invariably exhibit hierarchical genetic structure, with substantial genetic differences apparent among regions, neighbouring catchments within regions and even tributaries within the same river (Dionne *et al.*, 2008; Bourret *et al.*, 2013). Some of this genetic divergence is thought to reflect adaptations to local environments (Garcia de Leaniz *et al.*, 2007), although the magnitude of local adaptation varies with spatial scale (Fraser *et al.*, 2011). If continued one-way gene flow occurs from farm to wild salmon populations at high rates, then genetic differences (both among wild populations and between wild and farm populations) could rapidly erode, although some populations may be less susceptible to 'genetic invasion' than others (Glover *et al.*, 2012, 2013).

Introgressive hybridisation between farm and wild salmon can also lead to a drop in mean individual fitness in the wild. Experimental studies involving artificial crosses between wild and farm fish have provided evidence that offspring with one or two farm parents display lower survival than those with two wild parents (McGinnity *et al.*, 1997, 2003; Skaala *et al.*, 2012). Larger, more aggressive farm and hybrid fish may also displace native fish or force them into suboptimal habitats, which increases average mortality (McGinnity *et al.*, 1997, 2003; Fleming *et al.*, 2000). These studies suggest that repeated introductions of farm fish may depress the productivity of wild populations through both ecological and genetic mechanisms, in addition to fostering genetic homogenisation (Skaala *et al.*, 2006; Glover *et al.*, 2012) and potential loss of local adaptations. Most studies of the effects of artificial immigration of non-native fish (whether from farms or hatcheries), however, tend to emphasise group-level performance differences and typically overlook family-level variation in performance (but see Skaala *et al.* 2012). Information on families can minimise analytical bias and yield important insights; for example, certain non-native or hybrid families may fortuitously perform much better than others in natural environments and therefore contribute

92 disproportionately to introgression of non-native alleles/traits into wild populations (Garant *et*
93 *al.*, 2003).

94 A recent Norwegian study found substantial among-family differences in the freshwater
95 growth and survival of Atlantic salmon in a natural stream setting, with progeny of farm
96 parents exhibiting a broader range of survival rates (in addition to a lower mean survival)
97 than hybrid or wild progeny (Skaala *et al.*, 2012). As noted by these authors, patterns of
98 variation in the performance of farm, wild and hybrid families are likely to vary across space
99 and through time, given that rivers vary in habitat characteristics and performance depends on
100 an interaction between genes and environment. The extent of genetic divergence between
101 wild and farmed salmon (and hence the potential threat of outbreeding depression) is also
102 expected to vary among locales depending on the farm strains used, patterns of differentiation
103 in the local wild populations, and the extent of any prior gene flow from farm to wild
104 populations. Additional data on family differences in survival and fitness-related traits (e.g.
105 size-at-age) of farm salmon and farm-wild hybrids (particularly F2 hybrids and backcrosses,
106 which were not included in the Skaala *et al*. 2012 study and which provide extra information
107 on the genetic basis of farm-wild differences and multi-generation consequences of
108 interbreeding) from other geographic locations therefore would be highly valuable to estimate
109 evolutionary consequences of introgression. On a more practical level, data on families can
110 reveal if overall differences in mean performance between farmed, wild and hybrid groups
111 are driven by one or two outlier families. Moreover, if phenotypic data is collected on related
112 individuals (e.g. half-siblings), the resulting pedigree can be exploited to estimate quantitative
113 genetic parameters such as trait heritabilities and genetic correlations, for which there are still
114 very few estimates from wild salmonid populations (Carlson and Seamons, 2008).
115 Information on the extent to which variation in fitness-related traits (e.g. size-at-age) is

transmitted from parents to offspring is also crucial to predicting the genetic and demographic consequences of introgression

Here we reanalyse data from two experiments conducted in the west of Ireland (McGinnity *et al.*, 1997, 2003) where survival and size-at-age of Atlantic salmon progeny originating from experimental crosses between farm and wild parents (in three different cohorts) were measured in a natural stream under common garden conditions. We have three primary objectives: (1) To reanalyse these data with modern mixed-effects models that account for kin structure to test properly for group-level differences in mean survival and size-at-age, and to check whether patterns were driven by outlier families; (2) To test whether farm or hybrid families exhibited different patterns of variation in survival and size-at-age relative to wild families (i.e. variance heterogeneity with respect to groups). For example, farm families may exhibit higher variance than wild families (Skaala *et al.*, 2012), while outcrossing can lead to changes in additive genetic and residual (non-additive genetic and environmental) variance in hybrid groups (Lynch and Walsh, 1998; Debes *et al.*, 2014). (3) To exploit the pedigree structure inherent in the experimental designs to estimate quantitative genetic parameters of interest in a wild setting. Effects of egg size on offspring performance, assumed to reflect environmental maternal effects, are also tested and controlled for statistically at different offspring ages.

Methods

Study area and experimental design

The experiments were undertaken in the Burrishoole system in the west of Ireland (Figure 1). A number of afferent rivers flow into Lough Feeagh (one of two major lakes in the

139 catchment), one of which (the Srahrevagh River, hereafter 'experiment river') was used for
140 the freshwater stages of the experiment and was equipped with a trap ('experiment trap')
141 capable of capturing all downstream moving juveniles and upstream migrating adults. The
142 first experiment involved artificial crosses between farm adults (a derivative of the
143 Norwegian Mowi strain established in Ireland in 1983, which became known as the 'Fanad'
144 strain) and wild adults captured in the Burrishoole system in December 1992 and December
145 1993. By 1983, the Mowi strain had already experienced circa 15 years (3-5 generations) of
146 domestication in Norway, and thereafter the selection trajectory of the Fanad strain, which
147 has never received inputs from Irish wild strains, was likely different from that of the farm
148 strains in Norway. Four cross-types (hereafter simply 'groups') were made, involving pure
149 farm, pure wild and both reciprocal hybrids (Table 1). The families established from the
150 December 1992 broodstock, which hatched in spring 1993, are referred to as the 1993 cohort;
151 similarly, the families established from the December 1993 broodstock, which hatched in
152 spring 1994, are referred to as the 1994 cohort. To produce both the 1993 and 1994 cohorts,
153 each farm dam was mated to one farm sire and one wild sire, and vice versa; thus all dams
154 and sires were mated twice. For full details on the experimental design for the 1993 and 1994
155 cohorts, see (McGinnity *et al.*, 1997) and Appendix 1 (which includes a schematic on the
156 mating design).

157 In the autumn of 1997, returning F_1 hybrid Atlantic salmon, which had been ranched (i.e.
158 released to the ocean as hatchery-reared smolts) from the 1994 cohort and had spent two
159 winters at sea (2SW), were captured at the sea-entry traps (Fig.1). These were then used to
160 produce F_2 hybrids and BC_1 backcrosses, while a new set of farm and wild adults were used
161 as broodstock to produce pure and F_1 hybrids (Table 1). Families thus established, which
162 hatched in spring 1998, are referred to as the 1998 cohort. The mating design for the 1998

cohort was slightly different from the 1993 and 1994 cohorts (Appendix 1). For full details on the experimental design for the 1998 cohort, see (McGinnity et al. 2003).

For each cohort, families were first mixed at the eyed-egg stage and then planted out to the experiment river in artificial redds (Donaghy and Verspoor, 2000). Juveniles were then sampled from the experiment river by electrofishing in August 1993, August 1994 and August 1998. The experiment trap was also inspected daily from 30 April 1993 to 20 April 1995, and from 24 April 1998 to 30 June 2011. A random subset of parr and smolts from the experiment river caught in the experiment trap during these periods were sacrificed and preserved in 95% ethanol. Fish in their first calendar year of life were denoted as 0+ and in their second calendar year as 1+. For the 1993 and 1994 cohorts, sub-samples of eggs from each family (250 eggs per family for 1993 cohort, 200 eggs per family for 1994 cohort, eggs measured at this point) were retained in the hatchery and reared to the smolt stage, denoted as 'hatchery controls' (measured prior to being released to the ocean as smolts, and hence termed 'pre-smolts'). A sample of 0+ parr from the 1993 cohort hatchery control group was sampled in August 1993, while further samples of mature male parr and pre-smolts were taken from the hatchery controls in November 1993 and March 1994, respectively. A sample of hatchery pre-smolts was also taken from the 1994 cohort in March 1995, just prior to their release to sea. In total, sampling of the 1993, 1994 and 1998 cohorts yielded 14 different datasets on size-related traits and survival. DNA profiling techniques based on minisatellite (1998 cohort) or microsatellite (1993 and 1994 cohorts) marker loci were used to assign sampled offspring back to their parents with close to 100% power, which allowed individuals to be grouped into families (see McGinnity et al. 1997, 2003 for full details on the molecular methods and parentage assignment).

Statistical analyses

1. Representation

As the number of fish per family in some samples is determined by both emigration from the experiment stream and survival, counts are referred to simply as 'representation', following McGinnity et al. (1997, 2003, 2004). A series of generalised linear mixed effects models (GLMMs) were constructed to examine variation in family-level representation at different life/sampling stages. Mixed effects models are a powerful statistical technique for making inferences about explanatory variables of interest (typically the fixed effects, i.e. terms for which regression coefficients are estimated) while properly accounting for any sources of non-independence or hierarchical structure (random effects, i.e. terms for which an estimate of the variance is obtained) in the data; GLMMs are used when the response variable is non-normal (Bolker *et al*., 2009). The GLMMs were fitted in R version 3.0.2 (R Core Development Team 2008) using the glmer function from the lme4 package (Bates *et al*., 2012). The binomial response variable considered in these models was a concatenated vector of the number of individuals represented per family and the number not represented (the initial number of eggs per family planted out minus the number of individuals represented) and a logit link function was used. 'Dam' and 'sire' (unique identifier codes for each mother and father) were included as random effects, which accounts for the kinship structure inherent in the data (full-sibs nested within half sibs) and also provides estimates of the variance attributable to each parent.

For each model, fixed effects of group as a factor (i.e. separate levels for each cross type) and eyed-egg diameter (mean-centred) were included. The latter was a single value per family (see McGinnity *et al*., 1997, 2003 for details on how this was measured) and was used as an index of maternal effects mediated via egg size (Einum and Fleming, 1999). Dam fork length

(L_F) and egg mass were also measured but both were strongly correlated with egg diameter (r > 0.5 in all cohorts), so to avoid problems with collinearity of explanatory variables only egg diameter was included in the models. Backwards model selection (Zuur *et al.,* 2009) was performed on the fixed effects, by dropping each in turn and retaining only significant terms (as assessed using likelihood ratio tests, LRTs) in the final model, while retaining the random effects of sire and dam regardless of their significance (which was necessary to properly account for kin structure in the data). Multiple contrasts with univariate *P* values were then used to test whether each group differed significantly from the pure wild group (the reference group).

The existence of outlier families was checked by visually examining the family-level representation data. If a potential outlier was identified, its influence on the overall results was checked by re-running the analysis for that particular sample excluding that family and determining whether the results were changed qualitatively. To test for variance heterogeneity across groups in the raw representation data, the non-parametric Figner-Killeen Test of Homogeneity of Variances was used. The null hypothesis was that all groups had equal variance; the alternative hypothesis was that the variance differed for at least two of them. Finally, to test whether representation was consistent from the 0+ to 1+ parr stages, representation of 1+ parr per family (sampled in June 1995) were plotted against representation of 0+parr per family (sampled in August 1994) and a standard regression performed.

2. Size-at-age

A series of linear mixed effects models (LMMs) were constructed to examine variation in the L_F and mass of juveniles at different life stages (note that for some datasets, mass was not

measured). Using LMMs is appropriate as 'family' can be fitted as a random effect, which accounts for non-independence of measurements taken on individuals belonging to the same family (i.e. accounts for 'genetic pseudoreplication'). Failure to account for family structure can lead to inflated statistical significance of treatment (here group) effects, as the effective sample size per treatment level is lower than the number of observations per level (Zuur *et al.*, 2009). The goals of these LMMS were to test for (1) group differences in mean L_F and mass, (2) environmental maternal effects mediated via egg size (eyed-egg diameter), and (3) heterogeneity among groups in between-family variance and within-family variance. These goals were achieved by fitting a series of hierarchical models in two steps. In the first step, the most appropriate random effects structure was determined while including all candidate fixed effects, regardless of their statistical significance (Zuur *et al.*, 2009). In the second step, backwards model selection was performed on the fixed effects (while retaining the best random effects structure identified in the first step) to determine which were significant. For each model, fixed effects of group and eyed-egg diameter were included. The response variables (L_F, mass) were natural log-transformed, which ensured that model residuals were normally distributed.

To determine the most appropriate random effects structure and test for variance heterogeneity across groups (e.g. whether the variation in farm fish was less than that of wild fish), five different (increasingly complex) models were compared for each response variable. First, a common residual variance only was estimated using generalised least squares (the gls function in the R library nmle). Second, a random effect of family (common to all groups) was included (using the lme function). Third, the random effect of family was stratified by group, which allowed for different between-family variances for each group. Fourth, a common random effect of family (i.e. not stratified by group) was fitted and the residual variance was stratified by group (which allowed for different within-family variances for

each group). Fifth, both the random effect of family and the residual variance were stratified by group (which allowed for heterogeneity in both between- and within-family variance). The model with the lowest AIC was then chosen as the most appropriate model in terms of the random effects. To reduce the number of parameters to be estimated, all mixed ancestry groups were merged into a single 'hybrids' group when stratifying the family or residual variance by group. That is, 'group' was a three-level factor (pure, wild and hybrids) when included in the random effects part of the model, whereas hybrid groups were distinguished as separate levels when 'group' was fitted as a fixed effect. Significance of the fixed effects were then tested via backwards selection, with P-values calculated by comparing models with and without the fixed effect of interest (fit by maximum likelihood) using LRTs.

For the above LMMs, we focussed on size-at-age variation in the electrofishing and hatchery control samples only, where all individuals were measured on the same day. Variation in size-at-age was not examined for parr, pre-smolts and smolts caught in the experiment trap, as these fish were caught at different times of year and hence size differences could simply reflect age differences (age not being known accurately). The sample sizes were also insufficient to support more complex analyses of family variation in growth trajectories (e.g. random regression) for the trap sample data. As for the representation analyses, the existence of outlier families was checked by visually examining the family-level size-at-age data. If a potential outlier was identified, its influence on the overall results was checked by re-running the analysis for that particular sample excluding that family and determining whether the results were changed qualitatively.

3. Quantitative genetic analyses

283 A Bayesian animal model approach was taken to estimate quantitative genetic parameters of
284 interest, using the R package MCMCglmm (Hadfield, 2010). The animal model is a particular
285 form of linear mixed effects model in which the breeding value, or 'additive genetic merit',
286 of each individual is treated as a random effect. An estimate of the additive genetic variance
287 (V_A), and in the case of multivariate models, also the additive genetic covariance (COV_A) can
288 be obtained by combining phenotypic data with a pedigree. In our case, sampled offspring
289 were assigned back to their parents with almost complete certainty, as there were no
290 unknown parents (see McGinnity et al. 1997, 2003). The resulting pedigree gives an
291 expectation of how breeding values should co-vary among individuals of different genetic
292 relatedness (in this case full-sibs and half-sibs; note that parental phenotypes were not
293 measured at the same age and hence could not be included in the analysis), which then allows
294 V_A and COV_A to be solved for algebraically (Kruuk, 2004; Hadfield, 2010).

295 While it would have been possible to pool data from all groups to estimate quantitative
296 genetic parameters, we chose not to, as outcrossing genetically divergent groups (i.e. farm
297 and wild fish) leads to changes in non-additive genetic components of variance (dominance
298 and epistasis) in the hybrids (Lynch and Walsh, 1998). The data and pedigree structure were
299 not sufficiently informative to separate out these non-additive components (which otherwise
300 end up in the residual variance, V_R) and hence obtaining clean estimates of heritability with
301 the pooled data would be problematic, as both V_A and V_R are expected to vary among cross-
302 types (groups). We therefore ran animal models separately for the pure wild and pure farm
303 groups only and only for samples where at least 50 individuals were measured. Egg size was
304 included as a continuous fixed effect in all cases to test for environmental maternal effects
305 mediated via egg size.

306 Bivariate animal models were used to analyse variation in L_F and mass simultaneously. Fixed
307 effects of egg size were estimated for each trait in the same model (by including a trait × egg

size interaction), and the phenotypic variance-covariance matrix was decomposed into an additive genetic matrix and a residual (environmental) matrix (Hadfield, 2010). The distribution of both traits was modelled as Gaussian and weakly informative inverse Wishart priors were used (posterior distributions were robust to alternative prior specifications). Samples were taken from the posterior distributions of the parameters every1000 iterations of the Markov chain, after an initial burn-in of 2.5×10^4 iterations, for a total of 1000 samples. In all cases this was sufficient to achieve good convergence and acceptably low (<0.1) autocorrelation between adjacent MCMC samples. Posterior distributions of the narrow-sense heritability h^2 of each trait (for wild and farm groups separately) were calculated by dividing the posterior distribution of V_A by the sum of the posterior distributions of V_A and V_R, and the mode and 95% credible intervals (CI) of these posterior h^2 distributions are then presented. Posterior distributions of the genetic correlation between L_F and mass were calculated as the posterior distribution of the genetic covariance divided by the square root of the product of the posterior distributions of the genetic variances. General maternal environmental effects not accounted for by egg size effects were also tested for in all models by including an additional random effect of 'mother identity', but in all cases this variance component was estimated at close to zero (and the deviance information criterion did not drop by >2 units) and hence was not included in the final models.

Results

1. Representation

Overall group-level differences in representation were consistently found for 0+ parr in the electrofishing samples from each cohort, and for 1+ parr in the 1994 cohort (Table 2, full statistical results presented in Appendix 2). In the 1993 cohort 0+ parr electrofishing sample,

the WF group was significantly over-represented relative to the WW reference group but the other groups were equally represented (Table 2, Fig.2). For the 1994 cohort, both 0+ and 1+ electrofished parr were significantly under-represented in the FF group relative to the WW group, while 0+ parr were also under-represented in the FW group (Table 2, Fig.2). There was one obvious outlier in the WW group for the 1994 cohort 0+ parr electrofishing (family 49, Fig.2A); when this outlier was excluded, the results were qualitatively unchanged. Egg size had a significantly positive effect on representation of 0+ parr in the 1993 and 1994 cohort electrofishing samples and on the representation of 1+ parr in the June 1995 (1994 cohort) electrofishing sample (Appendix 2, Table A2.1 and supplementary figure1).

For the 1994 cohort, representation of 1+ parr per family in June 1995 was positively correlated with representation of 0 + parr per family in August 1994 (Fig. 3; $r = 0.674$, $P < 0.001$; no differences between groups in this relationship). A single outlier family (family 49, Fig. 2) had a large influence on this relationship; however, the positive correlation remained significant when excluding this family ($r = 0.383$, $P = 0.012$).

For the 1998 electrofishing sample, egg size did not have a significant effect on representation per family, but all groups were under-represented relative to the WW group, with the FF group having the lowest representation (Table 2, Fig.2). The other groups were approximately equally represented, but lower on average than the WW group (Table 2, Fig. 2). There was one obvious outlier in the F_2Hy group (family 162, Fig.2A), but excluding this family did not change the results qualitatively. The variances attributable to dam effects and sire effects for all representation models are given in Appendix 2 (Table A2.2).

Parr belonging to the 1993 cohort were under-represented in the experiment-trap in the FW and FF groups relative to the WW group (Table 2, supplementary figure 2A). Pre-smolts and smolts originating from this cohort were marginally under-represented in the FW and FF

356 groups relative to the WW group (Table 2, supplementary figure 2B). The latter result was
357 robust to excluding one outlier family (family 4, supplementary figure 2B). For the 1994
358 cohort, parr were under-represented in the experiment-trap in the WF, FW and FF groups (in
359 this order: WW>WF > FW > FF; Table 2, supplementary figure 2A). Results were
360 qualitatively the same when a single outlier family belonging to the WW group (family 49,
361 supplementary figure 2A) was excluded.

362 There were no significant differences among groups in representation of pre-smolts and
363 smolts from the 1994 cohort in the experiment-trap (Table 2). For the 1998 cohort, parr were
364 under-represented in the experiment-trap in the BC_1W, F_2Hy, BC_1F and FF groups (in this
365 order: WW > BC_1W > F_2Hy > BC_1F > FF; Table 2, supplementary figure 2A). There were
366 no significant differences among groups in representation of pre-smolts and smolts from the
367 1998 cohort in the experiment-trap (Table 2), and this result was robust to excluding one
368 outlier family (family 162, supplementary figure 1B). Egg size did not have a significant
369 effect on representation in any of the experiment-trap samples (Appendix 2).

370 For the 1993 cohort, there were no significant representation differences between groups in
371 the hatchery control 0+ parr August 1993 sample (Table 2, supplementary figure 3A). In the
372 hatchery control mature male parr sample, the WF and FF groups were under-represented
373 relative to the WW group (Table 2, supplementary figure 3B). There were no significant
374 representation differences among groups in terms of smolts in the hatchery control groups for
375 the 1993 and 1994 cohorts (Table 2, supplementary figure 3C and 3D). Egg size did not have
376 a significant effect on representation in any of the hatchery control samples (Appendix 2).

377 For most of the samples considered, no variance heterogeneity with respect to group was
378 found (Appendix 2, Table A2.3), apart from a few exceptions. For the 1998 cohort
379 electrofished 0+parr, the Fligner-Killeen test showed that at least two of the group variances

were different (median chi-squared = 11.65, df = 4, $P = 0.020$). The raw variance in representation (i.e. not correcting for egg-size variation) was highest for the F_2Hy group (8.2×10^{-5}), intermediate for the BC_1W (2.6×10^{-5}) and BC_1F (2.7×10^{-5}) groups and lowest for the FF (1.6×10^{-5}) and WW (1.3×10^{-5}) groups. Excluding the outlier in the F_2Hy group (family 162, Fig.2A), the variance for this group dropped considerably (to 2.2×10^{-5}), but the Fligner-Killeen test still showed that at least two of the groups were heterogeneous (median chi-squared = 9.92, df = 4, $P = 0.042$). For the 1993 cohort trapped parr, the Fligner-Killeen test showed that at least two of the group variances were different (median chi-squared = 11.93, df = 3, $P = 0.008$). The raw variance in representation was highest for the WW (7.6×10^{-5}) and WF groups (8.2×10^{-5}), and lower for the FW (2.7×10^{-5}) and FF (8.1×10^{-6}) groups. For the 1998 cohort trapped parr, the Fligner-Killeen test showed that at least two of the group variances were different (median chi-squared = 56.6, df = 4, $P < 0.001$). The raw variance in representation was highest for the WW group (5.8×10^{-5}), intermediate for the BC_1W group (4.0×10^{-5}) and lowest for the BC_1F (6.5×10^{-6}), F_2Hy (6.3×10^{-6}) and FF (2.8×10^{-6}) groups.

2. Size-at-age variation

For the 1993 and 1994 cohorts, electrofished 0+ parr assigning to the FF group were significantly larger (L_F) than those assigning to the WW group, while the hybrid groups (WF and FW) were intermediate (Table 2, Fig. 4A,B). A similar pattern was found for electrofished 0+ parr from the 1998 cohort, with FF parr being larger than WW parr and the BC_1W, F_2Hy and BC_1F groups being intermediate in size (Table 2, Fig. 4D). The general pattern was an increase in L_F of 0+ parr with an increase in the expected fraction of farm genes (i.e. the order was WW < hybrids < FF). L_F of 0+parr was also positively associated

404 with egg size in all three cohorts (Appendix 3, supplementary figure 4). Mass of 0+ parr
405 showed similar patterns to L_F, with farm fish being heavier than pure wild and hybrids
406 intermediate (supplementary figure 5). Egg size also had a positive effect on mass of 0+ parr
407 in all three cohorts (Appendix 3). L_F and mass of 1+ parr in the 1994 cohort were also higher
408 in the FF group compared with the WW group, with hybrids again intermediate (Table 2,
409 Fig.4C for L_F and supplementary figure 5B for mass). Egg size did not have a significant
410 effect on L_F or mass of 1+ parr (Appendix 3). There were no obvious outlier families in terms
411 of L_F and mass of electrofished parr (Fig. 4).

412 Growth patterns were less consistent for 0+ parr measured in the hatchery controls (1993
413 cohort): FF fish were significantly larger than WW fish, as were WF fish, but FF fish were no
414 larger than WF fish (standard errors largely overlapping, Table 2 and Appendix 3). Parr from
415 the FW group were not significantly larger than WW parr (Table 2, Fig.5A). Egg size did not
416 have an effect on L_F or mass of 0+parr in the hatchery controls (Appendix 3). No significant
417 differences in L_F of mature male parr in the 1993 cohort hatchery controls were apparent
418 (Table 2, Fig.5B), nor did egg size influence L_F of mature male parr in the hatchery
419 (Appendix 3). For the 1993 cohort hatchery controls, FF pre-smolts were significantly larger
420 and heavier than WW pre-smolts (Table 2, Fig.5C) but FW and WW pre-smolts were not
421 significantly larger than WW pre-smolts (Table 2, Fig.5C). Egg size had no effect on the L_F
422 and mass of pre-smolts in the 1993 hatchery controls (Appendix 3).

423 For the 1994 cohort hatchery controls, WF, FW and FF pre-smolts were all significantly
424 larger than WW pre-smolts, with FF being the largest and the two hybrid groups each
425 intermediate between WW and FF (Table 2, Fig.5D). Egg size had only a marginally
426 significant positive effect on the L_F of pre-smolts in the 1994 cohort hatchery controls
427 (Appendix 3, supplementary figure 4). The patterns for mass in the hatchery controls were

very similar to those for L_F (Appendix 3, supplementary figure 6). There were no obvious outlier families in terms of L_F (Fig. 5) and mass of hatchery control juveniles (supplementary figure 4).

For most of the samples considered, no variance heterogeneity in L_F or mass with respect to group was found (Appendix 3), apart from a few exceptions. In the 1994 cohort electrofished 1+ parr sample, there was heterogeneity among groups in the within-family variance in L_F and mass; this variance was highest in the WW group (raw variance = 10.39 g^2) and lower in the other three groups (WF = 6.66 g^2; WF = 7.58 g^2; FW = 7.34 g^2). In the 1993 cohort hatchery controls (supplementary figure6), the variance in mass of pre-smolts was higher in the FF group (raw variance = 242.93 g^2) compared to the other groups (WW = 102.06 g^2; WF = 85.59 g^2; FW = 112.29 g^2). In the 1994 cohort hatchery controls (Fig.5D), the variance in L_F of pre-smolts was higher in the WW group (raw variance = 8.95 mm^2) compared to the other groups (WF = 1.51 mm^2; FW = 2.71 mm^2; FF = 4.03 mm^2).

3. Quantitative genetic analyses

Moderate heritabilities were estimated for L_F and mass, with a general trend for higher h^2 estimates in the wild group than in the farmed group (Table 3). For L_F, modal h^2 estimates in the pure wild group ranged from 0.21 (Bayesian 95% CI: 0.07-0.75) in the June 1995 electrofished 1+ parr sample to 0.89 (CI: 0.23-0.96) in the August 1998 electrofished 0+ parr sample, whereas model h^2 estimates in the pure farm group ranged from 0.10 (CI: 0.03-0.44; June 1995 electrofished 1+ parr sample) to 0.31(CI: 0.04-0.86; August 1998 electrofished 0+ parr sample). For mass, modal h^2 estimates in the pure wild group ranged from 0.20 (CI: 0.09-0.77) in the June 1995 electrofished 1+ parr sample to 0.53 (CI: 0.15-0.94) in the August 1994 electrofished 0+ parr sample, whereas model h^2 estimates in the pure farm group ranged from 0.08 (CI: 0.03-0.43; June 1995 electrofished 1+ parr sample) to 0.17 (CI: 0.05-

0.86; August 1998 electrofished 0+ parr sample). The credible intervals for each h^2 estimate were quite large, reflecting the relatively low samples sizes and simple pedigree structure. The genetic correlations between L_F and mass of electrofished (0+ or 1+) parr were estimated to be very high (posterior modes of >0.85, with credible intervals not overlapping zero) in both the 1994 and 1998 cohorts, as were the environmental correlations (save for mass of August 1994 electrofished 0+ parr, where r_E was low; Table 3).

Discussion

Re-analysis of group-level performance differences accounting for family structure

The performances of individuals sharing one or two parents are not independent because of effects of shared genes and possible parental environmental effects. Earlier analyses of these experimental data (McGinnity *et al.* 1997, 2003) did not account for this family structure, but reassuringly the current results were largely congruent in terms of significant group-level differences (compare Table 2 here with Table 2 in McGinnity *et al.* 1997 and with Fig.2 in McGinnity *et al.* 2003) when hypothesis testing of parental genotypic effects was based on families rather than individuals (the former being tantamount to avoiding 'genetic pseudoreplication'). Minor differences, however, were noted. For example, with the electrofishing August 1993 0+ parr sample, McGinnity *et al.* (1997) reported that the FF group was significantly under-represented relative to the WW group, whereas here that difference was not significant. However, the qualitative conclusions were largely unchanged when kin structure was accounted for, suggesting that either the kin structure was not strong enough for genetic pseudoreplication to be a major issue, and/or that covariation in the performance of individuals sharing one or two parents was relatively weak due to moderate trait heritabilities.

476 Focusing analyses in to the family level allowed us to uncover interesting biological patterns
477 of variation and covariation in representation. Families highly represented at the 0+ parr stage
478 in the experiment stream (caught by electrofishing) were also highly represented at the 1+
479 parr stage (Fig. 3), implying consistent performance differences in the wild underpinned by
480 genetic differences or persistent maternal effects. Outlier families were also obvious in some
481 samples. For example, in the August 1994 0+ parr electrofishing sample, one pure wild
482 (WW) family (family 49, see Fig.2A and Fig.3) was represented by 59 parr, which compares
483 with an average representation of 11.4 parr per family excluding this family. Nevertheless,
484 the overall group-level differences in this sample remained statistically significant after
485 removing the outlier, instilling further confidence that the lower representation of offspring
486 with one or two farm parents was a robust, biologically meaningful result, not driven simply
487 by one or two highly performing wild families. Similarly, in the August 1998 0+ parr
488 electrofishing sample, one F_2 hybrid family (family 162, Fig.2A) was anomalously highly
489 represented relative to all other families, but the inferences regarding group-level differences
490 were robust to excluding this family. We can only speculate on the reasons as to why these
491 particular families were so highly represented, but in the case of the F_2 hybrid family,
492 recombination between the divergent wild and farm parental genomes could have produced
493 rare offspring genotypes that were fortuitously well-adapted to the local conditions through
494 hybrid vigour, or heterosis.

495 **Performance of farm and hybrid families: more or less variable than wild families?**

496 Overall genetic diversity may be considerably lower in farm salmon compared to wild
497 populations (Norris *et al.*, 1999; Skaala *et al.*, 2004), at least when considering highly
498 polymorphic genetic markers, because of low effective population sizes in the farm and/or
499 strong directional selection on target traits, which can deplete genetic variation (Lynch and
500 Walsh, 1998). *A priori*, therefore, one might expect that offspring produced by farm parents

should exhibit reduced phenotypic variation in the wild and therefore less variable survival rates compared to wild families. Skaala *et al.* (2012), however, reported the opposite: a larger range in survival rates (a ratio of 38:1 between the lowest and highest survival rates) in farm families compared to hybrid (7:1) or wild (8:1) families in a natural stream setting. In our case, however, no variance heterogeneity in representation with respect to group was found for most of the samples considered (Appendix 3). In a few samples, we did find variance heterogeneity but the patterns were inconsistent; for example, in the 1998 cohort electrofished 0+parr, survival variation was greatest among F_2Hy families (perhaps due to rare advantageous recombinants), while for the 1993 and 1998 cohort trapped parr samples, variance in representation was highest for pure wild families and lowest for pure farm families (as one would predict if farm families are genetically depauperate), with hybrid families being generally intermediate. While Skaala *et al.* used Mowi strain salmon in one of their experimental cohorts, the Fanad Mowi strain used by us is likely to be divergent from theirs in its genetic make-up (due to lower broodstock numbers and a separate breeding programme); thus differences in genetic background and selection trajectories of the farm strains may explain the inconsistent results, in terms of variance in performance, between Skaala *et al.* (2012) and the current study.

For offspring L_F and mass, no heterogeneity in between-family variance was found (Appendix 3), suggesting that each group had similar levels of additive genetic variance for these traits. In terms of within-family (residual) variance, which largely reflects environmental influences on the phenotype, no differences among groups were apparent in nine out of fourteen samples (Appendix 3). In the other five samples, the residual variance was either highest in the WW group (e.g. mass of 1994 cohort electrofished 1+ parr sample) or the FF group (e.g. L_F and mass of pre-smolts in 1993 cohort hatchery controls). Intriguingly, Debes et al. (2014) found that within-family variation in body size of Atlantic

salmon (measured in a hatchery setting) diminished with increasing generations of domestication (see also Solberg, Skaala *et al.*, 2013). Under fully wild conditions, variance differences between wild, farmed and hybrid families may be largely unpredictable and context-dependent, given that our findings did not match those of Skaala *et al.* (2012) despite very similar study designs (but different genetic backgrounds). One possibility is that farmed fish may lose their environmental sensitivity (i.e. degree to which their phenotypes or performance is buffeted by prevailing conditions) in hatchery environments, but not wild environments, as they are only selected in the former.

Genetic basis of group and family differences in size-at-age

Directional selection in farm strains has resulted in higher intrinsic growth rates of farm salmon, which in a hatchery environment can grow up to three times faster than wild salmon (Glover *et al.*, 2009; Solberg, Skaala, *et al.*, 2013, Solberg, Zhang, *et al.*, 2013). However, these growth rate differences seem to be less pronounced in wild stream environments (Skaala *et al.*, 2012) and in hatchery conditions simulating a semi-natural environment with restricted food (Solberg, Skaala, *et al.*, 2013). In our experiments, size-at-age differences between wild and farm offspring measured in the wild were statistically significant but moderate in magnitude (Table 2), with electrofished farm parr being on the order of 5-20% larger and heavier than wild parr, consistent with the findings of these previous studies. However, size differences between farm and wild juveniles were similar in the hatchery environment as in the wild (Table 2), which contrasts with the above-cited studies. This presumably reflects the fact that the Fanad farm strain used in our study had experienced a different selection trajectory in Ireland up until our experiments were carried out in the 1990s than the Norwegian farm strains used in the more recent Norwegian studies (Glover *et al.*, 2009; Skaala *et al.*, 2012; Solberg, Skaala, *et al.*, 2013, Solberg, Zhang, *et al.*, 2013) had. The latter had also undergone more generations of targeted artificial (and/or inadvertent

551 domestication) selection than our farm strain. These differences in historical selection
552 regimes, as well as possible founder effect differences, may explain why we found only
553 moderate size differences between our farm and wild groups in both the hatchery and wild
554 environments, whereas the Norwegian studies observed much larger differences in hatchery
555 environments (where their higher genetic growth potential is likely more easily realised) that
556 were attenuated in the wild (where environmental influences on growth are likely larger and
557 selection against extreme phenotypes also stronger). Interestingly, genetically-based somatic
558 growth differences between the farm and wild strains used in the Norwegian studies seem to
559 be more important after the onset of exogenous feeding, with alevin lengths being similar
560 once egg size differences between farm and wild strains are corrected for (Solberg *et al*.
561 2014).

562 V_A is a crucial parameter influencing the rate of microevolution and thus the potential for
563 genetic adaptation to a changing environment, while in the case of multivariate selection,
564 COV_A among characters determine the extent to which they can evolve along independent
565 trajectories. Typically, V_A is scaled relative to the phenotypic variance V_P, which gives a
566 measure of heritability h^2, while COV_A is scaled relative to the square root of the product of
567 the V_A in each trait to give a measure of the genetic correlation (r_G). Estimates of both
568 heritabilities and genetic correlations (including the sign of the latter) may depend, however,
569 on the quality of the environment experienced by measured individuals, which can affect both
570 V_A and V_R (i.e. the residual, or environmental variance) (Charmantier and Garant, 2005). In
571 the case of salmonid fishes, quantitative genetic parameter estimates calculated under farm or
572 hatchery conditions may have limited relevance for wild populations, given the
573 environmental-sensitivity of these parameters. Carlson and Seamons (2008) reported that
574 only 2% of published h^2 estimates in salmonids were from wild-reared populations, while no
575 estimates of r_G were available at the time for wild salmon reared in the wild. Since then, a

few additional studies have been published that estimated quantitative genetic parameters in wild settings (Saura *et al.*, 2010; Serbezov *et al.*, 2010; Letcher *et al.*, 2011) and our current study adds to this small list. For the pure wild group, our estimates of h^2 of L_F and mass (electrofished parr samples) were generally in the range of 0.20 to 0.50 (Table 3), which compares with a median h^2 of 0.29 and 0.32 for length-at-age and mass-at-age, respectively, reported in Carlson and Seamons (2008). Saura *et al.* (2010) estimated the h^2 of adult length (and also adult mass) of Atlantic salmon to be 0.32, while Serbezov *et al.* (2010) report h^2 estimates between 0.16 and 0.31 for length-at-age for wild-living juvenile brown trout (*Salmo trutta*). Body size of salmon juveniles is positively related to their ability to acquire and defend feeding/nursing territories and has previously been shown to be under positive natural selection (Einum and Fleming, 2000). Thus estimates of the h^2 of size-at-age traits obtained under natural conditions are of evolutionary importance; moreover, these traits are known to vary between farm and wild populations and hence understanding how they are inherited can improve predictions of likely genetic consequences of introgression.

We also found that the modal h^2 estimates for L_F and mass were generally lower for the pure farm (FF) group, compared with the pure wild group (Table 3), although the uncertainty associated with each h^2 estimate was relatively large and the posterior distributions for the wild and farm groups overlapped considerably. Because these traits were first natural-log transformed before running the animal models, the V_A values reported in Table 3 (multiplied by 100) can also be interpreted as evolvabilities (i.e. mean standardised additive genetic variances on the untransformed scale, see Hansen *et al*., 2011). Evolvability measures the expected proportional evolutionary change in a trait under a unit strength of selection and in many ways is a better measure of evolutionary potential that h^2, particularly when comparing groups or populations that have very different V_P. Thus for example, when the mean standardised selection gradient is 1 (i.e. very strong selection), the expected evolutionary

601 response in L_F for the wild 0+parr based on the August 1998 electrofishing sample would be
602 0.4% (i.e. an evolvability of 0.4% for the WW group), while that for the farmed parr would
603 be only 0.2% (Table 3). In general we found that V_A (and hence evolvability) was lower in the
604 FF group compared with the WW group, which is in line with previous findings that genetic
605 variation in farm salmon strains are often lower than in wild strains (Norris *et al.*, 1999;
606 Skaala *et al.*, 2004). Interestingly, Solberg, Zhang *et al.* (2013) reported reduced heritability
607 of juvenile mass in farm-provenance Atlantic salmon, compared to progeny of wild parents,
608 when both were reared under standard hatchery conditions with unrestricted access to food.
609 This pattern was reversed , however, when access to food was restricted, possibly reflecting
610 selective mortality against the slowest-growing wild genotypes (Solberg, Zhang, *et al.*, 2013).
611 We also found strong positive genetic correlations between L_F and mass (>0.85 in all
612 samples), which is higher than the median r_G of +0.71 reported by Carlson and Seamons
613 (2008) for pairs of morphological traits. Hence positive selection on body size would be
614 predicted to result in population-level increases in both mean L_F and mean mass (Lynch and
615 Walsh, 1998). We controlled for environmental maternal effects as far as possible by
616 including egg size as a covariate (fixed effect) in the animal models. Larger females tend to
617 produce larger eggs (as do farm females, see Table 1), and larger eggs can result in larger size
618 of fry at emergence and higher early-life survival (Einum and Fleming, 1999; Heath *et al.*,
619 1999). We found that egg size had a significant positive effect on the L_F and mass of 0+ fry
620 caught by electrofishing, whereas no egg size effect was found for electrofished 1+parr
621 (Appendix 3), consistent with previous findings in salmonids that egg size effects tend to
622 attenuate with offspring age (Heath *et al.*, 1999). However, positive effects of egg size on the
623 representation (i.e. survival) of both 0+ and 1+ electrofished parr were also found (Appendix
624 2, supplementary figure 1). Future salmonid studies that disentangle maternal genetic and

environmental effects from additive genetic effects in wild stream environments would be very revealing.

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References

Bates D, Maechler M, Bolker B (2012). lme4: Linear mixed-effects models using S4 classes.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, *et al.* (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* **24**: 127–135.

645 Bourret V, Kent MP, Primmer CR, Vasemägi A, Karlsson S, Hindar K, *et al.* (2013). SNP-
646 array reveals genome-wide patterns of geographical and potential adaptive divergence
647 across the natural range of Atlantic salmon (Salmo salar). *Mol Ecol* **22**: 532–551.

648 Carlson SM, Seamons TR (2008). A review of quantitative genetic components of fitness in
649 salmonids: implications for adaptation to future change. *Evol Appl* **1**: 222–238.

650 Charmantier A, Garant D (2005). Environmental quality and evolutionary potential: lessons
651 from wild populations. *Proc R Soc B Biol Sci* **272**: 1415–1425.

652 Clifford SL, McGinnity P, Ferguson A (1998). Genetic changes in an Atlantic salmon
653 population resulting from escaped juvenile farm salmon. *J Fish Biol* **52**: 118–127.

654 Debes PV, Fraser DJ, Yates M, Hutchings JA (2014). The between-population genetic
655 architecture of growth, maturation, and plasticity in Atlantic Salmon. *Genetics* **196**:
656 1277–1291.

657 Debes PV, Hutchings JA (2014). Effects of domestication on parr maturity, growth, and
658 vulnerability to predation in Atlantic Salmon. *Can J Fish Aquat Sci*.

659 Dionne Mel, Caron F, Dodson JJ, Bernatchez L (2008). Landscape genetics and hierarchical
660 genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation.
661 *Mol Ecol* **17**: 2382–2396.

662 Donaghy MJ, Verspoor E (2000). A new design of instream incubator for planting out and
663 monitoring Atlantic salmon eggs. *North Am J Fish Manag* **20**: 521–527.

664 Einum S, Fleming IA (1997). Genetic divergence and interactions in the wild among native,
665 farmed and hybrid Atlantic salmon. *J Fish Biol* **50**: 634–651.

666 Einum S, Fleming IA (1999). Maternal effects of egg size in brown trout (*Salmo trutta*):
667 norms of reaction to environmental quality. *Proc R Soc Lond B Biol Sci* **266**: 2095–
668 2100.

669 Einum S, Fleming IA (2000). Selection against late emergence and small offspring in
670 Atlantic salmon (*Salmo salar*). *Evolution* **54**: 628–639.

671 Fiske P, Lund RA, Hansen LP (2006). Relationships between the frequency of farmed
672 Atlantic salmon, *Salmo salar* L., in wild salmon populations and fish farming activity
673 in Norway, 1989–2004. *ICES J Mar Sci J Cons* **63**: 1182–1189.

674 Fleming IA, Agustsson T, Finstad B, Johnsson JI, Björnsson BT (2002). Effects of
675 domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo*
676 *salar*). *Can J Fish Aquat Sci* **59**: 1323–1330.

677 Fleming IA, Hindar K, MjÖlnerÖd IB, Jonsson B, Balstad T, Lamberg A (2000). Lifetime
678 success and interactions of farm salmon invading a native population. *Proc R Soc*
679 *Lond B Biol Sci* **267**: 1517–1523.

680 Fleming IA, Jonsson B, Gross MR, Lamberg A (1996). An experimental study of the
681 reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo*
682 *salar*). *J Appl Ecol*: 893–905.

683 Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011). Extent and scale of local
684 adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106**: 404–420.

685 Garant D, Fleming IA, Einum S, Bernatchez L (2003). Alternative male life-history tactics as
686 potential vehicles for speeding introgression of farm salmon traits into wild
687 populations. *Ecol Lett* **6**: 541–549.

688 Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, *et al.*
689 (2007). A critical review of adaptive genetic variation in Atlantic salmon:
690 implications for conservation. *Biol Rev* **82**: 173–211.

691 Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquac res*, 31: 25-33.

692 Gjøen HM, Bentsen HB (1997). Past, present, and future of genetic improvement in salmon
693 aquaculture. *ICES J Mar Sci J Cons* **54**: 1009–1014.

694 Glover KA, Otterå H, Olsen RE, Slinde E, Taranger G.L, Skaala Ø (2009). A comparison of
695 farmed, wild and hybrid Atlantic salmon (*Salmo salar* L.) reared under farming
696 conditions. *Aquaculture*, 286: 203-210.

697 Glover KA, Quintela M, Wennevik V, Besnier F, Sørvik AG, Skaala Ø (2012). Three
698 decades of farmed escapees in the wild: a spatio-temporal analysis of Atlantic salmon
699 population genetic structure throughout Norway. *PLoS One* **7**: e43129.

700 Glover KA, Pertoldi C, Besnier F, Wennevik V, Kent M, Skaala Ø (2013). Atlantic salmon
701 populations invaded by farmed escapees: quantifying genetic introgression with a
702 Bayesian approach and SNPs. *BMC Genet* 14:74.

703 Hadfield JD (2010). MCMC methods for multi-response generalized linear mixed models:
704 the MCMCglmm R package. *J Stat Softw* **33**: 1–22.

705 Hansen LP, Jacobsen JA, Lund RA (1999). The incidence of escaped farmed Atlantic
706 salmon, *Salmo salar* L., in the Faroese fishery and estimates of catches of wild
707 salmon. *ICES J Mar Sci J Cons* **56**: 200–206.

708 Heath DD, Fox CW, Heath JW (1999). Maternal effects on offspring size: variation through
709 early development of chinook salmon. *Evolution*: 1605–1611.

Houde ALS, Fraser DJ, Hutchings JA (2010). Reduced anti-predator responses in multi-generational hybrids of farmed and wild Atlantic salmon (*Salmo salar* L.). *Conserv Genet* **11**: 785–794.

ICES (2010). Report of the ICES Advisory Committee, Book 10: North Atlantic Salmon Stocks, as reported to the North Atlantic Salmon Conservation Organization.

Kruuk LE (2004). Estimating genetic parameters in natural populations using the 'animal model'. *Philos Trans R Soc Lond B Biol Sci* **359**: 873–890.

Letcher BH, Coombs JA, Nislow KH (2011). Maintenance of phenotypic variation: repeatability, heritability and size-dependent processes in a wild brook trout population. *Evol Appl* **4**: 602–615.

Lynch M, O'Hely M (2001). Captive breeding and the genetic fitness of natural populations. *Conserv Genet* **2**: 363–378.

Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Incorporated.

McGinnity P, Prodöhl P, Ferguson A, Hynes R, ó Maoiléidigh N, Baker N, *et al.* (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. *Proc R Soc Lond B Biol Sci* **270**: 2443–2450.

McGinnity P, Stone C, Taggart JB, Cooke D, Cotter D, Hynes R, *et al.* (1997). Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES J Mar Sci J Cons* **54**: 998–1008.

732 Naylor R, Hindar K, Fleming IA, Goldburg R, Williams S, Volpe J, *et al.* (2005). Fugitive
733 salmon: assessing the risks of escaped fish from net-pen aquaculture. *BioScience* **55**:
734 427–437.

735 Norris AT, Bradley DG, Cunningham EP (1999). Microsatellite genetic variation between
736 and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture*
737 **180**: 247–264.

738 Saura M, Moran P, Brotherstone S, Caballero A, Alvarez J, Villanueva B (2010). Predictions
739 of response to selection caused by angling in a wild population of Atlantic salmon
740 (*Salmo salar*). *Freshw Biol* **55**: 923–930.

741 Serbezov D, Bernatchez L, Olsen EM, Vøllestad LA (2010). Quantitative genetic parameters
742 for wild stream-living brown trout: heritability and parental effects. *J Evol Biol* **23**:
743 1631–1641.

744 Skaala Ø., Høyheim B., Glover K., & Dahle G. (2004). Microsatellite analysis in
745 domesticated and wild Atlantic salmon (*Salmo salar* L.): allelic diversity and
746 identification of individuals. *Aquaculture*, **240**: 131-143.Skaala Ø, Wennevik V,
747 Glover KA (2006). Evidence of temporal genetic change in wild Atlantic salmon,
748 *Salmo salar* L., populations affected by farm escapees. *ICES J Mar Sci* **63**:1224-1233.

749 Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, *et al.* (2012).
750 Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a
751 natural river environment. *Can J Fish Aquat Sci* **69**: 1994–2006.

752 Solberg MF, Skaala Ø, Nilsen F, Glover KA (2013). Does domestication cause changes in
753 growth reaction norms? A study of farmed, wild and hybrid Atlantic Salmon families
754 exposed to environmental stress. *PloS One* **8**: e54469.

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, Fjelldal PG, Nilsen F, Glover KA (2014). Hatching time and alevin growth prior to the onset of exogenous feeding in farmed, wild and hybrid Norwegian Atlantic salmon. *PloS one* 9: e113697.

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009). *Mixed effects models and extensions in ecology with R*. Springer.

Figure 1

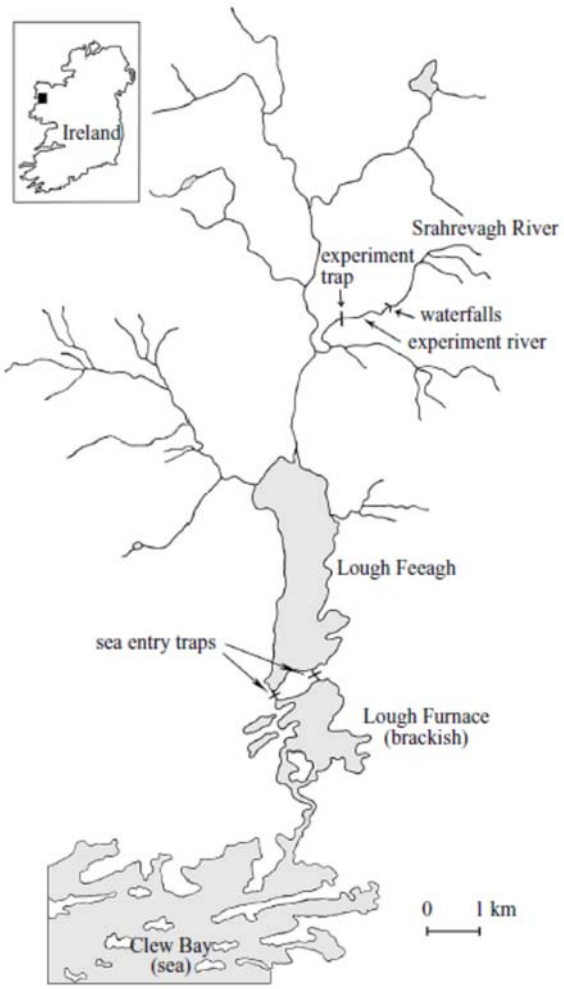

Fig.1 Map of the Burrishoole river system showing location of experiment river, experiment-trap and sea-entry traps.

Figure 2

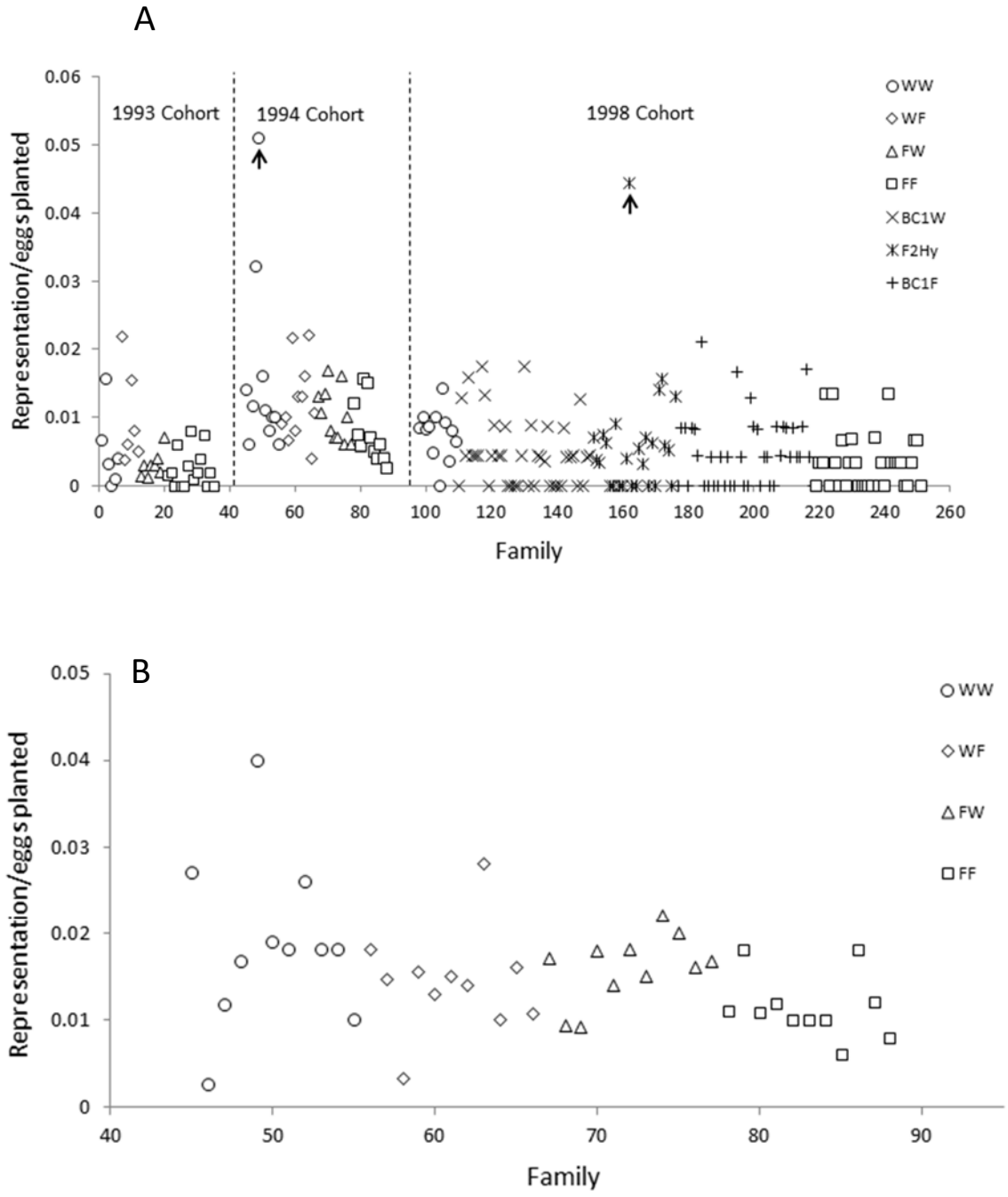

Fig.2 (a) Representation of 0+ parr in the August electrofishing samples for the 1993, 1994 and 1998 cohorts, scaled by the number of eyed-eggs planted per family. (b) Representation (scaled by eggs planted) of 1+ parr in the June 1995 electrofishing sample for the 1994 cohort. Families are labelled arbitrarily in each panel and family labels for 1994 cohort correspond between (a) and (b). Arrows indicate outlier families.

Figure 3

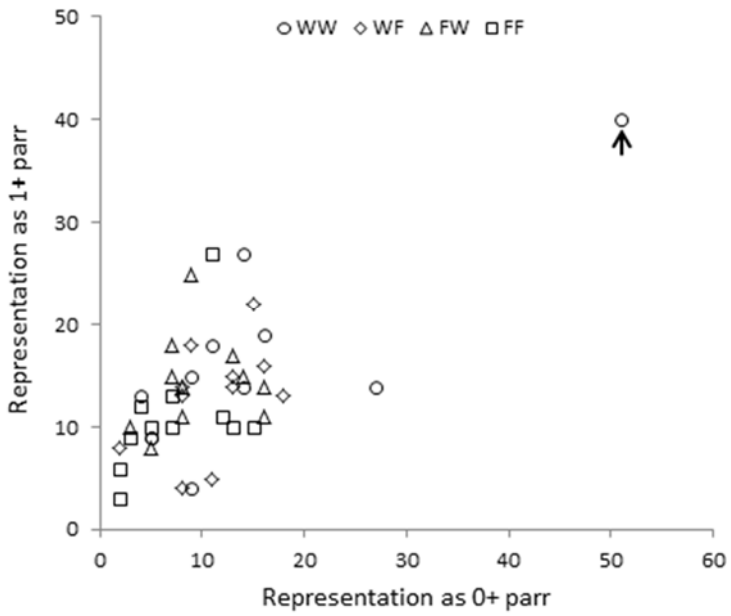

Fig.3 Representation of 1+ parr in the June 1995 electrofishing sample plotted against representation of 0+ parr in the August 1994 electrofishing sample (1994 cohort). Each data point is a family. The outlier family indicated with an arrow is family 49, which corresponds to the same outlier family identified in Fig. 2A.

Figure 4

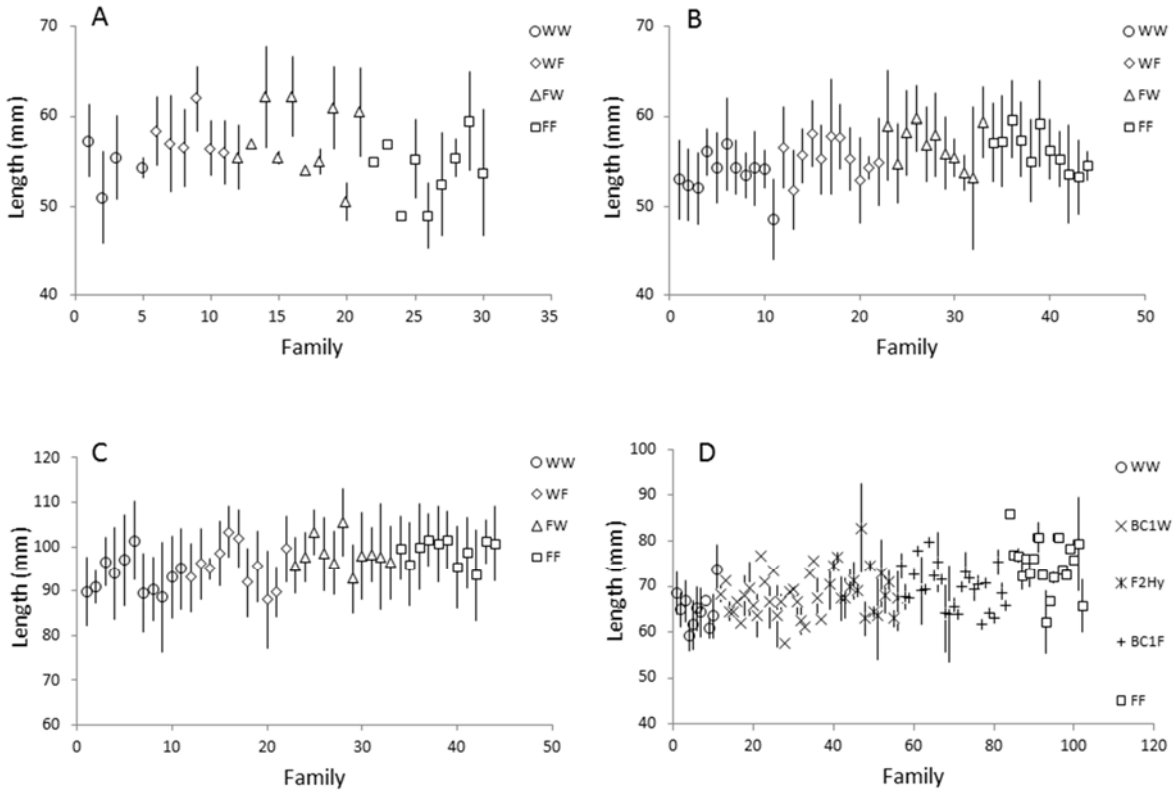

Fig.4 Fork length of (a) 0+ parr in August 1993 electrofishing sample (1993 cohort), (b) 0+ parr in August 1994 electrofishing sample (1994 cohort) (c) 1+ parr in June 1995 electrofishing sample (1994 cohort), and (d) 0+ parr in August 1998 electrofishing sample (1998 cohort). Error bars are standard deviations around the mean per family.

Figure 5

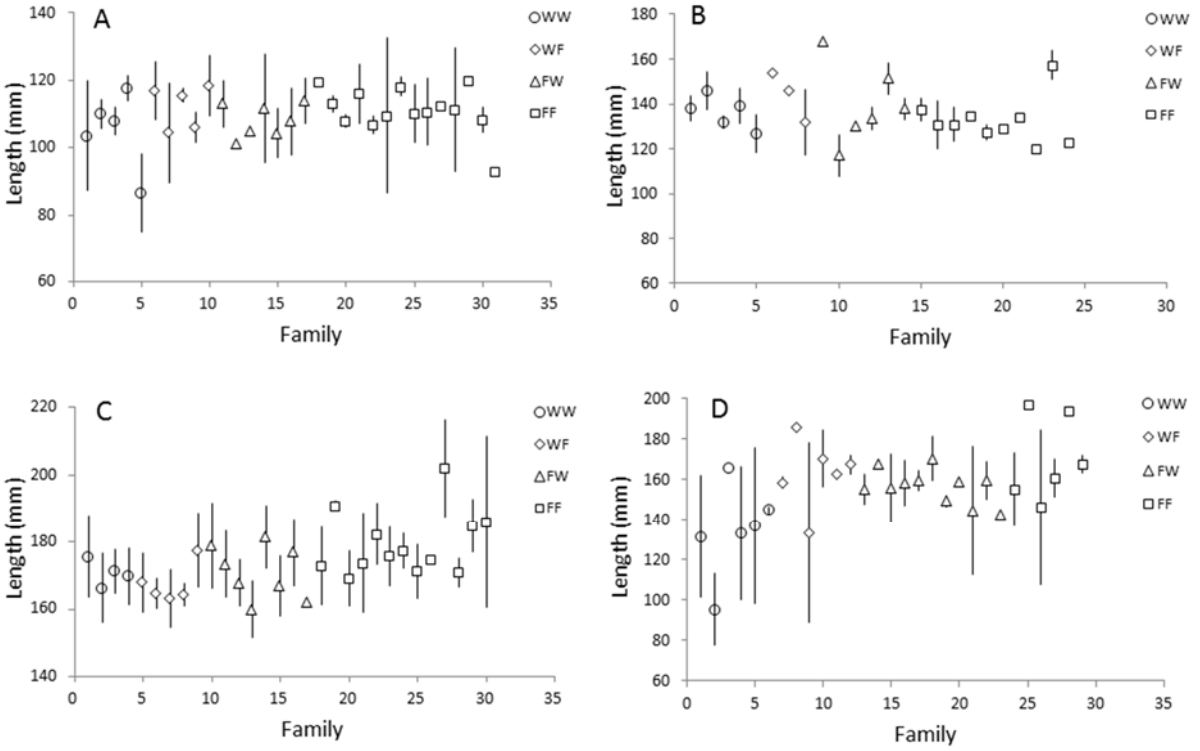

Fig.5 Fork length of (a) 0+ parr in August 1993 hatchery control sample (1993 cohort), (b) mature male parr in November 1993 hatchery control sample (1993 cohort) (c) pre-smolts in March 1994 hatchery control sample (1993 cohort), and (d) pre-smolts in March 1995 hatchery control sample (1994 cohort). Error bars are standard deviations around the mean per family.

Table 1 Experimental groups of Atlantic salmon in the 1993, 1994 and 1998 cohorts. Number of eggs = number of eyed-eggs planted out in the experiment river. Final column gives the expected percentage of farm genes per group. D = dam. S = sire.

Cohort	Group	Group code	No. dams	No. sires	No. families	No. eggs	Mean egg size (mm ± SD)	% Farm genes
1993	Wild D x Wild S	WW_93	6	6	6	5273	0.60 (0.04)	0
	Wild D x Farm S	WF_93	6	6	6	5886	0.60 (0.04)	50
	Farm D x Wild S	FW_93	8	8	8	8659	0.61 (0.03)	50
	Farm D x Farm S	FF_93	15	15	15	14997	0.61 (0.04)	100
1994	Wild D x Wild S	WW_94	11	11	11	10537	0.61 (0.04)	0
	Wild D x Farm S	WF_94	11	11	11	10537	0.61 (0.04)	50
	Farm D x Wild S	FW_94	11	11	11	10537	0.64 (0.05)	50
	Farm D x Farm S	FF_94	11	11	11	10537	0.64 (0.05)	100
1998	Wild D x Wild S	WW_98	4	5	12	8787	0.61 (0.02)	0
	F_1 hybrid x Wild	BC_1W_98	14	5	41	9549	0.61 (0.02)	25
	F_1 hybrid x F_1 hybrid	F_2Hy_98	14	2	26	8337	0.61 (0.02)	50
	F_1 hybrid x Farm	BC_1F_98	14	5	42	9928	0.60 (0.03)	75
	Farm D x Farm S	FF_98	7	5	33	9832	0.61 (0.02)	100

Table 2 Mean representation (number of fish in the sample divided by the initial number of eggs planted out/retained in hatchery) and mean fork length (L_F) for each group for the 1993, 1994 and 1998 cohorts. Also shown in parentheses are the means expressed relative to (divided by) the means for the WW reference group, and whether groups differ significantly (based on the GLMM for representation and the LMM for L_F, both of which control for egg size variation) from the WW group: *P* value bands: †: 0.1 - 0.05; * 0.05 – 0.01; ** 0.01 – 0.001; *** <0.001.

Cohort	Sample	Representation					Fork length (L_F)				
		WW	*WF*	*FW*	*FF*		*WW*	*WF*	*FW*	*FF*	
1993	Electrofishing Aug 1993 0+parr	0.005 (1.00)	0.01* (1.97)	0.003 (0.61)	0.002 (0.49)		54.50 ± 0.96 (1.00)	57.43 ± 0.58* (1.05)	54.78 ± 1.00 (1.01)	59.24 ± 0.92** (1.09)	
1994	Electrofishing Aug 1994 0+parr	0.016 (1.00)	0.012† (0.76)	0.010*** (0.65)	0.008*** (0.48)		54.13 ± 0.33 (1.00)	55.67 ± 0.43* (1.03)	56.89 ± 0.43 * (1.05)	57.41 ± 0.52** (1.06)	
1994	Electrofishing Jun 1995 1+parr	0.019 (1.00)	0.014 (0.74)	0.016 (0.84)	0.011** (0.58)		94.02 ± 0.71 (1.00)	96.46 ± 0.69 (1.03)	98.09 ± 0.66* (1.04)	98.69 ± 0.78** (1.05)	
1993	Trapped parr May 1993-May 1994	0.017 (1.00)	0.014 (0.82)	0.008** (0.47)	0.005*** (0.29)						
1993	Trapped pre-smolts + smolts Sep 1994-Apr 1995	0.007 (1.00)	0.006 (0.86)	0.003† (0.43)	0.003† (0.43)						
1994	Trapped parr May 1994-May 1995	0.023 (1.00)	0.011** (0.48)	0.012** (0.52)	0.005*** (0.22)						
1994	Trapped pre-smolts + smolts Sep 1995-Apr 1996	0.003 (1.00)	0.002 (0.67)	0.003 (1.00)	0.002 (0.67)						
1993	Hatchery controls 0+parr Aug 1993	0.013 (1.00)	0.012 (0.92)	0.009 (0.69)	0.01 (0.77)		106.14 ± 2.72 (1.00)	114.09 ± 2.23* (1.07)	109.64 ± 2.28 (1.03)	111.41 ± 1.54* (1.05)	
1993	Hatchery controls mature 0+parr Nov 1993	0.018 (1.00)	0.006* (0.33)	0.011 (0.61)	0.005* (0.28)		133.78 ± 1.77 (1.00)	135.89 ± 5.07 (1.02)	137.81 ± 3.18 (1.03)	133.47 ± 2.48 (0.99)	
1993	Hatchery controls smolts Mar 1994	0.023 (1.00)	0.013 (0.57)	0.019 (0.83)	0.019 (0.83)		170.35 ± 1.72 (1.00)	168.16 ± 2.03 (0.99)	173.21 ± 1.73 (1.02)	178.56 ± 1.68* (1.05)	
1994	Hatchery controls smolts Mar 1995	0.015 (1.00)	0.014 (0.93)	0.013 (0.87)	0.014 (0.93)		136.34 ± 5.29 (1.00)	162.80 ± 2.44*** (1.19)	156.14 ± 3.11** (1.15)	170.20 ± 3.61*** (1.25)	
		WW	*BC₁W*	*F₂Hy*	*BC₁F*	*FF*	*WW*	*BC₁W*	*F₂Hy*	*BC₁F*	*FF*
1998	Electrofishing Aug 1998 0+parr	0.008 (1.00)	0.005* (0.65)	0.006† (0.81)	0.005† (0.66)	0.003*** (0.44)	64.60 ± 0.61 (1.00)	66.91 ± 0.63 (1.04)	69.99 ± 0.91* (1.08)	70.49 ± 0.73** (1.09)	73.77 ± 0.74*** (1.14)
1998	Trapped parr May 1998-May 1999	0.011 (1.00)	0.007* (0.64)	0.002*** (0.18)	0.001*** (0.09)	0.001*** (0.09)					
1998	Trapped pre-smolts + smolts 1998 cohort	0.003 (1.00)	0.003 (1.00)	0.005 (1.67)	0.004 (1.33)	0.003 (1.00)					

Table 3 Quantitative genetic parameter estimates for size-at-age traits based on bivariate Bayesian animal models. EF = electrofished. WW = pure wild group. FF = pure farm group. L_F = fork length. V_P = raw phenotypic variance. V_A = additive genetic variance. h^2 = narrow sense heritability. r_P = raw phenotypic correlation between L_F and mass. r_G = additive genetic correlation between L_F and mass. r_E = residual correlation between L_F and mass. For V_A, h^2, r_G and r_E, estimates are posterior modes, with credible intervals in parentheses. L_F and mass were natural log-transformed in all models.

Cohort	Variable and sample	Group	V_P	V_A	h^2	r_P	r_G	r_E
1994	EF Aug 1994 0+parr L_F	WW	6.2×10^{-3}	1.8×10^{-3} ($0.3\text{-}8.1\times10^{-3}$)	0.29 (0.11-0.89)	0.95	0.96 (0.73-0.98)	0.94 (0.78-0.97)
1994	EF Aug 1994 0+parr mass	WW	0.065	0.019 (0.005- 0.085)	0.53 (0.15-0.94)	-	-	-
1994	EF Aug 1994 0+parr L_F	FF	6.7×10^{-3}	6.0×10^{-4} ($0.2\text{-}5.7\times10^{-3}$)	0.15 (0.03-0.65)	0.94	0.90 (0.27-0.97)	0.06 (0.03-0.09)
1994	EF Aug 1994 0+parr mass	FF	0.069	0.008 (0.002- 0.060)	0.11 (0.03-0.63)	-	-	-
1994	EF Jun 1995 1+parr L_F	WW	0.01	0.002 (0.001-0.01)	0.21 (0.07-0.75)	0.98	0.95 (0.82-0.99)	0.97 (0.94-0.98)
1994	EF Jun 1995 1+parr mass	WW	0.09	0.019 (0.005-0.091)	0.20 (0.09-0.77)	-	-	-
1994	EF Jun 1995 1+parr L_F	FF	0.008	7.0×10^{-4} ($0.2\text{-}4.2\times10^{-3}$)	0.10 (0.03-0.44)	0.97	0.82 (0.49-0.98)	0.97 (0.95-0.98)
1994	EF Jun 1995 1+parr mass	FF	0.06	0.008 (0.002-0.033)	0.08 (0.03-0.43)	-	-	-
1998	EF Aug 1998 0+parr L_F	WW	0.006	0.004 (0.001-0.009)	0.89 (0.23-0.96)	0.89	0.92 (0.47-0.97)	0.56 (0.22-0.96)
1998	EF Aug 1998 0+parr mass	WW	0.069	0.025 (0.006-0.105)	0.39 (0.16-0.095)	-	-	-
1998	EF Aug 1998 0+parr L_F	FF	0.007	0.002 (0.001-0.009)	0.31 (0.04-0.86)	0.93	0.90 (0.38-0.98)	0.93 (0.71-0.98)
1998	EF Aug 1998 0+parr mass	FF	0.065	0.009 (0.003-0.084)	0.17 (0.05-0.86)	-	-	-