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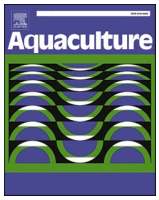

Impact of freshwater rearing on saltwater performance: A genotype-environment interaction study in Atlantic Salmon (*Salmo salar*)

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ABSTRACT

Atlantic salmon, *Salmo salar,* have traditionally been reared in net-pens in freshwater (FW) lochs up to smoltification, with subsequent transfer to saltwater (SW) cages for grow-out. Recently, interest in recirculating aquaculture systems (RAS) has grown due to environmental and husbandry benefits. To investigate the impact of RAS on their production cycle, we conducted an experiment under commercial conditions, raising a group of salmon in either a FW-RAS or -loch system. The study evaluated the effects of FW-rearing on SW performance by investigating phenotypic performance, genetic architecture, and genotype-environment interactions (GxE), which describe how the effects of different genotypes on traits change with environmental variation, potentially impacting performance across systems. We co-reared salmon for approximately nine-months before splitting them: half remained in FW-RAS and half transferred to FW-loch, where they were separated for about eight weeks. Both groups were then transferred to a SW cage-site. We sampled fish at the end of FW-rearing as smolts and three-months post-SW transfer as post-smolts, taking fin clips for genotyping. Results indicate that RAS-reared smolts were smaller in FW but demonstrated enhanced growth and lower trait variance post-transfer. Sexually dimorphic growth was observed in the loch population. Heritability of morphological traits increased post-SW transfer in the loch population but decreased in RAS. GxE for SW morphological traits were minimal, though significant genotype re-ranking was observed for SW growth. Genetic correlations between FW and SW morphological traits were high, except for whole-body weight in the loch population. These findings indicate that RAS-origin post-smolts, despite smaller FW size, showed faster growth and reduced phenotypic variance in SW compared to loch-origin fish. Differences in heritability estimates and genotype re-ranking for SW growth suggest that breeding programs may need to refine selection strategies for varied rearing environments.

1. Introduction

Atlantic salmon, *Salmo salar* (L. 1758), is a species whose aquaculture is important economically both globally, as well as more locally within the UK where the predominance occurs in Scotland (FAO, 2023; Regan et al., 2021). Traditionally, commercial production has involved rearing fish in hatcheries before transfer to FW net-pens for smoltification (Clarke and Bostock, 2017; Ellis et al., 2016). This is a key stage for the development where fish acquire morphological, physiological, and behavioural adaptations that facilitate their anadromous life cycle (McCormick, 2012; Stefansson et al., 2008). After this stage, post-smolts are typically transferred to SW cages for a grow-out period until they

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reach harvest size at ~3-5 kg (Ellis et al., 2016). However, within the industry, there is an increasing trend to shift away from the use of FW lochs to land-based systems, such as RAS (Bergheim et al., 2009; Ebeling and Timmons, 2012).

One concern to implementing RAS for salmons FW development is that the environment provided differs from other culture environments, potentially impacting their performance once transferred to SW for grow-out. For example, it has been suggested that use of RAS affects smolts development in FW, with physiological and molecular differences observed in these fish relative to those reared in alternative culture systems (e.g. Roque d'Orbcastel et al., 2009; Kolarevic et al., 2014; van Rijn et al., 2020; Lai et al., 2024). These differences have been thought to impact fish's osmoregulatory ability once transferred to the SW environment. Alternatively, Lai et al. (2024) suggested that the relative stability of this environment does not appropriately prepare smolts for the heterogeneous environment of SW cages. As a potential result of these factors, poorer performance in SW of RAS-reared fish has been observed.

The impact of the FW environment on SW performance can be studied by identification of GxE which forms when the genotypic performance is environmentally sensitive, causing potential re-ranking of families across environments (Falconer, 1952; Falconer and Mackay, 1996; Mulder et al., 2006; Sae-Lim et al., 2016). This is often quantified as the genetic correlation -which indicates how much two traits are underpinned by the same genes for the same trait measured in the same population but across distinct environments (Mulder et al., 2006). Importantly, where this value falls below 0.7, selective gains may not be realised across environments, and use of environment-specific breeding programs could be of benefit (Robertson, 1959; Mulder and Bijma, 2005; Sae-Lim et al., 2013).

For a commercial population, the impact of FW-RAS rearing relative to that in an ambient loch system on Atlantic salmon smolt morphometric traits, as well as their genetic architecture and GxE, was previously investigated in Tollervey et al. (2024). Following the same population at three-months post-SW transfer, this study aims to investigate if FW-rearing impacts SW performance. The objectives include (1) evaluating the impact of FW-origin on SW performance, (2) investigating the genetic architecture and GxE interactions underlying SW traits, and (3) to assess how well FW performance predicts SW outcomes through GxE quantification.

2. Materials and methods

2.1. Ethical approval

Animal handling and collection in this study was carried out following the UK Animals (Scientific Procedures) Act 1986 Amended Regulations (SI2012/3039) and the work was approved by the University of Stirling Ethics Committee (Animal Welfare and Ethics Review Board; AWERB202148713775). This project was also part of a larger BBSRC/NERC funded project, ROBUSTSMOLT, which also received ethical approval (AWERB/1819/063/New ASPA).

2.2. Experimental design

This study continues from that presented in Tollervey et al. (2024), where more detailed information on the study population and FW-rearing can be found. For a visual representation of experimental design, see Supplementary Fig. A.1.

In brief, the study population originated from the broodstock of the Mowi Ireland 2022 Generation (i.e. generation put out to sea in 2022). As the objectives of the study was to evaluate the impact of FW-rearing systems on SW performance under standard commercial aquaculture conditions, as implemented by the industry partners, all data were collected observationally without modifying or intervening in the farms' established operational procedures. Approximately 250,000 eyed eggs were co-reared in RAS in the northwest of Scotland for ~nine-months. During this time parr underwent re-distribution amongst tanks several times and ~10 % bottom cull. After this, the population was phenotypically sexed using ultrasound. Approximately half (91,708 fish) of the remaining population was transferred to floating net-pens in a FW-loch for smoltification, with the other half (91,713 fish) remaining in RAS. Phenotypically male and female fish were separately reared in both FW environments. In the loch, all males and females were reared in a single net-pen each, whereas in RAS phenotypic sexes were split across three tanks each. Fish were stocked at ~306 and ~22 fish/m^3 in RAS and loch respectively. Mean temperature was 13.21 °C in RAS and 10.86 °C in loch. In RAS, parr were initially held under 12 h light/darkness (LND) cycles before exposure to continuous light (LL) for approximately one month. Both populations were fed Mowi Proteus diets.

After ~eight weeks in their FW environments, both populations - RAS and loch net-pen - were transferred to the SW site for grow-out. Due to biomass limitations at the SW site, only fish from phenotypically female tanks/pen in the RAS and loch populations could be maintained separately. Specifically, the phenotypically male fish from both FW environments were transferred and pooled into the same cage. On the same day, the female pen from the loch environment was transferred to a single cage. The following week, all three female tanks from the RAS site were pooled and transferred to a single cage. The three populations of fish were housed in SW cages, each 120 m wide, 16 m deep, and 18,000 m^3 in volume. In total, 44,908 females were transferred from RAS, and 31,384 from loch environment, corresponding to a density of ~2.49 and ~1.74 fish/m^3 for RAS and loch populations at the sea-cage site, respectively.

Fish in SW were exposed to the same husbandry conditions, were initially fed NEPTUNE 50 adapt (from SW transfer for approximately 50 days) before transfer to NEPTUNE 200 adapt diet; and underwent a sea lice treatment using BioMar feed (Medi Adapt smolt LR 75, 3 mm pellet). The number of mortalities or moribund fish removed from each pen was recorded from approximately the day of transfer until the onset of SW sampling by Mowi site staff. Biomass limitation required the female population be pooled prior to harvest. Post-SW transfer, temperature (°C), salinity (‰), and water clarity were recorded daily. For each, the average and standard deviation (s.d) was measured across the study period.

2.3. Sample collection

The population was sampled twice, at the end of FW-rearing (referred to as 'smolts'), and approximately three-months post-SW transfer (referred to as 'post-smolts'). To distinguish between husbandry environments, fish sampled in FW as smolts will be referred to as RAS- or loch-reared, respectively. Correspondingly, fish sampled in SW as post-smolts will be referred to as RAS- or loch-origin. At both sampling points, 1000 fish per FW environment (RAS and loch) were sampled. Sampling occurred by random netting of fish from tanks/pens in FW and cages at SW. In the latter fish were attracted to the surface for sampling with feed. At both sampling points, measurements of whole-body weight (WBW) and length (tip of the head (snout) to the deepest point of the fork in the caudal fin) (cm) were taken. Fin clips were taken from adipose fin and stored in 2 ml of 95 % ethanol.

2.4. Genotyping, pedigree reconstruction, and population structure

The parental broodstock was previously genotyped by Mowi collaborators (non-public Axiom array, 97 NOFSAL03). FW and SW offspring were genotyped by IdentiGEN Ltd. (non-public Axiom array, SALMOWI). Single nucleotide polymorphisms (SNPs) were called based on major allele frequency with Applied Biosystems – Analysis Power Tools (APT) v2.11.6 (ThermoFisher Scientific, 2024). Genotypic sex was assigned based on sex-determining region Y gene expression. Pedigree reconstruction and family assignment were performed by Mowi using

the sequenced genotypes and their in-house software which employs an opposite homozygosity method (as reported in Tollervey et al., 2024). Fish which failed to be assigned to sire, dam, or both were removed from the analysis.

Parental and FW-SW offspring (combined samples from FW and SW) separately underwent quality control (QC) using PLINK v1.9 (Purcell et al., 2007) as in Tollervey et al. (2024). A list of common SNPs to both the parental and FW-SW offspring populations was then gathered from the overlap in the online Venn Diagrams resource at BioTools.Fr (https://www.biotools.fr/misc/venny). This list was used to merge the two populations in PLINK v1.9 (Purcell et al., 2007), which was formatted into .txt file using *genio* package (Ochoa, 2023) in R v4.2.2 (R Core Team, 2023).

The genomic relationship matrix (GRM) between all individuals was computed using GCTA software (Yang et al., 2011). The first two components of principal component analysis (PCA) on the GRM were visualised separately for FW and SW offspring using *ggplot* package (v3.4.2) in R v4.2.2 (R Core Team, 2023) to assess the presence of any population structure. The number of full and half-sib (sire and dam families) were investigated per location (FW and SW) and FW-origin (RAS and loch), with only those common across all four populations (i.e. FW-RAS, FW-loch, SW-RAS and SW-loch) retained for analysis.

2.5. Morphological traits

Of the fish sampled at the FW stage, only those in pens maintained separately by FW-origin once transferred to SW were retained for further analysis (i.e. phenotypically female populations). Samples with missing phenotypic data were removed. The FW WBW (WBW_{FW}) and length ($length_{FW}$), and SW WBW (WBW_{SW}) and length ($length_{SW}$) were recorded. Condition factor (K) at both time points (K_{FW} and K_{SW}) was also calculated following Fulton (1904), as

$$K = 100^{*}\left(WBW_{grams}/L_{cm}^{3}\right). \quad (1)$$

2.6. Growth traits

For the SW population, average daily gain (ADG), measured in a change in trait value per day (Δ/day) was calculated as

$$(X_F - X_I)/\text{days in SW}, \quad (2)$$

where X_F is the final trait value measured in SW and X_I is the initial trait value measured at the end of the FW-rearing period (Crane et al., 2020). Also, specific growth rate (SGR) was calculated as

$$100^{*}e^{((\log(X_F)-(\log(X_I))/\text{days in SW})-1}. \quad (3)$$

Units were measured in percent change in trait value per day (%Δ/day), with X_F and X_I as before, as in Crane et al. (2020). ADG and SGR were calculated for both WBW (ADG_{WBW} and ADG_{LENGTH}) and length (SGR_{WBW} and SGR_{LENGTH}). Of note, as fish were not tracked, the initial trait values (X_I) were based on the average family value at the end of FW rearing (i.e. WBW_{FW} and $length_{FW}$), calculated separately for the RAS and loch population. To allow methodology differences to be accounted for in statistical analysis, 'days in SW', the number of days between the date of SW transfer and SW sampling, as well as 'extra FW days', i.e. the number of days between the date of FW sampling and SW transfer (days since FW sampling-days in SW), were recorded (Supplementary Table S1).

2.7. Statistical analysis

All statistics were performed in R v4.2.2 (R Core Team, 2023). Mean and s.d were calculated for each trait in the SW population overall, as well as by FW environment and sex within each FW environment. The effect of FW environment, genotypic sex, and interaction between FW environment and sex on SW traits were investigated through 2-way-ANOVA (Type II sequential) with post hoc Tukey test (P-value $<$ 0.05). Validity of test assumptions was assessed visually via plotting a histogram as well as post-statistical testing q-q plots. Where test assumptions were invalid, non-parametric Kruskal–Wallis test was performed to separately assess the effect of FW environment and sex (P-value $<$ 0.05).

2.8. Quantitative analysis

Quantitative genetic analyses were performed using the BLUPF90 family of programs, due to their computational efficiency in handling large-scale mixed models and accommodation of complex pedigree and genomic information (Misztal et al., 2002).

2.8.1. Within environment genetic parameters

In the SW population, genetic parameters of traits were estimated separately for the loch- and RAS-origin populations from univariate animal mixed models (Eq. 1) fit via the use of a restricted maximum likelihood (REML) approach.

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \quad (4)$$

In this, **y** was a vector of trait values, **μ** the overall mean, **X** a design matrix linking individuals to a vector of fixed effects, **b**. **Z** was a design matrix linking individuals to a vector of additive genetic effects **u**, fitted using single-step genomic evaluation. In this, **u** has a multivariant normal distribution (MVN) with mean zero and variance V_g*$\mathbf{H}$ where V_g is additive genetic variance, * is the tensor product, and H is a matrix that combines information from both pedigree (**A** matrix) and SNP data (**GRM**), as defined in Legarra et al. (2014) ($u \sim MVN(0, V_g{}^{*}\mathbf{H})$). Lastly, **e** was a vector of residual effects with $\sim MVN(0, V_r{}^{*}\mathbf{I})$, where **I** was the identity matrix and V_r was residual variance. Full pedigree and SNP data were provided to each model but with only phenotypic data from environment-specific populations.

Depending on the population and trait of interest, fixed effects (**b**) changed. For the SW-RAS population growth traits genotypic sex (2 levels, 1 = male, 2 = female), SW days (3 levels, Supplementary Table S1) and extra FW days (2 levels, Supplementary Table S1) were included. For SW fish of loch-origin, **b** omitted extra FW days as only a single level existed within this population (Supplementary Table S1). For SW morphological traits (WBW_{SW}, $length_{SW}$, and K_{SW}) the initial FW trait value (i.e. X_I) was additionally fitted as a covariate.

For each trait, narrow sense heritability (h^2), coefficient of V_p (CV) and coefficient of V_g (CGV) were calculated as follows (Cheung, 2020; Falconer and Mackay, 1996):

$$h^2 = V_g/V_p, \quad (5)$$

$$CV = \left(SD_p/\overline{X}\right)^{*}100, \quad (6)$$

$$CGV = \left(\sqrt{V_g}/\overline{X}\right)^{*}100, \quad (7)$$

where V_p, $\overline{X}$, SD_p are the phenotypic variance, mean and s.d., respectively.

2.8.2. GxE

GxE was estimated using a multi-trait approach in which the same trait measured in different environments was treated as two independent traits (Mulder and Bijma, 2005; Sae-Lim et al., 2014). This was implemented by use of bi-variant animal mixed models,

$$\begin{pmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{pmatrix} = \begin{pmatrix}\mathbf{X_1}\ \mathbf{0}\\ \mathbf{0}\ \mathbf{X_2}\end{pmatrix}\begin{pmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{pmatrix} + \begin{pmatrix}\mathbf{Z_1}\ \mathbf{0}\\ \mathbf{0}\ \mathbf{Z_2}\end{pmatrix}\begin{pmatrix}\mathbf{u_1}\\ \mathbf{u_2}\end{pmatrix} + \begin{pmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{pmatrix} \quad (8)$$

fit via the use of a REML approach.

In this, **y1** and **y2** are the same trait in environment one and

environment two, respectively. Random and fixed effects followed that of the univariate analysis. Residual covariance was set to zero (Sae-Lim et al., 2016). The strength of GxE was quantified by the genetic correlation (r_{gxe}) between the two traits (Mulder and Bijma, 2005; Sae-Lim et al., 2014). Specifically, this approach was used to estimate r_{gxe} between (1) the same SW trait and measured in the RAS- and Loch-origin populations (SW GxE), and (2) between the same trait measured in populations of the same FW-environment, but between FW and SW time points (FW-SW GxE) (Fig. 1).

3. Results

3.1. SW environmental description

Environmental data of the post-SW transfer stage is shown in Supplementary Fig. A.2. The average SW temperature was 9.41 °C (s.d 1.26), clarity 7.55 (s.d 1.03) and salinity 34.42 ‰ (s.d 0.83). While salinity and clarity fluctuate around the mean, temperature decreased gradually with time from ~12 °C to 8 °C.

3.2. Population description, genotyping and QC

After transfer to SW, mortality was low with little difference seen between FW-origins, at 0.32 % for SW-RAS and 0.37 % for SW-loch populations. A total of 4000 fish were sampled in combined FW-SW offspring. Genotypic information was available for a total of 3919 individuals. In the parental population 254 individuals were genotyped. Raw parental and FW-SW offspring genotypes contained 55,357 SNPs, and 65,7745 SNPs, respectively, with 53,157 and 53,788 remaining after QC (Supplementary Table S2). There were 41,127 SNPs common to both (Supplementary Table S3, Supplementary Data S1). Based on filtered SNPs and GRM, there was little indication of population structure, with results from FW and SW populations highly similar (Supplementary Fig. A.3).

After QC, only FW smolts belonging to tanks which were separately maintained by FW-origin post-transfer were used in further analysis. This left 3429 of the FW-SW offspring. Pedigree re-construction identified 90 sire-, 163 dam-, and 166 full-sib families. Thirty-seven samples could not be assigned to either a sire or a dam, with a further 28 not assigned to a sire only. Across FW-RAS, FW-loch, SW-RAS and SW-loch populations, 124 full-sib families were shared. A full categorical breakdown of this remaining population can be seen in Table 1. For FW smolts, both average family size and its variance were similar in the RAS (5.24, s.d 2.73) and loch (5.24, s.d 2.94) populations. These values were approximately two fish lower than the family size of sampled post-smolts. Specifically, average family size and variance of the SW

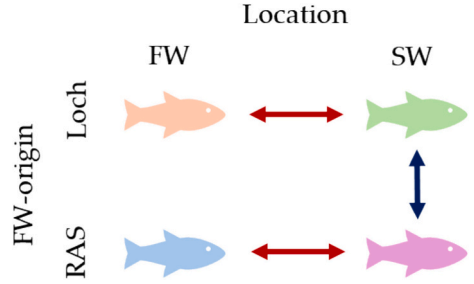

Fig. 1. Representation of GxE analysis performed. Four population across FW-origins and Locations as follows: orange is the FW-loch population, blue the FW-RAS population, green the FW-loch population once transferred to SW (i.e. SW-loch) and pink the FW-RAS population once transferred into SW (i.e. SW-RAS). Blue line indicates the populations between which SW GxE were calculated. Red lines indicate the populations between which FWSW GxE was calculated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
The number of fish of each genotypic sex and freshwater-rearing environment (RAS or loch) for both freshwater (FW) and saltwater (SW) populations.

	FW		SW	
	RAS	Loch	RAS	Loch
Male	432	172	324	262
Female	234	478	586	622

population were similar between RAS- (7.34, s.d 3.92) and loch- origin (7.13, s.d 4.17) families. Despite this, across all four populations, the total family size was highly variable, ranging from 5 to 47.

3.3. Phenotypic parameters

All SW traits approximated normality, except K_{SW} (Supplementary Figs. A.4–5).

3.3.1. SW morphological traits

Phenotypic and genetic parameters of SW post-smolts sampled can be seen in Table 2. On average SW post-smolts were 781.97 g (s.d 152.58 g) and 37.97 cm (s.d 2.04 cm), with a K_{SW} of 1.41 (s.d 0.13). CV % was 19.51 %, 5.37 %, and 9.33 %, for WBW_{SW}, $length_{SW}$, and K_{SW}, respectively. Fish of loch-origin were significantly heavier (degrees of freedom = 1/1790, F-value = 162.57, P-value < $2e^{-16}$) and longer (degrees of freedom = 1/1790, F-value = 204.45, P-value < $2e^{-16}$) than RAS counterparts (Table 2, Fig. 2, Supplementary Data S2, and Supplementary Fig. A.4).

Sex (WBW_{SW}: degrees of freedom = 1/1790, F-value = 16.07 P-value = $6.36e^{-5}$, $length_{SW}$: degrees of freedom = 1/1790, F-value = 13.25, P-value = $2.81e^{-4}$) and sex by FW environment (WBW_{SW}: degrees of freedom = 1/1790, F-value = 17.87, P-value = $2.48e^{-4}$, $length_{SW}$: degrees of freedom = 1/1790, F-value = 22.38, P-value = 2.42^{-6}) had a significant effect on WBW_{SW} and $length_{SW}$ (Fig. 2). Specifically, female fish were on average 29.19 g (s.d 14.31 g) lighter and 0.35 cm (s.d 0.19) shorter than males. Within environments, there was only a significant difference between the sexes when measured in the loch environment (Fig. 2). Neither FW environment (degrees of freedom = 1, Chi-squared = 2.57, P-value = 0.11) nor sex (degrees of freedom = 1, Chi-squared = 3.21 P-value = 0.07) had a significant effect on K_{SW}. Morphological traits, except for K_{SW}, were less variable in RAS-origin post-smolts (Table 2).

3.3.2. SW growth traits

Estimates of post-smolt growth rate over the first three-months in SW, as well as their genetic parameters, can be seen in Table 3. On average, growth rate was 1.88 g/day (s.d 0.26 g/day), 0.59 cm/day (s.d $8.25e^{-2}$ cm/day), 6.18 %g/day (1.29 %g/day) and 0.16 %cm/day (s.d $2.08e^{-2}$%cm/day) for ADG_{WBW}, ADG_{LENGTH}, SGR_{WBW}, and SGR_{LENGTH} respectively. The CV was 13.98 %, 14.01 %, 20.88 %, and 12.68 % for ADG_{WBW}, ADG_{LENGTH}, SGR_{WBW}, and SGR_{LENGTH}, respectively. ADG_{WBW} was not significantly affected by FW-origin (degrees of freedom = 1/1790, F-value = 0.066, P = 0.80). However, both sex (degrees of freedom = 1/1790, F-value = 12.02, P = $5.38e^{-4}$) and sex by environment interaction (degrees of freedom = 1/1790, F-value = 14.79, P = $1.24e^{-4}$) had a significant impact on this trait. Specifically, in males WBW was increasing at a faster rate than females, with a difference in sex performance only seen in the loch environment (Fig. 2).

In contrast, FW-origin had a significant impact on ADG_{LENGTH} (degrees of freedom = 1/1790, F-value = 731.00, P-value < $2e^{-16}$), SGR_{WBW} (degrees of freedom = 1/1790, F-value = 1533.59, P-value < $2e^{-16}$), and SGR_{LENGTH} (degrees of freedom = 1/1790, F-value = 2231.47, P-value < $2e^{-16}$) where fish of RAS-origin appeared to be growing at an increased rate compared to loch counterparts (Table 3, Fig. 2, Supplementary Data S2, and Supplementary Fig. A.4). Sex did not significantly affect ADG_{LENGTH} (degrees of freedom = 1/1790, F-value =

Table 2
Phenotypic and genetic parameters of morphological trait of post-smolts sampled three-months post-saltwater transfer, where WBW is whole-body weight, K is condition factor, CV% is coefficient of phenotypic variance, CGV% is coefficient of genetic variance, V_p is phenotypic variance, V_g is additive genetic variance, V_r is residual variance, h^2 is narrow sense heritability, s.d the standard deviation, and s.e standard error.

	WBW_{SW}		$Length_{SW}$		K_{SW}	
	RAS	Loch	RAS	Loch	RAS	Loch
Mean (s.d)	738.9 g (119.82)	826.25 g (169.19)	37.33 cm (1.65)	38.63 cm (2.19)	1.41 (0.14)	1.41 (0.13)
CV%	16.21 %	20.48 %	4.42 %	5.66 %	9.76 %	8.86 %
V_p *(s.e)*	14,061 (740.30)	26,952 (1513)	2.61 (0.13)	4.52 (0.25)	$0.19e^{-1}$ ($0.93e^{-3}$)	$0.15e^{-1}$ ($0.81e^{-3}$)
V_g *(s.e)*	3428.3 (859.44)	9227.7 (1803.6)	0.53 (0.14)	1.57 (0.30)	$0.30e^{-2}$ ($0.95e^{-3}$)	$0.37e^{-2}$ ($0.89e^{-3}$)
V_r *(s.e)*	10,636 (720.63)	17,734 (1286.0)	2.08 (0.13)	2.95 (0.22)	$0.16e^{-1}$ ($0.97e^{-3}$)	$0.12e^{-1}$ ($0.77e^{-3}$)
CGV%	7.93 %	11.62 %	1.95 %	3.37 %	3.88 %	4.31 %
h^2 *(s.e)*	0.24 ($0.55e^{-1}$)	0.34 ($0.56e^{-1}$)	0.20 ($0.50e^{-1}$)	0.35 ($0.56e^{-1}$)	0.16 ($0.48e^{-1}$)	0.24 ($0.51e^{-1}$)

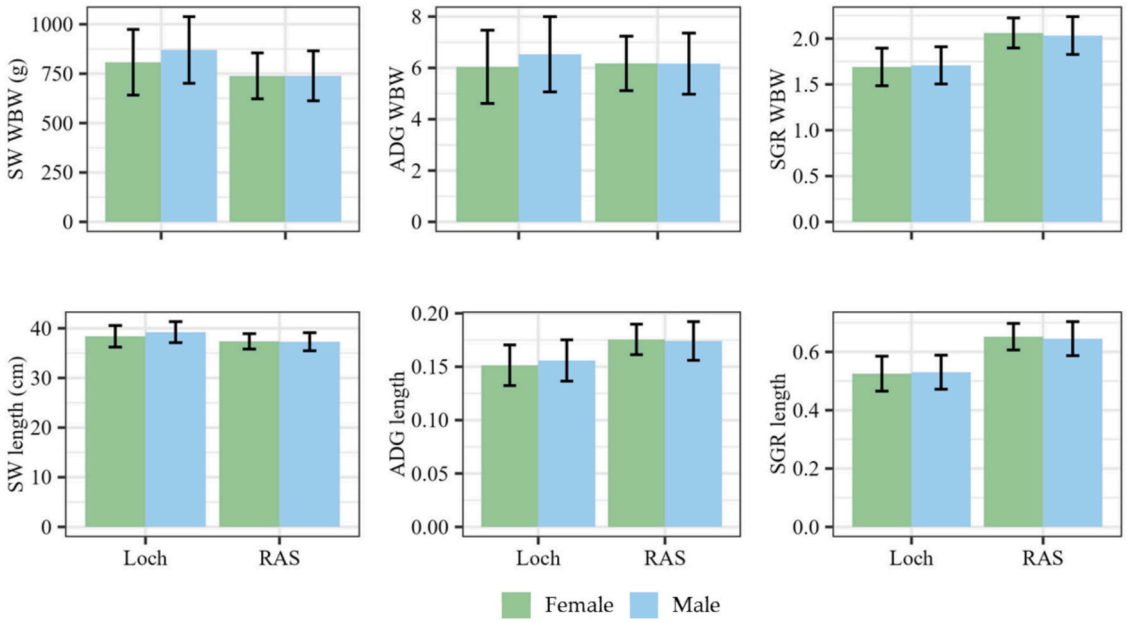

Fig. 2. Mean and standard deviation (s.d) for SW traits, split by environment (RAS and loch) and sex, where green is females, and blue is males. WBW is whole-body weight, ADG is average daily gain, and SGR is specific growth rate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Phenotypic and genetic parameters for growth traits of post-smolts sampled three-months post-saltwater transfer, where ADG is average daily gain, SGR is specific growth rate, CV% is coefficient of phonetic variance, CGV% is coefficient of genetic variance, V_p is phenotypic variance, V_g is additive genetic variance, V_r is residual variance, h^2 is narrow sense heritability and s.d the standard deviation and s.e the standard error.

	ADG_{WBW}		ADG_{LENGTH}		SGR_{WBW}		SGR_{LENGTH}	
	RAS	Loch	RAS	Loch	RAS	Loch	RAS	Loch
Mean (s.d)	6.17 (1.11)	6.19 (1.45)	0.18 ($1.58e^{-2}$)	0.15 ($1.92e^{-2}$)	2.05 (0.18)	1.70 (0.20)	0.65 ($5.05e^{-2}$)	0.53 ($5.95e^{-2}$)
CV%	17.96 %	23.51 %	9.00 %	12.60 %	8.80 %	12.06 %	7.78 %	11.92 %
V_p *(s.e)*	1.25 ($0.65e^{-1}$)	2.08 (0.12)	$0.25e^{-3}$ ($0.13e^{-4}$)	$0.38e^{-3}$ ($0.22e^{-4}$)	$0.34e^{-1}$ ($0.20e^{-2}$)	$0.42e^{-1}$ ($0.26e^{-2}$)	$0.26e^{-2}$ (0.16e-3)	$0.35e^{-2}$ ($0.22e^{-3}$)
V_g *(s.e)*	0.29 ($0.75e^{-1}$)	0.72 (0.14)	$0.62e^{-4}$ ($0.14e^{-4}$)	$0.16e^{-3}$ ($0.22e^{-4}$)	$0.16e^{-1}$ ($0.26e^{-2}$)	$0.22e^{-1}$ ($0.32e^{-2}$)	$0.12e^{-2}$ ($0.20e^{-3}$)	$0.21e^{-2}$ ($0.27e^{-3}$)
V_r *(s.e)*	0.97 ($0.64e^{-1}$)	1.36 ($0.98e^{-1}$)	$0.19e^{-3}$ ($0.13e^{-4}$)	$0.22e^{-3}$ ($0.17e^{-4}$)	$0.18e^{-1}$ ($0.15e^{-2}$)	$0.21e^{-1}$ ($0.17e^{-2}$)	$0.13e^{-2}$ ($0.11e^{-3}$)	$0.14e^{-2}$ ($0.13e^{-3}$)
CGV%	8.73 %	13.71 %	4.37 %	8.43 %	6.17 %	8.72 %	5.33 %	8.65 %
h^2 *(s.e)*	0.23 ($0.54e^{-1}$)	0.35 ($0.56e^{-1}$)	0.24 ($0.50e^{-1}$)	0.42 ($0.55e^{-1}$)	0.46 ($0.57e^{-1}$)	0.51 ($0.52e^{-1}$)	0.47 ($0.55e^{-1}$)	0.59 ($0.49e^{-1}$)

2.17, P-value = 0.14), SGR_{WBW} (degrees of freedom = 1/1790, F-value = 0.55, P-value = 0.46), or SGR_{WBW}: degrees of freedom = 1/1790, F-value = 0.20, P-value = 0.66). However, sex by FW environment did affect ADG_{LENGTH} (degrees of freedom = 1/1790, F-value = 11.14, P-value = $8.62e^{-4}$), SGR_{WBW} (degrees of freedom = 1/1790, F-value = 5.50, P-value = $1.92e^{-2}$) and SGR_{WBW} (degrees of freedom = 1/1790, F-value = 4.50, P-value = $3.41e^{-2}$) (Fig. 2). Specifically, in post-smolts of loch-origin, males appeared to be growing at a faster rate than females (Fig. 2). These within environment differences were significant for ADG_{LENGTH}, but not SGR_{WBW} and SGR_{LENGTH}. Across traits, estimates were less variable in RAS-origin post-smolts (Table 3).

3.4. Genetic parameters

For the H matrix, mean allele frequency was 0.28, with 0.40 % of genotypes missing. The correlation between GRM and A-matrix diagonal elements –which represents how well individual genetic variance estimates align between pedigree and SNP data- was 0.42, marginally below

the 0.5 threshold. The correlation of the off-diagonal elements –which represents how well genetic relatedness amongst individuals aligns between pedigree and SNP data- was greater at 0.71. For all SW traits, estimates of V_p and V_r were greater in the loch- than RAS-origin fish (Tables 2–3). This was at the exception of K_{SW}. Estimates of V_g were also greater in the loch population. This translated into consistently greater h^2 estimates for loch-origin post-smolts.

3.5. GxE

3.5.1. SW GxE

Estimates of GxE, expressed as r_{gxe}, between FW-rearing environments for SW traits are presented in Table 4. High r_{gxe} were observed (>0.80) for morphological traits (WBW_{SW}, $length_{SW}$ and K_{SW}) indicating their strong genetic consistency across environments.

For growth-related traits, r_{gxe} for ADG_{WBW} was also high (r_{gxe}=0.87 ± 9.00e^{-2}), while ADG_{LENGTH} showed a moderate correlation (r_{gxe}=0.6 ± 0.13). In contrast, both SGR_{WBW} and SGR_{LENGTH} showed lower estimates of r_{gxe} suggesting potential re-ranking of genotypes across environments and a higher sensitivity to environmental variation.

3.5.2. FW-SW GxE

Estimates of GxE between morphological traits (WBW, length and K) measured at the end of FW-rearing and three-months post-SW transfer are shown in Table 5. For both the loch and RAS groups r_{gxe} were high (>0.7). This is at the exception of WBW measured in the loch population (r_{gxe} = 0.57 ± 0.24), suggesting re-ranking of family performance for this trait pre- and post-SW transfer.

4. Discussion

In our previous study, RAS-reared smolts showed lower WBW and length but exhibited higher h^2 estimates and improved K compared to loch-reared counterparts (Tollervey et al., 2024). These differences were underpinned by a comparatively greater component of V_r in the loch-reared population, contributing to increased trait heterogeneity. Furthermore, while only weak GxE were observed between RAS- and loch-reared fish for length and K, significant estimates for WBW suggested their consideration in future breeding decisions could help improve selective gains (Mulder et al., 2006; Sae-Lim et al., 2016). Building upon this, the present study follows the same population, three months after SW transfer, to evaluate the effects of FW-origin on SW performance and investigate the genetic architecture and GxE of SW traits.

4.1. SW phenotypic and growth performance

Here, results showed that loch-origin fish were still larger in terms of WBW and length, although no difference in K was now seen, contrasting with FW where this was lower in the loch population. As K typically decreases with elongation of body form during smoltification, this lower FW K may indicate a comparatively greater state of preparedness of SW transfer in the loch population (McCormick et al., 1998; McCormick, 2012). In agreement, previous studies have found that smolt status prior to transfer can be shaped by the FW-rearing environment, underpinned by molecular and physiological differences, and has been related to poorer osmoregulation post transfer in RAS-reared fish (Kolarevic et al., 2014; Lai et al., 2024; van Rijn et al., 2020). The lower K reported in loch-reared fish at the end of FW-rearing may therefore indicate a physiological state more suited for SW transfer, potentially enabling better SW performance and larger size observed in terms of WBW and length.

In contrast, RAS-origin post-smolts, despite their smaller size, appear to be growing at a greater rate. This may indicate compensatory growth where the RAS environment limited total biomass, restricting individual fish growth, which then improved on SW transfer (Ellis et al., 2002). Similar compensatory growth has been observed in salmon and linked to specific environmental factors such as food deprivation (Hvas et al., 2022; Stefansson et al., 2009) and density changes (Refstie and Kittelsen, 1976). Considering this, FW stocking density differed substantially between FW-rearing environments, with higher densities in RAS reflecting standard industry practice (Thorarensen and Farrell, 2011). This suggests that their smaller SW size potentially linked to smaller initial size and not smoltification state.

Importantly, however, it has been previously demonstrated that early rearing environments can lay the basis for later life performance (Jonsson and Jonsson, 2014; Lai et al., 2024). Therefore, the enhanced growth of RAS post-smolts may have implications for the entire production cycle, with the RAS-reared population able to 'catch-up' to those reared in the loch environment. The implication of their smaller size at the end of FW-rearing may therefore have limited effect across the whole production cycle. However, fish growth patterns typically follow a sigmoid shape, while AGD and SGR assume linear and exponential growth, respectively. This means the measurements taken here for the initial period post-transfer may not be applicable throughout production (Dumas et al., 2010; Lugert et al., 2016). Furthermore, when evaluating the difference in growth between RAS- and loch-origin populations, SGR showed a greater difference compared to ADG. While this may reflect biological variation, it could also result from methodological artifact. For example, SGR estimates cannot be applied across life stages and its calculation influenced by stocking density and initial size (Aunsmo et al., 2014; Dumas et al., 2010; Lugert et al., 2016). Thus, while it was not possible to separately rear RAS and loch populations beyond three-months post-transfer, measurements of growth through to harvest would be of interest to collect in the future to verify if the FW-rearing environment continues to have an impact at more distal time periods.

4.2. Trait heterogeneity

Estimates of V_p, which represent how much a trait differs between individuals in a population, for SW traits were generally lower in RAS-origin fish, except for K. This is consistent with FW results, and estimates fall within the range previous reported (Gonzalez et al., 2022; Neira et al., 2004; Quinton et al., 2005). Considering this, it has been

Table 5
Genetic correlation (r_{gxe}) and standard error (s.e) for phenotypic traits whole-body weight (WBW) (g) length (cm) and condition factor (K) for the RAS- and loch-reared populations measured as smolts at the end of freshwater-rearing and as post-smolts approximately three-months post-saltwater transfer.

	WBW (s.e)	Length (s.e)	K (s.e)
RAS	0.82 (0.11)	0.82 (0.13)	0.78 (0.15)
Loch	0.57 (0.24)	0.78 (0.34)	0.85 (0.49)

Table 4
Genetic correlations (r_{gxe}) and standard error (s.e) for saltwater (SW) morphological (WBW_{SW}, $length_{SW}$, and K_{SW}) and growth (ADG_{WBW}, ADG_{LENGTH}, SGR_{WBW}, and SGR_{LENGTH}) traits. WBW is whole-body weight, K is condition factor, ADG is average daily gain, and SGR is specific growth rate.

	WBW_{SW}	$Length_{SW}$	K_{SW}	ADG_{WBW}	ADG_{LENGTH}	SGR_{WBW}	SGR_{LENGTH}
r_{gxe}	0.86	0.97	0.93	0.87	0.60	0.21	0.17
s.e	0.10	9.00e^{-2}	0.15	9.90e^{-2}	0.13	0.11	0.10

suggested that the increased environmental regulation offered by RAS creates more stable husbandry environments which favours reductions in trait heterogeneity (Vu et al., 2021). While this may have been a possible driver of the decreased variance in FW, the results presented here demonstrated this was maintained even after the RAS population entered the more varied SW environment.

Conversely, sex and/or sex-by-environment interaction significantly affected SW traits at the expectation of K. However, a clear difference was observed only in the loch population, where males were either larger or growing faster than females, in agreement with previous studies (Gjerde et al., 1994; Leclercq et al., 2010; Thorland et al., 2020). Although sexual maturation was not assessed, its link to growth suggests potential implications, particularly as males can mature earlier than females (Good and Davidson, 2016; Klemetsen et al., 2003). As the onset of sexual development depends on reaching certain size or condition threshold (Thorpe, 1989; Thrope, 1994), the greater FW size of loch-origin fish may indicate that this environment favoured the development of sexual dimorphism. In considering this finding, the difference in sex ratio between FW environments should, however, also be noted.

Considering this, FW conditions, including higher water temperature and continuous light, have been linked to enhanced growth and higher rates of early maturing males (Berrill et al., 2003; Imsland et al., 2014). While these conditions describe the environment fish were reared under in FW-RAS, in FW, loch-reared smolts experienced a low density, which has also been associated with early male maturation (Berg et al., 1996; McLay et al., 1992; Pino Martinez et al., 2021). Therefore, just as the FW-RAS environment could have limited total biomass per tank, the restriction this placed on individual growth could have also restricted the development of sexually dimorphic growth, further contributing to the lower V_p observed in this population.

4.3. Genetic parameters

Estimates of h^2 -which indicates the proportion of trait variation attributable to additive genetic differences passed from parents of offspring- for SW morphological traits (WBW_{SW}, $length_{SW}$, and K_{SW}) were low-to-moderate, falling within the range previously reported in salmonids (Correa et al., 2018; Gonzalez et al., 2022; Neira et al., 2004; Powell et al., 2008; Quinton et al., 2005). While to our knowledge there are no h^2 estimates for growth rate in salmonids, a wide range have been reported in other fish species, e.g. *Dicentrarchus labrax, Solea solea*, *Oreochromis niloticus* or *Piaractus mesopotamicus* (~0.05–0.60) (Dupont-nivet et al., 2010; Vandeputte et al., 2010; Mas-Muñoz et al., 2013; Trong et al., 2013; Freitas et al., 2021), with the estimates presented here (0.49 to 0.59) falling on the upper end of this.

FW-rearing environment was found to influence h^2 of both morphological and growth-related traits, with consistently higher estimates in loch- compared to RAS-origin fish. Importantly, the SW estimates contrast with the FW findings where loch-reared smolts had approximately half the h^2 of RAS fish. Considering both results, since transfer to SW, h^2 of morphological traits (WBW, length and K) appear to have increased in the loch but decreased in the RAS population. Of note, h^2 can be decomposed into several different components, the relative size of which indicates how much they control phenotypic expression. Specifically, as h^2 is calculated from a ratio between V_p and V_g, and V_p is itself the sum of V_g and V_r, h^2 can vary due to changes in V_g, V_r, or both (Falconer, 1952; Falconer and Mackay, 1996; Gjedrem, 2000). Considering this, in the loch-reared population greater estimates of V_r were returned for WBW and length at both time points. In contrast, estimates of the CGV showed that in FW, RAS-reared fish returned higher estimates than loch counterparts, with the opposite true in SW.

One factor potentially contributing to this observed change in the (relative) size of V_g is biased sampling (Lage and Kornfield, 2006). Previous studies have demonstrated this can arise due to a size-based distribution (Folkedal et al., 2012; Nilsson et al., 2013), size-based variability in capture (Vindas et al., 2016), and chance effects (Kritzer et al., 2001). Additionally, bias could have been introduced at either FW or SW sampling and may have differed between husbandry environments (Nilsson and Folkedal, 2019). However, retention of only families shared across the four populations sampled (i.e. Fig. 1) for analysis may have minimised these effects.

Alternatively, experimental design could have impacted the observed changes in h^2 (Supplementary Fig. A.1). Specifically, at transfer to SW, all three tanks containing phenotypically female fish from the FW-RAS environment were pooled into a single sea cage. While FW estimates were derived from fish in only one of the three tanks estimates were based on fish sampled from across all three. This contrasts with fish of loch-origin, where in both FW and SW environments, the population was only housed in a single tank/pen. This difference between populations may have helped drive the relative increase and decrease in h^2 observed in the loch and RAS populations post-transfer, respectively, similar to that described by Thorland et al. (2020). Unfortunately, without tracking individual fish, FW tank of origin could not be considered in SW analysis.

4.4. GxE

GxE for SW morphological traits (WBW, length and K) were insignificant ($r_{gxe} > 0.7$), indicating minimal re-ranking of family performance between RAS and loch populations. Compared to FW findings, these r_{gxe} for SW traits had increased. For example, though significant GxE was detected for WBW at the end of FW-rearing, this was not seen in WBW_{SW} $r_{gxe}=0.86 \pm 0.10$), suggesting the magnitude decreased since transfer. In agreement, previous studies have highlighted that the time spent in a shared relative to separate rearing environments can influence the observed strength of GxE. Such that, longer periods of communal rearing, or greater distance from a period of separate rearing, GxE effects may diminish. For example, Dupont-nivet et al. (2010) reported greater r_{gxe} in a group of *D. labrax* which shared a longer periode of communal rearing, and, conversly, Agha et al. (2018) reported greater estimates of GxE (lower r_{gxe}) in genetically improved farmed tilapia, results attributed to the fact the experimental population never shared an environment and instead separately reared from hatching.

A notable exception to these high r_{gxe} estimates was, however, SW GxE estimates for growth traits (except for ADG_{WBW}). These suggested that, unlike morphological traits, family growth performance in SW was impacted by the FW-origin. Considering this, the strength of GxE estimates can also depend on the trait of interest. Importantly, while morphological traits reflect the accumulated environmental experienced since fertilization, growth traits can be calculated across specific periods. As a result, previous studies have reported significant GxE for growth traits that are not seen in the overall morphometric performance (Dupont-Nivet et al., 2010, 2008; Omasaki et al., 2016). While these studies have previously only assessed growth during separate rearing phases, here it was done once populations had been returned to a shared environment. Interesting, despite this difference, these findings confirmed that growth, but not morphological traits, demonstrated GxE. Notably, this effect was more pronounced for SGR than AGD, possibly reflecting a genuine biological difference. However, as mentioned above, factors like initial stocking weight, which can bias SGR estimates, may have influenced these results (Aunsmo et al., 2014).

While the performance of Atlantic salmon reared in RAS throughout the production cycle, relative to those exposed to both RAS and sea-cage environments has been investigated (Correa et al., 2018; Gonzalez et al., 2022), the influence of the FW environment on later life performance was yet to be thoroughly explored. In this study, the estimated r_{gxe} for FW-SW interactions was high (> 0.8) in both RAS and loch populations, meaning that the best-performing families remained consistent pre- and post-SW transfer. This was at the exception of the correlation between WBW measured in FW- and SW-loch populations ($r_{gxe} = 0.57 \pm 0.24$).

Consideirng this, a further factor known to impact the strength of GxE estimates is the degree of difference between environments (Mas-Muñoz et al., 2013; Sae-Lim et al., 2013; Turra et al., 2016). As the FW-loch environment was more similar to the SW sea-cage site in terms of temperature and stocking density, weaker FW-SW GxE, relative to the RAS population, may have been expected. However, due to the limited environmental parameters that were recorded and shared between FW and SW sites, other variables, which cannot be considered here, could have differed between environments and contributed to the greater restructuring of family WBW performance in the loch population post-transfer.

It is worth noting that r_{gxe} estimates could also have been biased due sampling size. Specifically, based on suggestions in Sae-Lim et al. (2010), while number of families was sufficient (124), both the number of fish sampled per environment (650–910) and number of fish per family (~6) were below recommendations. Consequently, the quantification of GxE effects presented here could have been downwardly biased. In evaluation of GxE for growth traits, it should also be considered that as derived/composite traits, they may be more likely to express GxE, as has been suggested previously (Evans and Langdon, 2006).

4.5. Aquacultural implications

It was previously demonstrated that at the end of FW-rearing, RAS-reared smolts were smaller than their loch counterparts. This may have been a result of environmental differences between them. Despite this, RAS-origin fish showed enhanced growth rate post-SW transfer. Given previous suggestions that early SW growth maintains the same trajectory to harvest (Lai et al., 2024), this highlights that the size difference between the RAS and loch populations may diminish with time. Furthermore, while SW successes and survival has been associated with larger smolt size (Russell et al., 2012), here low mortality rates were observed for both populations despite a size difference. Together, this suggests the smaller size of RAS-reared smolts may have limited production implications.

Homogeneous RAS growth seen in FW was maintained after the population was transferred to the SW environment, with indications of sexually dimorphic growth also present in those of loch-origin. This lower trait variance in the RAS population is particularly advantageous commercially as trait homogeneity can improve animal welfare, increase produce value, and reduce production costs (Janhunen et al., 2012; Marjanovic et al., 2016; Vu et al., 2021). While this could be both genetically and environmentally regulated, it is suggested here that the same RAS-specific variables that favoured their small size at the end of FW rearing may have also encouraged trait homogeny. Management of FW-rearing environment should then also consider the balance between FW size and trait variance.

Estimated parameters and GxE quantification indicated different patterns of V_g between time points dependent on FW-rearing. Firstly, h^2 estimates for the loch population increased from pre- to post-transfer, but a low r_{gxe} between FW and SW WBW was identified. The opposite was true of the RAS population. These results could be used to help improve breeding strategies. For example, the difference in h^2 estimates suggest selection should target, for include in breeding goals, traits measured at different time points depending on the FW-rearing environment employed. In line with results presented here, this would be SW traits for the loch-reared population, and those at the end of FW-rearing for the RAS fish. Of note, because of the higher r_{gxe} between FW and SW traits in the RAS populations, selection applied at FW would be expected to also produce a large response in SW traits. However, investigation into how much of a difference this targeted approach would have on phenotypic response to selection would be of interest.

Lastly, growth performance appears to be environment-specific, with moderate-to-strong GxE identified between SW-RAS and -loch populations. This suggests that targeting environment specific growth in a program of selection could improve post-SW transfer performance.

5. Conclusions

Though smaller at the end of FW-rearing, RAS-reared post-smolts were growing faster in SW than loch-origin counterparts. These fish also demonstrated lower trait variance than loch counterparts who also showed indications of sexual dimorphic growth. In the loch population, h^2 increased from pre- to post-SW transfer, while the opposite was true in RAS. In addition, while insignificant GxE was generally observed, significant family re-ranking was identified for SW growth traits. Altogether, the results suggest that further improvement in breeding programs accommodating for the observed effects on h^2estimate and GxE are warranted.

CRediT authorship contribution statement

Mette J. Tollervey: Visualization, Methodology, Formal analysis, Writing – original draft, Project administration, Investigation, Data curation. **Saif Agha:** Methodology, Writing – review & editing. **Michaël Bekaert:** Investigation, Supervision. **Almas A. Gheyas:** Supervision, Writing – review & editing. **Ross D. Houston:** Conceptualization, Project administration. **Andrea Doeschl-Wilson:** Supervision, Conceptualization, Writing – review & editing, Funding acquisition. **Ashie Norris:** Conceptualization, Resources. **Herve Migaud:** Resources, Investigation, Conceptualization, Supervision, Project administration, Funding acquisition. **Alejandro P. Gutierrez:** Project administration, Supervision. **Monica B. Betancor:** Supervision, Writing – review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Microsoft Copilot in order to suggest alternative sentence structure. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Declaration of competing interest

H.M is currently employed by Mowi Scotland, A.N by Mowi Genetics AS, M.B by Cooke Aquaculture, A.P.G by the Centre for Aquaculture Technology, and R.D.H by Benchmark Genetics, though this did not influence or bias their work. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742892.

Data availability

Data will be made available on request.

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