Genotype-by-environment interaction in production traits of yellowtail kingfish (*Seriola lalandi*) reared in RAS and flow through systems

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A R T I C L E I N F O

A B S T R A C T

Seriola lalandi (Yellowtail Kingfish or Haku in te reo Māori) is emerging as an ideal species for aquaculture in New Zealand. The majority of *S. lalandi* production occurs in sea cages, however there is increased interest in using Recirculating Aquaculture Systems (RAS) that provide increased environmental control in the face of climate change, improved biosecurity, reduced water usage, and better waste capture. It is currently unknown whether a breeding program designed to generate genetic improvement in RAS would produce similar genetic gain in sea cages or if genotype-by-environment (GxE) interactions would limit the transfer of genetic improvement between growing systems. In this study we investigate the GxE interaction for both harvest length and harvest weight between *S. lalandi* reared in land-based RAS and flow through (FT) systems. We used genotyping-by-sequencing (GBS) SNP genotypes to estimate the genomic relationship matrix (GRM) for all individuals and used the GRM to estimate the genetic correlation of the same trait between the two systems. We found genetic correlations of 0.57 ± 0.1 and 0.54 ± 0.1 for harvest length and harvest weight respectively between the two systems. These lower-to-moderate genetic correlations indicate moderate-to-strong GxE because genotypes rank differently across the two systems for the same trait. We hypothesize that this GxE is primarily due to the large temperature and stocking density differences between the RAS and flow through systems. We also aimed to assess the relationship between the level of GBS SNP coverage and the standard errors of our genetic correlation estimates to determine possibilities for developing low or moderate density genotyping methods to reduce genotyping costs for future GxE experiments. Reducing the number of SNPs by 98.3 % from 167 K to 2.8 K only increased the standard error of the genetic correlations between environments from 0.10 to 0.13 in harvest weight and from 0.10 to 0.12 in harvest length. Therefore, it should be possible to reduce per-sample sequencing costs for future GxE experiments by using low or moderate density genotyping methods.

1. Introduction

The genus *Seriola* contains nine fish species commonly referred to as amberjacks or kingfish. *S. quinqueradiata*, *S. lalandi*, *S. dumerili*, and *S. rivoliana* are used for aquaculture production due to their rich texture, whole-fillet value, and popularity as raw sashimi grade products. In 2020, over 160,000 metric tonnes of these four species were produced globally with over 85 % attributed to Japanese sea cage farming of *S. quinqueradiata* (Statistics Team of the Fisheries and Aquaculture Division, 2021).

The New Zealand National Institute of Water and Atmospheric Research (NIWA) identified *S. lalandi*, known indigenously as haku and commonly as yellowtail kingfish, as an excellent candidate species for aquaculture in New Zealand in the 1990s due to its culturability, fast growth rate in temperate climates, superior flesh quality, and high market demand (Symonds et al., 2014). From the late 1990s through the early 2010s, several research teams studied the reproductive physiology and early larval and juvenile rearing of yellowtail kingfish to achieve consistent seed production and commercial grow-out success (Abbink et al., 2012; Moran, 2007; Moran et al., 2007, 2010).

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While the vast majority of global *Seriola* are farmed in sea cage systems, there is growing interest in rearing these species in Recirculating Aquaculture Systems (RAS). RAS are typically land-based, self-contained farming systems that repeatedly recycle up to 99 % of the water in fish growing tanks and use water filtration and treatment systems to concentrate and remove solid and dissolved fish waste products (Badiola et al., 2018; Rurangwa and Verdegem, 2015). While RAS can incur higher investment and operational costs than simpler production systems, its use in yellowtail kingfish production is attractive for several reasons: 1) RAS allow greater control over environmental conditions including water quality parameters and photoperiod while being insulated from the volatility of the marine environment. 2) RAS are almost completely isolated from the marine environment and therefore offer higher biosecurity than traditional sea cages where pathogens can easily spread between cages and wild fish populations. 3) In contrast to sea cage farming where fish waste can pollute nearby marine environments, RAS waste is completely contained and can be properly captured for treatment or re-use. 4) The physical containment of RAS significantly reduces the risk of escape and therefore of genetic introgression from captive stocks into to wild *Seriola* populations.

The future of yellowtail kingfish aquaculture will likely include both sea cage and RAS production, but it is currently unknown whether a breeding program designed to produce genetic improvement in RAS would produce similar genetic gain in sea cages. Genotype-by-environment interactions (GxE) could limit the transfer of genetic improvement between system-types. Genotype-by-environment interactions (GxE) occur when separate genotypes respond differently to varying environmental conditions (Falconer and Mackay, 1996). GxE is typically quantified as the genetic correlation between the same trait in separate environments, with a low genetic correlation indicating strong GxE because genotypes rank differently in the two systems. If GxE is high, it may be necessary to split the breeding program into two, with one focused on genetic improvement in RAS and the other in sea cages or to construct a selection index that targets an acceptable compromise in genetic gain for both systems.

Many studies have estimated GxE between aquaculture production systems that differ in key environmental parameters such as temperature (Gulzari et al., 2022; Sae-Lim et al., 2013), salinity (Domingos et al., 2021; Setyawan et al., 2022), and dissolved oxygen level (Mengistu et al., 2020). While several production systems have been evaluated for GxE, comparatively few studies include RAS. Li et al. (2019) examined GxE for production traits in olive flounder (*P. olivaceus*) farmed in RAS vs flow through (FT) systems. Harvest weight and total length had a genetic correlation of 0.65 ± 0.13 and 0.33 ± 0.12 respectively between the two farming environments. Fernandes et al. (2019) estimated the genetic correlation for body weight as 0.67 between Nile Tilapia in RAS and freshwater cage environments when selection took place at 225 days. Sae-Lim et al. (2013) estimated the genetic correlation of final harvest weight in rainbow trout between RAS and a high-elevation FT farm to be 0.40 ± 0.12. All three of these RAS GxE studies used the pedigree-based numerator relationship matrix (or **A**-matrix) to estimate variance components and genetic correlations. Tollervey et al. (2024) investigated GxE for growth traits in genotyped Atlantic salmon smolts reared in a recirculating aquaculture system (RAS) and a freshwater loch. The genetic correlations between the loch and RAS for body weight, body length, and condition factor were 0.62 ± 0.14, 0.78 ± 0.15, and 0.85 ± 0.17 respectively. Premachandra et al. (2017) used eight microsatellite markers to estimate the genetic correlation between body weight in yellowtail kingfish reared in a sea cage and an indoor RAS tank and found a genetic correlation of 0.92 ± 0.49. The RAS tank operated at around the same temperature as the sea cage and the same stocking density of 6 kg per m³. Commercial RAS typically operate at higher temperatures and stocking densities than in this study, potentially making the GxE estimate an overestimation in addition to its large standard error.

The goal of our study was to quantify GxE for two growth-related traits: harvest weight (HW) and harvest length (HL) in *S. lalandi* reared in two different production systems: 1) a RAS system and 2) a FT system which was intended as a proxy for a sea cage environment. Given the low genetic correlations observed in previous studies between RAS and other rearing systems, we hypothesized that there would be a large GxE effect (low genetic correlation) between the RAS and FT system. Because fish were produced using group spawning, traditional tag-based parentage tracking was impractical, and we used genotyping-by-sequencing (GBS) to both assign parentage and estimate the genomic relationship matrix (GRM) between experimental fish. We also aimed to assess the relationship between the number of SNPs used to estimate the GRM and the standard errors of our genetic correlation estimates typically used to quantify GxE to see whether it is possible to reduce genotyping costs for future GxE experiments.

2. Materials and methods

### 2.1. Production of experimental fish and fish husbandry

We produced experimental fish from thirteen group spawning (batch) events between October 15 and November 19, 2020, using 49 genotyped males and 24 genotyped females of yellowtail kingfish. The broodstock were sourced from six tanks, each containing a unique, non-overlapping set of parents (see Supplemental 2, Fig. 1).

Each broodstock tank contributed between one to three spawning batches within a four-day period, collectively classified as a "hatch group" for that tank. The only exception was hatch group 5, which included eggs from two different broodstock tanks that spawned on consecutive days. Each batch was incubated separately. To optimize space and minimize within-tank variation in larval size, larvae from batches that hatched within one day of each other, regardless of their broodstock tank of origin, were combined in a single nursery tank. These nursery-reared larvae, distributed across six nursery tanks, were later consolidated into two larger larval flow-through (FT) tanks: Tank L1 (10 m³): containing ten spawning batches from four broodstock tanks (hatch groups 1–4), with hatch dates ranging from October 16 to October 31, 2020. Tank L2 (5 m³): containing three spawning batches from two broodstock tanks (hatch group 5), with hatch dates on November 20 and November 21, 2020.

To minimize the effect of hatching date on size at the start of Stage 2 (Fig. 1) and ensure comparable initial body weights, we regulated growth through temperature control after weaning in the nursery tanks until tagging (see Supplemental 2, Fig. 1). Earlier-hatched groups were reared at lower temperatures, while later-hatched groups were kept at higher temperatures, all well within their tolerance range (18.5–24.0 °C). Growth was monitored through periodic weight measurements, and the weight distribution at tagging followed a normal distribution.

Prior to Stage 2, we PIT-tagged and collected fin clips from fish (average of 165 g) in both tanks (L1 and L2). We assigned parentage using GBS, thus allowing us to deduce the broodstock tank of origin and estimate the hatching date of each hatch group to within two days based on the range of spawn dates recorded for each tank. For example, a fish from a tank from which eggs were collected between 15/10/2020 and 19/10/2020 would have an estimated fertilization date of 17/10/2020, and an average hatch date of 19/10/2020 as the kingfish eggs hatch after two days of incubation.

After tagging before the start of Stage 2, we measured the weight and length of all fish from L1 and L2 (see Supplemental 2, Table 1) and proportionally split them into one 10 m³ circular flow through tank (FT1) ($n = 306$) at 8.34 kg/m³ and one 25m³ circular RAS tank (RAS1) ($n = 1820$) at 20.31 kg/m³ (Stage 2). FT1 and RAS1 fish were reared for 230–231 and 195–197 days respectively until we recorded final phenotypes (Stage 3). On days 131–132 of Stage 2, RAS1 fish were split evenly into two equally sized tanks (RAS1 and RAS2) when the stocking density reached 104.87 kg/m³.
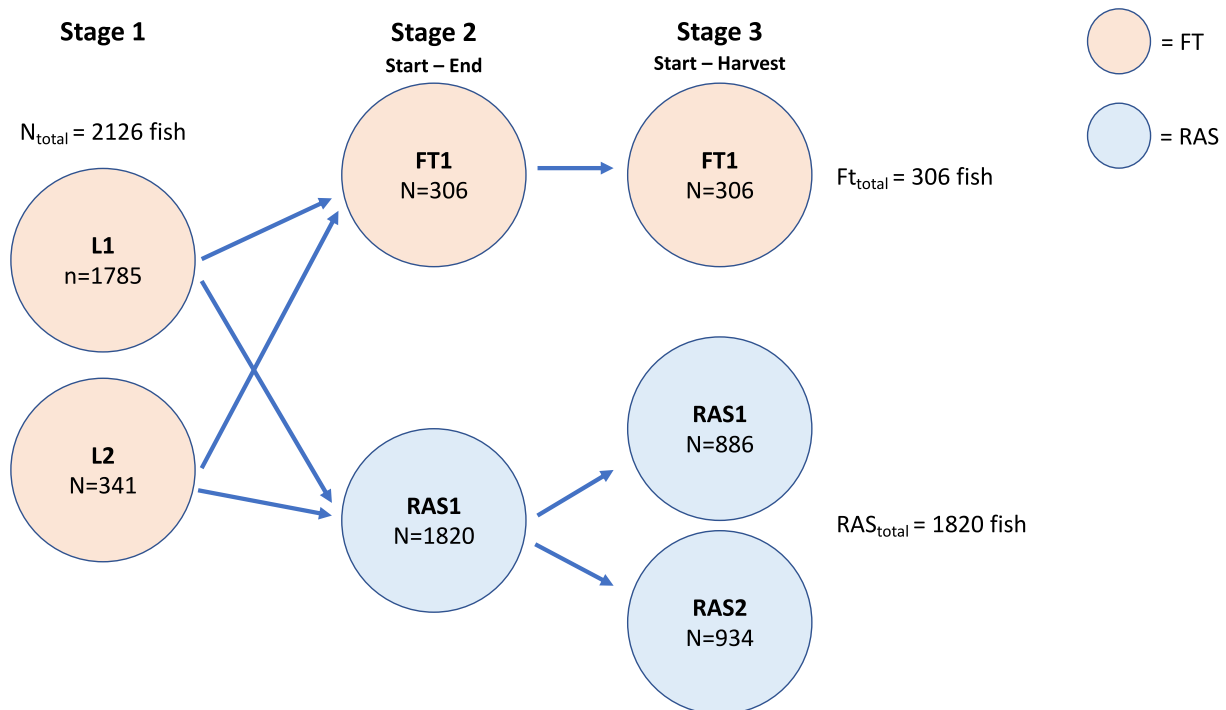
Fig. 1. Paths of fish movement through each tank in the experiment. FT tanks are labeled in red and RAS tanks are shown in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Number of recorded fish (N), mean of the trait, and coefficient of variation (CV) of phenotypes and rearing length in degree days measured from the start of stage 2 to Harvest. * means for the same trait between environments were significantly different by two-sample Student's *t*-test. #start weight indicates the weight of fish at the beginning of stage 2 when they were first separated into FT and RAS systems.

Trait	Time point/ period	Environment	N	Mean	CV
Start Weight[#] (kg)	Start Stage 2	RAS	1820	0.28	0.20
Start Weight[#] (kg)	Start Stage 2	FT	306	0.27	0.19
HW (kg)	Harvest	RAS	1820	1.89*[a]	0.19
HW (kg)	Harvest	FT	306	1.51*[a]	0.16
HL (cm)	Harvest	RAS	1820	49.50*[b]	0.06
HL (cm)	Harvest	FT	306	45.00*[b]	0.05
Age (days)	Harvest	RAS	1820	377.81*[c]	0.03
Age (days)	Harvest	FT	306	411.58*[c]	0.03
Degree Days (days * temperature)	Start Stage 2 -Harvest	RAS	1820	4234	
Degree Days (days * temperature)	Start Stage 2 -Harvest	FT	306	3711	

The flow through water is sourced from a pipe approximately 500 m offshore in Bream Bay, NZ at a depth of 10 m. This seawater is mechanically filtered with glass-sand, sterilized using UV treatment, and passed through a 5 μm mesh filter prior to entering the tanks. The RAS contains mechanical drum filtration, moving bed biofiltration, a protein skimmer, and UV treatment. RAS tanks used a 24 h light photoperiod, whereas FT1 used an ambient photoperiod. Fish in RAS were automatically fed 10 times per day over 24 h and fish in FT1 were automatically fed hourly during daylight hours (approximately 10–12 feeding bouts). Fish in both system types were fed to a ration based on NIWA's size and temperature-dependent growth model (unpublished).

Temperature in the RAS tanks averaged 21.6 °C (range 18–26.6 °C) while FT1 had an average of 16.1 °C (range 14–19.9 °C) over the growing period. Fig. 2 shows a time series comparing temperatures between tanks. Final harvest stocking densities in Stage 3 were 67.11

kg/m^3, 70.00 kg/m^3, and 46.20 kg/m^3 for RAS1, RAS2, and FT1 tanks respectively (Table 2).

2.2. Phenotypic data collection

We collected phenotypic data at the end of Stage 3 for both harvest weight (HW) and harvest length (HL) on all experimental fish after 378 days and 412 days of total growth for RAS and FT fish respectively. RAS fish were harvested when average weight exceeded ~1.9 kg which is within the range of a typical commercial harvest weight. FT fish grew more slowly and were harvested when the average weight reached ~1.5 kg which is the minimum acceptable commercial harvest weight. A total of 1820 fish in RAS and 306 fish in FT were phenotyped at harvest. Data was collected on one day for FT fish and over the course of two days for RAS fish. Before harvest phenotyping, fish were anaesthetized in 30 ppm AQUI-S (AQUI-S New Zealand Ltd, Lower Hutt, NZ). We measured each fish to the nearest 5 mm and 10 g and collected a 3 mm square fin clip which we stored in 100 % ethanol. Fin clip samples were sent to AgResearch (Lincoln, New Zealand) for genotyping and quality control. Downstream data analyses and processing was conducted using R software (R Core Team, 2021).

2.3. Genotyping, parentage assignment, and genomic relationship matrix

The DNA extraction and GBS were performed by GenomNZ from AgResearch, NZ (www.genomnz.co.nz). The DNA was extracted from ~3 mm tissue punch from fin clips preserved in ethanol using a proteinase K ethanol precipitation method (Clarke et al., 2014). Using *Pst*I/ *Msp*I restriction enzymes, 100 ng of DNA was digested for GBS library construction according to the methods outlined in Elshire et al. (2011) with modifications as outlined in (Dodds et al. (2015). Each GBS library included negative control samples (no DNA), and each library underwent size selection using a Pippin Prep (SAGE Science, Beverly, Massachusetts, United States) to select fragments in the size range of 193–318 bp (genomic sequence plus 123 bp of adapters). Each library consisted of 376 individually barcoded samples that were run on one
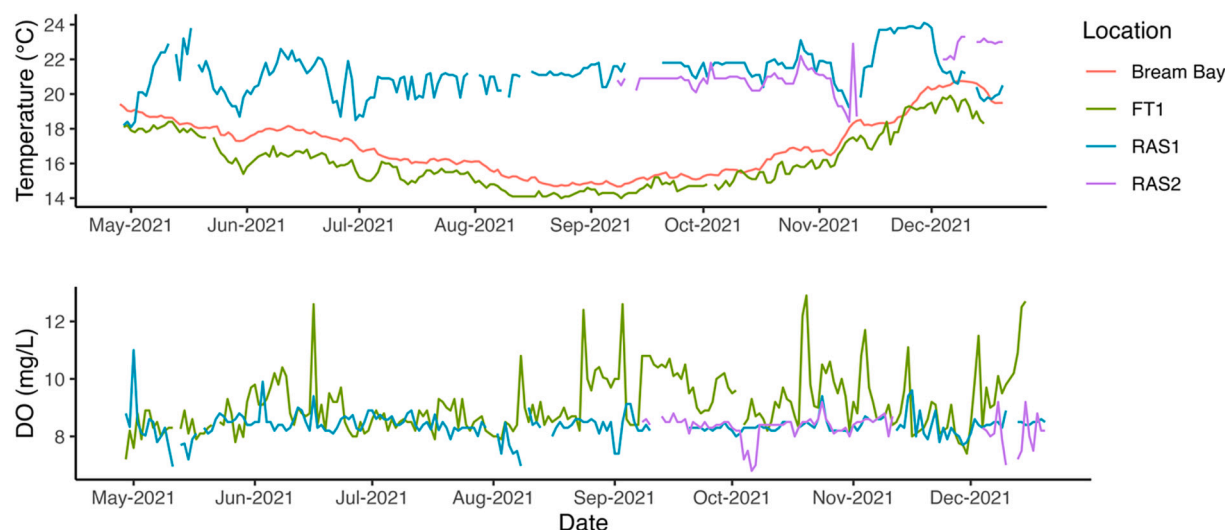
Fig. 2. Daily temperatures (upper plot) and dissolved oxygen (lower plot) for each system type from Stage 2-Stage 3. Line gaps indicate missing data. The blue line shows RAS1, the purple line shows RAS2, the green line shows FT1, and the red line shows the average sea surface temperature of Bream Bay where the FT1 water was sourced. Bream Bay temperature data was taken from the NOAA ERDAPP database (https://coastwatch.pfeg.noaa.gov/erddap/index.html). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Stocking densities at the start and end of Stages 2 and 3 for all tanks. RAS = Recirculating Aquaculture System, FT = Flow Through System.

	Density (kg/m^3)				
	Stage 2			Stage 3	
Tank	Start	End		Start	End
FT1	8.3	–		–	46.2
RAS1	20.3	104.9		52.5	67.1
RAS2	–	–		52.5	70

lane of an Illumina HiSeq2500 utilizing v4 chemistry and single-end sequencing (1 × 101 bp; ~ 22–24 Gb data). Raw fastq files were quality checked using a custom qc pipeline (available at https://github.com/AgResearch/DECONVQC). As one of the qc steps raw fastq files were quality checked using FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Following QC, reads were demultiplexed using GBSX v1.3 (Herten et al., 2015) with settings '-mb 0 -me 0 -n false'.

The adapters were removed using cutadapt v2.9 (Martin, 2011) with settings '-a AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG -m 40'. Using a custom Python script, internal *PstI* cut sites were detected, to identify potential chimeric reads. These reads were virtually digested and only the first fragment (5 prime end) was kept for further analysis. Processed reads shorter than 40 bp were discarded. The clean reads were then aligned to the yellowtail kingfish (*S. lalandi*) genome GCA_003054885.1 available at (https://www.ncbi.nlm.nih.gov/assembly/GCA_003054885.1), using bwa mem v0.7.17-r1188 (Li and Durbin, 2009) with default settings. The alignments were further filtered using samtools v1.9 (Li et al., 2009) with settings '-q 30'. For variant detection, bcftools v1.0 was used (Li, 2011) with settings 'mpileup –max-depth 8000 –skip-indels -a AD', 'call -cv', 'view -M2'.

We further filtered the raw GBS vcf file containing 374,149 SNPs using the KGD package in R (Dodds et al., 2015) prior to estimating the genomic relationship matrix (G). We removed individual fish samples with a mean sample depth of <0.3 reads/SNP, duplicate genotyping records, incomplete parentage assignment, and sample IDs not found in the GBS data. SNPs observed to be mono-allelic, Hardy-Weinberg disequilibrium values < −0.05 (observed frequency of reference allele homozygote minus its expected frequency), or sample depth < 0.01

were removed. After filtering, 167,795 SNPs and 2186 individuals (2115 of 2126 experimental fish and 71 of 73 parents) remained. This left 304 fish in FT and 1811 in RAS. The full GRM was calculated using all SNPs with the calcG() function in the KGD package. To estimate relatedness between individuals, we used the KGD package which calculates kinship using a depth adjustment (KGD) method to account for depth (including zero depth) of genotype calls (Dodds et al., 2015). This makes KGD well suited for estimating the GRM from GBS data which often contains missing or low call depth for genotypes.

We assigned individuals to parents using the GBSPed()function from the KGD package (Dodds et al., 2019), and used this parentage information to build a shallow A-matrix which contained only information regarding experimental fish and their assigned parents. The inverted A-matrix was constructed using the ainverse() function in ASReml-R version 4.1.0.160 (Butler et al., 2018). Individuals missing one or both parental assignments were discarded.

2.4. Animal model, heritabilities and genetic/phenotypic correlations

We estimated genetic parameters for the two traits HL and HW in both RAS and FT environments using the resulting 2115 experimental fish. Genetic parameters, heritabilities, and genetic correlations were estimated by creating a 4-trait animal model in ASReml-R (Butler et al., 2018). In this model, the same trait measured in different environments were considered as separate traits. Hatch Group, and Tanks nested within Environment were used as fixed effects:

$$y_{i,j,k,l} = u_i + \text{HatchGroup}_j + \text{Tanks(Env)}_{i,k} + r_l + e_{i,j,k,l}$$

In the animal model, $y_{i,j,k,l}$ is a vector of phenotypic values of the four

traits (HW-RAS, HL-RAS, HW-FT, and HL-FT); u_i is a vector containing the mean for the four traits used ($i = 1$–4 for HW-RAS, HL-RAS, HW-FT, and HL-FT); HatchGroup is a fixed effect to account for differences in average hatching dates between five hatch groups of experimental fish ($j = 1$–5, Date-1, Date-2, Date-3, Date-4, Date-5); Tanks(Env)$_{i,k}$ is the fixed effect for the combination of stage 1 and stage 3 tanks, nested within each environment (k = 1–2 for FT, 3–6 for RAS, Fig. 1); r_l is random additive genetic effect of the l-th individual; $e_{i,j,k,l}$ is a vector of random residuals for each individual l.

The random animal additive genetic effects (r) were distributed as:

$$r \sim \left(\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, G \begin{bmatrix} \sigma^2_{a,R1} & r_{a,R1R2}\sigma_{a,R1}\sigma_{a,R2} & r_{a,R1F1}\sigma_{a,R1}\sigma_{a,F1} & r_{a,R1F2}\sigma_{a,R1}\sigma_{a,F2} \\ r_{a,R2R1}\sigma_{a,R2}\sigma_{a,R1} & \sigma^2_{a,R2} & r_{a,R2F1}\sigma_{a,R2}\sigma_{a,F1} & r_{a,R2F2}\sigma_{a,R2}\sigma_{a,F2} \\ r_{a,F1R1}\sigma_{a,F1}\sigma_{a,R1} & r_{a,F1R2}\sigma_{a,F1}\sigma_{a,R2} & \sigma^2_{a,F1} & r_{a,F1F2}\sigma_{a,F1}\sigma_{a,F2} \\ r_{a,F2R1}\sigma_{a,F2}\sigma_{a,R1} & r_{a,F2R2}\sigma_{a,F2}\sigma_{a,R2} & r_{a,F2F1}\sigma_{a,F2}\sigma_{a,F1} & \sigma^2_{a,F2} \end{bmatrix} \right),$$

where G is the genomic relationship matrix, σ^2_a is the additive genetic variance of a given trait; r_a is the genetic correlation between two traits. R1 = trait 1 in RAS, R2 = trait 2 in RAS, F1 = trait 1 in FT, and F2 = trait 2 in FT. The residual effects (e) were distributed as:

$$e \sim \left(\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, I \begin{bmatrix} \sigma^2_{e,R1} & r_{e,R1R2}\sigma_{e,R1}\sigma_{e,R2} & 0 & 0 \\ r_{e,R2R1}\sigma_{e,R2}\sigma_{e,R1} & \sigma^2_{e,R2} & 0 & 0 \\ 0 & 0 & \sigma^2_{e,F1} & r_{e,F1F2}\sigma_{e,F1}\sigma_{e,F2} \\ 0 & 0 & r_{e,F2F1}\sigma_{e,F2}\sigma_{e,F1} & \sigma^2_{e,F2} \end{bmatrix} \right),$$

where I is the identity matrix, σ^2_e is the residual variance of a given trait. r_e is the residual correlation between two traits. Residual correlations for traits measured in different systems were fixed at 0 because for individual fish reared in a single system, the model produces only one residual value. Residual correlations between different traits within the same system type were allowed to differ from 0 as both traits were measured on the same fish, and the model estimates two residuals. We determined the heritability for each trait as the additive genetic variance (σ^2_a) divided by the phenotypic variance (σ^2_p) corrected by fixed effects, $h^2 = \frac{\sigma^2_a}{\sigma^2_p}$. GxE was estimated through the genetic correlation $r_{a,RjFj}$ of the same trait j in different systems and calculated as: $r_{a,RjFj} = \frac{COV(\sigma^2_{a,Rj}, \sigma^2_{a,Fj})}{\sigma_{a,Rj}\sigma_{a,Fj}}$. A high value (close to unity) of $r_{a,RjFj}$ would be indicative of a low genotype-by-environment interaction, while a value lower than 0.8 is generally considered indicative of significant GxE (Robertson, 1959).

2.5. Generating lower density SNP subsets

To investigate the effect of lower-density genotyping on parameter estimates, we created GBS datasets with a smaller number of SNPs to cover the whole genome. This smaller SNP panel could be the result of using a different restriction enzyme combination, a targeted GBS approach like that of Martinez et al., 2023, which captures fewer genome segments for sequencing, or the development of a lower density SNP chip to reduce genotyping costs. Because SNPs in GBS often occur in clusters due to the specificity of the enzyme digestion and size-selection that results in DNA fragments for sequencing, we used a custom script "GBSsnpClusterThinKeep.R" (Supplemental 1) to randomly sample these DNA fragments ("SNP clusters") rather than individual SNPs.

Clusters were defined as SNPs within 340 bp on either side of a randomly sampled SNP taken from the full KGD-filtered vcf file containing 167,795 SNPs. We aimed to make subsets for the following target number of SNPs with the desired number of replicates in parenthesis: 100,000(10), 50,000(10), 25,000(10), 10,000(10), 5000(10), 2500(10), 2000(15), 1000(15), and 500(30). From each SNP subset we generated a new GRM and used it in the same four-trait animal model described above. We also used our shallow A-matrix in the four-trait animal model to serve as a base reference level for standard errors of genetic correlation estimates. The genetic correlations between the same traits in different environments and their standard errors were recorded from each replicate. The standard errors were used to compare the accuracies of these genetic correlation estimates from each SNP subset.

3. Results

3.1. Descriptive statistics

There was no significant difference in the mean weight between FT and RAS fish when they were first stocked into their respective systems at the start of Stage 2 (Table 1). However at harvest, weight and length were significantly different ($p < 0.0001$) between FT and RAS with RAS having a higher average harvest length and weight. This difference existed even though RAS fish were harvested at an average age of 378 days while FT fish were harvested at a higher average age of 412 days. The coefficient of variation was similar between all like traits across the two systems. RAS fish were on average reared for 523 degree days more than FT fish which equates to 24 extra days of growth at 21.6 °C (average RAS temperature in stages 2 and 3).

3.2. Parental contributions

Parentage assignment was successful for all 2115 genotyped experimental fish that passed genotype filtering and identified 187 full-sib families. The contribution of each family to the total pool of experimental fish is shown in Fig. 3. The smallest number of parents were found in hatch group 1 (October 19, 2020), which was produced by 5 males and 6 females.

3.3. Genetic parameters

The genetic variances for each trait in RAS were approximately double the genetic variance of the same trait in FT (Table 3), but error variances in RAS were around triple for the same traits in FT. This resulted in RAS having lower heritability estimates than FT. Overall, heritability estimates for all traits in both systems were relatively high. The standard errors of the heritability estimates in RAS were around one
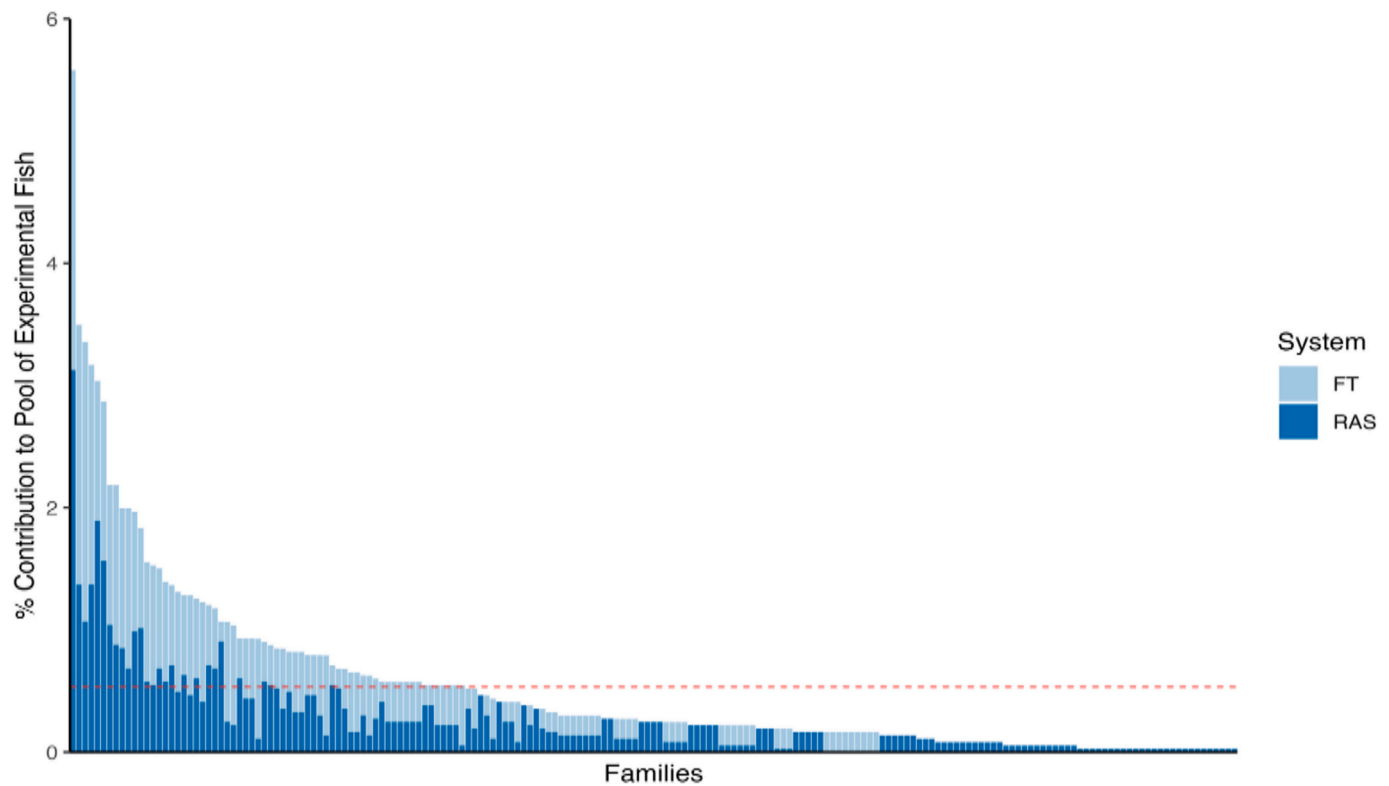
Fig. 3. Relative contribution (percentage) from each of the 187 families in Flow Through (FT) and RAS. The horizontal red line indicates the mean contribution of all families (0.53 %). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Additive genetic variance (σ_a^2), residual variance (σ_E^2), phenotypic variance (σ_P^2), and heritability (h2) for both traits in both environments estimated with the G from the full GBS dataset. Standard errors are shown in parenthesis, HL = Harvest Length, HW = Harvest Weight, RAS = Recirculating Aquaculture System, FT = Flow Through System.

Trait	Environment	σ_a^2	σ_E^2	σ_P^2	$h^2 \pm$ se
HL	RAS	3.96 (0.40)	3.63 (0.20)	7.59 (0.33)	0.52 (0.03)
HW	RAS	0.066 (0.006)	0.047 (0.003)	0.11 (0.005)	0.59 (0.03)
HL	FT	2.54 (0.62)	1.60 (0.39)	4.14 (0.39)	0.61 (0.11)
HW	FT	0.034 (0.007)	0.015 (0.004)	0.05 (0.005)	0.70 (0.10)

Table 4

Genetic correlations (se) (below diagonal), phenotypic correlations (se) (above diagonal), and heritabilities (se) (on diagonal) for both traits in both environments. n.e. = not estimable phenotypic correlations due to fish being in different environments. HL = Harvest Length, HW = Harvest Weight, RAS = Recirculating Aquaculture System, FT = flow through system.

	HL-RAS	HL-FT	HW-RAS	HW-FT
HL-RAS	0.52 (0.03)	*n.e.*	0.90 (0.01)	*n.e.*
HL-FT	0.57 (0.10)	0.61 (0.11)	*n.e.*	0.88 (0.01)
HW-RAS	0.92 (0.01)	0.46 (0.10)	0.59 (0.03)	*n.e.*
HW-FT	0.54 (0.10)	0.88 (0.04)	0.54 (0.10)	0.70 (0.10)

third of those of FT which was expected given the larger number of RAS fish in the study.

Phenotypic correlations between HL and HW within each environment were very high (Table 4). Genetic correlations between HL and HW within each environment were also high at 0.92 for RAS and 0.88 for FT. Genetic correlations between the same trait in different environments were much lower. The estimates were similar for the two traits HW (0.54 ± 0.10) and HL (0.57 ± 0.10). These estimates indicate a strong genotype-by-environment interaction for both traits and we observed substantial reranking of parental estimated breeding values for the same

trait in both environments (Fig. 4). However, for both HW and HL, parents with the most favorable breeding values showed large changes in scale but limited reranking. When looking at the performance of the top 10 % of all parents, RAS and FT shared 6 of the top 7 individuals for HW and 5 of the top 7 for HL. There was more reranking among parents with moderate breeding values.

3.4. SNP subsets

The accuracy of estimated genetic correlations between the same trait in RAS and FT decreased when we used fewer SNPs to build the GRM (Fig. 5). Using fewer SNPs also resulted in an increase in the variation in SE between replicates. Reducing the number of SNPs by 98.3 % from 167 K to 2.8 K only increased the mean S.E. from 0.10 to 0.13 in HW and from 0.10 to 0.12 in HL. Further reductions in SNP numbers below 2.8 K give increasingly larger SE. To achieve a level of accuracy comparable to the cross-environment genetic correlation estimate based on a shallow pedigree, the average number of SNPs needed was ~1.7 K for HW and ~2.1 K SNPs for HL respectively. Statistics on SNP subset groups can be seen in Table 5.
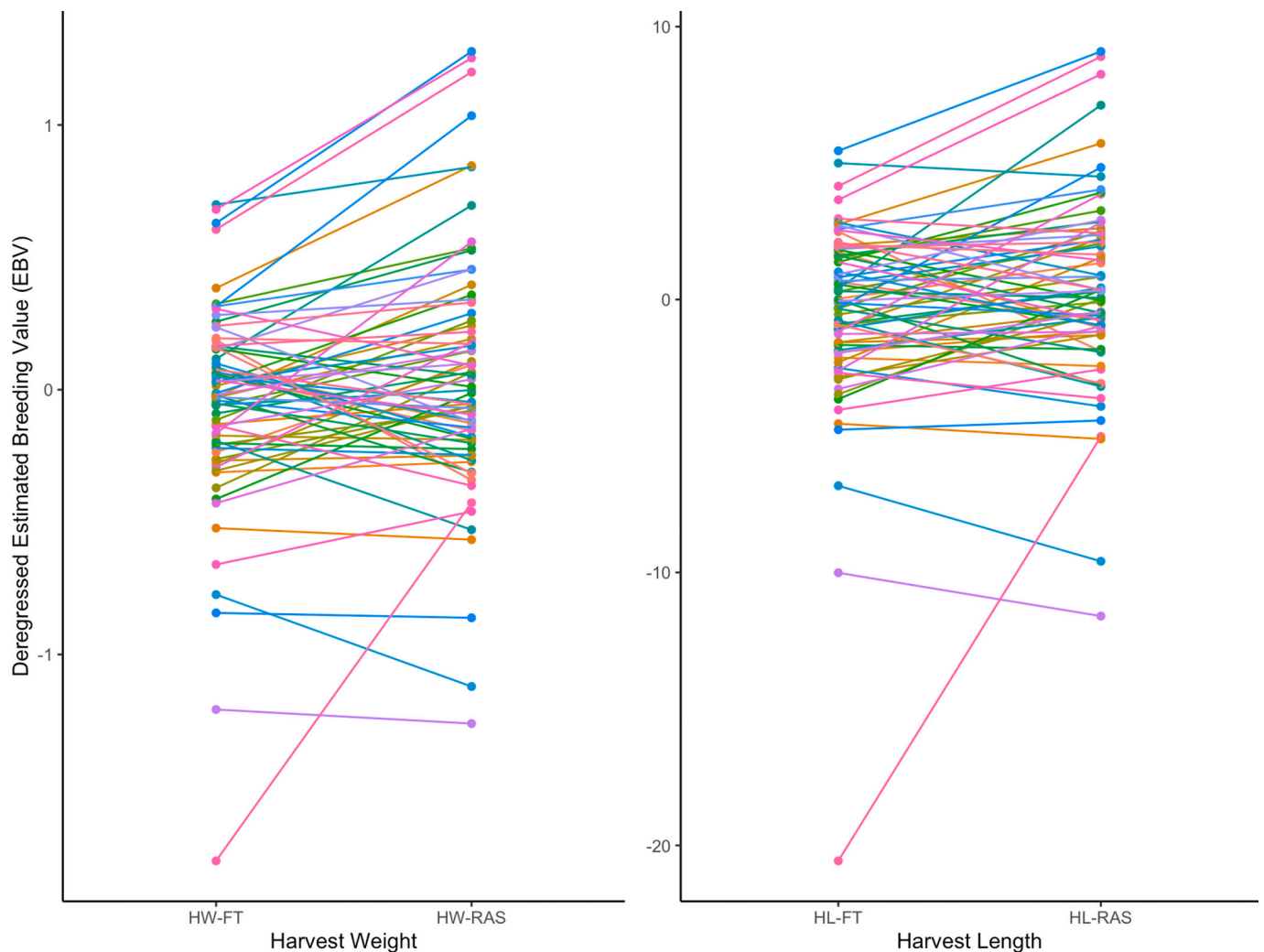
Fig. 4. Ranking of deregressed parental estimated breeding values for harvest weight and harvest length in both RAS and flow through environments.

4. Discussion

Understanding the genotype-by-environment interaction for production traits has important implications for breeding program design. If GxE is sufficiently strong, particularly if the between environment genetic correlations are close to zero or negative, it would be necessary to have separate breeding programs for different rearing environments or use a selection index to appropriately balance selection between them. In this study, we estimated GxE between RAS and flow through (FT) systems for harvest weight and harvest length in yellowtail kingfish. The FT system was meant to approximate a sea cage environment with fluctuating temperature and photoperiod, and thus the results of this study could help yellowtail kingfish breeders to optimize a breeding program that results in genetic gain for both RAS and sea cages.

The genetic correlations of 0.54 for HW and 0.57 for HL between the RAS and FT indicate moderate-to-strong GxE, and suggest that fish derived from a RAS-focused breeding program will only realize 54 % and 57 % of the genetic gain for HW and HL respectively when raised in FT. Given that some of the environmental variables (mainly weather and mechanical/UV pre-filtration) in the FT system were more similar to a RAS than a true sea cage, it is likely that the GxE between RAS and sea cages is even stronger than between RAS and FT. The moderate to strong GxE observed for harvest weight and length indicates that the genetic architectures responsible for the same trait in RAS and flow through environments are substantially different. Strong GxE results in substantial re-ranking (Fig. 4) which leads to a suboptimal selection of

breeding candidates and consequently decreased genetic gain in one or both environments. The majority of re-ranking occurred for individuals with moderate breeding values and is limited among the top individuals (Fig. 4). The limited re-ranking of top selection candidates in the current generation, however, does not guarantee that this will hold true in subsequent generations of selection.

Given the GxE observed, there are several options for breeding program design: 1) a single breeding program optimized for one system type with limited transfer of genetic gain to the other, 2) a balanced selection index designed for a single breeding program to favor generalist individuals that perform well "on average" across environments, and 3) separate breeding programs set up for each system type with the attendant costs of operating two breeding programs. Option 3 must be justified by the increased genetic gain achieved compared to option 2. The decision to use a balanced selection index vs separate breeding programs can be determined by finding the "break-even correlation". This is the genetic correlation where genetic gain is equal between the two breeding program designs when controlling for program cost (Mulder et al., 2006). The break-even correlation must be determined individually by a breeder depending on operational, genotyping, and phenotyping costs.

While our study was designed to estimate the overall effect of the two rearing environments rather than to identify the system-specific environmental effects that drive the observed GxE, we hypothesize that there are three likely variables: density, temperature, and photoperiod. It is possible to isolate the effects of these variables, however this requires
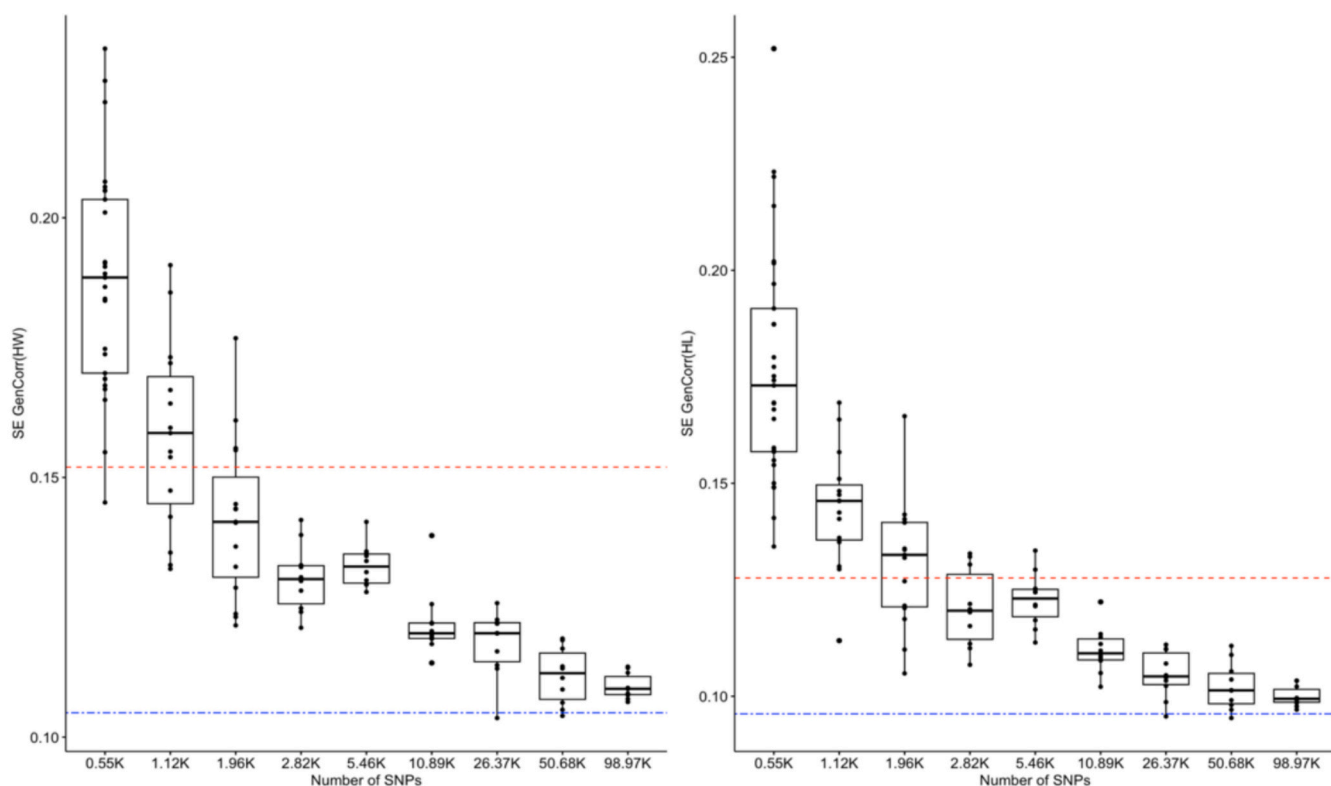
Fig. 5. Standard errors of genetic correlation estimates between RAS and FT for HW (left) and HL (right) estimated using between 10 and 29 different random subsets of the GBS SNPs. – – – = The standard error using the full SNP set, – – – = SE using the shallow A-matrix.

Table 5
Number of SNPs (#SNPs) and number of SNP clusters selected per replicate. SD = Standard Deviation, CV = Coefficient of Variation.

Average #SNPs	SNP clusters	Mean #SNPs	Min #SNPs	Max #SNPs	Range #SNPs	SD	CV	Number of replicates
0.59 K	122	594.1	549	693	144	49.0	0.08	29
1.12 K	245	1122.2	984	1204	220	74.7	0.07	15
1.96 K	430	1956.9	1768	2144	376	106.5	0.05	15
2.82 K	616	2822.5	2648	3050	402	126.9	0.04	10
5.46 K	1244	5456.5	5293	5644	351	114.6	0.02	10
10.89 K	2534	10,892	10,562	11,221	659	181.1	0.02	10
26.37 K	6727	26,373.9	26,039	26,732	693	211.7	0.01	10
50.68 K	15,116	50,679.2	50,328	51,140	812	307.8	0.01	10
98.97 K	42,410	98,968.4	98,578	99,374	796	206.6	0.002	10

carefully manipulating each variable independently to avoid confounding them with the system environment. Because commercial RAS operate at higher densities, temperature, and photoperiod, we intentionally grouped these effects into our system-type variable, so our findings have practical application for industry. It is still important to note how the FT and RAS systems differed in our experiment so that future studies can estimate the system-specific effects of these environmental variables.

Fish in RAS were stocked at the start of Stage 2 at 20.3 kg/m³, 146 % higher than FT fish. RAS densities peaked at 104.9 kg/m³, with a final density of 67.1 kg/m³ at harvest which was 45 % higher than in FT. While intensive rearing is a key feature of RAS, the limited staff availability due to the SARS-CoV-2 pandemic during the end of stage 2 resulted in higher rearing densities than originally planned. Since excessively high densities in any rearing system will have a negative impact on growth, e.g., in rainbow trout and tilapia (Gibtan et al., 2008; Holm et al., 1990), it is possible that the short period of higher than intended density in RAS imposed a penalty on growth even with sufficient feed.

S. lalandi has an optimal temperature range for growth from 24 to 26.5 °C when dissolved oxygen levels are adequate (Abbink et al., 2012; Bowyer et al., 2014). Our study took place primarily during the winter months in New Zealand where FT temperatures routinely dropped below 15 °C. The average RAS temperature of 21.6 °C was 4.5 °C warmer than the average FT temperature of 16.1 °C which could largely explain the differences in growth between the two system types. Importantly, the quantity of feed given to each tank was not based solely on fish biomass but also on tank temperature. Because RAS tanks were much warmer for most of the experiment, RAS fish were given more feed which almost certainly resulted in higher growth. The decision to feed fish in RAS more was not made to introduce bias but rather to more accurately mimic a RAS production environment which operates at a higher temperature. The FT system in our study was set up to reflect the temperatures of a sea cage environment and tracked the temperature of the oceanic environment extremely well (Fig. 2). While the Bream Bay sea surface temperature was on average slightly higher than the FT tank, it is likely an overestimation of the temperature in sea cages which typically extend some distance below the ocean surface.

Photoperiod is another factor that could influence growth. RAS fish in our study were kept under 24-hour light to increase the daily feeding

window while FT fish were kept under ambient photoperiod and were fed only during daylight hours. The higher number of feeding bouts in RAS could lead to more efficient feed utilization and weight gain as seen in other aquaculture fish species (Argüello-Guevara et al., 2018; Daudpota et al., 2016; Holm et al., 1990).

The aim of using a FT system was to approximate a commercial sea cage environment with ambient temperature and light profiles; however, for other parameters, our FT was more similar to a RAS. FT water was cleaner and contained fewer pathogens than raw seawater, as it was filtered and UV-sterilized before entering the facilities, making it effectively sterile in comparison. The FT tank structure itself is like a RAS tank, and other fluctuations in environmental conditions such as weather patterns, tides, and oceanic debris that affect sea cages were nearly eliminated. Because our FT environment is an intermediate environment between a RAS and sea cage, we believe our GxE findings are likely to be conservative estimates of what would be expected between a RAS and a true sea cage farm. Conversely, Premachandra et al. (2017) produced optimistic genetic correlations between sea cages and RAS for harvest weight (0.92 ± 0.47) and length (0.97 ± 0.67) by keeping RAS temperatures and densities similar to the sea cage. The RAS densities of 6 kg per m^3 were tenfold lower than in our study. Because commercial RAS operate at higher temperatures and densities than sea cages, the genetic correlations by Premachandra et al. (2017) likely underestimate GxE and, given their large standard errors, are less informative than the estimates in this study.

One complication of this study is that RAS and FT fish were harvested at different weights and ages with RAS fish being harvested earlier and at heavier weights. The animal model partially accounts for this because the fixed hatch-group and tank effects re-express the data as deviations from contemporary groups. Nevertheless, when comparing rearing time in degree days, RAS fish had 24 extra days of growth at their average RAS temperature of 21.6 °C. Hence, it is possible that the GxE observed is to some extent, a function of size and/or age rather than just the rearing environment. If the genetic correlation between weight and length at the two ages/sizes is low, then treating them as the same trait is unjustified. Thorland et al., 2020 found the genetic correlation between body weight at harvest and 4 months prior in Atlantic salmon to be 0.92. Turra et al., 2012 found a genetic correlation of 0.9 for weight between Nile tilapia at 130 and 200 days old which is towards the end of the growth cycle. He et al., 2017 found a genetic correlation of ~0.85 for body weight measured at 100 and 140 days in Nile tilapia. All three of these studies found that the genetic correlation between body weight decreased as the time between measurements increased. Harvest weight and length in RAS were measured on fish that were 34 days younger in absolute growth days and 24 days older when considering degree days. These age differences are smaller or similar to the differences reported above. Therefore, we don't expect that these age and weight differences had a large impact on our estimates of genetic correlation for traits between the two systems.

Another potential concern is the smaller sample size in FT ($n = 304$) compared to RAS ($n = 1811$), which could reduce the precision of genetic parameter estimates and potentially introduce bias to the resulting genetic correlation estimates used to assess GxE. Sae-Lim et al. (2010) simulated bias in genetic correlation estimates and found that for low-heritability traits ($h^2 = 0.1$), small fish family sizes (<10) led to downward bias. For moderate-heritability traits ($h^2 = 0.3$), slight downward bias occurred when the number of fish per environment was low (300−1000) and the true genetic correlation was high ($r_g = 0.8$). However, this bias was minimal and not reported as statistically significant, and the standard errors of their genetic correlations were high ranging from 0.18 to 0.32. In other simulations with small numbers of fish, no bias was observed when the true r_g was 0 or 0.5. Given that our traits have high heritabilities ($h^2 = 0.52–0.70$), moderate genetic correlation estimates (0.54–0.57), and low standard errors (0.10), we believe our genetic correlation estimates fall outside the range of scenarios at risk of downward bias described in Sae-Lim et al. (2010).

Nonetheless, further research with larger FT sample sizes would help confirm our findings.

Genotyping-by-sequencing was found to be a powerful tool to generate more accurate estimates of genetic correlations based on genomic relationships in comparison to pedigree-based estimates, however for our application, as well as others (Kriaridou et al., 2020), the number of SNPs produced by our genotyping workflow is potentially excessive. We found that randomly reducing our full SNP dataset by 98.3 % to 2.8 K SNPs (616 GBS SNP clusters) only increased the S.E. from 0.10 to 0.13 in HW (genetic correlation = 0.54) and from 0.10 to 0.12 in HL (genetic correlation = 0.57). The average number of SNPs needed to outperform the pedigree-based estimates was ~1.7 K for HW and ~2.1 K SNPs for HL respectively. We used a shallow pedigree matrix where only the parents and the experimental animals were included. Most breeding programs have pedigrees with many more generations which will result in more accurate relationship matrices and better estimates of genetic parameters.

For most practical applications, it is convenient for breeders to use the same genotyping data to estimate both breeding values and variance components. Therefore it is important to know the minimum number of markers necessary to obtain both accurate breeding values and variance components. Kriaridou et al., 2020 found that using as few as 1000 randomly sampled SNPs gave a genomic prediction accuracy comparable to the full SNP panel for four different aquaculture species and traits. Fraslin et al., 2023 found that 3000 randomly selected SNPs yielded breeding value accuracies comparable to a 28 K SNP panel for *Flavobacterium columnare* resistance in rainbow trout. Song and Hu (2021) found that pruning to 3000 SNPs based on linkage disequilibrium resulted in the same accuracy of genomic breeding value prediction as using high density SNP panels ranging from 12 K–57 K for four aquaculture species and traits.

While the relationship between SNP array density and accuracy of genomic prediction of breeding values is well studied, to our knowledge, no one has researched the relationship between the number of SNPs and the confidence intervals of genetic correlation estimates used to quantify GxE.

The GBS fragment size in our study was only 680 bp which likely produces high linkage disequilibrium between SNPs within clusters. This means that the number of randomly selected clusters in our simulations is approximately comparable to the number of randomly selected SNPs in a SNP array, where SNPs are typically included based on being evenly spread across the genome. The 2.8 K SNPs or 616 random SNP clusters is enough to estimate genetic correlations with limited loss of accuracy and therefore the 1000–3000 SNP needed for accurate breeding value estimation (Fraslin et al., 2023; Kriaridou et al., 2020) should also be enough to effectively estimate genetic correlations. One assumption of our SNP sampling was that SNPs retained the same sequencing depth regardless of the number of sampled SNPs. Standard GBS techniques may not be able to greatly reduce the number of SNPs in GBS without lowering sequencing depth; however, recently developed genotyping methods such as 3D-GBS (de Ronne et al., 2023), RAD capture (Rapture) (Ali et al., 2016), and GT-seq (Campbell et al., 2015) offer cost saving opportunities by utilizing unique restriction enzyme combinations, biotinylated oligonucleotide baits, or SNP-targeted oligonucleotides, respectively, to reduce genome coverage and produce less markers than traditional GBS at the same sequencing depth. Alternatively, our data could be used to develop a low or moderate density SNP array. Both approaches could lower genotyping cost and facilitate genotyping more individuals for the same budget.

5. Conclusions

In this study, moderate-to-strong genotype-by-environment interactions (GxE) were found for harvest weight and harvest length in yellowtail kingfish reared in flow through and recirculating aquaculture systems. Providing both production systems with a breeding program

will require a balanced selection index for performance in both system types or two separate breeding programs for each system type. The number of SNPs used to estimate genetic correlations between the same trait in different environments can be significantly reduced from standard SNP arrays that usually contain 30 K or more SNPs. Reducing genome representation in GBS, or using lower coverage SNP panels, will have a limited impact on accuracy of genetic correlation estimates and should reduce genotyping costs.

Abbreviations

FT	flow through
RAS	Recirculating Aquaculture System
HW	Harvest Weight
HL	Harvest Length
GxE	genotype-by-environment interaction
GBS	genotyping-by-sequencing

CRediT authorship contribution statement

Nicholas K. Jacob: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Mark Camara:** Supervision, Data curation, Conceptualization. **Alvin Setiawan:** Writing – review & editing, Data curation, Conceptualization. **John W.M. Bastiaansen:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: NIWA funded and hosted this research and employs both co-authors Mark Camara and Alvin Setiawan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742592.

Data availability

The data that has been used is confidential.

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