1 Unravelling the complex genetic basis of growth in New 2 Zealand silver trevally (*Pseudocaranx georgianus*)

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19 **Abstract**

20 Growth directly influences production rate and therefore is one of the most important and
 21 well-studied trait in animal breeding. However, understanding the genetic basis of growth
 22 has been hindered by its typically complex polygenic architecture. Here, we performed
 23 quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) for
 24 10 growth traits that were observed over two years in 1,100 F₁ captive-bred trevally
 25 (*Pseudocaranx georgianus*). We constructed the first high-density linkage map for trevally,
 26 which included 19,861 single nucleotide polymorphism (SNP) markers, and discovered
 27 eight QTLs for height, length and weight on linkage groups 3, 14 and 18. Using GWAS, we
 28 further identified 113 SNP-trait associations, uncovering 10 genetic hot spots involved in
 29 growth. Two of the markers found in the GWAS co-located with the QTLs previously
 30 mentioned, demonstrating that combining QTL mapping and GWAS represents a powerful
 31 approach for the identification and validation of loci controlling complex traits. This is the
 32 first study of its kind for trevally. Our findings provide important insights into the genetic
 33 architecture of growth in this species and supply a basis for fine mapping QTLs, marker-
 34 assisted selection, and further detailed functional analysis of the genes underlying growth
 35 in trevally.

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38 Introduction

39 Growth facilitates essential functions such as reproduction or the ability to adapt to
 40 environments. Although there are exceptions, increase in body size is usually positively
 41 correlated with numerous fitness traits such as higher mating success and fecundity,
 42 increased offspring quality and lengthened longevity (Gebhardt-Henrich and Richner 1998,
 43 Dmitriew 2011). Most vertebrates exhibit a finite amount of growth. In fish, however, the
 44 process of growing is indeterminate, or indefinite, and continues throughout their life
 45 (Weatherley 1972), although the rate tends to decline as body size increases (Pedersen
 46 and Jobling 1989). This highly complex process is the result of interactions between
 47 environmental effects and genetic differences. Factors including sex (e.g. Imsland and
 48 Jonassen 2003), age or food availability (e.g. Jones 1986) influence growth rate, as well
 49 as abiotic factors changing seasonally such as temperature (e.g. Karås and Klingsheim
 50 1997, Imsland, Schram et al. 2007), photoperiod (e.g. Imsland and Jonassen 2003) or
 51 oxygen levels (e.g. Brett and Groves 1979). The genetic basis of growth traits is typically
 52 highly polygenic (Wellenreuther and Hansson 2016). Functional relationships between
 53 genetic variations and physiological parameters of growth have been described in
 54 commercially relevant species such as cod (*Gadus morhua*) (see review by: Imsland and
 55 Jónsdóttir 2002), Atlantic salmon (*Salmo salar*) (Tsai, Hamilton et al. 2015) or tilapia
 56 (*Tilapia mossambica*) (Liu, Sun et al. 2014), but are still poorly understood in many non-
 57 model species.

58 With the development of next generation sequencing technologies (NGS) the cost of high-
 59 density genotyping has drastically decreased, therefore enabling the more widespread
 60 application of quantitative trait locus (QTL) mapping experiments. Many variants
 61 associated with complex phenotypes have since been found in a variety of non-model
 62 species. However, QTL mapping approaches only allow the identification of genetic
 63 regions that are polymorphic between two parents, and relevant in a particular
 64 environment, therefore potentially missing some common variants associated with the
 65 traits (Mackay 2001). For this reason the location and effects of detected QTLs can vary
 66 between mapping populations. For example, in the Atlantic salmon, QTLs involved in
 67 growth have been found on different linkage groups according to different studies
 68 (Baranski, Moen et al. 2010, Tsai, Hamilton et al. 2015, Besnier, Solberg et al. 2020).

69 Recently, genome-wide association studies (GWAS) have been widely employed to detect
70 QTLs both in captive (Palaikostas, Kocour et al. 2018) and natural populations (Santure
71 and Garant 2018). This technique identifies associations between markers and
72 phenotypes based on linkage disequilibrium (LD) (Meuwissen and Goddard 2010).
73 Compared with QTL mapping, GWAS can be conducted on genetically diverse, unrelated
74 individuals and is particularly advantageous when controlled crossing and generation of
75 large segregating populations is difficult. GWAS has proved useful for the identification of
76 loci associated with numerous growth traits in fish (e.g. Yang, Wu et al. 2020). However,
77 the power of GWAS relies on the number of markers in relation to the extent of linkage
78 disequilibrium in the population (Newell, Cook et al. 2011). When applied to natural
79 populations or collections of outcrossing individuals, characterised by a rapid linkage
80 disequilibrium decay, tens of thousands of markers are often required to obtain an
81 adequate level of resolution. Because of the intrinsic limitations of statistical methods
82 comparing thousands of tests, GWAS has a higher frequency of false discovery of loci
83 than QTL mapping (Hayes, Lewin et al. 2013). A final disadvantage of GWAS is its low
84 power for detecting rare allelic variants. However, their frequency can be increased in
85 controlled crosses and therefore captured with QTL mapping. Hence, combining GWAS
86 and QTL mapping often gives more complete and reliable results than using one of the
87 methods alone (Fraslin, Quillet et al. 2020).

88 Aquaculture is the fastest growing food-production sector in Aotearoa New Zealand.
89 Currently, the local industry relies almost exclusively on the farming of three species:
90 Greenshell™ mussels (*Perna canaliculus*), Pacific oysters (*Crassostrea gigas*) and only
91 one finfish, king/chinook salmon (*Oncorhynchus tshawytscha*), which is an introduced
92 species (Camara and Symonds 2014, Symonds, Clarke et al. 2019). Hence, there is a
93 strong interest in diversifying the range of species available for aquaculture. The native
94 finfish silver trevally (*Pseudocaranx georgianus*, Cuvier 1833) has been identified as a
95 suitable candidate for New Zealand aquaculture. In Aotearoa, its Māori name is araara.
96 Indigenous Māori people have a strong cultural connection to trevally, where it is
97 considered as taonga (i.e. has value, or is treasured). Trevally is a shoaling pelagic
98 species found throughout the coastal waters of southern Australia and around New
99 Zealand (Smith-Vaniz and Jelks 2006). It is most common at depths of approximately 80
100 m, although its range is thought to be 10–238 m and can reach over 40 years of age

(Ministry for Primary Industries 2014, Bray 2020). In many regions of New Zealand, trevally is a major component of recreational and commercial fisheries (Ministry for Primary Industries 2014). For it to be suitable for commercial aquaculture, however, trevally's growth rate must be improved, which can be done using selective breeding (Valenza-Troubat, Montanari et al. 2021).

In this study, we investigated the genetic architecture of 10 growth traits in a new population of trevally. More specifically, we genotyped and phenotyped 1,100 F₁ trevally to 1) generate a high-density linkage map, 2) detect rare QTLs associated with growth using linkage mapping in a sub-family comprising 89 individuals, 3) identify common SNPs associated with growth using GWAS on the whole population, and 4) overlap the results and annotate supported regions.

Methods

Study population

The trevally population used in this study was generated as part of a breeding programme started at the Plant and Food Research (PFR) finfish research facility in Nelson, New Zealand. The full description of holding conditions and pedigree are described in Valenza-Troubat *et al.* (2021). Briefly, the population comprised of 13 wild caught F₀ broodstock and 1,100 F₁ captive-bred offspring. In 2015, induced mass spawning of the F₀ generation in a single tank was used to produce the offspring F₁ generation. This resulted in a complex pedigree, including a combination of unrelated, full- and half-siblings in the F₁ generation. F₁ offspring were held in a single tank from hatch receiving the same feeding regime, light, water flow and aeration until the end of this experiment. The seawater tanks in the facility are located on the seaward side of Port Nelson and receive ambient seawater from an underground bore which is filtered using mesh filters and UV treatment.

Phenotyping and trait estimation

Ten growth traits were used in the current study, namely peduncle length (PL), height at 25 (H25), 50 (H50) and 75% (H75) of the PL, estimated weight (EW) and related net gain traits (Δ PL, Δ H25, Δ H50, Δ H75 and Δ EW, respectively) (Valenza-Troubat, Montanari et al. 2021). These measurements were recorded on three occasions throughout the

experiment, at the beginning (November 2017), in the middle (October 2018), and at the end (November 2019), when the fish were a little over two, three and four years old, respectively. Using the Morphometric SoftwareTM (<https://www.plantandfood.co.nz/page/morphometric-software-home/>), the outline of each fish was extracted from images and morphometric were located, and then used to make measurements. PL was measured by assessing the distance between the upper lip and narrowest cross-section of the tail. Height was measured at three positions along the fish: 25, 50, and 75% of the PL. The weight estimations (EW) were done following a Bayesian hierarchical approach as described in Froese et al. (2014). The net gain for each trait was calculated as the difference from the initial measurement (November 2017) with subsequent ones. The normality of the data was assessed visually using Quantile-Quantile (QQ) plots generated in the R statistical environment version 3.2.3 (R Development Core Team 2016).

Genotyping and variant calling

Samples of fin tissue from 13 tagged F_0 and 1,100 F_1 individuals were collected and stored as described in Valenza-Troubat et al. (2021). Total DNA was extracted as described in Ashton et al. (2019) with minor modifications, and then quantified and quality-checked by fluorescence, spectrophotometry and agarose gel electrophoresis. The 13 F_0 individuals were whole genome sequenced (paired-end, 125 bp reads) over three lanes of the HiSeq 2500 platform at the Australian Genome Research Facility (AGRF, Melbourne, Australia). The F_1 were genotyped using a modified GBS approach (Elshire, Glaubitz et al. 2011), as described in Valenza-Troubat *et al.* (2021). A total of 12 pools of 96 samples each were prepared and sent to AGRF for sequencing on a HiSeq 2500 platform (single-end, 100 bp reads). Sequencing data quality for both F_0 and F_1 generations were checked using FastQC v0.11.7 (Andrews 2010). Raw reads from the F_0 were trimmed using trimmomatic v0.36 (Bolger, Lohse et al. 2014) (using the parameters HEADCROP: 9, TRAILING: 10, SLIDINGWINDOW: 5:20, MINLEN: 75). The F_1 samples were de-multiplexed from the 12 sequencing libraries using the process_radtags module available in the STACKs version 2.1 pipeline (Catchen, Hohenlohe et al. 2013) and the reads were trimmed using Fastq-mcf in ea-utils v1.1.2-806 (minimum sequence length = 50, quality threshold causing base removal = 33) (Aronesty 2013). Read groups were added to all sequences and bam files were sorted and indexed using Picard toolkit (Broad_Institute 2015). Reads were then

mapped to the reference genome (Catanach, Ruigrok et al. 2021) using the Burrows-Wheeler Aligner (BWA) v0.7.17 (Li & Durbin, 2009) and variants were called jointly with the parallel module of freebayes v1.3.1 (Garrison and Marth 2012), with minimum of five observations and minimum mapping quality of 10. The SNPs with single-sample sequence coverage (sequencing depth) < 3 were removed to reduce the number of putatively erroneous genetic variants, and missing data and minor allele frequency (MAF) were set to < 20% and > 0.05, respectively.

Linkage map construction and QTL identification

The parents of each F_1 individual in the dataset were identified with Sequoia v2.0.7 (Huisman 2017) as reported in Valenza-Troubat *et al.* (2021). The full SNP dataset (i.e. before the stringent filtering performed for the parentage analysis) was filtered for Mendelian errors (> 5%), and checked for distorted segregation using a chi-square test with $\alpha=0.05$. The linkage map was constructed in Lep-MAP v3.0 (Rastas, Paulin et al. 2013), using the largest family. Markers were separated into linkage groups (LG) with the SeparateChromosomes module (logarithm of odds (LOD) limit = 14, minimum markers per LG = 50). The order of the markers was computed with the OrderMarkers module. Single markers at the end of each LG were removed if they were more than 3 cM apart from the next closest marker. MapChart v2.32 (Voorrips 2002) was used to visualise the genetic map. As the F_0 were assumed to be outbred, the linkage map and the genotypes of the mapping family were input as a 4-way cross in the R package R/qtl version 1.47-9 (Broman, Wu et al. 2003) for interval mapping. Standard interval mapping was performed and a genome-wide permutation test (Doerge and Churchill 1996) with 1000 permutations was used to determine the LOD significance thresholds (p -value = 0.05).

Genome-Wide Association Study

GWAS was carried out on the entire genotyped F_1 population ($n=1100$). SNPs were removed if the call rate was smaller than 0.8, MAF < 0.01, if Mendel error rate > 5% (based on trios identified with the parentage analysis carried out above), and they were LD-pruned using an $r^2 > 0.80$ in a 50kb sliding window with five variants. Association analysis were performed using the Fixed and random model Circulating Probability Unification (FarmCPU) method (Liu, Huang et al. 2016) implemented in GAPIT3 v3.1.0 (Wang and Zhang 2021). A Bayesian information criterion (BIC)-based model selection

was used to find the optimal number of principal components (PCs) for each time measure, to account for family and population structure. The cut-off for significant association was a False Discovery Rate (FDR) adjusted p -value = 0.05 (Benjamini and Hochberg 1995), to control for multiple testing. To assess how well the model used in GWAS accounted for population structure and family relatedness, results of the GWAS were visualised with QQ plots implemented in GAPIT3, which depicted the distribution of the actual p -values compared with the theoretical ones. Manhattan plots were used to visualise the SNPs associated with the different phenotypes, using the physical position of the markers on the reference genome.

Ethics statement

All research carried out in this study was reviewed and approved by the animal ethics committee of Victoria University of Wellington in New Zealand (Application number 25976).

Results

Phenotypic values of growth traits

All offspring were phenotyped at the first measurement (November 2017), while numbers decreased at the two subsequent time points because of natural mortality that occurred during the study. All traits showed a normal distribution and exhibited large levels of phenotypic variation, as discussed in Valenza-Troubat et al. (2021). A summary of the mean values and standard deviations of the 10 growth phenotypes is shown in Table 1. In both the family used for QTL mapping and in the entire population used for GWAS, a normal distribution of the residuals was observed for the ten traits investigated (Figure 1). Transgressive lines (i.e. offspring that have more extreme phenotypes than the parents) were observed in the population.

High-density linkage map

Sequencing of the 13 parental fishes generated 1.23 billion reads, corresponding to an average genome coverage of 13x per individual. Of the 1,100 offspring, 1094 F1 were successfully genotyped via GBS, resulting in 0.42x genome coverage per individual fish. Variant calling and basic quality filtering generated a dataset of 171,923 SNPs. Overall, 21 families from 10 out of the 13 sequenced F0 fishes were identified by Valenza-Troubat et

al. (2021) when reconstructing the pedigree of the population. The largest family included 87 offspring and was used to assemble the sex-averaged linkage map. A total of 21,665 SNPs were polymorphic in this family and passed the chi-square test, and 19,861 were successfully mapped to 24 Linkage groups, 1,830 after removal of the co-mapping loci (Figure 2). LG numbers were randomly assigned, as no previous reference had been published. The genetic map spanned 1,335.46 cM, with an average marker distance of 0.73 cM. The largest gap was on LG14 and was 4.10 cM long. The longest and shorter Linkage groups were 17 (82.89 cM) and 8 (48.14 cM), respectively (Table 2).

QTL mapping revealed rare variants associated with growth

The genome-wide significant thresholds for QTL mapping were established at LOD values between 4.60 and 4.81 for the ten phenotypic traits across three years (Table 3). In total, eight significant QTLs were identified on four locations in three linkage groups (Figure 3). Six of these QTLs were for traits recorded during the first measurement (November 2017), and two for the last measurement (November 2019). In particular, three QTLs detected for H25, PL and EW measured at the first time point co-mapped on LG3 (between 23.2 and 24.9 cM). A second QTL was mapped to LG3 at 52.1 cM for H75 from the first measurement. Additionally, the traits PL and EW from 2017 were associated to a second locus, at the top of LG18. The percentage of phenotypic variance explained (PVE) by the 2017 QTLs ranged from 22.1 to 25.3%. In the last measurement two QTLs for Δ H75 and Δ PL were found to co-map to LG14 (between 17 and 17.1 cM), with PVE of 39.7 and 31.1, respectively (Table 3). No QTLs were found for the middle measurement (October 2018) and for all other traits in 2017 and 2019.

Genome-wide association found strongly associated SNPs

A total of 1,024 F1 individuals had both genotypic and phenotypic data for all measurements. After filtering based on Mendelian errors, MAF and LD pruning, 107,067 SNPs were left and used in the GWAS. Model selection in GAPIT resulted in no PCs to be used as co-variates in any of the traits (Figure 4A). QQ plots showed that FarmCPU adequately accounted for the confounding effects of family and population structure (Figure 4B). A total of 93 SNPs were significantly associated ($-\log_{10}(p) > 7.03$) with at least one of the ten traits measured at each year of phenotyping (Figure 4C, Table S1). Only Δ H75 in October 2018 and November 2019 had no significant association. Of the 93

SNPs, 15 were associated with more than one trait. These included four, seven and four SNPs associated with measurements at the first, second and third time point, respectively. No common SNPs were identified among different years. Significant associations were in some cases found for SNPs less than 0.5 Mb apart, highlighting hot spots on chromosomal regions. A total of 10 hot spots were identified: two on LG1, one on LG2, two on LG3, two on LG5, one on LG6, one on LG10 and one on LG18. Six of these included associations for traits measured in different years (Table S1).

Discussion

We constructed the first genetic map for trevally, and we were able to use this map to determine genomic regions associated with phenotypic growth traits. Our map included 19,861 SNPs across 24 linkage groups and confirmed the 24 mega-scaffolds of the trevally reference genome (Catanach, Ruigrok et al. 2021). A linkage map built for yellowtail kingfish (*Seriola lalandi*), the closest species to trevally for which a map has been constructed, was also resolved into 24 linkage groups (Nguyen, Rastas et al. 2018), consistent with our findings. The map built here for trevally was 1,335.46 cM in length, which is within the range of map length expected for many teleost fish. For example, the map assembled here is longer when compared to the yellowtail kingfish map, similar in length to the map of the Australasian snapper (*Chrysophrys auratus*) (Ashton, Ritchie et al. 2019) and shorter when compared to the European sea bass (*Dicentrarchus labrax*) (Griot, Allal et al. 2021).

Eight significant QTLs related to growth were mapped to three of the trevally linkage groups. Other studies in teleost have found multiple QTLs affecting growth such as in Atlantic salmon (Tsai, Hamilton et al. 2015, Besnier, Solberg et al. 2020), Australasian snapper (Ashton, Ritchie et al. 2019), spotted sea bass (*Lateolabrax maculatus*) (Liu, Wang et al. 2020) or yellowtail kingfish (Nguyen, Rastas et al. 2018), supporting the hypothesis of a polygenic regulation. No significant QTLs were identified with QTL mapping for the second time point, October 2018. In the segregating family used for QTL mapping, the number of offspring decreased from 87 in 2017 to 68 in 2018 and 67 in 2019 as the result of natural mortality. Although some statistically significant QTLs were found in the measurements made in the latter period of the experiments, the smaller sample sizes in the two last measurements reduced the power of the study, which could explain the

284 absence of QTLs detected in October 2018. Indeed, sample size is known to influence the
285 power of a study to detect QTLs (Hong and Park 2012), and is regularly discussed as one
286 of the most important concerns when designing a mapping experiment (Ashton, Ritchie et
287 al. 2017). Interestingly, two traits (Peduncle Length and Estimated Weight) shared the
288 same QTLs on two linkage groups in the first measurement period (November 2017). This
289 is consistent with the level of high genetic and phenotypic correlations reported for body
290 length and body weight (Valenza-Troubat, Montanari et al. 2021). These findings suggest
291 that selection applied on easily measurable traits such as length will result in the
292 concomitant improvement of more difficult to assess, yet valuable, growth traits (such as
293 weight) in the breeding of trevally.

294 GWAS identified 113 associations with growth, corresponding to 93 different SNPs
295 spanning 22 LGs, further supporting the hypothesis of a polygenic basis of growth-related
296 traits in trevally. Growth is considered as a complex trait and has been found to be
297 polygenic across the tree of life, in very diverse taxa from plants, like in the model species
298 *Arabidopsis thaliana* (Wieters, Steige et al. 2021), to vertebrates like humans (Sinnott-
299 Armstrong, Tanigawa et al. 2021) and other fish species (e.g. Liu, Sun et al. 2014, Yang,
300 Wu et al. 2020, Debes, Piavchenko et al. 2021). In our study, a lower number of loci were
301 found with the QTL mapping experiment than with the GWAS, which can be explained by
302 the smaller amount of genetic variation represented in the single F₁ family compared with
303 the overall breeding population, which was derived from thirteen parental individuals. Four
304 QTLs and 15 SNPs were found to have a significant association with more than one trait.
305 This was expected, as the different phenotypes were all deduced from the Peduncle
306 Length and they all then measured the same process underlying growth.

307 For each trait, the markers found in association were different from a year to another, both
308 in QTL mapping and in GWAS. These differences could be due either to significant loci
309 changing over time, by switching on or off, or to environmental variation, as it was
310 observed in other QTL mapping studies that investigated traits highly affected by the
311 environment (Sun, Niu et al. 2017, Ashton, Ritchie et al. 2019). However, there were some
312 hot spot regions of 0.5 Mb that contained SNPs associated to different traits and different
313 years. Noteworthy is the chromosomal region at the top of LG5, where two hot spots were
314 found, one between 0 and 0.5 Mb (for Δ EW and PL in 2018, and H75 and ES in 2019) and
315 one between 1.4 and 1.8 Mb (for Δ EW, H25, H50 and PL in 2019). Future studies should

investigate these further. In addition to these hot spots, we also identified SNP regions that were significant in both QTL and GWAS analyses. Specifically, by comparing the relative physical positions of the SNPs, two regions found with QTL mapping appeared to be in close proximity with two significant SNPs identified by GWAS. In particular, the QTLs for H25, PL, and EW measured in 2017 spanned the 4.45-4.50 Mb region on chromosome 3 (corresponding to 23.20-24.90 cM on LG3) and SNP trevally000114_4956408, located at 4.96 Mb on chromosome 3, was found to be significant for PL in 2019 with GWAS; and the QTLs for Δ H75 and Δ PL in 2019, encompassing the 25.18-25.21 Mb region on chromosome 14 (17.02-17.59 on LG14), are close to SNP trevally001025_25939455, located at 25.94 Mb on chromosome 14 and significant for Δ H50 and Δ PL in 2019 (Figure 5, Table 3 and Table S1). Being identified with two different statistical analyses and for multiple traits across two years, these two regions are then of particular interest for the understanding of the genetic determinism of growth in trevally. A BLAST search of the 100 kb regions flanking those markers against the NCBI nucleotide database did not return any sequence similarities, indicating that they are located in non-coding or in non-annotated regions. Intergenic regions can still have a functional role in gene expression and regulation (Wyrick and Young 2002). For example, these SNPs could be located in an intron that acts as a regulatory region (promoter, enhancer, silencer or insulator).

Compared with the heritability estimates found in Valenza-Troubat et al. (2021), the present analysis still seems underpowered. The range of heritability estimates was moderate to high (0.67 ± 0.05 to 0.76 ± 0.06) for the measured traits (H25, H50, H75, PL and EW) and moderate (ranging from 0.28 ± 0.07 to 0.68 ± 0.07) for the net gain traits (Δ H25, Δ H50, Δ H75, Δ PL and Δ EW), and it remained consistent throughout the experiment. In the present study, PVEs ranged from 22.1% to 47.36% for the QTL mapping study and from 4.74% to 46.81% for GWAS, indicating that neither techniques were able to capture all of the genetic components of growth traits (Table 4). Additional genetic interactions, other than additive effects, should be considered. While heritability is a key feature of a trait indicating its potential for improvement via selection, polygenic traits are often influenced by non-additive genetic effects such as dominance or epistasis (Glover, Solberg et al. 2017). Understanding the genetic mechanisms that underlie a trait is an important part of explaining phenotypic diversity. This is particularly relevant when looking at traits related to fitness (e.g. growth, shyness, foraging or predator awareness) in

348 populations that are undergoing domestication but still occasionally interbreed with wild
349 conspecifics (e.g. when new broodstock is caught and added).

350

351 **Future directions**

352 In this study, the combination of QTL mapping and GWAS enabled the identification of
353 genomic regions that control growth in a large captive trevally breeding population. From a
354 farming perspective, parameters such as stocking density (Irwin, O'halloran et al. 1999) or
355 feed availability and quality (Holm, Refstie et al. 1990) can be carefully managed to
356 accelerate fish growth in a land-based aquaculture facility. However, when aiming to
357 develop new species for aquaculture, it is fundamental to understand the genetic
358 architecture of commercially important traits that can potentially be enhanced through
359 selective breeding. The findings of this study provide a useful framework for determining
360 the genetic basis for growth traits in trevally. The identification of multiple QTLs through
361 QTL mapping and genetic markers commonly involved in growth-related traits via GWAS,
362 represents an essential step for the establishment of marker-assisted selection (MAS)
363 breeding programme for trevally. Fine mapping, confirmation and annotation of relevant
364 regions will bring a deeper understanding of the genetic architecture of growth.

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367 **Data Availability Statement**

368 Table S1 contains a list of SNP-trait associations detected with GWAS for height at 25%,
 369 50% and 75% of the peduncle length (H25, H50 and H75 respectively), Peduncle Length
 370 (PL), Estimated Weight (EW) and net gain traits (Δ H25, Δ H50, Δ H75, Δ PL, Δ EW) in
 371 November 2017 (Nov17), October 2018 (Oct18) and November 2019 (Nov19). For each
 372 SNP the table show the physical position on the linkage group (LG), the p-value, the minor
 373 allele frequency (MAF), the False Discovery Rate (FDR) adjusted p-value and the
 374 percentage of variance explained (PVE).

375 Trevally (araara) are a taonga (treasured) species to Māori, the Indigenous people of
 376 Aotearoa New Zealand. All genomic data obtained from taonga species have whakapapa
 377 (genealogy that includes people, plants and animals, mountains, rivers and winds) and are
 378 therefore taonga in their own right. These data are tapu (sacred) and tikanga (customary
 379 practices, protocols, and ethics) determine how people interact with these data. Thus, all
 380 the genomic data have been deposited in a managed repository that controls access. Raw
 381 and analyzed data are available through the Genomics Aotearoa data repository at
 382 <https://repo.data.nesi.org.nz/>. This was done to recognise Māori as important partners in
 383 science and innovation and as inter-generational guardians of significant natural resources
 384 and indigenous knowledge.

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552 Tables

553 Table 1: Summary of the phenotypes height at 25%, 50% and 75% of the body (H25, H50
554 and H75 respectively), Peduncle Length (PL), estimated weight (EW) and net gains traits
555 associated (Δ H25, Δ H50, Δ H74, Δ PL and Δ EW respectively) across the whole F1
556 population and for the largest family. Included are the number offspring phenotyped (n),
557 mean, and standard deviation (SD).

	Nov 2017		Oct 2018		Nov 2019	
<i>All</i>	n = 1,094		n = 719		n = 694	
	Mean	SD	Mean	SD	Mean	SD
H25	49.71	9.02	66.94	13.51	90.10	15.70
H50	59.45	11.02	79.99	16.39	105.53	18.65
H75	48.58	9.91	61.26	12.69	85.63	15.80
PL	156.79	23.92	200.64	35.36	264.09	40.44
EW	89.90	41.85	191.93	99.89	426.34	177.93
Δ H25			15.85	7.80	39.08	11.21
Δ H50			18.75	8.42	44.34	12.36
Δ H75			11.08	6.77	35.54	10.19
Δ PL			40.03	18.00	103.59	27.31
Δ EW			95.83	64.45	330.78	147.12
<i>Family</i>	n = 87		n = 68		n = 67	
	Mean	SD	Mean	SD	Mean	SD
H25	52.21	7.95	73.71	11.32	96.52	12.64
H50	63.10	9.29	86.87	13.41	111.90	14.77
H75	52.85	8.99	66.72	9.75	92.36	12.39
PL	167.59	21.32	218.19	30.04	280.78	33.07
EW	107.43	37.85	238.01	89.61	498.52	159.82
Δ H25			20.43	6.53	42.78	9.21
Δ H50			22.46	6.92	46.84	9.89
Δ H75			12.88	5.54	37.79	8.33
Δ PL			47.91	15.91	109.31	23.15
Δ EW			125.99	60.54	384.63	135.00

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559

560 Table 2: Marker statistics of the linkage map constructed from the largest trevally family.

LG	Number of markers ^a	Length (cM)	Average distance between markers (cM)
1	85	61.27	0.72
2	80	58.41	0.73
3	81	57.26	0.71
4	68	49.34	0.73
5	81	56.69	0.70
6	65	51.72	0.80
7	87	60.09	0.69
8	70	48.14	0.69
9	78	54.43	0.70
10	84	57.22	0.68
11	82	61.88	0.75
12	71	49.30	0.69
13	79	59.00	0.75
14	64	53.51	0.84
15	67	52.84	0.79
16	67	56.27	0.84
17	108	82.89	0.77
18	82	54.95	0.67
19	80	53.82	0.67
20	71	55.04	0.78
21	65	52.83	0.81
22	73	49.29	0.68
23	63	45.39	0.72
24	79	53.88	0.68
Total	1,830	1.335.46	0.73
^a count after removal of co-mapping			

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562

563 Table 3: List of significant Quantitative Trait Loci (QTL) detected for height at 25% and
 564 75% of the peduncle length (H25 and H75 respectively), Peduncle Length (PL), Estimated
 565 Weight (EW) and net gain for H75 (Δ H75) and PL (Δ PL) in November 2017 (Nov17) and
 566 November 2019 (Nov19), using a high-density linkage map. For each QTL the position on
 567 the linkage group (LG), the significance threshold, the LOD at the peak, the SNP name
 568 and the percentage of variance explained (PVE) are shown.

Time	Trait	LG	Peak position (cM)	SNP at peak	LOD threshold	LOD at peak	PVE (%)
Nov17	H25	3	23.2	trevally000114_4454829	4.71	4.73	22.1
Nov17	PL	3	24.9	trevally000114_4498123	4.71	4.83	22.6
Nov17	EW	3	24.9	trevally000114_4498123	4.60	5.39	24.8
Nov17	H75	3	52.1	trevally000114_23355470	4.64	5.50	25.3
Nov17	PL	18	0.0	trevally001200_2242455	4.71	4.88	22.8
Nov17	EW	18	0.0	trevally001200_2242455	4.60	4.82	22.5
Nov19	Δ PL	14	17.0	trevally001025_25205374	4.81	5.41	31.0
Nov19	Δ H75	14	17.6	trevally001025_25180484	4.69	7.36	39.7

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570

571 Table 4: Comparison of heritability estimates found in Valenza-Troubat et al. (2021) and
 572 Percentage of Variance Explained (PVE) by Quantitative Trait Loci (QTL) found in interval
 573 mapping and markers from the Genome Wide Association Study (GWAS). Data are shown
 574 for phenotypes height at 25%, 50% and 75% of the body (H25, H50 and H75 respectively),
 575 Peduncle Length (PL), estimated weight (EW) and net gains traits associated (Δ H25,
 576 Δ H50, Δ H75, Δ PL and Δ EW respectively) in November 2017 (Nov17), October 2018
 577 (Oct18) and November 2019 (Nov19).

Time	Trait	Heritability	PVE QTL (%)	PVE GWAS (%)
Nov17	H25	0.67±0.05	22.1	18.4
Nov17	H50	0.75±0.05	--	24.2
Nov17	H75	0.73±0.05	25.3	38.5
Nov17	PL	0.74±0.05	45.3	28.8
Nov17	EW	0.75±0.05	47.4	42.3
Oct18	H25	0.74±0.05	--	38.6
Oct18	H50	0.75±0.05	--	24.2
Oct18	H75	0.68±0.05	--	4.7
Oct18	PL	0.70±0.05	--	40.6
Oct18	EW	0.70±0.05	--	5.3
Oct18	Δ H25	0.46±0.05	--	10.9
Oct18	Δ H50	0.52±0.05	--	43.5
Oct18	Δ H75	0.28±0.05	--	--
Oct18	Δ PL	0.47±0.05	--	46.8
Oct18	Δ EW	0.63±0.05	--	18.0
Nov19	H25	0.73±0.05	--	20.3
Nov19	H50	0.72±0.05	--	41.6
Nov19	H75	0.68±0.05	--	31.2
Nov19	PL	0.69±0.05	--	26.4
Nov19	EW	0.72±0.05	--	21.4
Nov19	Δ H25	0.56±0.05	--	16.1
Nov19	Δ H50	0.54±0.05	--	15.2
Nov19	Δ H75	0.43±0.05	39.7	--
Nov19	Δ PL	0.51±0.05	31.0	21.7
Nov19	Δ EW	0.68±0.05	--	11.9

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579

Figures

Figure 1: Distribution of the peduncle length measurements in November 2017 (n = 1,094), October 2018 (n= 719) and November 2019 (n = 694).

Figure 2: Visualisation of the sex-averaged map built for the largest segregating family (n = 87). The 24 linkage groups represent the expected 24 *Pseudocaranx georgianus* chromosomes

592

593 Figure 3: Eight significant Quantitative Trait Loci (QTL) associated with growth traits were
594 found in trevally, across three linkage groups (LG3, LG14 and LG18). In November 2017,
595 two QTLs were found associated with peduncle length (PL_1_2017 and PL_2_2017, on
596 LG3 and LG18 respectively), which were also associated with estimated weight
597 (EW_1_2017 and EW_2_2017). In the same year, two more QTLs were found associated
598 with height at 25% (H25_2017) and 75% (H75_2017) of the peduncle length, both on LG3.
599 Finally, two QTLs were found in November 2019, one for net gain in peduncle length
600 (Δ PL_2019) and one for net gain in height at 75% of the peduncle length (Δ H75_2019),
601 both on LG14.

602

603

604 Figure 4: GWAS results for the net gain in peduncle length (Δ PL) in November 2019. (A)
605 Summary of Bayesian information criterion (BIC) for the optimal number of Principal
606 Components (PCs) to use in the model. (B) Quantile-Quantile (QQ)-plot of p-values. On
607 the Y-axis is the observed negative base 10 logarithm of the p-values, and on the X-axis
608 the expected observed negative base 10 logarithm of the p-values under the assumption
609 that the p-values follow a uniform [0 to 1] distribution. The grey area shows the 95%
610 confidence interval for the QQ-plot under the null hypothesis of no association between the
611 SNP and the trait. (C) Manhattan plot of the results of the GWAS. On the X-axis is the
612 physical position of the SNPs on the genome divided by chromosomes, and on the Y-axis
613 is the negative log base 10 of the p-values. The horizontal green line represents the
614 significance threshold.

615

616 Figure 5: Comparison of Quantitative Trait Loci (left) and SNP associated (right) with the
617 net gain in peduncle length (ΔPL) in November 2019.