Genetic diversity and heritability of economically important traits in captive Australasian snapper (*Chrysophrys auratus*)

David T. Ashton[a],*, Elena Hilario[a], Peter Jaksons[a], Peter A. Ritchie[b], Maren Wellenreuther[a]

[a] *The New Zealand Institute for Plant & Food Research Limited, Nelson, New Zealand*
[b] *School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand*

ABSTRACT

Aquaculture is the fastest growing animal production sector in New Zealand but low species diversity is a barrier to long-term growth. Snapper (*Chrysophrys auratus*) has been identified as a promising candidate for aquaculture development and an initial population has been established at the New Zealand Institute for Plant & Food Research Limited. The aim of the present study was to combine high-throughput Genotyping by Sequencing (GBS) and trait data from this population to reconstruct the pedigree, measure the degree of inbreeding across generations, and determine the heritability of 11 traits of interest within the breeding programme, in particular growth-related traits. Likelihood of parentage values showed that the pedigree consisted of a complex mixture of full- and half-sib individuals, with skewed contributions across parents. Average inbreeding did not change significantly between generations, but dramatic inbreeding differences were detected between F_2 descendants from the two independent starting (F_0) cohorts and between F_2 offspring from either full-sib, half-sib, or unrelated F_1 parents. Trait heritability ranged from 0.03 to 0.63, with growth related traits being situated around 0.27 and 0.10 in the first and third year, respectively. These results suggest that selection for higher growth could result in 4.6–15.7% and 1.4–4.9% improvement per generation, in the first and third years, respectively.

1. Introduction

Aquaculture has a fundamental role in meeting current and future global food needs (Bernatchez et al., 2017). In contrast to agricultural animals, many aquaculture species are not domesticated and have not been genetically enhanced through selective breeding programmes. Consequently, selective breeding programmes have the potential to yield significant gains (e.g. faster growth, greater disease resistance) in aquaculture. This is particularly relevant for marine finfish, because there are a large number of novel species being explored for commercial aquaculture, which have no history of captive rearing. The development of marine fish species, which are suitable for aquaculture, is a strong focus in the South Pacific area around Australia and New Zealand. This is primarily because of the presence of large coastal areas, but a limited number of marine species that have been domesticated for marine aquaculture (Camara and Symonds, 2014; Gentry et al., 2017).

Breeding programmes can benefit greatly from the insights provided by genetic information. Genetic approaches can be used to reconstruct pedigrees, determine the contributions of individual parents, measure inbreeding and genetic diversity of a population, and identify traits with a suitable genetic basis for enhancement through selective breeding. Until fairly recently, the high cost of genetic methods has limited the application of these tools beyond well-established species, such as salmon (Gjedrem et al., 2012). However, the recent development of high throughput sequencing is beginning to remove this limitation. Large volumes of genetic data can now be generated for species with no or limited prior information at much lower costs than were previously possible (e.g. Genotyping by Sequencing) (Elshire et al., 2011). This is opening the opportunity for these genetic tools that were previously limited to well-established species to be applied to a wide array of new species (Ellegren, 2014).

For long-term breeding programmes it is important to understand the pedigree structure of the population and the effects this has on genetic diversity. Loss of genetic diversity can lead to inbreeding, which can adversely affect important phenotypic traits and the long-term suitability of the population (Wang et al., 2002). Three key components to review in aquaculture populations are the number of individuals in the population, relatedness among individuals, and the contribution of each individual to subsequent generations (Falconer and Mackay, 1996). Contribution distortion is especially important to monitor in species that participate in group or otherwise undocumented breeding behaviours, as it can increase the rate at which genetic diversity is lost.

It is also important to note that all genetic variation enters a breeding programme through the founding individuals. Once this variation is lost it can only be replaced by the introduction of new founding individuals, which significantly reduces the effectiveness of selective breeding programs.

Understanding the heritability of commercially relevant phenotypic traits is another important application of genetic tools to breeding programmes (Wan et al., 2017). Heritability can include either broad-sense heritability (phenotypic variation due to all genetic variation) or narrow-sense heritability (phenotypic variation due to additive genetic variation). In the case of selective breeding programmes, narrow sense heritability is the most important because selection response depends on this (Wray and Visscher, 2008). Traits with a higher narrow sense heritability are better candidates for enhancement through selective breeding, while those with lower heritability are more suitable for enhancement via other factors (e.g. environment, feed, or other manipulations). While heritability estimates are population specific (Wray and Visscher, 2008), the heritability of many traits is often consistent across populations (Visscher et al., 2008).

In this study, we applied genetic tools to a newly formed population of the marine finfish snapper (*Chrysophrys auratus* also *Pagrus auratus*) that is being developed for aquaculture. Snapper is a valuable commercial and recreational fish species located around the coasts of Australia, New Zealand, and several Pacific Islands. It is closely related to *Pagrus major*, a major aquaculture species in Japan (Murata et al., 1996). Despite snapper's recreational and commercial importance in New Zealand, genetic investigations on this species have been relatively scarce. Most of what is known comes from relatively low-powered studies carried out in the wild populations (Adcock et al., 2000; Hauser et al., 2002; Le Port et al., 2017; Smith et al., 1978), but almost no genetic work has been conducted so far on the captive population (prior to more recent research e.g. Ashton et al., 2018; Wellenreuther et al., 2019). The specific goals of this study were to 1) reconstruct the pedigree for the population using genomic markers, 2) investigate the genetic diversity and inbreeding rates of each generation, and 3) finally, estimate the heritability for a number of target traits, to determine their potential for enhancement through a selective breeding programme.

2. Materials and methods

2.1. Snapper pedigree information and holding conditions

The pedigree investigated in this study was a new population of the snapper that is being developed as part of a finfish breeding programme at the Maitai Seafood Research Facility in Nelson, New Zealand (41°25′44.96″S, 173°28′11.46″E). The Seafood Research Facility is located on the seaward side of Port Nelson and seawater is pumped into the facility from an underground bore. The water flowing to the early life stage section of the hatchery is further filtered using mesh filters and UV treatment.

The original population was founded from wild sourced F_0 individuals ($n = 50$), the wild F_0 individuals were originally captured in two cohorts: the first cohort in 1994 and 1995 ($n = 25$) and the second in 2006 (n = 25). The first cohort of F_0 individuals (w1) were caught from several sites around the Tasman Bay, New Zealand (41°03′33.3″S, 173°15′01.3″E). The second cohort of F_0 individuals (w2) were caught from a single site within the Tasman Bay, New Zealand (41°03′33.3″S, 173°15′01.3″E). At the time of this study only the F_0 individuals from the second 2006 cohort were alive and available for sampling. An F_1 generation was produced over multiple years from either the wild F_0 individuals caught in 1996 (F_1 year classes: 2004) or the wild-caught individuals caught in 2006 (F_1 year classes: 2006, 2008, 2009, and 2010). F_1 individuals ($n = 70$) were combined into a single population in 2013 and subsequently produced an F_2 generation ($n = 577$).

All breeding events were tank-based spawning, with equal sex ratios and all individuals able to mate freely with other individuals in the population. Prior to the spawning season parents were fed a specialized diet containing fresh fish and oil supplements. Fertilized eggs were collected from the tank outlet during the first two weeks of November. Individuals were observed to be spawning during the evenings and eggs were collected early in the morning and placed in hatchery tanks. Eggs from a single day were used for each F_1 year class, but fertilized eggs from five consecutive days were used for the F_2 generation.

Eggs from the five days were placed into four tanks with identical lighting, oxygen, water flow, and tank setups. The larvae were fed a combination of rotifers and *Artemia salina*. At 1 month old, the juveniles from all four tanks were combined into a single tank. The juveniles were then fed a combination of *Artemia*, dry crumb, and wet diet (minced fresh fish). At 1 year old, the juveniles were split into four tanks with identical lighting, oxygen, water flow, and tanks setups. After 6 months of age, all fish were fed a combination of dry pellets and wet diet (fish mince or chunks of fresh fish). All research carried out in this study was approved by the Animal Ethics Committee of Victoria University of Wellington: Application number 2014R19.

2.2. Generation of molecular data from the pedigree

Samples of fin tissue were collected from each individual at the beginning of the study (sample sizes; $F_0 = 25$, $F_1 = 70$, and $F_2 = 577$). Each sample was directly placed into chilled 96% ethanol, heated to 80 °C for 5 min within 1 h of collection, and then stored at -20 °C until needed. DNA was isolated using a modified salt extraction method (Aljanabi and Martinez, 1997). Quantification of DNA was carried out using Hoescht 33,258 fluorescent dye. Fragmentation of the extracted DNA was checked by gel electrophoresis. Samples with moderate (~25%) amounts of fragments below 10 kbp were re-extracted and if needed fresh samples were recollected.

Genotyping libraries were prepared for each sample following modified Genotyping By Sequencing (GBS) protocol (Elshire et al., 2011). To build one library, one microgram of genomic DNA was double digested with the restriction enzymes *PstI* and *MspI*. The adaptor ligation step was done after digestion, without drying out the DNA/adaptor mixture. The barcoded adaptors designed by Deena Bioinformatics (van-Gurp, 2011) were associated with the *PstI* cut sites. Adaptors were annealed according to the method of Ko et al. (2003). A high fidelity enzyme was used for amplifications (AccuPrime Taq DNA polymerase High Fidelity, Life Technologies). Each library was amplified separately and its efficiency assessed by capillary electrophoresis (Fragment Analyzer, Advanced Analytical). The GBS libraries were prepared in parallel in plates. Duplicate or triplicate samples were prepared for each of the parent and grandparents and single samples for each of the offspring (except three individuals with poorer DNA quality, for which duplicate samples were prepared). Each plate containing 96 individual libraries were pooled, cleaned up, quantified and sent to the Australian Genome Research Facility (AGRF) in Melbourne, Australia, for sequencing. Each pool was sequenced on a single lane from the Illumina HiSeq 2500 platform in single end (SE) mode, with read length of 100 bases. In total, eight pools of libraries were sequenced in eight lanes.

2.3. Processing of pedigree genotyping data

FastQC was used to conduct an initial check of the sequencing data quality. Sequences were then de-multiplexed and cleaned. Adapters and primers were removed and the sequencing data were cleaned using Fastq-mcf in the ea-utils package (Aronesty, 2011). Genotyping was carried out on the cleaned datasets using the STACKs pipeline (Catchen et al., 2013). The samples were demultiplexed from the eight sequencing libraries using the process_radtags module. Sequencing reads for the duplicate or triplicate samples were concatenated into a single file, after which the reads were trimmed using Fastq-mcf (minimum sequence length = 50, quality threshold causing base removal = 33).

Bowtie 1.0 was used to align the GBS data to an initial Snapper genome assembly being developed at Plant & Food Research (allowed mismatches = 3, reported alignments = 10). The pstacks module was then run (minimum coverage = $7\times$), followed by cstacks and sstacks; preset parameters were used for all these modules. The population module was used to output the data to a Genepop file (minimum minor allele frequency = 0.05, allowed missing data = 0.25, additional commands = –write_random_snp).

2.4. Molecular pedigree reconstruction

The parents for each individual were identified using Cervus (Kalinowski et al., 2007) and a subset of SNPs (n = 2174) that were present in > 98% of individuals. The parents for each individual were selected by Cervus as the two closest matches, which passed the 95% confidence of assignment using simulation testing. The mother and father were designated based on known sex information about the parents. A network displaying the pedigree structure was constructed based on these relatedness scores using custom code in the R statistical environment (version: 3.2.3) (R Core Team, 2013).

2.5. Calculation of inbreeding value for the pedigree

A subset of SNP markers (n = 6441) that had been successfully placed on a linkage map (unpublished data) and were present in > 80% of the individuals were used to calculate a method-of-moments F coefficient (F_H) for inbreeding for each individual. This statistics is calculated as (observed homozygotes – expected homozygotes) / (total observations – expected homozygotes) (Kardos et al., 2015) and is equal to Nei's F_{IS} statistic, but is calculated using a different formula. Inbreeding (F_H) was calculated for each individual which had contributed offspring or was part of the final generation using the software package PLINK 1.9 (Purcell et al., 2007). The distribution of inbreeding values was then visualized using ggplot2 library in the R statistical environment (version: 3.2.3) (R Core Team, 2013; Wickham, 2009). Welch two-sample *t*-tests was used to compare the mean inbreeding values between the three generations and between groups within the F_2 offspring from the first and second F_0 cohort lineages. The F_2 individuals were grouped by grandparent type (w1-w1, w1-w2, and w2-w2) and parent type (full-sib, half-sib, and unrelated).

2.6. Phenotyping and trait correlations

A total of 11 phenotypic traits were measured for the F_2 individuals including fork length, peduncle length, weight, relative height at 0.25, 0.50, and 0.75 along the peduncle length from the nose (after correcting for length), number of nostrils, sex, survival from one to three years old, number of external blue spots, and external skin darkness (Fig. 1). Images were taken for each individual in the F_2 generation at year one (464–467 days old) and year three (1045–1048 days old), respectively. Due to time constraints during these short measurement intervals we were only able to capture weight for around half of the fish. All phenotypes, except sex and weight, were extracted from these images either manually or using the image analysis library OpenCV 2.0 through custom Python 3.0 scripts. Lighting variability in some of the images that were taken precluded us from measuring colour with high certainty in all individuals measured, and this trait was thus unable to be quantified for all.

Survival from year one and year three was determined by the presence or absence of an individual in the first and second set of images. The sex of each fish was determined by checking if it was producing milt or eggs during the middle of the breeding season (January–February) after the individuals had reached three years of age in 2016. Individuals that were not obviously producing milt or eggs were assumed to be female, because stripping eggs from females is more difficult. Weight was measured by placing individuals on scales. The

correlations between individual traits was measured using a Pearson's correlation matrix which was constructed using all phenotypic measurements (year one and three) in Python 2.7 using the Numpy library (McKinney, 2010).

2.7. Genetic correlations, trait heritability, and selection potential

Variance and covariance components were estimated using linear mixed animal models and restricted maximum likelihood methods with ASREML (Gilmour et al., 2009) in the R statistical environment (version: 3.2.3) (R Core Team, 2013). The narrow sense heritability for each trait was estimated in a univariate analysis, while genetic (co)variances were estimated in a series of bivariate analyses. Appropriate (co)variances for the trait combinations were then used to estimate genetic correlations and their standard error. Two different heritability models were used, including a model for continuous traits and a model for binomial traits. For each model the target trait was predicted using a fixed intercept effect and the tank and pedigree as random effects. The binomial traits were tested using the logit link function, however a higher log likelihood was attained in the model when fitting these traits as continuous. Sex and origin of the F_0 populations were tested as fixed effects, but did not have a significant effect on the results. The heritability models were run for data in year one and year three, while the genetic (co)variances were run only for data in year one.

The selection potential for each continuous trait was calculated based on the heritability and trait distribution - using the selection response formula ($R = h^2S$). In this formula h^2 is narrow sense heritability and S is the trait difference between the average parent and the average of the selected parents. The trait distributions from the F_2 individuals in year one were used for all calculations and the top 10% of individuals for a given trait were assumed to be the parents. The selection differential was calculated as the upper 95% confidence interval for each trait (mid-point of the upper 10%) minus the mean of the trait for the population. The response to selection was converted to a percentage by dividing by the mean of the population.

3. Results

3.1. Sequencing data quality and quantity

A total of 1.6 billion reads were generated from the eight sequencing lanes with approximately two, four, or six million reads generated for each single, duplicate, or triplicate library, respectively (Supplementary Fig. 1). Coverage of reads was consistent across all samples, with few having noticeable lower or higher coverage. All samples were included in further analysis. FastQC results indicated that the read quality was very high throughout the entire read (Illumina quality scores above 30 throughout reads) (Supplementary Fig. 2). From the STACKS pipeline a total of 249,468 SNPs were identified among 672 samples with > $7\times$ sequence coverage; of which 20,311 were present in 75% of individuals in the population and had a minor allele frequency (MAF) > 0.05.

3.2. Pedigree reconstruction based on genomic markers

Parents were identified for 93% of the individuals in the F_1 and F_2 generations. The remaining 7% were mainly located in the F_1 generation and belonged to the year classes produced from the missing wild F_0 cohort. The top two potential parents assigned for each individual using CERVUS relatedness scores were shown to be consistently male-female. Visualization of the pedigree showed that a large number of individuals had contributed from the F_0 to F_1 generation and from the F_1 to F_2 generation, but that the contributions were highly skewed, with some individuals contributing many more offspring than others (Fig. 2). A closer look at these contributions in the F_1 parents showed that the highest producing female and male produced 39% and 16% of the
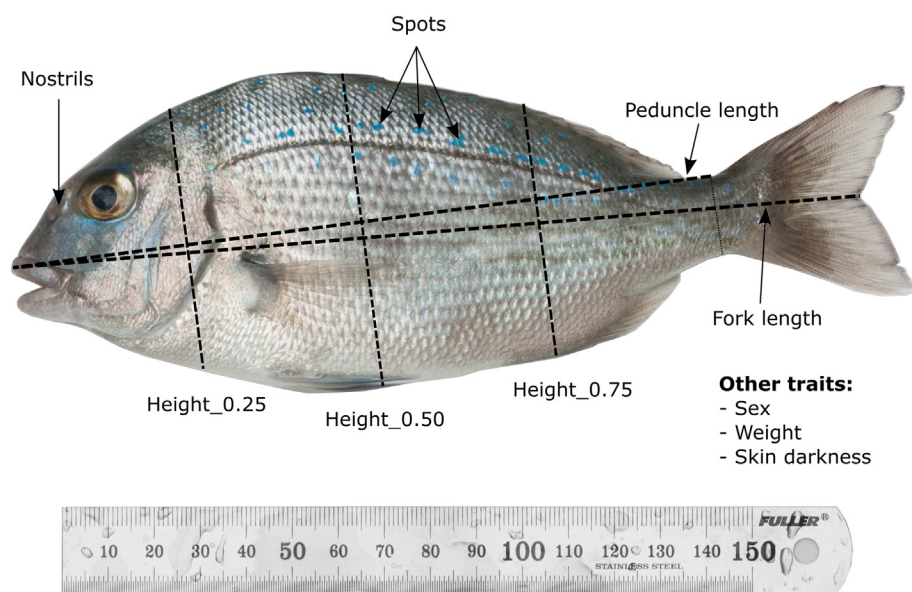
Fig. 1. The 11 phenotypic traits measured in the Australasian snapper (*Chrysophrys auratus*). Nostrils, sex, and weight were measured manually and all the others were measured using custom image analysis scripts. The ruler in picture was used to convert all the length measurements to mm. Height measurements were measured relative to length. Skin darkness was measured by comparing the average pixel values along the edge of the fish in comparison with the background.

offspring, respectively. The F_1 and F_2 generations contained a complex mixture of full and half siblings sharing either their mother, father, or both parents.

3.3. Inbreeding in the pedigree

The inbreeding F statistic (F_H) was calculated for 611 individuals in the dataset, which were either parents or offspring with known parents. The values ranged from a minimum of -0.57 to a maximum of 0.55 with a median of 0.02. Variation in the inbreeding values was lowest in the wild-caught F_0 generation, but this group also contained the fewest individuals (Fig. 3A). In the F_1 generation the variation in inbreeding values increased dramatically (-0.57 to 0.25) over those in the wild-caught F_0 individuals, but the median did not change significantly (p-value = .7410, Fig. 3a, Supplementary Table 1A). In the F_2 generation, the variation decreased from that observed in the F_1 generation, but was still higher than in the original wild-caught F_0 individuals (Fig. 3A). In the F_2 generation there was also a skewed distribution towards higher inbreeding values (Fig. 3A). Subdividing the F_2 offspring into those that were the product of the first wild broodstock (w1), second wild broodstock (w2), or a combination (w1-w2) it was found that all groups were significantly different from each other (p-value < .001, Fig. 3B, Supplementary Table 1B). Further subdividing F_2 individuals from the W2 group into offspring resulting from full-sib, half-sib, and unrelated crossing events showed a clear relationship between the degree of parental relatedness and the coefficient of inbreeding (p-value < .05, Fig. 3C, Supplementary Table 1C). Offspring from full sibling crosses were the most inbred, followed by half-sibling crosses, and the offspring of unrelated individuals had the lowest coefficient of inbreeding.

3.4. Trait values and phenotypic correlations

Phenotype data were recorded for 11 traits in the F_2 generation and a full list of means, standard deviations, and measurement counts for each trait are shown in Table 1. The number of measurements per individual and year differed depending on the availability of the individuals and access to the individuals within the tanks. A drop in sample size (568 to 314 individuals) occurred between year one and year three as a result of natural mortality. High variation was observed in growth-specific traits including fork length (year one: 160.1 mm ± 15.0, year three: 257.8 mm ± 20.1), peduncle length (year one: 132.1 mm ± 12.3, year three: 214.5 mm ± 17.0), and weight (year

one: 89.8 g ± 23.9, year three: 361.9 g ± 82.3). Relative to fish length, the weight and height increased disproportionately over the two years between measurements. Skin darkness of the fish also increased from year one to year three. Sex ratios measured during year three identified a slightly skewed sex ratio based on the ability to strip milt or not (female: 245, male: 182), assuming those without milt were female. The number of external blue spots was highly varied, but remained consistent between year one and year three (year one: 43 ± 9.6, year three: 43.7 ± 8.5).

Based on Pearson's correlation coefficients (Table 2), strong phenotypic correlations were observed between all the growth traits (> 0.93 for fork length, peduncle length, and weight). Moderate correlations (0.44–0.80) were found between the three relative height traits. None or weak correlations were found between sex and other traits (< 0.15). Weak correlations (< 0.23) were found between the remaining traits in the dataset.

3.5. Genetic correlations, trait heritability, and selection potential

Genetic correlations were estimated between all of the traits in year one (Table 2). Strong genetic correlations were found between the three growth traits (> 0.96). Moderate to strong genetic correlations were found between the three measures of relative height (0.71–0.99). Interestingly, sex was found to be moderately to highly correlated with two of the relative height measurements (relative height_50 = -0.84 ± 0.33 and relative height_75 = 0.99 ± 0.24), skin darkness (-0.99 ± 0.16), and number of blue spots (0.83 ± 0.23). Relative height_25 was also found to be highly correlated with the number of blue spots (0.99 ± 0.08). Moderate correlations were found for many of the remaining traits, but also coincided with large standard errors (greater than or close to the correlation values).

Narrow sense heritability was estimated for all phenotypic traits in both year one and year three using a model for either continuous or binomial traits (Table 3). The trait heritability varied widely depending on the trait and year it was measured (0.09–0.63). Growth traits all had similar heritability, averaging 0.27 in year one and 0.10 in year three. The heritability for relative height increased as the measure moved from the front of the fish towards the tail (Fig. 1, Table 3). Skin darkness was not heritable in year one (0.03 ± 0.03), but had moderate heritability in year three (0.22 ± 0.18). Number of blue spots was the most heritable trait in the data set and had a higher heritability in year three (year one = 0.45 ± 0.13 and year three = 0.63 ± 0.18). The
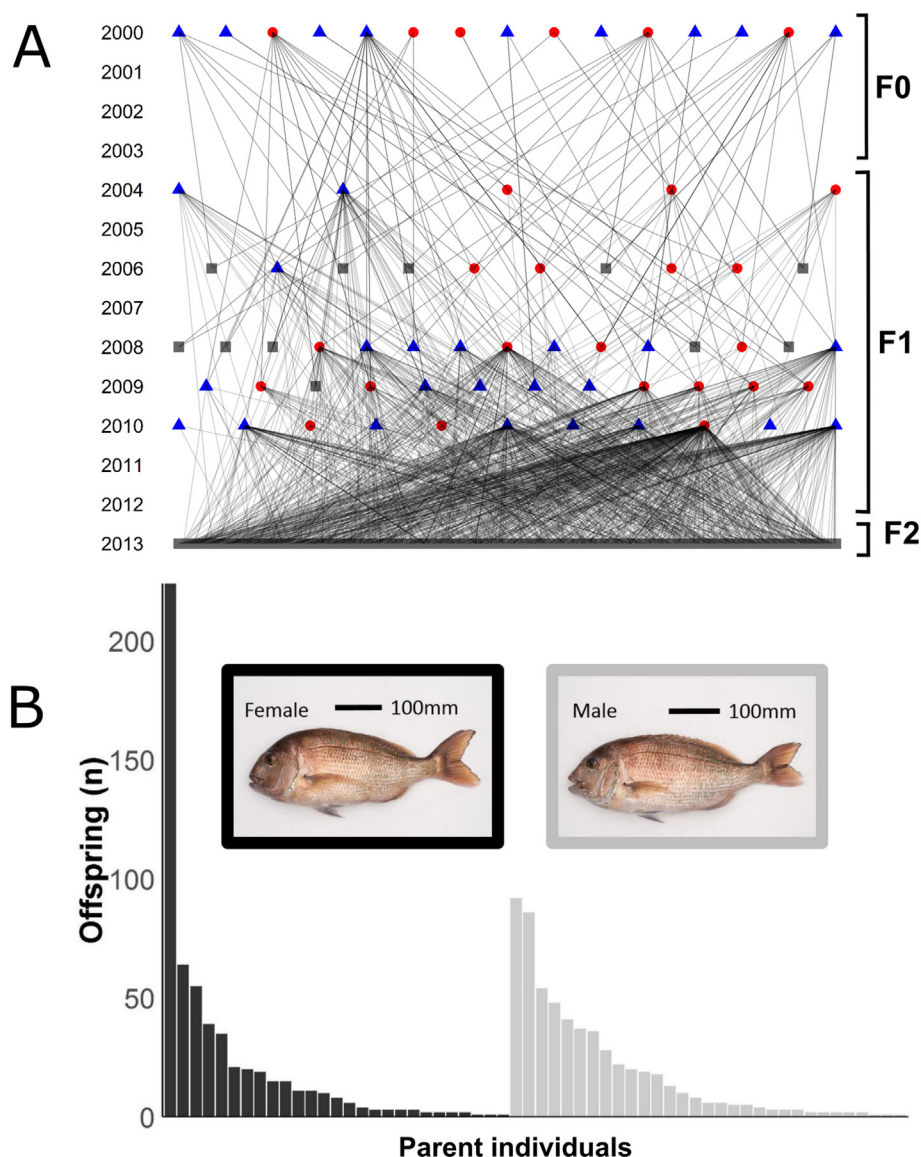
Fig. 2. Panel A shows the pedigree structure of the study population of *Chrysophrys auratus*. In the pedigree, each individual is represented either as male (blue triangle), female (red circle), or as unknown gender (grey square). Individuals are placed into rows based on the year hatched, and are connected to their respective parents by lines. The generations F$_0$, F$_1$, and F$_2$ are displayed. Panel B shows the number of offspring produced by each parent in the F$_1$ generation divided into females (dark grey) and males (light grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

three binomial traits were only recorded once. Nostrils, sex, and survival all had low to moderate heritability (0.34 ± 0.12, 0.16 ± 0.09, and 0.08 ± 0.06, respectively).

Percentage gains per year based on heritability and trait distribution were calculated in both year one and year three (Table 3). The percentage gains for growth rate traits (lengths and weight) ranged from 4.6 to 12.2 in year one and 1.4 to 4.9 in year three. The number of blue spots had the highest predicted percentage gains per year (year one: 18.8, year three: 23.8). The remaining traits had percentage gains ranging from 0.7 to 3.3 for years one and year three.

4. Discussion

In this study we applied genetic tools to a newly formed snapper population. These tools were used to reconstruct the pedigree, investigate the genetic diversity and inbreeding rates in each generation, and calculate the heritability of important phenotypic traits. The results of this study will help the development of this new population by providing information about what has occurred during the initial

generations and informing future controlled breeding work.

Pedigree reconstruction based on the genomic markers indicated that many of the individuals in the F$_0$ and F$_1$ generations contributed to offspring in the next generation (Fig. 2A); however, the contributions were highly skewed (Fig. 2B). Skewed contributions have been observed in a wide range of captive fish populations including Asian seabass (*Lates calcarifer*) (Liu et al., 2012), gilthead seabream (*Sparus aurata*) (Chavanne et al., 2014), and flounder (*Paralichthys olivaceus*) (Sekino et al., 2003). Contribution distortion can be problematic in a long-term breeding programmes because of the negative effects that it has on genetic diversity. Because this study was carried out on post-juvenile fish, two main explanations could account for the skewed distribution. Firstly, the parents may have contributed unevenly to breeding events. Secondly, the parents may have contributed evenly, but their progeny had different survival rates. Further work is needed to clarify what is occurring during this stage of the snapper production cycle.

Some interesting patterns of inbreeding were observed which stemmed originally from the two F$_0$ wild cohorts. The F$_0$ grandparents

Fig. 3. Shown are the F statistics (F_H) for *Chrysophrys auratus* individuals grouped based on A) generation, B) grandparent types (F_1 only), or C) parent types (w2-w2 only). Visuali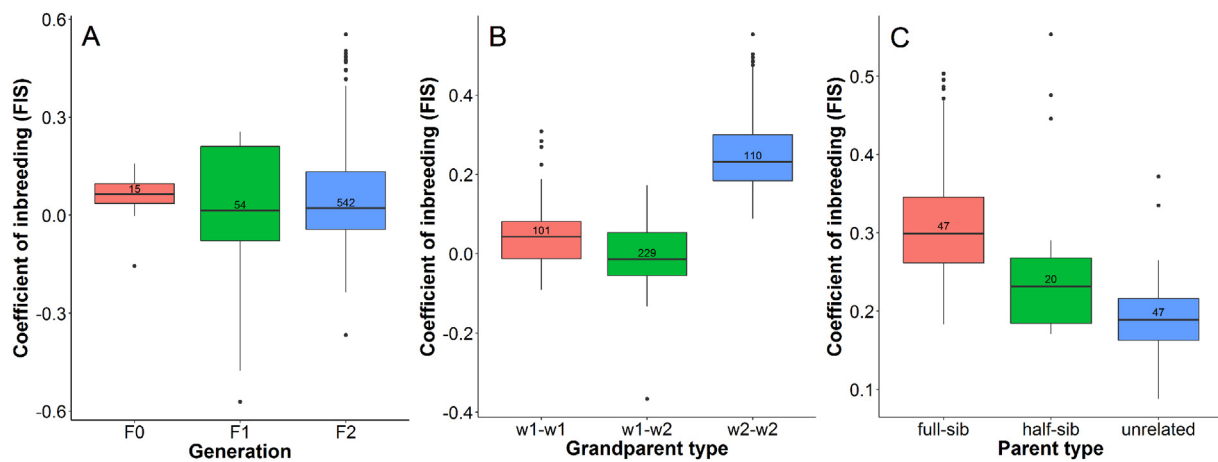zed are the 1st, 2nd (median), and 3rd quartile and whiskers extending 1.5 times the interquartile range from the median (~95% confidence interval). Comparison between groups indicated that significant differences ($p < .01$) were found between the mean value of w2-w2 and the other two grandparental types. Significant differences ($p < .05$) were found between the mean values for all of the different parent types. See supplementary Table 1 for significance results.

Table 1

Shown are the mean, standard deviation (Stdv), and count for each continuous trait for year one and year three, and the mean and count for a subsample of these measurements from the highest 10% in year three. The number of measurements does not always reflect the number of individuals because for some traits an individual may have been measured more than once (e.g. multiple images).

Year	Trait	Mean	Stdv	Samples
One	Fork length (mm)	160.1	15.0	568
One	Peduncle length (mm)	132.1	12.3	568
One	Weight (g)	89.8	23.9	280
One	Height_0.25 (pixels)	377.2	20.4	568
One	Height_0.5 (pixels)	460.9	19.2	568
One	Height_0.75 (pixels)	388.4	20.2	568
One	Skin darkness	89.6	10.0	503
One	Spots	44.7	9.5	568
Three	Fork length (mm)	257.8	20.1	314
Three	Peduncle length (mm)	214.5	17.0	314
Three	Weight (g)	361.9	82.3	247
Three	Height_0.25 (pixels)	381.9	14.2	314
Three	Height_0.5 (pixels)	460.9	19.6	314
Three	Height_0.75 (pixels)	384.2	21.4	314
Three	Skin darkness	111.3	8.4	122
Three	Spots	43.5	8.4	314

were sourced from the wild and as such represent a baseline for other inbreeding statistics. Unfortunately, genetic samples were available only for the second F_0 wild cohort. However, we can gain some idea about the absent F_0 cohort through their progeny in the F_1 and F_2 generations. The average inbreeding (F_H) for the second F_0 wild cohort (0.055) was within ranges observed for other marine fish from wild populations, including orange clown fish (*Amphiprion percula*, 0.018) (Salles et al., 2016), brook trout (*Salvelinus fontinalis*, 0.098) (Pilgrim et al., 2012), winter flounder (*Pseudopleuronectes americanus*, 0.169–0.283) (O'Leary et al., 2013), and gilthead seabream (*Sparus aurata*, 0.00–0.319) (Zeinab et al., 2014). It is worth noting that the above study in winter flounder was reporting severe inbreeding for a wild population (O'Leary et al., 2013). While the average inbreeding rates in the current study did not change significantly between generations, the distribution of inbreeding values was noticeable different (Fig. 3). Some of the changes to the variation could be explained by differences in sample size between the generation (15 vs 54 vs 542); however, further analysis indicated that F_1 individuals with low values (outbred) were primarily located in the offspring of the first F_0 cohort (W1) and those with high values (inbred) where located in the offspring

of the second F_0 cohort (W2). These results suggest that some structure may be present in the source population and that while the second F_0 cohort was sourced from a single population, the first set were sourced from multiple populations, which have subsequently produced outbred offspring. These results are particularly interesting because most previous studies have suggested that minimal structure is present in the wild snapper population around New Zealand (Bernal-Ramírez et al., 2003; Paul and Tarring, 1980; Smith et al., 1978) and none would be expected over the range from which these two F_0 wild cohorts were sourced. The differences in inbreeding continued into the F_2 generation with individuals that were the product of solely the first F_0 cohort (W1-W1, Fig. 3b) being significantly (*p*-value < .001) less inbred than those produced solely from the second F_0 cohort (W2 – W2, Fig. 3B). F_2 individuals that were the product of crossing between the two F_0 wild cohorts (W1 – W2) had the lowest average inbreeding values for any group. As expected, further subdivision based on parent type indicated that F_2 individuals that were from unrelated individuals had significantly lower inbreeding values than those from half-sib or full-sib parents (Fig. 3C).

Trait values and heritability differed largely across the 11 traits investigated (Tables 1 and 2). Growth traits (e.g. weight and length) are some of the most commonly reviewed traits for aquaculture selective breeding programmes. This is because they directly affect production rates and are often moderately heritable; for example, heritability of growth traits (weight or length) was 0.2 to 0.4 in mirror carp (*Cyprinus carpio*) (Hu et al., 2017), 0.4 in gilthead seabream (*Sparus aurata*) (Fernandes et al., 2016), 0.21 to 0.362 in half-smooth tongue sole (*Cynoglossus semilaevis*) (Liu et al., 2016), and 0.31 to 0.34 in Atlantic cod (*Gadus morhua*) (Kristjánsson and Arnason, 2016). In the current study, growth traits had a heritability of ~0.27 in year one and ~0.10 in year three. Additionally, because the three growth traits (fork length, peduncle length, and weight) were all highly correlated (phenotypic > 0.97, genetic > 0.95), selection for one should also affect the others. This could prove useful in a breeding programme because length can often be more easily measured using high-throughput methodologies than weight.

Body shape measurements are another commonly measured group of traits; for example, heritability for shape traits ranged from 0.18 to 0.289 in Nile tilapia (*Oreochromis niloticus*) (de Oliveira et al., 2016), 0.24 to 0.58 in gilthead seabream (*Sparus aurata*) (Boulton et al., 2011), and 0.34 in common sole (*Solea solea*) (Blonk et al., 2010). By comparison heritability for relative height traits in the current study ranged from 0.14 to 0.30 for both years measured. However, it should be noted that shape measurements are often more specific to an individual

Table 2

Shown are the Pearson's (above diagonal) and genetic (below diagonal) correlations between all traits in year one. Included are the mean and standard error. "Failed" indicates genetic correlations for which the (co)variance models failed to converge. FL = fork length, PL = peduncle length, WGT = weight, H25 = height_25, H50 = height_50, H75 = height_75, DRK = darkness, SP = spot number, NOS = nostrils, SEX = sex, SUR = survival.

	FL	PL	WGT	H25	H50	H75	DRK	SP	NOS	SEX	SUR
FL	–	0.99 ± 0.00	0.94 ± 0.03	−0.07 ± 0.06	−0.05 ± 0.05	−0.08 ± 0.06	−0.14 ± 0.03	0.22 ± 0.03	−0.06 ± 0.00	−0.04 ± 0.00	−0.16 ± 0.00
PL	0.99 ± 0.00	–	0.94 ± 0.03	−0.07 ± 0.07	−0.07 ± 0.07	−0.09 ± 0.07	−0.16 ± 0.04	0.23 ± 0.03	−0.06 ± 0.00	−0.05 ± 0.00	−0.16 ± 0.00
WGT	0.96 ± 0.01	0.97 ± 0.02	–	0.04 ± 0.06	0.14 ± 0.05	0.10 ± 0.06	−0.14 ± 0.03	0.20 ± 0.02	−0.08 ± 0.00	−0.01 ± 0.00	−0.12 ± 0.00
H25	0.30 ± 0.34	0.30 ± 0.34	0.33 ± 0.35	–	0.53 ± 0.03	0.44 ± 0.04	−0.10 ± 0.02	0.00 ± 0.02	0.00 ± 0.00	−0.02 ± 0.00	0.04 ± 0.00
H50	0.35 ± 0.30	0.35 ± 0.29	0.51 ± 0.27	0.77 ± 0.18	–	0.80 ± 0.03	−0.02 ± 0.03	0.06 ± 0.02	−0.08 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
H75	0.28 ± 0.31	0.30 ± 0.30	0.50 ± 0.27	0.71 ± 0.22	0.99 ± 0.02	–	0.01 ± 0.02	0.04 ± 0.02	−0.10 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
DRK	−0.01 ± 0.56	−0.10 ± 0.56	−0.63 ± 0.62	Failed	0.85 ± 0.60	0.48 ± 0.63	–	−0.07 ± 0.04	0.02 ± 0.00	0.03 ± 0.00	0.08 ± 00
SP	−0.15 ± 0.27	−0.15 ± 0.27	−0.18 ± 0.31	0.99 ± 0.08	0.31 ± 0.28	0.36 ± 0.28	0.56 ± 0.76	–	−0.17 ± 0.00	0.14 ± 0.00	−0.12 ± 0.00
NOS	0.30 ± 0.28	0.33 ± 0.28	−0.11 ± 0.63	−0.41 ± 0.27	−0.21 ± 0.41	−0.00 ± 0.42	Failed	0.17 ± 0.40	–	−0.10 ± 0.06	0.04 ± 0.04
SEX	−0.35 ± 0.49	−0.30 ± 0.46	−0.63 ± 0.62	Failed	−0.84 ± 0.33	0.99 ± 0.24	−0.99 ± 0.16	0.83 ± 0.23	Failed	–	–
SUR	−0.35 ± 0.36	−0.29 ± 0.37	0.08 ± 0.45	−0.28 ± 0.44	0.14 ± 0.41	0.29 ± 0.37	−0.88 ± 0.50	−0.05 ± 0.37	0.25 ± 0.39	–	–

Table 3

Shown is the heritability of each trait at year one and year three in the current study and the potential selection gains (as a percent) based on a selective breeding programme in which only the top 10% of individuals were used. The three binomial traits were only measured once for each individual.

Trait		Heritability		Selection gains %	
Category	Name	Year one	Year three	Year one	Year three
Continuous	Fork length	0.25 ± 0.10	0.10 ± 0.08	4.6	1.5
Continuous	Peduncle length	0.25 ± 0.10	0.09 ± 0.08	4.6	1.4
Continuous	Weight	0.30 ± 0.14	0.11 ± 0.10	15.7	4.9
Continuous	Height_0.25	0.15 ± 0.09	0.14 ± 0.09	1.6	1.0
Continuous	Height_0.50	0.25 ± 0.11	0.20 ± 0.12	2.0	1.7
Continuous	Height_0.75	0.26 ± 0.11	0.30 ± 0.14	2.7	3.3
Continuous	Skin darkness	0.03 ± 0.03	0.22 ± 0.18	0.7	3.3
Continuous	Spots	0.45 ± 0.13	0.63 ± 0.18	18.8	23.8
Binomial	Nostrils	0.34 ± 0.12		–	–
Binomial	Sex	0.16 ± 0.09		–	–
Binomial	Survival	0.08 ± 0.06		–	–

species than growth traits. As might be expected, moderate correlation (phenotypic > 0.44, genetic > 0.71) was found between the three relative height measurements.

Skin pigmentation traits (skin darkness and spots) were found to be weakly to highly heritable across the two years (0.03 and 0.63, respectively). Because external skin colour is an important consumer requirement for snapper, the moderate heritability for some of these this skin pigment traits indicates they could be a potential target for selective breeding. However, the low heritability for some traits, such as skin darkness in year one, suggests other non-genetic factors should also be investigated. The number of nostrils was found to be moderately heritable (0.34 ± 0.12). However, previous research at PFR (unpublished) has indicated that this trait may be affected by tank-specific conditions within the first few months of life. Interestingly, it was notable that while skin darkness exhibited a lack of heritability in year 1, a positive heritability and a correlation with sex was found in year 3, indicating that skin colouration may be a secondary sex trait linked to male or female maturation. Indeed, we have observed that sexually reproductively active snapper exhibit a darkened skin and body colour whereas females stay more or less the same colour year-round.

The final two of the 11 traits that we investigated in this study, sex and survival, both had low heritability (0.16 ± 0.09 and 0.08 ± 0.06, respectively, Table 3). If sex in snapper was chromosomally determined then the pedigree information should predict none of the trait variation (i.e. H2 = 0) and the genomic information of the offspring should predict all of the variation of the trait (H2 = 1). In the current study the heritability was slightly different than zero which could indicate a multifactorial basis to sex determination. However, the low heritability value and the possible error rate when phenotyping for sex could indicate that this none zero heritability value is random. More data on sex determination at different ages and work to identify a chromosomal sex determining region could help resolve this. In fish, survival for specific viral and bacterial diseases often has moderate to high heritability (e.g. 0.12 to 0.52, Antonello et al., 2009; Flores-Mara et al., 2017; Shoemaker et al., 2017), but survival for non-specific or highly multifactorial diseases and events often have low heritability (see introduction by Vehviläinen et al., 2008). In this study mortalities occurred for a variety of reasons over the course of the study rather than being related to a specific disease event, which may explain our low heritability estimates for survival (0.08, Table 3). Interestingly, despite sex not being significantly phenotypically correlated with other traits in the dataset it was moderately to strongly correlated with the relative height and colour traits in the dataset (> 0.84, Table 2).

The percentage gains per generation for a selective breeding programme can be calculated using the selection response formula

(R = h²S). The degree of change in this formula is affected by both the heritability of the trait and the difference in mean value between the selected and non-selected parents, which is in turn affected by the degree of selection pressure applied and the distribution of the trait. Results from this study suggested that gains for weight in year one would be around 15.7% per generation if the top 10% of individuals were used. This is comparable to that suggested rate in sea bream (*Sparus aurata*, 15.6%) (Thorland et al., 2006) and near the average of 13% suggested across a wide range of fish species (Gjedrem and Rye, 2016). However, the gains at three years of age were much lower, at around 4.9% per generation. The reason for this drop in gains at year three is unknown and needs further investigation. Probably, another non-genetic factor is increasingly becoming the driver of growth as the fish age. One possibility could be size-based tank effects. One final note on these calculations is that although weight and length had similar heritability (year one: ~0.26, year three: ~0.11, Table 3), gains for length were less than half of those expected for weight. This is because length had a much narrower trait distribution than weight: 257.7 ± 21.0 vs 363.1 ± 85.0 for fork length and weight, respectively (Table 1). Gains for the remaining traits were all low (3.3 or less), except for spots, which had the highest gains of any trait in the study (year one: 18.8%, year three: 23.8%).

4.1. Future directions

This study represents the most in-depth genetic investigation of this new snapper population to date. The resources developed will be useful for a wide range of future investigations and applications to support the selective breeding of this species. Specifically, we were able to demonstrate the likely effects of selective breeding and the operational use of the latest genomic tools to investigate pedigrees and inbreeding. Importantly, our study indicated that snapper show potential for genetic improvement of economically important growth traits. As part of future development of this population, the genome-wide markers developed in the present study will allow estimation of genomic breeding values (BVs). These breeding values can then be used in conjunction with traditional selection to increase the rate of genetic improvement (Van Eenennaam et al., 2014). Overall, these genomic resources will facilitate further domestication of snapper in a way that enhances economically important traits while maintaining genetic variation and reducing any potentially negative effects of inbreeding.

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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.2019.02.034.

References

Adcock, G.J., Bernal Ramázrez, J.H., Hauser, L., Smith, P., Carvalho, G.R., 2000. Screening of DNA polymorphisms in samples of archived scales from New Zealand snapper. J. Fish Biol. 56, 1283–1287. https://doi.org/10.1111/j.1095-8649.2000. tb02141.x.

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25, 4692–4693.

Antonello, J., Massault, C., Franch, R., Haley, C., Pellizzari, C., Bovo, G., Patarnello, T., de Koning, D.-J., Bargelloni, L., 2009. Estimates of heritability and genetic correlation for body length and resistance to fish pasteurellosis in the gilthead sea bream (Sparus aurata L.). Aquaculture 298, 29–35. https://doi.org/10.1016/j.aquaculture.2009.10. 022.

Aronesty, E., 2011. ea-utils: Command-line Tools for Processing Biological Sequencing Data.

Ashton, D.T., Ritchie, P.A., Wellenreuther, M., 2018. High-density linkage map and QTLs for growth in snapper (Chrysophrys auratus). bioRxiv 376012. https://doi.org/10. 1101/376012.

Bernal-Ramírez, J.H., Adcock, G.J., Hauser, L., Carvalho, G.R., Smith, P.J., 2003. Temporal stability of genetic population structure in the New Zealand snapper, Pagrus auratus, and relationship to coastal currents. Mar. Biol. 142, 567–574. https://doi.org/10.1007/s00227-002-0972-9.

Bernatchez, L., Wellenreuther, M., Araneda, C., Ashton, D.T., Barth, J.M.I., Beacham, T.D., Maes, G.E., Martinsohn, J.T., Miller, K.M., Naish, K.A., Ovenden, J.R., Primmer, C.R., Young Suk, H., Therkildsen, N.O., Withler, R.E., 2017. Harnessing the power of genomics to secure the future of seafood. Trends Ecol. Evol. 32, 665–680. https://doi.org/10.1016/j.tree.2017.06.010.

Blonk, R.J.W., Komen, J., Tenghe, A., Kamstra, A., van Arendonk, J.A.M., 2010. Heritability of shape in common sole, Solea solea, estimated from image analysis data. Aquaculture 307, 6–11. https://doi.org/10.1016/j.aquaculture.2010.06.025.

Boulton, K., Massault, C., Houston, R.D., de Koning, D.J., Haley, C.S., Bovenhuis, H., Batargias, C., Canario, A.V.M., Kotoulas, G., Tsigenopoulos, C.S., 2011. QTL affecting morphometric traits and stress response in the gilthead seabream (Sparus aurata). Aquaculture 319, 58–66. https://doi.org/10.1016/j.aquaculture.2011.06.044.

Camara, M.D., Symonds, J.E., 2014. Genetic improvement of New Zealand aquaculture species: programmes, progress and prospects. N. Z. J. Mar. Freshw. Res. 48, 466–491. https://doi.org/10.1080/00288330.2014.932291.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140. https://doi.org/10.1111/mec.12354.

Chavanne, H., Parati, K., Cambuli, C., Capoferri, R., Jiménez, C.A., Galli, A., 2014. Microsatellites markers to depict the reproductive and genetic patterns of farmed gilthead seabream (Sparus aurata): illustration by a case study on mass spawning. Aquac. Res. 45, 577–590. https://doi.org/10.1111/are.12013.

Core Team, R., 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

de Oliveira, C.A.L., Ribeiro, R.P., Yoshida, G.M., Kunita, N.M., Rizzato, G.S., de Oliveira, S.N., dos Santos, A.I., Nguyen, N.H., 2016. Correlated changes in body shape after five generations of selection to improve growth rate in a breeding program for Nile tilapia Oreochromis niloticus in Brazil. J. Appl. Genet. 57, 487–493. https://doi.org/10.1007/s13353-016-0338-5.

Ellegren, H., 2014. Genome sequencing and population genomics in non-model organisms. Trends Ecol. Evol. 29, 51–63. https://doi.org/10.1016/j.tree.2013.09.008.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E., 2011. A robust, simple Genotyping-by-Sequencing (GBS) approach for high diversity species. PLoS One 6, e19379. https://doi.org/10.1371/journal.pone. 0019379.

Falconer, D., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th ed. Longman, Burnt Mill, England.

Fernandes, T., Herlin, M., Dolores López Belluga, M., Ballón, G., Martinez, P., Toro, M., Fernández, J., 2016. Estimation of genetic parameters for growth traits in a hatchery population of gilthead sea bream (Sparus aurata L.). Aquacult. Int. 25. https://doi. org/10.1007/s10499-016-0046-5.

Flores-Mara, R., Rodríguez, F.H., Bangera, R., Lhorente, J.P., Neira, R., Newman, S., Yáñez, J.M., 2017. Resistance against infectious pancreatic necrosis exhibits significant genetic variation and is not genetically correlated with harvest weight in rainbow trout (Oncorhynchus mykiss). Aquaculture 479, 155–160. https://doi.org/10.1016/j.aquaculture.2017.05.042.

Gentry, R.R., Froehlich, H.E., Grimm, D., Kareiva, P., Parke, M., Rust, M., Gaines, S.D., Halpern, B.S., 2017. Mapping the global potential for marine aquaculture. Nat. Ecol. Evol. 1, 1317–1324. https://doi.org/10.1038/s41559-017-0257-9.

Gilmour, A., Gogel, B., Cullis, B., Thompson, R., 2009. ASReml User Guide, 3rd ed. VSN International Ltd, Hemel Hempstead (HP1 1ES, UK).

Gjedrem, T., Rye, M., 2016. Selection response in fish and shellfish: a review. Rev. Aquacult. https://doi.org/10.1111/raq.12154. n/a-n/a.

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. Aquaculture 350–353, 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008.

Hauser, L., Adcock, G.J., Smith, P.J., Bernal Ramírez, J.H., Carvalho, G.R., 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus). Proc. Natl. Acad. Sci. U. S. A. 99, 11742–11747. https://doi.org/10.1073/pnas.172242899.

Hu, X., Li, C., Shang, M., Ge, Y., Jia, Z., Wang, S., Zhang, Q., Shi, L., 2017. Inheritance of growth traits in Songpu mirror carp (Cyprinus carpio L.) cultured in Northeast China. Aquaculture 477, 1–5. https://doi.org/10.1016/j.aquaculture.2017.04.031.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x.

Kardos, M., Luikart, G., Allendorf, F.W., 2015. Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. Heredity 115, 63–72. https://doi.org/10.1038/hdy.2015.17.

Ko, W.-Y., David, R.M., Akashi, H., 2003. Molecular phylogeny of the Drosophila melanogaster species subgroup. J. Mol. Evol. 57, 562–573. https://doi.org/10.1007/s00239-003-2510-x.

Kristjánsson, T., Arnason, T., 2016. Heritability of economically important traits in the Atlantic cod Gadus morhua L. Aquac. Res. 47, 349–356. https://doi.org/10.1111/

are.12496.

Le Port, A., Montgomery, J.C., Smith, A.N.H., Croucher, A.E., McLeod, I.M., Lavery, S.D., 2017. Temperate marine protected area provides recruitment subsidies to local fisheries. Proc. R. Soc. B Biol. Sci. 284, 20171300. https://doi.org/10.1098/rspb.2017.1300.

Liu, P., Xia, J.H., Lin, G., Sun, F., Liu, F., Lim, H.S., Pang, H.Y., Yue, G.H., 2012. Molecular parentage analysis is essential in breeding Asian seabass. PLoS One 7. https://doi.org/10.1371/journal.pone.0051142.

Liu, F., Yang, Y., Li, Y., Guo, H., Dai, H., Gao, J., Zhang, Y., Ma, J., Liu, Y., Wei, Z., Shao, C., Chen, S., 2016. Phenotypic and genetic parameter estimation of juvenile growth and bottom color traits in half-smooth tongue sole, Cynoglossus semilaevis. Acta Oceanol. Sin. 35, 83–87. https://doi.org/10.1007/s13131-016-0888-8.

McKinney, W., 2010. Data structures for statistical computing in Python. In: Proceedings of the 9th Python in Science Conference, pp. 51–56.

Murata, O., Harada, T., Miyashita, S., Izumi, K.-I., Maeda, S., Kato, K., Kumai, H., 1996. Selective breeding for growth in red sea bream. Fish. Sci. 62, 845–849.

O'Leary, S.J., Hice, L.A., Feldheim, K.A., Frisk, M.G., McElroy, A.E., Fast, M.D., Chapman, D.D., 2013. Severe inbreeding and small effective number of breeders in a formerly abundant marine fish. PLoS One 8, e66126. https://doi.org/10.1371/journal.pone.0066126.

Paul, L.J., Tarring, S.C., 1980. Growth rate and population structure of snapper, Chrysophrys auratus, in the East Cape region, New Zealand. N. Z. J. Mar. Freshw. Res. 14, 237–247. https://doi.org/10.1080/00288330.1980.9515866.

Pilgrim, B.L., Perry, R.C., Keefe, D.G., Perry, E.A., Dawn Marshall, H., 2012. Microsatellite variation and genetic structure of brook trout (Salvelinus fontinalis) populations in Labrador and neighboring Atlantic Canada: evidence for ongoing gene flow and dual routes of post-Wisconsinan colonization. Ecol. Evol. 2, 885–898. https://doi.org/10.1002/ece3.200.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.

Salles, O.C., Pujol, B., Maynard, J.A., Almany, G.R., Berumen, M.L., Jones, G.P., Saenz-Agudelo, P., Srinivasan, M., Thorrold, S.R., Planes, S., 2016. First genealogy for a wild marine fish population reveals multigenerational philopatry. Proc. Natl. Acad. Sci. U. S. A. 113, 13245–13250. https://doi.org/10.1073/pnas.1611797113.

Sekino, M., Saitoh, K., Yamada, T., Kumagai, A., Hara, M., Yamashita, Y., 2003. Microsatellite-based pedigree tracing in a Japanese flounder Paralichthys olivaceus

hatchery strain: implications for hatchery management related to stock enhancement program. Aquaculture 221, 255–263. https://doi.org/10.1016/S0044-8486(02)00667-1.

Shoemaker, C.A., Lozano, C.A., LaFrentz, B.R., García, J.C., Soto, E., Xu, D.-H., Beck, B.H., Rye, M., 2017. Additive genetic variation in resistance of Nile tilapia (Oreochromis niloticus) to Streptococcus iniae and S. agalactiae capsular type Ib: is genetic resistance correlated? Aquaculture 468, 193–198. https://doi.org/10.1016/j.aquaculture.2016.10.022.

Smith, P.J., Francis, R.I.C.C., Paul, L.J., 1978. Genetic variation and population structure in the New Zealand snapper. N. Z. J. Mar. Freshw. Res. 12, 343–350. https://doi.org/10.1080/00288330.1978.9515761.

Thorland, I., Papaioannou, N., Kottaras, L., 2006. The Kego Breeding Programs for Sea Bream (*Sparus aurata*) and Sea Bass (Dicentrarachus labrax). (in Greece).

Van Eenennaam, A.L., Weigel, K.A., Young, A.E., Cleveland, M.A., Dekkers, J.C.M., 2014. Applied animal genomics: results from the field. Ann. Rev. Anim. Biosci. 2, 105–139. https://doi.org/10.1146/annurev-animal-022513-114119.

van-Gurp, T., 2011. Deena Bioinformatics.

Vehviläinen, H., Kause, A., Quinton, C., Koskinen, H., Paananen, T., 2008. Survival of the currently fittest: genetics of rainbow trout survival across time and space. Genetics 180, 507–516. https://doi.org/10.1534/genetics.108.089896.

Visscher, P.M., Hill, W.G., Wray, N.R., 2008. Heritability in the genomics era—concepts and misconceptions. Nat. Rev. Genet. 9, 255–266. https://doi.org/10.1038/nrg2322.

Wan, S., Li, Q., Liu, T., Yu, H., Kong, L., 2017. Heritability estimates for shell color-related traits in the golden shell strain of Pacific oyster (Crassostrea gigas) using a molecular pedigree. Aquaculture 476, 65–71. https://doi.org/10.1016/j.aquaculture.2017.04.012.

Wang, S., Hard, J.J., Utter, F., 2002. Salmonid inbreeding: a review. Rev. Fish Biol. Fish. 11, 301–319. https://doi.org/10.1023/A:1021330500365.

Wellenreuther, M., Luyer, J.L., Cook, D., Ritchie, P.A., Bernatchez, L., 2019. Domestication and temperature modulate gene expression signatures and growth in the Australasian snapper Chrysophrys auratus. G3: Genes, Genomes, Genetics 9, 105–116. https://doi.org/10.1534/g3.118.200647.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data analysis, Use R!. Springer-Verlag, New York.

Wray, N., Visscher, P.M., 2008. Estimating trait heritability. Nat. Educ. 1, 1.

Zeinab, Z., Shabany, A., Kolangi-Miandare, H., 2014. Comparison of genetic variation of wild and farmed bream (Abramis brama orientalis; berg, 1905) using microsatellite markers. Mol. Biol. Res. Commun. 3, 187–195.