FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Testing established methods of early prediction of genetic merit in abalone broodstock

Phoebe Arbon, Dean Jerry, Jan M. Strugnell
May 2022

FRDC Project No 2017/220

ISBN **978-0-6454198-2-5**

Testing established methods of early predication of genetic merit in abalone broodstock
2017-220

2022

Researcher Contact Details
Name: Prof. Jan Strugnell
Address: James Cook University, Townsville, Qld 4811
Phone: 07 47816357
Fax: -
Email: jan.strugnell@jcu.edu.au

FRDC Contact Details
Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6122 2100
Email: frdc@frdc.com.au
Web: www.frdc.com.au

Contents

Tables

Figures

Acknowledgments

Funding for this project was provided by the Australian Abalone Growers Association (AAGA) and the Fisheries Research and Development Corporation (FRDC) on behalf of the Australian Government. Southern Ocean Mariculture (SOM) provided samples for this study. We acknowledge the technical support and sampling efforts of Mark Gervis, Hamish Ebery and Steve Farrell from SOM. We also thank James Cook University(JCU) researchers Dr Cecile Massault for assistance with quantitative genetic analysis, Dr David Jones, Dr Jose Domingos and Dr Catarina Silva for guidance with genetic data analysis, and Julie Goldsbury for assistance with the laboratory components of the project.

Abbreviations

AAGA: Australian Abalone Growers Association (AAGA)
DOC: days of culture
DNA: deoxyribonucleic acid
EBV: estimated breeding value
FRDC: Fisheries Research and Development Corporation
gc: Genetic correlation
gEBV: genetic estimated breeding value
JCU: James Cook University
RNA: ribonucleic acid
SNP: single nucleotide polymorphism

Executive Summary

What the report is about

This report provides an assessment of the utility of RNA/DNA ratio as a method for early prediction of high performing abalone broodstock. The study was carried out on farmed Greenlip Abalone (*Haliotis laevigata*) whereby families were produced and resulting progeny were reared using commercial protocols. RNA/DNA ratio and shell length were measured in post larvae, and shell length, shell width and total weight were measured in juveniles and harvest sized individuals. All individuals were genotyped, parentage was assigned and heritability and genetic correlation of traits was calculated. Analyses in post-larvae and juveniles could not estimate heritability of traits including RNA:DNA and shell length indicating that the additive genetic variance component of these traits were unable to be separated from non-genetic components (e.g., environment) at early production stages (i.e. post-larvae and juveniles). This indicates that further grow out would be required to assess broodstock quality for use in breeding programs. The project was carried out between 2019-2022 by Phoebe Arbon, under the supervision of Prof. Jan Strugnell and Prof. Dean Jerry, based within the Department of Aquaculture at James Cook University, Australia.

Background

There is a need in the abalone industry to improve production animals. However, abalone are relatively slow growing animals and take several years to reach harvest size. This means that during the establishment of foundation broodstock populations it may be several years before the relative genetic merit of each of the broodstock can be determined and the first selection decisions made. During this time, the hatchery manager will have to blindly spawn broodstock to stock the farm, often with broodstock possessing poor genetic merit and that produce slow growing animals. This comes at the additional cost of not being able to cull poor performing broodstock early in the establishment of the population and replacing them with new broodstock.

Through measuring performance of desirable traits within families of broodstock (genetic estimated breeding values, EBV) these cellular traits in larvae can be compared with those derived from the same progeny at harvest. Using this approach, a high genetic correlation would indicate that it is possible to predict genetic merit using these cellular traits within early-stage larvae. Studies have shown that the ratio between RNA and DNA in cells has a high heritability (necessary for the traits to have predictive power) and that it can be used to accurately predict the EBV of barramundi broodstock without the necessity of rearing progeny all the way through to harvest (genetic correlation (gc)>0.8). Therefore, using RNA/DNA as the trait to measure in barramundi larvae it is now possible to establish high performing broodstock foundation populations via mass-spawning broodstock, estimating their genetic merit based on larvae RNA/DNA, and then eliminating those broodstock with inferior EBV from the breeding population.

Several Australian barramundi hatcheries have applied this technique to assist in the selection of broodstock. This method is as yet untested in abalone, but if successful, has great potential in helping screen broodstock. This project will test the efficacy of this early prediction method in abalone. The impact of this early detection method would be to save costs by assisting in the selection of superior broodstock individuals which would produce faster growing offspring in the creation of the foundation population of a breeding program. Currently new abalone broodstock are unevaluated with regard to their genetic merit.

Aims/objectives

The aim of the project was to assess the utility of RNA:DNA ratio as a method for early prediction of high performing Greenlip Abalone broodstock.

Methodology

Greenlip Abalone broodstock (n = 16) were spawned to produce progeny via single pair crosses. The progeny were stocked communally and reared using standard commercial husbandry protocols. The ratio of cellular RNA to DNA and, shell length were measured in 62 day old post-larvae (n = 1030) from the cohort. Shell length, width and total weight were measured in juvenile progeny from the same cohort at 188 days of culture (DOC) (n = 997), and at 803 DOC in harvest size individuals (n = 935). All progeny were genotyped using the DArTag™ SNP panel described in Arbon et al. (2021) and parentage assigned using consensus between CERVUS 3.0.7 and APIS. Heritability and genetic correlation of the measured traits were calculated in ASREML 4.0.1 using an animal model with sire geographic origin as a fixed effect. Estimated breeding values (EBVs) were calculated for the broodstock using the measured traits at each life stage. The broodstock EBVs generated using the post-larval and juvenile progeny traits were compared to the EBVs generated in harvest size progeny of the same cohort to assess their predictive accuracy.

Results/key findings

There was no detectible heritability of post-larval traits including RNA:DNA and shell length. Therefore, the genetic potential of broodstock was not able to be predicted using progeny performance at the earlier life stages (i.e., post larvae or juveniles). This is likely to be due to a strong influence of environmental factors at early life stages. At harvest size, however, all production traits (shell length, width and animal weight) had a significant additive genetic component. Therefore, realisation of a genetic effect only occurred in the later harvest stage of production and was masked at the earlier stages of production (i.e., post-larvae and juvenile stages).

Implications for relevant stakeholders

The implications of this study are that grow out of progeny to harvest size (or close to) is currently still required to determine the genetic merit of abalone broodstock in selective breeding programs. Furthermore, future studies following the same individuals in a cohort through time are required to better understand the result that the genetic effect is only realised at the harvest stage. This work is required to better inform current grading practices. Furthermore, a moderate heritability for growth traits was detected and so there is also potential for farmers to improve growth of stock through selection.

Keywords

Broodstock, genetic merit, estimated breeding value, EBV, Greenlip Abalone, *Haliotis laevigata,* RNA:DNA, single nucleotide polymorphism (SNP)

Introduction

The achievement of continuous and sustainable improvement in aquaculture productivity is reliant on the widespread implementation of genetic improvement programs (Weller, 2006). In many cases, aquaculture species are intrinsically suited to achieve large genetic gains through selective breeding, due to their high fecundity, large phenotypic variance in commercial traits, and short generation intervals (Gjedrem, 1998). Despite this, compared to agricultural species, such as in dairy cattle (Weigel et al., 2017), the integration of genetic improvement programs into aquaculture has lagged considerably, with the vast majority of the aquaculture industry currently relying on wild/unevaluated stock, or broodstock only one or two generations removed from the wild (Gjedrem et al., 2012). In total, only approximately 10% of aquaculture production is based on genetically improved stocks, thus, many farmed species still incur high variability in growth rates, leading to inefficiencies in production (Gjedrem et al., 2012).

Progeny performance information is regarded as highly valuable for estimating the breeding values (EBV) of desirable traits of broodstock, and is used extensively within the dairy industry to rank and select superior bulls, yielding considerable increases in productivity (Gjerde, 2005; Hayes et al., 2009; Schaeffer, 2006). The estimation of broodstock genetic merit based on the performance of their progeny (e.g., individual progeny weight at harvest) allows for the selection and retention of broodstock which produce high quality progeny (e.g., fast growing) for the formation of a superior foundation broodstock population.

Generally, the majority of modern aquaculture breeding programs operate based on large scale family selection, where each breeder is only spawned once, removing the opportunity to produce consecutive cohorts of superior progeny from broodstock with desirable traits that are highly ranked (EBV) (Gjedrem, 1985). For many highly fecund, multiple spawning species, which have broad reproductive windows, progeny testing for evaluation of EBVs of desirable traits, retention, and repeated spawning of superior broodstock holds obvious potential (e.g., barramundi *Lates calcarifer*; Domingos et al., 2014). For such species, selective breeding programs founded with progeny tested broodstock can theoretically yield faster genetic gain than contemporary breeding programs (Macbeth and Palmer, 2011), permitting more immediate realisation of genetic potential (Domingos et al., 2014).

Traditional progeny testing requires extended periods to rear and evaluate the progeny at a size and age where differences in phenotypic performance can be measured, generally at harvest (Camara and Symonds, 2014; Domingos et al., 2014; Gjedrem, 1983). For some species this may be anywhere from 2-4 years after spawning, resulting in a long-lag time for breeders to be able to predict the EBV of their broodstock and eliminate poor-performers. This required duration could be dramatically reduced if evaluation could be made at an early stage of the production cycle such as during larval rearing within the hatchery. This would enable the highly ranked broodstock to be immediately respawned, producing superior performing progeny quickly for commercial production. Here, genetic correlations between economically important harvest traits (e.g., harvest weight) and larval traits could be identified and exploited to achieve early prediction, such that, if a larval trait is heritable and strongly genetically linked with a harvest trait, the progeny performances in the larval trait could be used to accurately estimate the long-term growth trajectory of the family, and thus the broodstock breeding value. This method would allow for genetic improvement in economically important growth traits as soon as the next spawning cycle, by spawning from the highest ranked broodstock based on their larval progeny performance, expediting genetic improvements. For example, in Barramundi, *L. calcarifer*, the cellular ratio of RNA to DNA (RNA: DNA) of 18 day old larvae has been found to accurately predict broodstock genetic merit (gc = 0.80), dramatically reducing the time to achieve genetic gains (Domingos et al., 2014).

The use of the RNA:DNA is founded on the principle that the quantity of cellular DNA in cells remains relatively constant, on average, and can be used as an index of cellular activity in protein production. Comparatively, the cellular RNA content increases in accordance with protein synthesis, requisite to growth (Holm-Hansen, 1969; Price, 2004). Thus, RNA:DNA can be applied as a standardised and instantaneous

index for the protein synthetic capacity of an organism (Buckley, 1984). Potential variability and interactions between growth rate, temperature, feed intake and RNA:DNA have been well documented in research conducted on wild fish larvae (Buckley, 1984, 2008). In these studies, RNA:DNA was found to explain variability in growth rate when other environmental factors, such as temperature and food availability, were not limiting. Considering these dynamics and relating them to aquaculture, where food availability and temperature is largely regulated, and remains relatively constant between individuals in communal rearing conditions, RNA:DNA could theoretically explain the majority of variability in growth rate and serve as an accurate stand-alone index. Previous studies have suggested that genetically determined expression of growth-related processes (such as the RNA:DNA) at early life stages, could also persist throughout later life stages, influencing total animal growth (Bang et al., 2006; Domingos et al., 2014; Hoie et al., 1999). As aforementioned, the ratio has recently been investigated for its applicability as an early predictor of long-term family growth (~ broodstock genetic merit) in Barramundi (Domingos et al., 2014). Here, high heritability was observed in the RNA:DNA ratio in 18 day old Barramundi larvae (h^2 = 0.51 ± 0.15). Estimation of the EBV of broodstock based on RNA:DNA in these 18 day old larvae highly genetically correlated with the traditionally derived EBV estimates based on harvest size of progeny (r^2 = 0.88 ± 0.10). This demonstrated that Barramundi broodstock genetic merit could be estimated as early as 18 days of progeny rearing and there was no need to wait till harvest size to estimate EBVs. Thus, the RNA:DNA ratio potentiates a highly valuable measure for estimating broodstock genetic merit, to expedite the establishment of a genetically superior foundation broodstock population, allowing for immediate realization of genetic potential. This quality is of particular importance to slow growing species with long generation intervals, as both traditional progeny testing at harvest size, and contemporary selection programs, comparatively delay the onset of genetic gains (Camara and Symonds, 2014; Domingos et al., 2014; Gjedrem, 1983).

Abalone exhibit high fecundity and are multiple spawners, allowing for high selection intensities and propagation of genetic gains to farmed stocks from breeding nuclei (Li, 2008). However, the generation interval of the Greenlip Abalone is relatively long (~3.5-5 yrs.), reducing the rate of both genetic and economic gain through traditional or contemporary selection strategies (Li, 2008). Consequently, the quantification of larval RNA:DNA to predict broodstock genetic merit early in the production cycle could potentially expedite the selection process in this species and greatly reduce costs associated with rearing poor performing progeny. This approach has not yet been examined in any mollusc species.

Accordingly, the overarching aim of this project was to determine whether the RNA:DNA could be used as an accurate tool to predict long-term family growth and the genetic merit early in the production cycle of Greenlip Abalone. To achieve this, heritability and genetic correlation estimates and quantification of the phenotypic distributions of post-larval RNA:DNA and shell length, and juvenile and adult weight, shell length and shell width, in commercially reared Greenlip Abalone, were derived and assessed.

Objectives

The objective of the project was to assess the utility of RNA:DNA ratio as a method for early prediction of high performing Greenlip Abalone broodstock.

Method

Experimental abalone

Sixteen Greenlip Abalone broodstock (seven dams and nine sires) were spawned through single pair crosses at Southern Ocean Mariculture (SOM), Port Fairy, Victoria, Australia, resulting in the production of 49 families. Broodstock originated from one geographic source (origin = Farm 1), with the exception of two out of the nine sires (origin = Farm 2). All broodstock were sampled post-spawn for retrospective genotyping. All families were communally reared from settlement. At 62 days of culture (DOC), 1047 post-larvae were sampled from the cohort. Sampling was initially conducted at 62 DOC as this was determined to be the earliest time at which the individuals were large enough (held ample genetic material) to conduct the genetic analysis (sequencing using DArTag™, Diversity Arrays Technology, Australia) and nucleic acid quantifications. The post-larvae samples were removed from the settlement plates, in small batches of approximately 100 individuals at a time, and placed into a petri dish with a small amount of water from their residing tank. This petri dish was also placed on an ice pack to keep the water temperature low (tank temperature at time of sampling ~19.5 °C, ambient temperature at time of sampling ~ 30°C). This precaution was taken to reduce potential handling stress prior to sample preservation in RNAlater (ammonium sulphate, UNIVAR), as an elevation in stress may have caused elevation in RNA expression (Tripp-Valdez et al., 2019), confounding the accuracy of the RNA:DNA measurements. At 188 DOC, 1003 juvenile individuals were sampled from the same cohort. Juvenile samples were removed from settlement plates in small batches of approximately 100 individuals at a prior to sample preservation in ~5 mL of ethanol. At 803 DOC, (2 years, 2.4 months), 940 adult individuals were randomly sampled from the experimental cohort. The adult abalone were stored frozen until sample measurements were completed.

Sample measurements

Post-larval shell measurement

The post-larvae samples were individually imaged using an Olympus® SZ61 stereomicroscope, in conjunction with an Olympus® DP26 microscope digital camera (www.olympus.com.au). The samples were retrospectively measured for shell length (mm) using ImageJ software (https://imagej.nih.gov/) and visually inspected for condition (complete or damaged shell). Only abalone with complete, undamaged shells were retained for further analysis of shell length.

Juvenile and adult harvest trait measurement

Three harvest traits were measured for all juvenile and adult samples. Individuals were weighed (total mass; g) and additionally imaged for retrospective shell length (mm) and width measurement (mm) with ImageJ software (https://imagej.nih.gov/). After weighing each individual, tissue samples were taken for genotyping.

Post-larval RNA and DNA quantification

The general protocol used to estimate the total RNA and DNA concentration of whole abalone homogenates in this study was first validated for use in whole Barramundi larvae (Domingos et al., 2014). The ratio between total RNA and DNA was analysed as it represents a more standardized metabolic index between individuals (Ferron and Leggett, 1994). Individual whole larval homogenates were used to quantify total RNA and DNA. All samples were assigned a unique plate location and identifying label, enabling tracking of the sample through RNA and DNA quantification, extraction, genotyping and parental

assignment. Briefly, individual larvae were transferred from RNAlater into tubes containing 1 mm diameter zirconia/silica beads (Daintree Scientific Australia – BioSpec) and 250 µl of 1 x TE (Tris-EDTA) buffer. The samples were homogenized using a BioSpec Mini-BeadBeater 96 for 2 min. To the homogenate, 5 µl of 10% sarcosyl solution (ionic detergent) was added and mixed using a rotary shaker for 30 min to disrupt the nuclear membrane and free the nucleic acid. After mixing, a 100 µl aliquot of the sample homogenate was set aside for nucleic acid extraction. To the remaining 150 µl of sample homogenate, 720 µl of cold 1 x TE buffer was added. The samples were then mixed again in the rotary shaker for 2 min and centrifuged at 14 000 rcf and 4°C for 15 min.

Immediately following homogenisation, total RNA and DNA were quantified on two separate 96 well microplates using the Quant-iT™ RNA, broad range, Assay Kit (www.thermofisher.com.au) and the AccuBlue® High Sensitivity dsDNA Quantitation Kit (https://biotium.com/), respectively, as per the manufacturer's instructions. Each microplate contained triplicates of each sample homogenate and all wells contained 50 µl of the Quant-iT™ RNA buffer and dye solution for RNA, or 100 µl of the AccuBlue® High Sensitivity dsDNA buffer and dye solution for DNA. *Escherichia coli* rRNA and dsDNA standards were diluted using 10% sarcosyl solution and included in triplicate on each plate to create a six point standard curve of known concentration for quantifying RNA and DNA concentrations in each sample (Equation 1.). Fluorescence was measured using an EnSpire® Multimode Plate Reader using standard luminescence (www.perkinelmer.com).

Equation 1: Calculation of individual sample DNA or RNA concentration (ng/ µL) from the triplicate sample fluorescence readings and standard curve equation, derived from the fluorescence readings of the DNA or RNA standards.

RNA or DNA (ng/ul) =

$$\frac{[(\text{average fluorescence value of sample triplicates}) - (\text{intercept value of the standard curve})]}{(\text{Slope of the standard curve})}$$

Nucleic acid extraction and genotyping

Post larval nucleic acid was extracted from the 100 µL of sample homogenate excluded from the RNA and DNA quantification analysis. This was conducted as previously described in Arbon et al., (2021) to yield 20 µL of eluted product. For the juvenile samples, 5-10 mg of muscular foot tissue was mechanically homogenized after sampling and incubated for 6 hr at 55 °C with 10 µl of proteinase K and 300 µl of Perkin Elmer™ lysis buffer solution (1a). DNA extraction was then conducted using the same method as for the post-larval samples. For the adult samples, muscular foot tissue (5-10 mg) was sampled and stored in ~98% ethanol for DNA extraction by Diversity Arrays Technology (DArT), Australia.

Broodstock, and adult tissue samples and, extracted post-larval and juvenile DNA samples were sent to Diversity Arrays Technology (DArT), Australia, for DNA extraction and sequencing using the DArTag™ panel containing 1,004 SNPs for Australian Greenlip Abalone (Arbon et al., 2021). Samples were subjected to nucleic acid quality control testing within DArT, resulting in the removal of twelve post-larval and four juvenile samples from further analysis. Genotypic data across the four sample groups was co-analysed and filtered using DartQC 2.0 (https://github.com/esteinig/dartqc) to retain SNPs with an average read depth > 5, call rate > 95 %, MAF > 0.02 and sequence similarity < 95 %. Data across a total of 706 SNPs were retained for pedigree reconstruction of the broodstock and progeny genotypes. Samples that were typed at less than half of the filtered 706 loci (n = 353 SNPs) were removed from further analysis.

Identification of families

Retrospective DNA parentage analysis to identify families was conducted using both CERVUS 3.0 (Kalinowski et al., 2007) and APIS 1.0.1 (Griot et al., 2020) with genotype data across the 706 SNPs

previously described. To increase the statistical confidence of subsequent quantitative genetic analyses, only families containing five or more assigned offspring (for each time point) were retained.

Statistical analysis

Cleaning the data

As the post-larvae samples were so small, a few negative calculated RNA and DNA values arose (due to insufficient quantity of RNA or DNA in the samples). These RNA or DNA values were removed from the dataset, in addition to values where the triplicate fluorescence readings for the sample had a coefficient of variance (CV) greater than 0.20.

Batch effect correction

Degradation of the RNA and DNA standards and dye over time resulted in minor systemic differences between batches, where a batch represented a single 96 well plate consisting of six standards and 24 samples, each in triplicate. To account for this, a batch correction method was developed and applied for both the quantified RNA and DNA values, separately. Sample collection was random (individuals were chosen at random from settlement plates throughout the rearing tank) and families (groups) were assumed to be distributed evenly throughout the tank and hence throughout the study batches. Prior to batch correction, trait value outliers were identified and removed using Equation 2, where k = 1.5.

Equation 2: Outlier detection using k*IQR method

$$If\ X > or < k * IQR, X\ is\ defined\ as\ an\ outlier$$

Each batch was zero centered to the batch mean ($\tilde{Y}_{ij}^{0}$) (Equation 3) and then all batches were mean centred to the common dataset mean ($\tilde{Y}_{ij}^{avg}$) (Equation 4).

Equation 3: Batch correction, zero centre trait values to the batch mean

$$\tilde{Y}_{ij}^{0} = Y_{ij} - \tilde{Y}_{\iota}\ where,\ \tilde{Y}_{\iota} = (\frac{1}{n_i}) \sum_{j=1}^{k} Y_{ij}\ and\ n_i = \sum_{j=1}^{k} n_{ij}$$

Where Y is the trait (quantified RNA or DNA) value, i = 1, … , K are the different batches, inclusive of the samples for each batch, j = 1, … , k, n is the number of samples and $\bar{Y}i$ is the mean trait value for each batch *i*.

Equation 4: Batch correction, mean centre trait values to common dataset mean

$$\tilde{Y}_{ij}^{avg} = \tilde{Y}_{ij}^{0} + \tilde{Y}$$

Where, $\tilde{Y}_{ij}^{0}$ is the zero centred trait value resulting from Equation 3 and $\tilde{Y}$ is the common dataset mean of the trait. Following batch correction, RNA:DNA far-outlier values were re-identified and removed using Equation 2, where k = 3.

Phenotypic data

Phenotypic data was assessed using R Software (R Core Team, 2021). Normality was assumed based on the central limit theorem, such that when a sample size is sufficiently large the central limit theorem ensures that the distribution of disturbance term will approximate normality. Additional visual inspection of data distributions using histograms and QQ plots was used for confirmation. For each stage and trait measured, the mean, standard deviation (SD) and coefficient of variance (CV) of the trait were calculated. One-way ANOVAs were used to assess significant differences in growth traits measured for each life stage between

progeny of different dams and different sires. Significant differences were further investigated using Tukey HSD post-hoc analysis.

Genetic parameter analysis

Genetic parameter analysis was conducted using an animal model in ASReml 4.0.1 software (Gilmour et al, 2002; Gilmour et al., 2015). Log likelihood ratio testing (Equation 6) informed the exclusion of sire and dam as random terms within the model, as they were not significant. Wald testing confirmed the inclusion of only sire origin (Farm 1 or 2) as a fixed effect in the model at the adult stage, given the realisation of significant apparent genetic structure at this stage. Genetic parameters, including heritability, genetic correlation, phenotypic correlation and estimated breeding values, were estimated for all traits measured at the post-larval, juvenile and adult stages using an animal model with sire origin as a fixed effect as described in Equation 5. The significance of heritability and genetic correlation estimates were determined by log likelihood ratio tests using Equation 6. For heritability, the alternative model was the animal model for estimating heritability including pedigree information and the null model was the animal model without pedigree information. For the genetic correlations, the alternative model was the bivariate model and the null model was the bivariate model where the covariance between traits was fixed at 0 (i.e. no covariance).

Using ASReml 4.0.1 (Gilmour et al, 2002; Gilmour et al., 2015), broodstock EBVs for each trait were generated. EBVs for post-larval and juvenile traits were plotted against adult trait EBVs and Pearson's correlation analysis was used to assess the covariation of the broodstock EBVs and hence the predictive power of the post-larval and juvenile traits.

Equation 5: Animal model used for genetic parameter analysis

$$y_{ijk} = \mu + \text{Origin}_j + \text{Animal}_{ijk}$$

Where; y is the vector of trait observations in the cohort (post larvae, juvenile or adult), μ is the trait mean across the cohort, $Origin_j$ is the fixed effect of sire genetic origin (Farm 1 or 2), and $Animal_{ijk}$ is the vector of additive genetic effects.

Equation 6: Likelihood ratio to estimate Chi-squared test statistic

$$LR = -2[ln(\theta_0) - ln(\theta)]$$

Where; θ_0 is the converged likelihood of the null model (subset parameter space) and θ is the converged likelihood of the alternative model (full parameter space). As per Wilk's theorem, as the sample size increases towards infinity, the test statistic generated by Equation 6 asymptotically approaches the chi-squared distribution. Hence, LR can be used as an approximate statistical alternative to the X^2 test statistic (Silvey, 2017; Wilks, 1938).

Results & Discussion

Pedigree establishment

Pedigree information was established for all retained post larval (n = 1030), juvenile (n = 997), and adult (n = 935) samples after filtering, achieving 100% assignment success with complete consensus between CERVUS and APIS assignments. A total of 48 families were identified from the post-larvae samples, with 34 of those families containing more than five sampled offspring (n = 996 offspring); 44 families were identified from the juvenile samples, with 33 of those families containing more than five sampled offspring (n = 961 offspring); 42 families were identified from adult samples, with 34 of those families containing more than five sampled offspring (n = 917 offspring).

Phenotypic traits

Phenotypic traits of the abalone with assigned parentage, are summarised in Figures 1-3. All length and width measurements conformed to a normal distribution at each life stage, as confirmed by QQ plots (R Core Team, 2021). Abalone weights and RNA:DNA were marginally skewed, however, their distributions were normal after square root transformation.

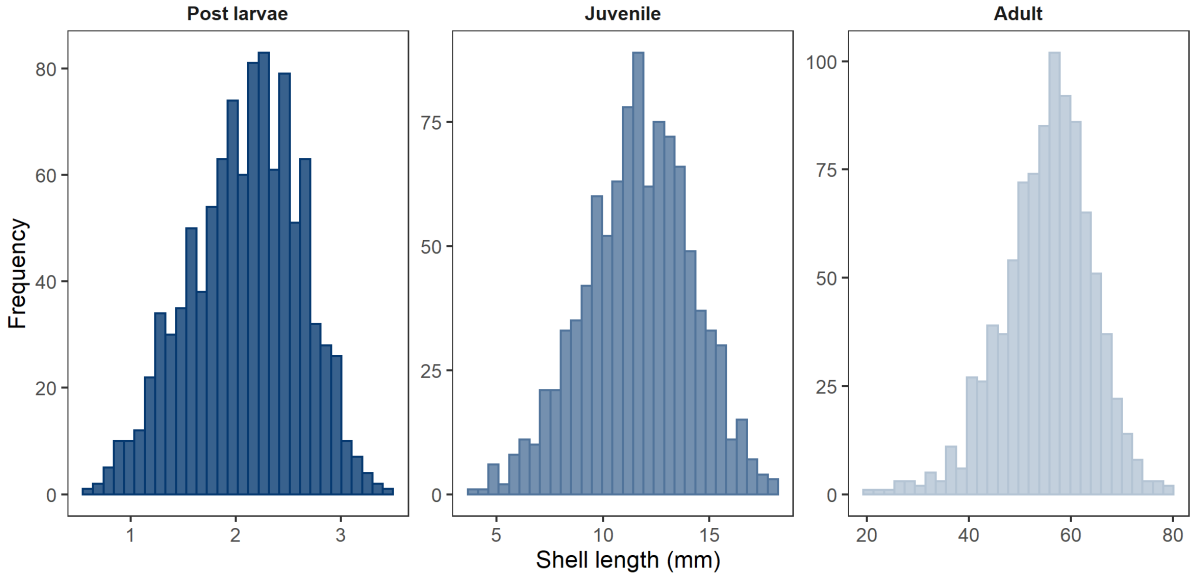

Figure 1. Histogram of shell lengths (mm) measured from post-larvae (62 days old), juvenile (188 days old) and adult abalone (803 days old)

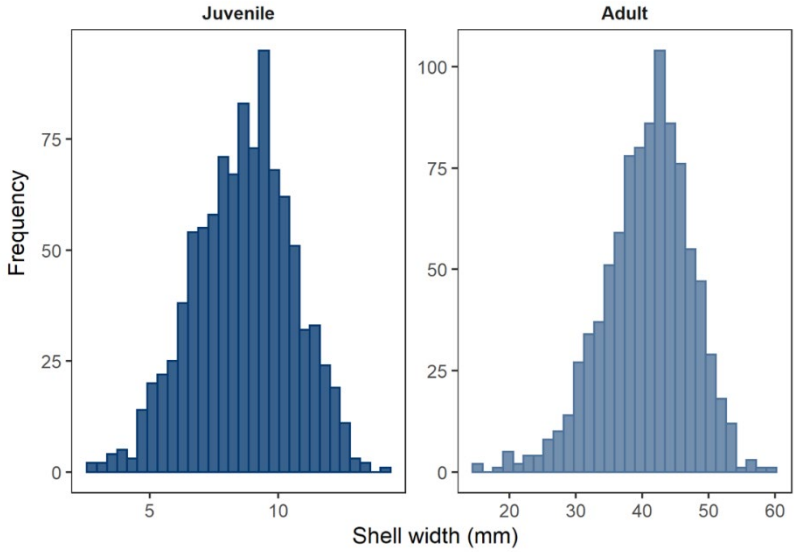

Figure 2. Figure 1. Histogram of shell widths (mm) measured from juvenile (188 days old) and adult abalone (803 days old)

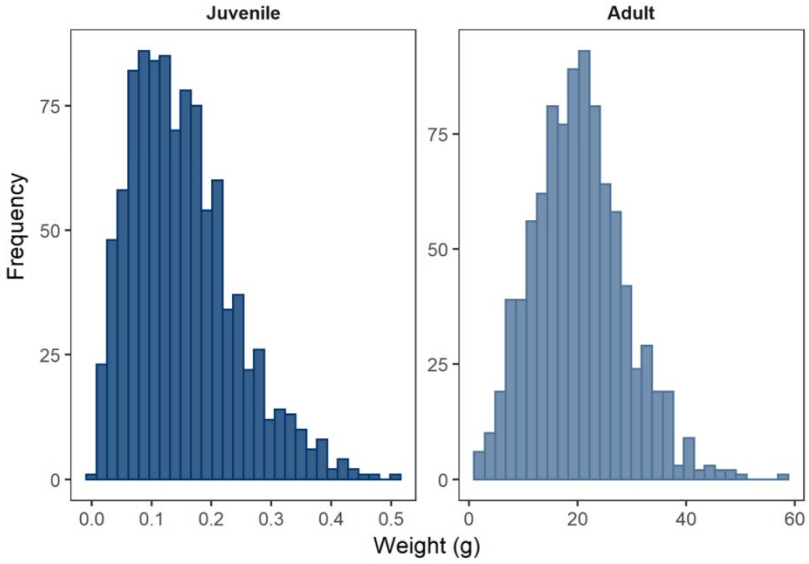

Figure 3. Histogram of abalone weights (total mass) (g) measured from juvenile (188 days old) and adult abalone (803 days old)

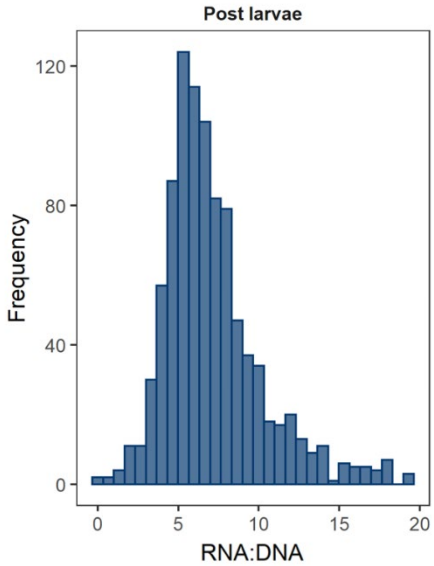

Figure 4. Histogram of abalone cellular RNA:DNA measured from post larvae (62 days old).

Distribution of traits between sires and dams

The variation of progeny RNA:DNA and shell length from the same dam was generally larger than the variation observed between progeny of different dams at the post larval stage (Figure 5). This variation indicated a negligible maternal genetic effect on the growth traits measured at this stage. At the juvenile stage progeny shell length, width, and total weight between dams was marginally statistically differentiated. This differentiation continued into the adult stage, although within-dam variation remained high (Figure 5). The variance in shell length and RNA:DNA at the post larval stage, and shell length, width and total weight at the juvenile stage from progeny of the same sire was generally larger than the variance between progeny of different sires (Figure 6). These results indicated a negligible paternal genetic effect on the growth traits at the earlier stages of production. However, at the adult stage progeny shell length, width and total weight became significantly differentiated by Sire, particularly between sires from the two genetic origins (Farm 1 and 2). Taken together, these results suggested the realisation of a genetic effect in the later adult stage of production, with the potential presence of a paternal effect yielding the more marked differentiation of progeny growth traits.

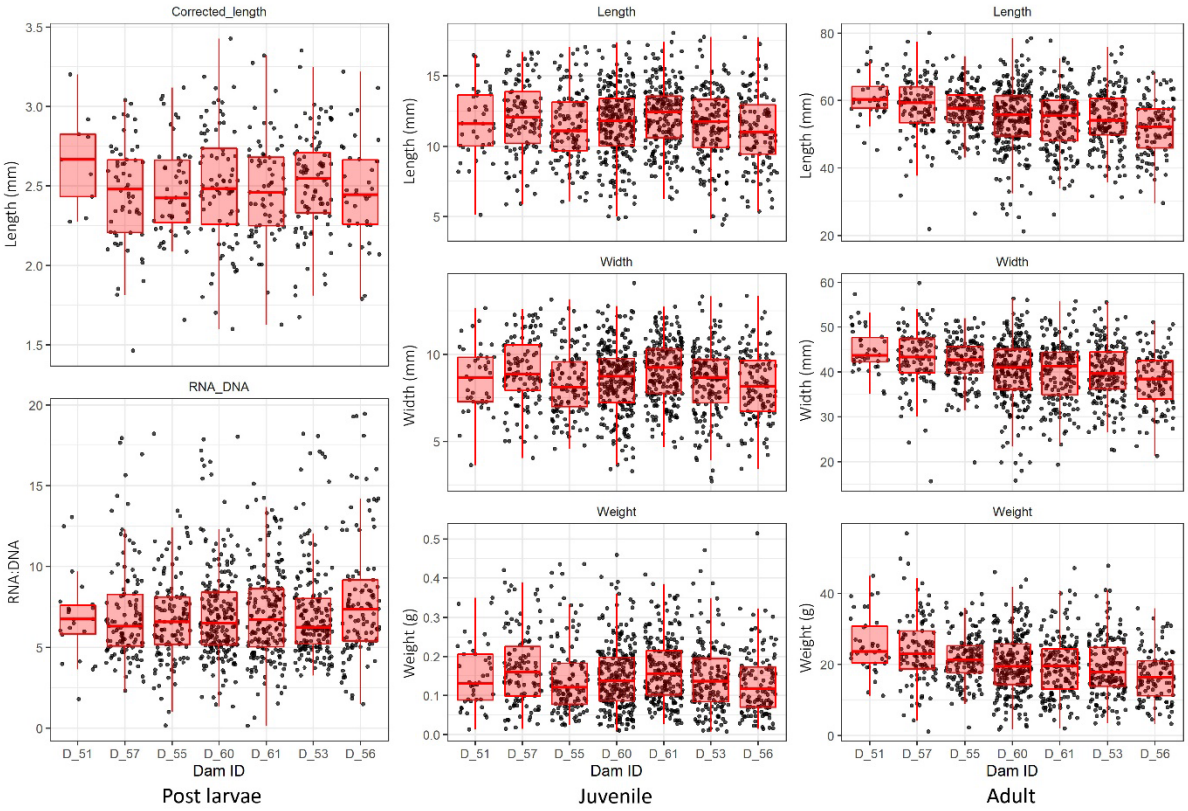

Figure 5. Phenotypic trait distributions for progeny by dam. Post larval RNA:DNA and shell length (mm) and juvenile and adult shell length (mm), width (mm) and weight (total mass; g) metrics are plotted for individual progeny (black points), grouped by dam.

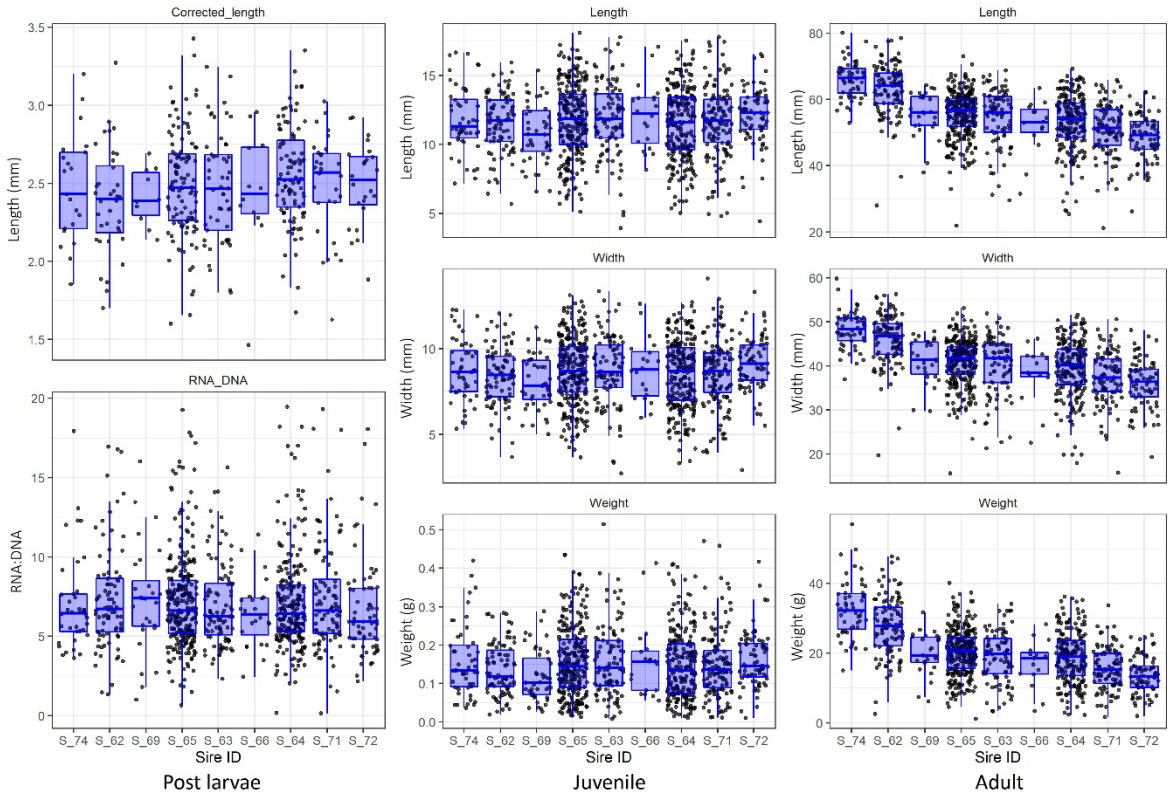

Figure 6. Phenotypic trait distributions for progeny by sire. Post larval RNA:DNA and shell length (mm) and juvenile and adult shell length (mm), width (mm) and weight (total mass; g) metrics are plotted for individual progeny (black points), grouped by sire.

Heritability

Analysis of the growth performance traits at the different stages revealed an apparent masking of additive genetic effect at the two earlier production stages, with trait heritability only becoming detectable at the adult stage (Table 1). Heritability of larval traits including RNA:DNA (0.00 ± 0.02) and shell length (0.03 ± 0.03) were not significantly different from zero. Heritability estimates of growth traits at the juvenile stage were significantly different from zero, however, were very low and accompanied by relatively large standard errors. In adults, all production traits measured had a significant additive genetic component, with heritability estimates of 0.27 ± 0.11, 0.25 ± 0.10 and 0.31 ± 0.12 for shell length, width and total animal weight, respectively.

*Table 1. Summary of trait measurements for the experimental greenlip abalone. T1 was 62 days of culture (DOC) and T2 was 188 DOC and T3 was 803 DOC. Statistical significance of each heritability estimate is denoted by ** for p-value < 0.05 and *** for p-value < 0.001.*

Stage	Variable	Age (days)	Number of samples	Mean ± SD	CV	Heritability ± SE
T1	Length[1] (mm)	62	393	2.49 ± 0.33	13.25 %	0.03 ± 0.03
T1	RNA:DNA	62	944	7.14 ± 3.05	42.72 %	0.00 ± 0.02
T2	Length (mm)	188	997	11.65 ± 2.51	21.55 %	0.05 ± 0.04 ***
T2	Width (mm)	188	997	8.64 ± 1.92	22.22 %	0.04 ± 0.03 **
T2	Weight (g)	188	997	0.15 ± 0.09	60.00 %	0.05 ± 0.04 **
T3	Length (mm)	803	935	55.31 ± 8.78	15.87 %	0.27 ± 0.11 ***
T3	Width (mm)	803	935	40.70 ± 6.56	16.12 %	0.25 ± 0.10 ***
T3	Weight (g)	803	930	20.33 ± 8.38	41.22 %	0.31 ± 0.12 ***

The heritability estimates reported in the present study for post larval length and juvenile traits are consistent with existing literature. For Greenlip Abalone, Kube et al. (2007) reported the heritability of shell length ranging from 0.00 to 0.04 ± 0.10 and total weight from 0.00 to 0.10 ± 0.10 up to ~1200 DOC (38 months). Li (2008) reported heritability of shell length and total weight at 930 DOC (~30 months) of a similar magnitude, at 0.07 ± 0.007 and 0.08 ± 0.006, respectively.

Heritability (h^2) estimates are derived from the statistical partitioning of the total observed phenotypic variance (Vp) that is explained by additive genetic variance (Va) (i.e h^2= Va/Vp); commonly through quantification of how phenotypic variance correlates with pedigree structure (Griffiths et al., 2000). A heritability value of 1 indicates that all of the phenotypic variation observed for the trait is explained by additive genetic variation passed from parents to progeny; however, a value close to zero indicates that very little phenotypic variation is attributable to additive genetic factors. For the post larvae and juvenile abalone traits examined in the present study, and the studies of Kube et al., (2007) and Li (2008), the low heritability estimates indicate that only a small proportion of the observed phenotypic variance could be apportioned to the pedigree structure and thus the additive genetic component. Previous studies have demonstrated that morphological differences in abalone are heavily influenced by environmental factors during the early life-stages, resulting in morphological plasticity which is not genetically fixed (Saunders et al., 2009; Kim et al., 2013; Padilla and Savedo, 2013; Pecl et al., 2011). Given that environmental condition gradients can occur readily in intensive rearing systems due to non-homogenous culture conditions (ie. DO, water flow, algal growth, water temperature and nitrogenous compounds), it is likely that the variation in growth traits measured were primarily under environmental influence which makes it difficult to estimate the underlying additive genetic variance. As such, the low heritability estimates observed suggest that environmental effects were masking the expression of additive genetic effects and reduced the ability to estimate the underlying additive genetic contribution to the traits.

For the growth traits measured at the adult stage, significant heritability estimates were observed (h^2 = 0.25-0.31). These results suggest that the additive genetic effects influencing progeny phenotypes were more evident at this later life stage, and further supports that the genetically driven phenotypic divergence

[1] Length of abalone with complete, undamaged shells only.

had not yet had sufficient time to be realized at the earlier life stages. The heritability values observed in the present study are larger than those reported in the studies of Kube et al. (2007) (~ 820 DOC; length h^2 = 0, h^2 = weight: 0.01 ± 0.05) and Li (2008) (930 DOC; length h^2 = 0.07 ± 0.007, h^2 = weight: 0.08 ± 0.007) at similar age ranges. The present study included analysis across a larger number of families (33), compared to the two prior studies by Li (2008) (14 families) and Kube et al., (2007) (17 families) and conducted retrospective DNA parentage analysis to determine pedigree structure, as opposed to physical tagging of individuals, benefitting from high accuracy pedigree reconstruction. These factors may have contributed to improved capacity to apportion and resolve the additive genetic component of the growth traits measured at the adult stage, yielding larger and more significant heritability estimates than previously reported.

Phenotypic and Genetic correlation

Genetic correlation values represent the proportion of variance between two traits that is genetically driven, such that genetic selection for one trait would result in the selection for (positive correlation) or against (negative correlation) of the other trait, given significant heritability of each trait. The post-larval traits of RNA: DNA and shell length had no significant genetic correlation with weight, shell length or shell width at harvest. Similarly, the juvenile traits measured had no significant genetic correlation with the same traits measured at harvest size (Table 2). These non-significant values, coupled with the low heritability estimates for these traits, further indicate the masking of genetic influence on growth traits by environmental influences at these early life stages. Significant genetic correlation was observed between traits measured within the juvenile stage and within the adult stage, as was expected given the strong phenotypic correlation between these traits within each life stage. The absence of significant genetic correlation between post-larval or juvenile traits and the harvest traits measured in adult progeny provide additional evidence for the low predictive capacity for these early life stage traits to predict broodstock genetic merit.

Table 2. Phenotypic and genetic trait correlations for all traits measured at the post larvae (62 DOC), juvenile (188 DOC) and adult (803 DOC) stages. Phenotypic correlations (rp ± standard error) are displayed in the upper right triangle, and genetic correlations (ra ± standard error) are displayed in the lower left triangle. Non-diagonal empty table values were unable to be calculated due to matrix singularity when the residual became limiting.

		Post larvae		Juveniles			Adult		
		RNA:DNA	**Length**	**Length**	**Width**	**Weight**	**Length**	**Width**	**Weight**
Post larvae	**RNA:DNA**						-0.1 ± 0.06	-0.1 ± 0.06	-0.13 ± 0.08
	Length			-0.01 ± 0.02	0.11 ± 0.16	-0.03 ± 0.04	-0.07 ± 0.12	-0.08 ± 0.11	-0.11 ± 0.16
Juveniles	**Length**		-0.91 ± 4.66		0.96 ± 0	0.94 ± 0	-0.06 ± 0.09	-0.06 ± 0.08	-0.02 ± 0.1
	Width		0.48 ± 0.46	0.99 ± 0.03 ***		0.93 ± 0	-0.07 ± 0.1	-0.06 ± 0.09	
	Weight		-0.79 ± 1.09	0.97 ± 0.04 ***	0.93 ± 0.07 ***		0.07 ± 0.1	0.07 ± 0.09	0.17 ± 0.11
Adult	**Length**	-0.98 ± 0.45	-0.9 ± 2.38	-0.35 ± 0.44	-0.32 ± 0.41	0.35 ± 0.44		0.98 ± 0	0.95 ± 0.01
	Width	-0.97 ± 0.45	-0.97 ± 1.63	-0.35 ± 0.43	-0.3 ± 0.41	0.35 ± 0.44	1 ± 0 ***		0.94 ± 0.01
	Weight	-0.98 ± 0.32	-0.57 ± 0.72	-0.12 ± 0.52		0.66 ± 0.29	0.94 ± 0.04 ***	0.98 ± 0.02 ***	

Estimated breeding values

Estimated breeding values were derived from pedigree relationships and progeny performance traits for each trait at post-larval, juvenile and adult stages. Adult 'harvest' stage EBVs were assumed to be the 'standard' to which the EBVs derived from the earlier life stage data were compared to. The EBVs derived from the post-larval traits of RNA:DNA and shell length, and the juvenile shell length, shell width and weight EBVs did not correlate strongly or significantly with the adult stage EBVs, suggesting a lack of predictive capacity of these early life stage traits to predict broodstock genetic merit (Figure 7). High trait heritability and significant genetic correlation to economically important harvest traits, for the derivation of

accurate parental EBVs, is obligatory for predictive power of long term, genetically driven growth (Domingos et al., 2014). In the present study, the low heritability and genetic correlation estimates of early progeny traits yielded EBVs with no accuracy for prediction of broodstock genetic merit.

The disparity of genetic estimates and ultimately predictive power for broodstock genetic merit of the larval traits in the present study and the study conducted by Domingos et al. (2014) on Barramundi likely stems primarily from life history differences between the two species. As previously discussed, deviations from optimum environmental conditions can commonly occur in both aquaculture and natural systems resulting in phenotypic variation driven by environmental variability rather than the expression of genetic variation. In the present study, the RNA:DNA of the post-larvae abalone was measured at 62 DOC, after the abalone had been stocked into a large outdoor rearing tank with considerable potential variability in conditions throughout the tank, including DO, temperature and food availability. The heterogeneous nature of the rearing tank may have resulted in significant environmentally influenced variability in the RNA:DNA (Buckley et al., 2008; Peck et al., 2003), and therefore poor ability to estimate heritability and genetic correlation. Conversely, in the seminal study of Domingos et al., (2013) which found the RNA:DNA in 18 day old Barramundi heritable as a strong predictor of final family harvest weight, larvae were measured at a time when they were still pelagic in a highly homogenous tank environment with abundant food and before behavioural confounding effects are expressed (i.e. due to cannibalism at day 25) (Domingos et al., 2013; Schipp et al., 2007). Here, high heritability estimates for growth related traits demonstrated that additive genetic effects significantly genetically correlated to barramundi growth at 18 days post hatch. The estimation of heritability at this stage was likely higher due to the innate large additive genetic effect of barramundi growth (Domingos et al., 2013), in addition to the highly continuous nature of the rearing environment, introducing reduced confounding phenotypic-environment interactions which would have lowered the heritability estimates if present. Consequently, for Barramundi the larval RNA:DNA served as an accurate predictor of long-term family growth, as the expression of RNA was primarily genetically determined, rather than environmentally influenced as was observed for abalone in this study (Domingos et al., 2013; 2014).

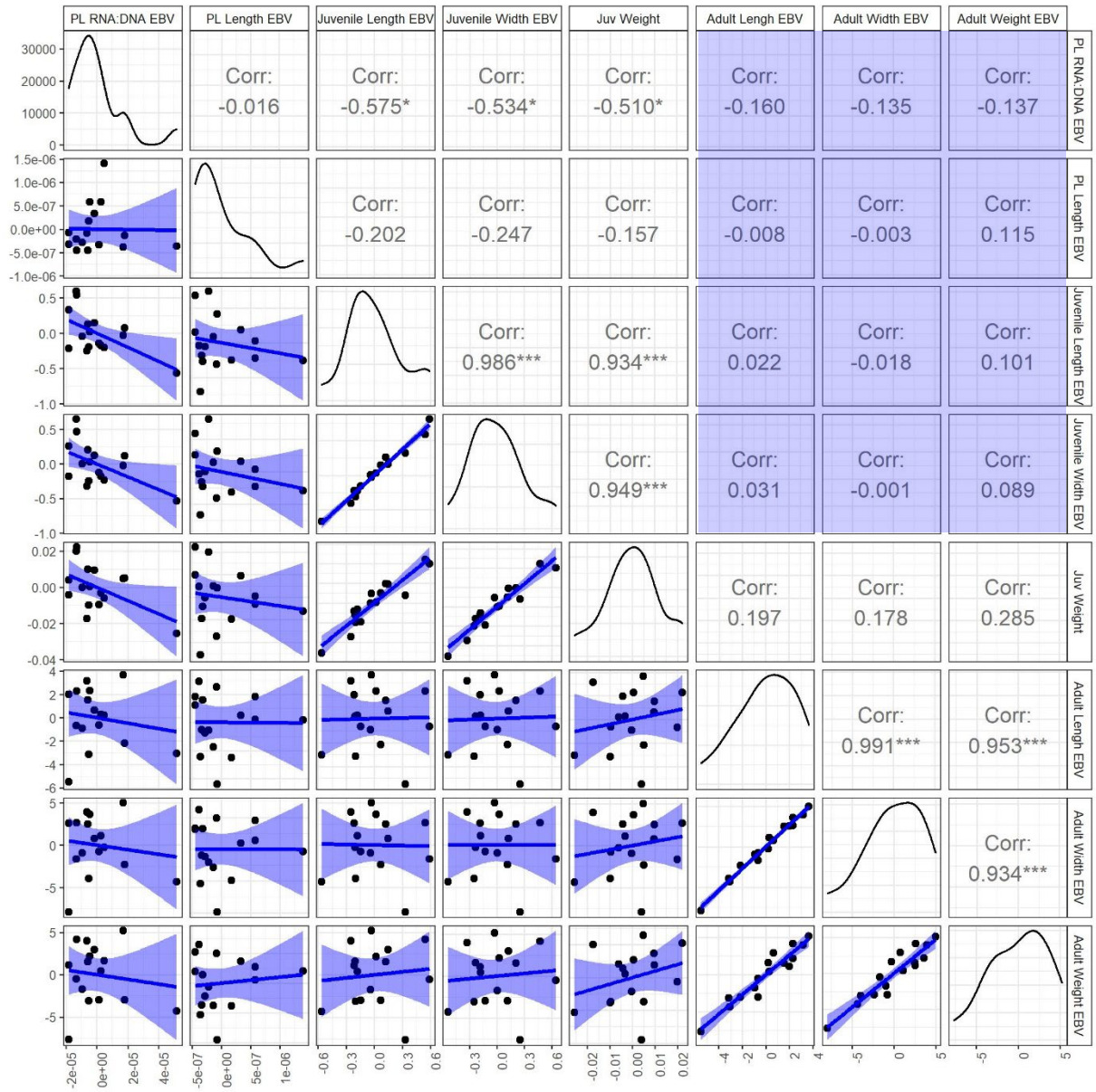

Figure 7. Pearson correlation matrix for broodstock EBVs derived from progeny trait measurements at each stage (post larvae of 62 days old, juveniles of 188 days old, and adults at 803 days old). Displayed in the upper right triangle is the Pearson's correlation coefficient of the EBVs derived from each trait. Significant correlations are indicated with asterisks. Displayed in the lower left triangle are the EBVs plotted against one another, with a linear regression line overlayed. Highlighted in the upper right is the correlation values for the early life stage trait EBVs with the adult 'harvest' stage EBVs.

Conclusion

The larval traits investigated in this study did not represent strong early predictors of broodstock genetic merit in Greenlip Abalone. Cellular RNA:DNA and shell length were found to have low heritability and genetic correlations to economically important traits measured in harvest size siblings and non-significant statistical correlation between larval trait EBVs and harvest trait EBVs. The limited capacity for these traits to act as broodstock genetic merit predictors is likely a result of a large environmental influence on abalone growth at the early life stages, resulting in a masking of the genetic contribution to the phenotype. Increased trait heritability observed at the harvest stage suggests that the genetic component of Greenlip Abalone growth is not realised and measurable until the later life stages.

Implications

- The additive genetic component of growth traits measured in this study was not realised until later life stages, indicating that the genetic potential of broodstock cannot be predicted using progeny performance at the earlier life stages.
- Selective breeding programs remain limited by a lengthy grow-out period of progeny to assess broodstock quality.
- At the adult stage, all production traits measured had a significant additive genetic component, yielding heritability estimates (EBV) of 0.27 ± 0.11, 0.25 ± 0.10 and 0.31 ± 0.12 for shell length, width and total animal weight, respectively. These EBV results confirm that improvements to commercial traits can be achieved through family-based selection programs.

Extension and Adoption

The project was communicated to the industry through this report. Very preliminary outcomes were presented to industry at the Australian Abalone Growers Association (AAGA) meeting in July of 2019. Further presentations will be given to AAGA at their invitation.

Project materials developed

This project formed a significant component of Phoebe Arbon's honours thesis:

Arbon, P.M. (2019) Early predication of broodstock genetic merit: A case study on Australian Greenlip Abalone. BSc hons. Aquaculture Department, James Cook University, Townsville, Australia.

The single nucleotide polymorphism (SNP) panel developed for use in this study is now published in a peer-reviewed journal:

Arbon, P.M., Silva, C.N.S., Jones, D.B., Jaccoud, D., Gervis, M., Jerry, D.R., Strugnell, J.M. (2021) Development and validation of a SNP-based genotyping tool for pedigree establishment in Australian Greenlip Abalone *Haliotis laevigata* Donovan, 1808 *Aquaculture Reports* 20: 100746.

Appendices

References

Arbon, P.M., Silva, C.N.S., Jones, D.B., Jaccoud, D., Gervis, M., Jerry, D.R., Strugnell, J.M., 2021. Development and validation of a SNP-based genotyping tool for pedigree establishment in Australian Greenlip Abalone *Haliotis laevigata* Donovan, 1808. Aquac. Reports 20, 100746. https://doi.org/10.1016/j.aqrep.2021.100746

Bang, A., Grønkjær, P., Clemmesen, C., Høie, H., 2006. Parental effects on early life history traits of Atlantic Herring (*Clupea harengus* L.) larvae. J. Exp. Mar. Bio. Ecol. 334, 51–63. https://doi.org/10.1016/J.JEMBE.2006.01.003

Buckley, L.J., 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80, 291–298. https://doi.org/10.1007/BF00392824

Buckley, L.J., Caldarone, E.M., Clemmesen, C., 2008. Multi-species larval fish growth model based on temperature and fluorometrically derived RNA:DNA ratios: Results from a meta-analysis. Mar. Ecol. Prog. Ser. 371, 221–232. https://doi.org/10.3354/meps07648

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

Domingos, J., Smith-Keune, C., Harrison, P., Jerry, D.R., 2014. Early prediction of long-term family growth performance based on cellular processes — A tool to expedite the establishment of superior foundation broodstock in breeding programs. Aquaculture 428–429, 88–96.

Domingos, J.A., Smith-Keune, C., Robinson, N., Loughnan, S., Harrison, P., Jerry, D.R., 2013. Heritability of harvest growth traits and genotype–environment interactions in Barramundi, *Lates calcarifer* (Bloch). Aquaculture 402–403, 66–75. https://doi.org/10.1016/J.AQUACULTURE.2013.03.029

Ferron, A., Leggett, W.C., 1994. An appraisal of condition measures for marine fish larvae. pp. 217–303. https://doi.org/10.1016/S0065-2881(08)60064-4

Gilmour, A., Gogel, B., Cullis, B., Thompson, R., 2009. ASReml User guide.

Gilmour, A.R., Gogel, B.J., Welham, S.J., 2015. ASReml User guide functional specification.

Gjedrem, T., 1998. Developments in fish breeding and genetics. Acta Agric. Scand. Sect. A - Anim. Sci.

Gjedrem, T., 1985. Improvement of productivity through breeding schemes. GeoJournal 10, 233–241.

Gjedrem, T., 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture 33, 51–72. https://doi.org/10.1016/0044-8486(83)90386-1

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

Gjerde, B., 2005. Design of Breeding Programs, in: Selection and Breeding Programs in Aquaculture. Springer-Verlag, Berlin/Heidelberg, pp. 173–195. https://doi.org/10.1007/1-4020-3342-7_12

Griffiths, A., Miller, J., Suzuki, D., 2000. An Introduction to Genetic Analysis: Quantifying heritbility, 7th Editio. ed. W. H. Freeman, New York.

Griot, R., Allal, F., Brard-Fudulea, S., Morvezen, R., Haffray, P., Phocas, F., Vandeputte, M., 2020. APIS: An auto-adaptive parentage inference software that tolerates missing parents. Mol. Ecol. Resour. 20, 579–590. https://doi.org/10.1111/1755-0998.13103

Hayes, B.J., Bowman, P.J., Chamberlain, A.J., Goddard, M.E., 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. J. Dairy Sci. 92, 433–443. https://doi.org/10.3168/JDS.2008-1646

Hoie, H., Folkvord, A., Johannessen, A., 1999. The influence of different parental combinations and incubation temperature on the RNA and DNA content of herring larvae at hatching: a pilot study. J. Fish Biol. 55, 110–118. https://doi.org/10.1111/j.1095-8649.1999.tb01049.x

Holm-Hansen, O., 1969. Algae: amounts of DNA and organic carbon in single cells. Science 163, 87–8.

Kalinowski, S., Taper, M., Marshall, T., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106.

Kim, T.W., Barry, J.P., Micheli, F., 2013. The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile Red Abalone. Biogeosciences 10, 7255–7262. https://doi.org/10.5194/bg-10-7255-2013

Kube, P.D.D., Appleyard, S.. A., Elliot, N.G.G., 2007. Selective breeding Greenlip Abalone (*Haliotis laevigata*): Preliminary results and issues. J. Shellfish Res. 26, 821–824. https://doi.org/10.2983/0730-8000(2007)26[821:sbgahl]2.0.co;2

Li, X., 2008. Abalone Aquaculture Subprogram: Selective breeding of farmed abalone to enhance growth rates (II). FRDC Project No. 2001/254. SARDI Publication No: F2008/000813-1. SARDI research Report Series no: 318.

Macbeth, G.M., Palmer, P.J., 2011. A novel breeding programme for improved growth in Barramundi *Lates calcarifer* (Bloch) using foundation stock from progeny-tested parents. Aquaculture 318, 325–334. https://doi.org/10.1016/j.aquaculture.2011.05.037

Padilla, D.K., Savedo, M.M., 2013.A systematic review of phenotypic plasticity in marine invertebrate and plant systems. Adv. Mar. Biol. 65, 67–94. https://doi.org/10.1016/B978-0-12-410498-3.00002-1

Peck, M., Buckley, L., Caldarone, E., Bengtson, D., 2003. Effects of food consumption and temperature on growth rate and biochemical-based indicators of growth in early juvenile Atlantic Cod, *Gadus morhua* and Haddock, *Melanogrammus aeglefinus*. Mar. Ecol. Prog. Ser. 251, 233–243. https://doi.org/10.3354/meps251233

Pecl, G., Ward, T., Doubleday, Z., Clarke, S., Day, J., Dixon, C., Frusher, S., Hobday, A., Hutchinson, N., Jennings, S., Li, X., Spooner, D., Stoklosa, R., 2011. Risk assessment of impacts of climate change for key marine species in south eastern Australia Part 2 : Species profiles.

Price, W.H., 2004. Phage formation in *Staphylococcus muscae* cultures: XI. The synthesis of ribonucleic acid, deoxyriboncleic acid, and protein in uninfected bacteria. J. Gen. Physiol. 35, 741–759. https://doi.org/10.1085/jgp.35.5.741

R Core Team, 2021. R: A language and environment for statistical computing.

Saunders, T.M., Connell, S.D., Mayfield, S., 2009. Differences in abalone growth and morphology between locations with high and low food availability: morphologically fixed or plastic traits? Mar. Biol. 156, 1255–1263. https://doi.org/10.1007/s00227-009-1167-4

Schaeffer, L., 2006. Strategy for applying genome-wide selection in dairy cattle. J. Anim. Breed. Genet.

Schipp, G., Bosmans, J., Humphrey, J., 2007. Barramundi Farming Handbook. Aust. Dep. Resour. North. Territ. 71.

Silvey, S.D., 2017. Statistical inference, Statistical Inference. Chapman and Hall. https://doi.org/10.1201/9780203738641

Tripp-Valdez, M., Bock, C., Lannig, G., Koschnick, N., Portner, H., Lucassen, M., 2019. Assessment of muscular energy metabolism and heat shock response of the Green Abalone *Haliotis fulgens* (Gastropoda: Philipi) at extreme temperatures combined with acute hypoxia and hypercapnia. Comp. Biochem. Physiol. Part B 227, 1–11.

Weigel, K.A., VanRaden, P.M., Norman, H.D., Grosu, H., 2017. A 100-Year Review: Methods and impact of genetic selection in dairy cattle—From daughter–dam comparisons to deep learning algorithms. J. Dairy Sci. 100, 10234–10250. https://doi.org/10.3168/JDS.2017-12954

Weller, J.I., 2006. Lessons for aquaculture breeding from livestock breeding, Israeli Journal of Aquaculture - Bamidgeh.

Wilks, S.S., 1938. The large-sample distribution of the likelihood ratio for testing composite hypotheses. Ann. Math. Stat. 9, 60–62. https://doi.org/10.1214/aoms/1177732360